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Toward a Theory of Help-Seeking Behavior for African American women survivors of intimate partner violence

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African American women overwhelmingly experience intimate partner homicide. Fundamental to improving their outcomes is understanding what precludes their help-seeking. This study employed constructivist grounded theory methodology to develop a model that explains the psychosocial processes of help-seeking among a vastly understudied, difficult-to-reach, marginalized population of African American women IPV survivors when they are seeking a solution to their abuse. Thirty in-depth interviews were conducted with women who were seeking assistance from the domestic violence service provision system and/or domestic violence ministries at their church. Data was collected during one interview between July and October 2019. The Transtheoretical Model of Change, Intersectionality and Agency were utilized as sensitizing concepts. Data was triangulated via demographic surveys, semi-structured interviews, and field notes. All eight techniques were employed to establish trustworthiness. Dedoose was utilized for data manaement. The Theory of Help Seeking Behavior (THSB) emerged from the data. This theory postulates that survivors' social context, specifically their experiences with racism, racial discrimination, and systemic racism, inform their perceptions about which supports were available to them. This nascent theory includes three constructs: 1. Social context; 2. Beliefs; and 3. Agency. To our knowledge, this is the first theory that seeks to provide a comprehensive understanding of African American IPV survivors' help-seeking process. Findings suggest that adverse interactions with providers within the domestic violence service provision system informed their evaluations about which services and supports were readily available to them. THSB is an initial step to developing culturally informed, comprehensive interventions designed to meet their nuanced needs.

About the National Institutes of Health Office of Research on Women's Health

Over 30 Years of Research for the Health of Women

Samia Noursi, PhD, *NIH Office of Research on Women's Health*

The NIH Office of Research on Women's Health (ORWH) serves as the focal point for women's health research at the National Institutes of Health (NIH). ORWH is the first Public Health Service office dedicated specifically to promoting women's health research within and beyond the NIH scientific community. The office also fosters the recruitment, retention, reentry, and advancement of women in biomedical careers. In partnership with the NIH Institutes, Centers, and Offices (ICOs), ORWH has been able to:

Transform the term "women's health" from a narrow conceptualization focused on reproductive health to a broader definition that considers all factors that influence the health of women, from head to toe, including internal and external factors across their life course.

Spearhead the development and implementation of the NIH Policy on Sex as a Biological Variable (SABV) to promote the study of female biology within preclinical research on vertebrate animals and humans. The policy requires investigators applying for NIH funding to incorporate sex into their research design or explain why it is not relevant.

Increase the inclusion of clinical research participants by sex and gender, race, ethnicity, and age to reflect the population that would ultimately benefit from medical interventions. ORWH guides scientists in the effective recruitment and retention of women participants in clinical studies, and now over half of participants in NIH-funded clinical trials are women. NIH has the ability to examine inclusion data by disease categories and provide these data in a publicly available database.

Accelerate research that supports the expanded view of the health of women by funding innovative mechanisms, such as the Specialized Centers of Research Excellence on Sex Differences (SCORE) program. SCORE focuses on sex differences and major medical conditions affecting women. Each center in the SCORE program serves as a national resource for translational research, at multiple levels of analysis, to identify the role of biological sex differences on the health of women. These NIH-supported Centers of Excellence are vital hubs for research on sex and gender that also provide pilot funding, training, and education. Through this support, established scientists conduct research that integrates basic, clinical, and translational approaches with a focus on sex and gender.

Issue a call to action on maternal morbidity and mortality (MMM) through enhanced research, as maternal health is a critical determinant of the health of women and future generations. ORWH has galvanized Federal and non-Federal stakeholders to promote research and collaborations related to MMM.

Enhance biomedical research by issuing supplemental funding to account for sex and gender influences in established and new research studies. ORWH has created mechanisms to encourage investigators representing all of NIH's mission areas to incorporate sex and gender considerations into their science.

Establish the U3 Administrative Supplement Program for research on **u**nderstudied**,** **u**nderrepresented, and **u**nderreported populations of women. Researchers focus on the effects of sex and gender influences at the intersections of social determinants of health, such as race, ethnicity, education, and socioeconomic status.

Women of Understudied, Underrepresented, and Underreported Populations

Discover best practices to support the advancement of women in biomedical careers, including mentoring. "Building Interdisciplinary Research Careers in Women's Health" (BIRCWH) is a mentored career-development program that connects junior faculty, known as BIRCWH Scholars, with senior faculty members who have shared interests in women's health and sex differences research.

Building Interdisciplinary Research Careers in Women's Health

Lead the NIH Working Group on Women in Biomedical Careers, which addresses barriers that hinder the advancement of women in science through innovative strategies.

Develop free interprofessional e-learning courses to promote the recognition of male–female differences during research and clinical training at all levels and across multiple disciplines. New courses include *Sex as a Biological Variable: A Primer*, with support from the National Institute of General Medical Sciences, and *Bench to Bedside: Integrating Sex and Gender to Improve Human Health, developed* in partnership with the Food and Drug Administration (FDA) Office of Women's Health.

Provide timely resources through a regularly updated website and a quarterly publication—both provide news on research, policy, legislation, and events related to the health of women.

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Sex Differences in the Effect of COX-2 Inhibition on Arterial Stiffness

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Cardiovascular (CV) disease occurs in females at an earlier stage of kidney disease compared to males. Cyclooxygenase (COX)-derived prostaglandins play an important role in the regulation of CV and kidney health. Animal studies suggest COX-2 inhibition is more detrimental to the male vasculature as compared to females, however whether that extends to humans is unknown. Therefore, we aimed to assess the effect of COX-2 inhibition on arterial stiffness, a validated marker of cardiovascular risk, in healthy males and females. Premenopausal females and males were studied at baseline and 14d after daily oral ingestion of 200mg celecoxib (COX-2 inhibitor). Arterial stiffness was measured using pulse-wave velocity (PWV) assessment of carotid and femoral arteries. Augmentation index (AIx) was calculated as the difference between the first and second aortic systolic peaks normalized to pulse pressure. Changes in PWV and AIx pre- and post- 14d celecoxib use were analyzed by sex. Thirteen females (mean±SD; age: 37.7±12.8 years, BMI: 24.9±3.9 kg/m^2) and 11 males (33.8±9.3 years, 26.2±3.4 kg/m^2) underwent assessment of arterial stiffness pre- and post- 14-day celecoxib use. Analyses are ongoing. AIx was significantly greater post- vs. pre-celecoxib use in males only (p=0.023). PWV did not differ pre-post celecoxib in either sex. The 14-day exposure change in baseline AIx was lesser (p=0.005) in females as compared to males. Female vasculature is more dependent on COX-2 prostaglandins than males. Kidney disease that interferes with prostaglandin production may contribute to the increased CV risk with early kidney disease in females. **Funding:** N/A

Serum Estradiol Levels and Cardiovascular Risk Associated with Gender- Affirming Hormone use in Transgender Women: A Systematic Review and Meta-Analysis

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Estrogen in the form of gender-affirming hormone therapy (GAHT) is associated with higher cardiovascular (CV) risk in transgender women (male at birth with female gender identity). However, it is not known whether these risks are associated with serum estradiol concentration. Our objective was to determine the association between serum estradiol levels and CV-related mortality, adverse CV events and surrogate markers of CV risk in transgender women. Three databases (MEDLINE, EMBASE, Web of Science) were searched from inception until present day for studies reporting on 1) transgender women and gender-diverse individuals; 2) estrogen GAHT independently or in conjunction with other GAHT; 3) reported serum estradiol levels; 4) reported CV outcomes (mortality, events, surrogate measures) and 5) are were original studies (control trial, cohort or cross-sectional study designs). Of the 7,213 articles identified, 22 articles (12 cohort, 10 cross-sectional) ranging from 1999 to 2020 representing 1,425 transgender women and 2 gender-diverse individuals aged 16 to 59 years old using oral (n=755), sublingual (n=23), transdermal (n=178), intramuscular (n=134) or unclear (n=335) routes of GAET prescriptions were included. The majority (64%) were Caucasian, with high rates of depression and anxiety (18%), and smoking (18%) reported. No articles reported on CV-related mortality or adverse events. Articles reported on blood pressure (n=11), lipid levels (n=17), body mass index (n=19), insulin resistance (n=5), fasting glucose (n=9) and insulin (n=5) levels ranging from 0 to 60 months post-GAHT commencement. Serum estradiol levels varied widely with prescription type, dose, route of administration and gender-identity goals. Meta-analyses and assessment of heterogeneity of surrogate markers of CV risk stratified by serum estradiol quintiles are currently underway. The majority of presented articles represent fair to poor-quality data. Through quantifying the association between serum estradiol levels and markers of CV risk, these results will inform the use of estrogen GAHT in transgender women and gender-diverse individuals. Funding: This study is unfunded.

The Maternal X Chromosome Impairs Cognition and Accelerates Brain Aging Through Epigenetic Modulation in Female Mice

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The X chromosome is enriched for cognition-related genes – and X-linked disruptions often cause intellectual disability. Female mammalian cells have two X chromosomes, one from mom and one from dad. During development, one X randomly inactivates. This renders either the maternal or paternal X active, causing X mosaicism, with some females showing considerable or complete skew. Parent-of-X-origin can modify epigenetics via DNA methylation and possibly gene expression; thus, mosaicism could buffer dysregulated processes in aging and disease. However, whether X skewing – or its mosaicism – alters functions in females is largely unknown. Using transgenic mice skewed toward the maternal X (Xm) and their wildtype (Xm+Xp) litter-mate controls, we tested whether the maternal X influences key functions of the body. Among cardiac, bone, metabolic, and brain functions, Xm selectively impaired cognition in female mice across the lifespan. Cognitive deficits were accompanied by Xm-mediated acceleration of aging of the hippocampus, a key center for cognition. Xm showed epigenetic imprinting of several genes within hippocampal neurons, suggesting silenced cognitive loci. Thus, Xm impaired cognition, accelerated brain aging, and silenced genes. Understanding how Xm impairs brain function could increase understanding of female heterogeneity in cognitive heath and unlock new X-derived pathways against cognitive deficits and brain aging. Funding: This study was funded by NIH grants NS092918 and AG068325, AFAR, and philanthropy to DBD.

Influence of Sex and Sex Hormones on a Mouse Model of Multi-Etiology Dementia

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Biological sex is a well-known risk factor for dementia however it varies depending on the dementia type. Indeed 2/3 of those with Alzheimer's disease are women however men are more affected by vascular contributions to cognitive impairment and dementia (VCID) throughout most of their lifespan. Up to 80% of AD patients suffer from VCID resulting in multi-etiology dementia (MED). In this study we examine sex differences in the effect of gonadal hormones using the App NL-F knock-in Alzheimer's disease mouse model that do not overexpress amyloid precursor protein but instead expresses human App mutations under the endogenous mouse promoter. 5-6 months old male and female App NL-F/NL-F mice were subjected to gonadectomy or sham surgery and left to recover for 3 weeks to clear any endogenous gonadal hormones. MED was modeled using chronic cerebral hypoperfusion (unilateral carotid artery occlusion). Control animals (AD only model) received a sham surgery. Mice were then subjected to a battery of behavioral tests before being euthanized and brains and serum were collected. Using an open field test, sex differences were found in activity levels but not anxiety like behavior, in which females traveled longer distances then males but spent equivalent time in the center regardless of dementia type or state of gonadectomy. However, MED males displayed better episodic-like memory than females in a novel object recognition test. These findings highlight the importance of studying sex as biological variable in preclinical models and the need to model endocrine aging in animal models of dementia. Funding: This study was funded by NINDS/NIA R01 NS110749

Mechanistic Heterogeneity in Human Male and Female Neutrophil Responses to a Chemorepellent

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A considerable amount is known about how eukaryotic cells move towards an attractant, while relatively little is known about chemorepulsion, where cells move away from a repellent signal. We previously found that the peptide SLIGKV, an agonist of Protease Activated Receptor 2, acts as a neutrophil chemorepellent in both human and mouse models. Our objective is to developed an inhaled neutrophil chemorepellent as a therapeutic for neutrophil-driven lung diseases, such as Acute Respiratory Distress Syndrome. To determine how SLIGKV induces chemorepulsion, human neutrophils were treated with inhibitors of potential signal transduction pathway components, and then assayed by videomicroscopy for chemorepulsion from SLIGKV. Surprisingly, inhibiting Rho-associated Kinases 1 and 2 or Cdc42 potentiated male neutrophil chemorepulsion from SLIGKV, but caused female neutrophils to be attracted to SLIGKV. Male and female neutrophils also showed differences in persistence of movement, cell adhesion, polymerized actin accumulation, and cytoskeletal actin and myosin light chain localization during chemorepulsion. Proteomics showed 36 proteins with significant differences in abundance between male and female neutrophils, including five proteins potentially associated with chemorepulsion pathways, and three of these five also show differences in localization in male and female neutrophils. In conclusion, we have identified human neutrophil chemorepulsion mechanisms and differences between human male and female neutrophils that cause a mechanistic heterogeneity in chemorepulsion, a motility mechanism vital to the innate immune system. **Funding:** This work is supported by NIH grant GM139486.

Characterization and Anti-Inflammatory Effects of a Novel Subtype of Regenerative Exosomes in Viral Myocarditis

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Myocarditis is an inflammatory cardiomyopathy commonly caused by viral infections. It is a leading cause of sudden death and can progress to other serious, chronic conditions like dilated cardiomyopathy and/or heart failure. More men than women develop myocarditis with a sex ratio of 3.5:1 male to female. In animal models of viral myocarditis, cardiac inflammation is more severe in male than female mice and males progress to more severe dilated cardiomyopathy. We published previously that heart failure based on a heart failure biomarker, soluble ST2, occurred more often in men under the age of 50, while women were more protected. With no current disease-specific therapies for myocarditis, there is high interest for novel therapy development. Because premenopausal women are more protected from myocarditis, we injected a premenopausal purified exosome product (pmPEP) into BALB/c male mice vs. PBS control intraperitoneally on day -1, 0, 1 of viral infection (day 0). Mice were harvested on day 10 at the peak of acute myocarditis. We found significantly less myocardial and pericardial inflammation with pmPEP than controls, indicating that the exosome treatment reduced myocarditis severity. Subsequent RT-qPCR analysis revealed a global downregulation of the immune response to infection with decreased T cells, macrophages, complement components, and mast cells. These data suggest that exosomes from the plasma of premenopausal women may be a novel treatment for the acute phase of viral myocarditis. Future studies will explore more clinically-relevant timelines for administration of the therapy.

Aging Awakens Transcription of the Inactive X Chromosome (Xi) in Cells of the Female Hippocampus

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In aging, females show advantage in lifespan and cognitive deficits that may be linked to a second X chromosome. One of the two X chromosomes in females (XX) randomly inactivates resulting in an active (Xa) and inactive (Xi) X. At baseline, some genes from Xi can escape leading to higher expression in female compared to male cells. Since aging induces loss of epigenetic repression of the genome, we wondered whether aging increases X dose in females by reactivating previously silenced Xi genes in the brain. To identify and characterize the transcriptional signatures of Xi and Xa during female brain aging, we used a mouse assay based on strain-specific detection of differing SNPs. Next, we performed single-nucleus RNA sequencing to profile the young and aged female mouse hippocampus, a brain region vulnerable to cognitive decline. We then used bioinformatic tools to assess patterns governing baseline gene escape and age-induced reactivation of Xi. We observed expected cell types of the hippocampus, which clustered in distinct transcriptomic profiles in both young and old female brains. Aging significantly increased expression of four Xi genes in a cell type-specific manner. Three of these genes cluster together on the X chromosome, suggesting common mechanisms of reactivation. Future studies will assess the top baseline and reactivated Xi genes using in vivo and in vitro models to test for their contribution to female resilience against age-related cognitive decline. Understanding how Xi may confer female advantage, and specifically how it is regulated throughout the lifespan, may lead to novel therapeutic targets to prevent brain aging in both sexes. Funding: This study was funded by the Bakar Aging Research Institute (CKS) and NIH grants NS092918 (DBD) and AG068325 (DBD), the Simons Foundation (DBD), the Coulter-Weeks and
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Sex Differences in the Effects of High Fat Diet on Neuroinflammation in a Mouse Model of VCID

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Neuroinflammation plays a central role in the progression of vascular contributions to cognitive impairment and dementia (VCID). Unfortunately, anti-inflammatory therapeutics have been insufficient at slowing the progression of VCID, maybe because broadly suppressing inflammation inhibits its beneficial functions. Instead, supporting endogenous inflammation resolving programs may prove more effective. Midlife metabolic disease is a major risk factor for VCID, particularly in women. We examined neuroinflammation at midlife in males and females with VCID. We modeled metabolic disease via long-term high fat (HF) diet, and VCID via unilateral carotid artery occlusion which results in chronic cerebral hypoperfusion. We hypothesized that metabolic disease would lead to more adverse cognitive and neuroinflammatory effects in middle-aged females than males with VCID. 8.5 month mice received a HF or control diet for 7 months. At month 3 they received VCID or sham surgery. At month 6 cognitive function was assessed. Brains were collected for histology and for qPCR analysis of inflammatory and pro-resolving mediators. Spatial memory was impaired in VCID males, regardless of diet; however, in females both HF diet and VCID (or a combination of the two) impaired spatial memory. In males, HF diet increased hippocampal microglia activity; HF diet with VCID increased expression of inflammatory and pro-resolving mediators. However, in females, HF diet suppressed hippocampal microglia activity and decreased inflammatory and pro-resolving mediators. In line with clinical findings, these data suggest that midlife metabolic disease increases cognitive deficits to a greater extent in females than males. Our data also suggest VCID with metabolic disease increases neuroinflammation and its resolution in males, but not females. This highlights the need for a better understanding of the sex differences in neuroinflammation following vascular injury. Funding: Funded by an American Heart Association Scientist Development Grant 12SDG2719002 (KLZ), NINDS/NIA R01NS110749 (KLZ), Albany Medical College Start Up Funds (KLZ)

Adolescent social isolation disrupts sex-specific food reward and developmental dopaminergic and thyroid hormone expression profiles in the medial amygdala of adult mice

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Adolescence is associated with the onset of sex differences in palatable food intake and an increased sensitivity to reward and stress. Given the role of dopamine and thyroid hormones in food intake and reward, transcriptional alterations within these systems may play a role. To test this hypothesis, we examined how adolescent social isolation (SI) influences sex-specific food reward behavior and developmental profiles of dopaminergic and thyroid hormone receptor gene expression (Drd1, Drd2, Thrα and Thrβ) in the medial amygdala (meA), a brain region crucial for regulating sex-specific food reward. Female and male mice were exposed to SI or group housed during adolescence (P22-P42) and then group housed until adulthood (P90). In adulthood, animals were tested in novelty suppressed feeding (NSF) and palatable food (PF) intake as measures of food reward-related behavior. Gene expression in the meA was measured via RT-qPCR from pre-adolescence into adulthood (P22, P32, P42 and P72). Preliminary data show that sex differences in NSF and PF intake (F>M) were lost following adolescent SI and that SI increased sex differences in Drd1 and Drd2 gene expression on P32 and flipped sex differences in Drd1 expression in adulthood. SI also suppressed/blocked sex-specific developmental increases in THRα and β. These data suggest that adolescent SI disrupts adult sex-specific food-reward-related behavior and developmental profiles of dopaminergic- and thyroid-receptor gene expression in the meAMY which may promote long-lasting synaptic and behavioral adaptations associated with overeating of palatable food.

Brain-Gut-Microbiome Signatures Differentiate Bowel Habit Subtypes In Women With IBS

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Alterations in the brain-gut-microbiome system (BGM) have been implicated in the pathophysiology of irritable bowel syndrome (IBS), a female-predominant disorder of gut-brain interaction. However, bowel habit related alterations in these interactions have not yet been elucidated. We apply a systems biology approach, leveraging clinical, neuroimaging, and fecal microbiome data to investigate our hypothesis: that IBS bowel habit subtypes have distinct BGM signatures in women. Fecal samples and resting state fMRI images were obtained from 102 premenopausal women: 36 Rome+ constipation-predominant IBS (IBS-C), 27 diarrhea- predominant IBS (IBS-D) and 39 healthy controls (HCs). sPLS-DA, ANOVA, and DeSeq were run to explore group differences, clinical variables, and microbiome differential abundance, respectively, with q-significant (FDR) corrected for age, BMI, and diet. An integrative analysis of the clinical, brain, microbiome, and metabolite data evaluated associations between the variables. Compared with IBS-C, IBS-D had higher connectivity involving regions of the central autonomic, emotional arousal, and central executive networks. Fecal histidine and glutamate pathway metabolites were significantly higher in IBS-C compared to HCs. Succinimide and N- acetylthreonine metabolites were associated with brain changes in the sensorimotor network in IBS-C, while 2-aminoadipate, 4-ureidobutyrate and fucose metabolites were associated with brain changes in the central executive, occipital, and default mode networks in IBS-D. Our findings suggest that the interactions between sensorimotor, emotional, and autonomic brain networks and selected gut metabolites may contribute to predominant IBS bowel habits in women, consistent with a bowel habit specific BGM model of IBS. Funding: This research was supported by grants from the National Institutes of Health including K23 DK106528 (AG), ULTR001881/DK041301 (UCLA CURE/CTSI Pilot and Feasibility Study; AG), U54 DK123755 (EAM/LC), P50 DK064539 (EAM), R01 DK048351 (EAM), P30 DK041301 (CURE), and pilot funds provided for brain scanning by the UCLA Ahmanson-Lovelace Brain Mapping Center.

The Effect of Targeted State Policies on Mental Health Distress among Sexual and Gender Minority Adults in the United States

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Background: Sexual and gender minority (SGM) adults carry a disproportionate U.S. burden of mental distress and related morbidity and mortality. Researchers theorize that stigma, in the form of sexual-orientation and gender-based policies, drives SGM mental health disparities. We evaluated the effect of targeted policies on mental health distress among SGM adults in the U.S. **Methods:** We aggregated acute mental health distress and covariate data on SGM adults from the 2019 Behavioral Risk Factor Surveillance System and linked these data to state-level policy scores from the Human Rights Campaign's 2018 State Equality Index. We used a causal inference framework to produce a targeted maximum likelihood (TMLE) estimate of the average treatment effect comparing those living in high vs. low SGM protections states. **Results:** Our final sample comprised 14,792 SGM adults across 30 states. The survey-weighted and adjusted TMLE estimate was borderline statistically significant, signaling a decreased mental health distress risk in 2019 for SGM adults living in states with high versus low SGM policy protections in 2018 (estimate: -1.5%, 95%CI: -3.2%,0.2%). **Discussion:** If identifiability assumptions hold, the mental distress risk would be 1.5% lower under an intervention in which all SGM adults lived in states with high versus low SGM policy protections, which could mean better mental health for over 275,000 SGM adults. Higher-powered, longitudinal analyses are needed to determine the extent to which protective SGM polices reduce mental distress related morbidity and mortality for SGM adults.

Mechanisms of Sex Differences in Changes in Cardiorespiratory Fitness with Exercise Training

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Increasing cardiorespiratory fitness ($\dot{V}O_2$peak) improves prognosis in patients with cardiovascular disease (CVD). Emerging data suggest females with CVD experience smaller increases in $\dot{V}O_2$peak after exercise training than males. Our review summarizes the potential physiological mechanisms of this sex disparity, an understudied topic. The pathways to increase $\dot{V}O_2$peak differ by sex; females rely more on peripheral adaptations (increased arterio-venous O_2 difference), while males on central adaptations (increased cardiac output, $\dot{Q}$). Inherent biological sex differences may enhance health benefits from exercise in males and lessen these improvements in females. For example, higher testosterone in males facilitates greater exercise-induced cardiac and skeletal muscle hypertrophy. Following menopause, females experience greater impairments in vascular (increased arterial stiffness, decreased eNOS), mitochondrial (decreased PGC-1α, AMPK; more reactive oxygen species) and cardiac (decreased left ventricular [LV] relaxation) function. The exercise-induced vasodilation and increase in LV hypertrophy and contractile function are blunted in older females than males. Females' expansion in blood volume is also smaller due to an impaired baroreflex sensitivity, reducing venous return at a given cardiac preload. When combined with a smaller increase in LV mass/size, females experience smaller improvements in the Frank-Starling curves and subsequent changes in stroke volume, $\dot{Q}$ and $\dot{V}O_2$peak with exercise training. Females' greater health impairments with aging may reduce their exercise trainability. We could speculate that females with CVD may need a higher exercise volume to counteract this natural phenomenon, which contrasts the conservative approach traditionally used to prescribe exercise in females with CVD. Clinical exercise research must take sex into consideration to further clarify sex-specific physiological mechanisms.
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Post-menopausal impairment in brain arteriolar K^+ channel function in a mouse model of Alzheimer's disease

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Cognitive decline is linked to decreased cerebral blood flow, particularly in post-menopausal women. Impaired cerebrovascular function occurs during Alzheimer's disease (AD), likely due to reduced endothelial function in parenchymal arterioles through incompletely described mechanisms. The goal of this study was to determine whether menopause impairs parenchymal arterioles function of wild-type (WT) and *5x-FAD* mice. Menopause was induced by the 4-vinylcyclohexene diepoxide (VCD) model; parenchymal arterioles were studied by pressure myography. Data are means ± SEM, vehicle vs VCD. In WT mice, VCS caused a significant increase in myogenic tone (% tone: 26.4 ± 3.3 vs. 33 ± 2.3%, n = 7 / 10; $p<0.05$), and a significant reduction in resting lumen diameter (35.7 ± 1.6 vs. 29.9 ± 1.9 µm, n = 9 / 11; $p<0.05$). We then tested endothelial function, focusing on K^+ channels, SK_{Ca} / IK_{Ca} and $K_{IR}2$. VCD did not affect arteriolar dilation to the SK_{Ca}/ IK_{Ca} activator NS-309 (at 1 µM, vasodilation: 23.49 ± 11.5 vs. 17.80 ± 1.7%, n = 8 / 8). Similarly, $K_{IR}2$ function was unchanged (vasodilation: 13.7 ± 3.5 vs. 17.9 ± 3.5%, n = 8 / 10). In *5x-FAD*, VCD did not alter myogenic tone (% tone: 23.49 ± 2.2 vs. 28 3.2%, n = 10 / 7), but significantly decreased resting lumen diameter (39.16 ± 2.6 vs. 27.79 ± 2.0 µm, n = 10 / 7 $p<0.05$). VCD significantly blunted response to NS-309 in *5x-FAD* (at 1 µM, vasodilation: 14.96 ± 2.9 vs. 9.42 ± 1.4%, , n = 7 / 4, $p<0.05$), without affecting $K_{IR}2$ (vasodilation: 16.02 ± 1.3 vs. 13.69 ± 2.3%, n = 9 / 6). In conclusion, menopause changes myogenic tone in WT mice without affecting endothelial K^+ channel function. However, in the *5x-FAD*, menopause impairs endothelial SK_{Ca}/IK_{Ca} channels independently of myogenic tone.

Optogenetic Stimulation of BNST Vasopressin Neurons Increases Sex-Specific Social Approach and Communication in Mice

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The neuropeptide arginine-vasopressin (AVP) has long been implicated in the regulation of social behavior and communication, often sex-specifically, but the source of AVP release relevant for behavior has not been precisely determined. AVP cells in the bed nucleus of the stria terminalis (BNST) are a major source of sex-different AVP expression in brain regions associated with social behavior. Consequently, to define the behavior-relevant sources, we bilaterally injected AAVs that express Cre-dependent channelrhodopsin-2 (ChR2; EF1a-DIO-hChR2(H134R)-YFP) for cell excitation or Cre-dependent fluorescent label only (YFP; EF1a-DIO-YFP) as a control, into the BNST of adult AVP-iCre+ male and female mice. After recovery, subjects underwent a total of four tests for social communication (scent marking, ultrasonic vocalizations) and social investigation in a three-chamber apparatus. Each subject received two test days with light stimulation and two test days without light stimulation with each stimulus type (male and female conspecifics). Finally, mice were tested on an elevated-zero maze (EZM) for anxiety-like behavior. Preliminary results indicate that, in male mice, stimulation of BNST AVP- expressing neurons increases social investigation of male and female conspecifics and increases male scent marking toward female conspecifics. Additionally, stimulation of these neurons decreases male anxiety-like behavior in the EZM. In females, stimulation of BNST AVP neurons did not alter any behaviors measured. These results point to differential involvement of BNST AVP neurons in male social behavior and communication. Similar sex differences in the neurochemical underpinnings of behavior may contribute to sex differences in disorders of social behavior and communication. Funding: Research supported by National Institute of Mental Health (NIMH) of the National Institutes of Health under award number [R01 MH121603]; Ruth L. Kirschstein National Research Service Award (NRSA) Individual Predoctoral Fellowship [F31MH125659]

Postnatal Developmental Trajectory of Sex-Biased Gene Expression in the Mouse Pituitary Gland

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The pituitary gland regulates biological processes which are sex-biased in their activity, including growth, stress response, metabolism, reproduction, and puberty. The clinical presentation and prevalence of pituitary-related disorders also differs between sexes. However, the mechanisms underlying sex biases in pituitary biology are not well understood. To address this, we profiled gene and microRNA expression in the mouse pituitary gland at five postnatal ages spanning pubertal transition. We found over 900 instances of sex-biased gene expression, with most sex differences occurring with the onset of puberty, including 18 genes that are predicted targets of sex-biased microRNAs at matched ages. By combining single-cell pituitary transcriptome with bulk gene expression profiles, we found that sex differences in pituitary cell-type proportions likely arise across puberty and can contribute to observed sex-biased gene expression in the pituitary. Overall, we report an emergence of sex bias in both gene regulation and cell-type proportions in the pituitary across puberty, underlying the mechanisms driving sex-biased pituitary biology observed later in life. We will next address the contributions of sex chromosomes and gonadal hormones to sex-biased pituitary cellular proportions and gene expression by profiling the pituitary of four-core genotype mice using single-cell RNA-seq. Funding: This work is supported by CIHR funding to MDW and MRP.

A comparison of prediction equations for estimating glomerular filtration rate in transgender individuals on gender affirming hormone therapy: A protocol

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Background: Estimated glomerular filtration rate (GFR) equations use sex/gender as a covariate and have been developed and validated in cisgender (sex assigned at birth aligning with gender identity) populations. Transgender (sex assigned at birth not aligning with gender identity) individuals are commonly treated with gender-affirming hormone therapy (testosterone or estrogen), resulting in changes in serum creatinine, a biomarker of kidney function used to estimate GFR. The accuracy of estimated GFR equations in the transgender population treated with gender-affirming hormone therapy is unknown. Purpose: To compare existing glomerular filtration rate prediction equations with the gold standard measure of glomerular filtration, iohexol clearance, in the transgender population using gender-affirming hormone therapy. Description: Transgender men and women □18 years using gender-affirming hormone therapy for >2 years will be recruited. Five equations to predict estimated GFR (24h urine creatinine clearance, 4 variable Modification of Diet in Renal Disease (MDRD), CKD-EPI creatinine, CKD- EPI cystatin-C, and the CKD-EPI creatinine-cystatin C equations) will be employed, and a result will be calculated for each using 1) sex assigned at birth and 2) gender identity. Each result will be assessed for precision, bias, and accuracy of predicting GFR, measured by iohexol clearance. Implications: This study will inform optimal estimation of kidney function in the transgender population, which will guide clinical decision-making including nephrology consultation, medication dosing, multidisciplinary kidney disease care, referral for kidney transplantation, and timing of dialysis initiation.

Systems biology of variation in *Drosophila* sex-differential fat storage

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Research into sex differences relies upon statistical tests of mean values measured in males and females, with results considered to be representative of all individuals within a species. However, in studies of model organisms where large numbers of genetic backgrounds are measured in the same environment, the magnitude and even the direction of sexual dimorphism can vary among genotypes. This pattern is the rule rather than the exception in the Drosophila Genetic Reference Panel (DGRP), a set of 200 inbred fly lines. These interactions between sex and genotype indicate that natural genetic variation exists for sexual dimorphism itself. Here, we use a systems biology approach to understand the biological basis for that variation. We focus in particular on body fat, a highly dimorphic trait in both flies and mammals, and a critical mediator of energy availability and expenditure. The metabolome, composed of small molecules that make up the molecular building blocks in the body, is often a better predictor of phenotypic variation than is genetic variation. This study aims to investigate whether the metabolome can explain and predict variation in sex-specific and sex-differential fat storage among DGRP genotypes. We measure triglyceride (TAG) levels of 100 DGRP lines, selecting groups of lines for which TAG levels are highly dimorphic (HD) or not significantly dimorphic (ND) between males and females. As body TAG levels are partly under neuronal control, we measure the head metabolome of HD and ND groups to identify pathways associated with variation in sex-differential fat storage. The findings of this study will contribute to a better understanding of how sex modifies adiposity, and thus may identify important considerations for future studies of sex differences in metabolic disorders across the lifespan. **Funding:** This study is funded in part by the Biological Mechanisms for Healthy Aging Training Grant NIH/NIA T32 AG066574.

Track-by-Day: A Standardized Approach to Estrous Cycle Monitoring in Biobehavioral Research

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The majority of preclinical neuroscience studies continue to use only male subjects despite well- documented sex differences in the brain and the clear value of female subjects in certain areas such as anxiety-related disorders, which disproportionally affect women. Concerns about female subjects increasing experimental variability are driven in part by conflicting reports of the effects of sex, and especially of the estrous cycle. Inconsistencies in the estrous cycle literature may arise from a lack of universal reporting standards, which also serves as a barrier to entry for researchers wishing to study females. There is an unmet need to standardize estrous cycle staging and reporting in rodents and increase accessibility for scientific disciplines outside of reproductive physiology. In this study, we collected and analyzed vaginal cytology for over one hundred female rats, across a variety of conditions, to assess variability and cyclicity of quantifiable features of the estrous cycle within and between subjects. Desquamation of keratinized vaginal epithelium is an established indicator of cytological turnover, traditionally marking the estrus phase. This phase lasts roughly one day and is rapidly identifiable and reliably cyclic across conditions. Therefore, we suggest standardization of the estrous cycle via tracking-by-day, using this cytological biomarker to indicate Day 1. This approach is further supported by our results from an aversive Pavlovian conditioning study (n=84), showing freezing levels are significantly lower in females on Day 3 of the estrous cycle, compared to males and females on other days of the cycle. Our method can be used to simplify experimental planning for those who wish to account for the estrous cycle, or to aid in the interpretation of female data post-hoc. We provide our recommendations for adoption of universal standards to report estrous staging data and hope to ultimately foster inclusion of female animals in neuroscience. Funding: This study is funded by a graduate fellowship grant from The Connecticut Institute of Brain and Cognitive Sciences and the National Science Foundation (NSF 2014862).

Sex Differences in Mitochondria during Viral Myocarditis

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Myocarditis is an autoimmune disease caused by viral infections that localize to the heart and affects 3.5 times more men than women. Mitochondria are abundant in cardiomyocytes and regulate processes beyond the energetic demands of the cell. Biological sex affects properties related to mitochondria; males have more mitochondrial content than females, but females have more robust anti-oxidant responses. Because mitochondria comprise a significant portion of cardiomyocytes, are essential for normal cardiac function, and have not been thoroughly explored in the context of viral myocarditis, we investigated how mitochondria may differ in male and female Balb/c WT mice during acute myocarditis. Using pathway enrichment analysis, we analyzed bulk-tissue RNA-sequencing data from the hearts of coxsackievirus B3 infected male and female mice and healthy controls. We found that hearts from infected female mice are enriched for pathways involving mitochondrial repair, homeostasis, and anti-oxidant responses compared to males with myocarditis. Upstream regulatory analysis with TRANSFAC suggested that estrogen-related receptors (ERRs) co-regulate a sex difference in mitochondrial respiratory complex assembly with PGC1alpha; both ERR1 and PGC1alpha are significantly more expressed in females compared to males with myocarditis. Mice with myocarditis have decreased mitochondrial size during acute myocarditis indicating fission, a process mediated by the protein DRP1 during mitochondrial stress. Western blots showed that males have higher activation of DRP1 and expression of Parkin, suggesting that male mitochondria are more damaged than females. These data demonstrate that sex differences exist in mitochondrial homeostasis during viral myocarditis. **Funding:** Funded by the National Institutes of Health [R21 AI145356, R21 AI152318, & NIH training grant TL1 TR002380]

Evaluating Endocrine-Immune Interactions on Social Behaviour in a Rodent Model of Maternal Immune Activation

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Maternal immune activation (MIA) can profoundly influence fetal neurological and behavioral development. In rodents, MIA triggered by prenatal administration of polyinosinic-polycytidylic acid (Poly IC) results in a sex bias where male mice demonstrate greater susceptibility than females to social behavior deficits. Here, we investigated the role of prenatal androgens in MIA-associated social deficits. In a pilot study, we asked whether social deficits following MIA in C57BL/6 mice would be greater during the prenatal androgen surge (embryonic day (E) 16.5) versus before the surge (E12.5). Neonatal ultrasonic vocalizations (USV) were recorded at postnatal day (PND) 6 in response to brief maternal separation. Analysis of vocalizations revealed that MIA at E16.5 resulted in greater alterations in call frequency compared to MIA at E12.5 and controls, suggesting a relationship between androgens and MIA for social deficits. In an ongoing experiment, we administered either an oil vehicle or testosterone propionate (TP) to dams or pups at E16, E18, and E20, and PND1 timepoints, and a saline vehicle or Poly IC at E12.5 or E17.5 to assess whether hyperandrogenization exacerbates MIA-associated social deficits. Our behavioral battery consists of neonatal USV recordings in response to maternal separation, juvenile USV in response to same and other sex conspecifics, marble-burying, and a three-chamber sociability test. If androgens interact with immune activation to increase neurodevelopmental deficits, TP administration following MIA is expected to increase social deficits in both sexes. These findings will determine whether prenatal androgen exposure increases social behavior deficits following MIA, which may contribute to our understanding of the role of sex and sex hormones in sex biased neurodevelopmental conditions such as autism spectrum disorder. **Funding:** This study was funded by a Discovery Grant from the Natural Sciences and Engineering Council of Canada (NSERC) to Dr. Ashlyn Swift-Gallant.

Sex Differences in Aging and in the Regenerative Effects of iPSC-Derived Immune Cells

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Several studies have demonstrated the ability of young blood or plasma to restore cognitive function in aged animals, and our lab has shown that bone marrow transplants from young to aged mice has beneficial effects on cognition and neural health. However, these strategies have significant practical drawbacks that limit their potential therapeutic value. Induced pluripotent stem cells (iPSCs) could provide an autologous therapy. Thus, we sought to identify the cell type responsible for the beneficial effects observed in studies using young plasma and bone marrow. We differentiated iPSCs into macrophages (iMACs) and administered these cells to aged, genetically immunocompromised, male and female NOD-scid-gamma (NSG) mice via tail vein injection. Our results demonstrate substantial sex differences in the effects of aging on cognitive performance, with male mice demonstrating greater age-associated deficits on spatial memory tasks. However, iMAC treatment significantly improved cognition, specifically in aged male mice. We also examined several key neuronal health indices and found that iMAC treatment and sex had effects on the synaptic transporter, VGLUT1, as well as on astrocyte and microglial numbers and morphology. These results demonstrate that iMACs have significant regenerative potential, offering a promising new therapeutic strategy. Importantly, the finding that the effects of both aging and iMAC treatment differ between males and females highlights the necessity of considering sex as a variable in therapeutic development and, more broadly, in aging research. **Funding:** Cedars-Sinai Center for Women's Health and Sex Differences; Cedars-Sinai Board of Governors Regenerative Medicine Institute

Sex differences in neural activation related to negative cognitive bias after chronic stress exposure

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Major depressive disorder (MDD) is more common in women than in men, and women have more severe symptoms of MDD. Cognitive symptoms of MDD include negative cognitive bias. Negative cognitive bias is an increased perception of neutral situations as negative. MDD can be modelled in rodents using a chronic unpredictable stress (CUS) paradigm. We examined whether there were sex differences in cognitive bias after CUS and measured neural activity in the amygdala and nucleus accumbens via c-Fos protein expression. After 16 days of training to discriminate between a shocked context (context A) versus a non-shock context (Context B), rats were placed in an ambiguous context (context C) and tested for cognitive bias based on the amount of freezing behavior. Male and female rats exposed to CUS displayed a greater negative cognitive bias (potentiated freezing behavior in an ambiguous context) compared to non-stressed (NS) rats. CUS males had higher neural activity in the amygdala compared to NS males, which was not seen in females. Furthermore, freezing behavior was associated with the higher neural activity in the central amygdala of NS males but in the lateral amygdala of CUS males. Females had higher neural activity in the nucleus accumbens compared to males regardless of CUS. These findings suggest a greater role of the nucleus accumbens in the cognitive bias females and of the amygdala in the negative cognitive bias of males after CUS. Our findings illustrate that sex must be considered when investigating the neurobiology of depressive-like endophenotypes. Funding: CIHR MOP 142328 held by LAMG

Binge Ethanol in Adolescence vs Adulthood: Differences in Spatial Memory, Ethanol Sedation, and Ethanol Metabolism

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While both adolescents and adults engage in binge drinking, adolescents show fewer adverse physiological effects while presenting greater memory deficits. Additionally, conflicting results regarding blood ethanol concentration (BEC) between the age groups have been reported. Sex also impacts BEC, with women showing higher BEC when given the same weight-adjusted volume as men, yet rodent studies have shown no sex effect. We hypothesize adolescent ethanol will negatively impact spatial memory, cognitive flexibility, and ethanol sedation while adult ethanol exposure will not. Sex will impact BEC with females showing higher BEC at both ages. Male and female adolescent DBA/2J mice were exposed to a intermittent binge ethanol model of either water or 4g/kg ethanol from postnatal day (PND) 29-42 (ae). Adults underwent the same dosing paradigm from PND 64-77 (AB). After a 3-week abstinence period, mice were tested for spatial memory, and cognitive flexibility via Barnes Maze. Another cohort was tested for ethanol sedation via loss of righting reflex (LORR), one month after the last dose. A behavioral and ethanol naïve cohort was given 4g/kg of ethanol via gavage at PND 35 or 65 and blood was collected over 4 hours. ae mice showed decreased spatial memory but no difference in cognitive flexibility, while AB mice showed no differences. Ethanol sedation showed females regardless of age slept less than males. BEC differed due to a timepoint X age interaction with ae mice regardless of sex showed a consistent decline in ethanol concentration over time. Adult mice showed an initial rise in ethanol concentration during the first hour, followed by a decline. This data suggests ethanol metabolism differs in mice by age but, despite higher BEC in adult mice, memory performance is unaffected. The higher BEC in adult females initially at 1 hour but the same ethanol levels at 4 hours suggests a sex difference in ethanol metabolism. Funding: Supported by NIAAA R01AA026347 (JTW) and NIAAA F31AA029305 (MAMB).

Serum Testosterone and Cardiovascular Risk in Adult Transgender Men: A Systematic Review and Meta-Analysis

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Transgender men (assigned female at birth who identify as men) are at increased cardiovascular risk. Elevated testosterone levels are associated with increased cardiovascular risk in cisgender women (assigned female at birth who identify as women), though whether this applies to transgender men is unknown. We aimed to determine the association between serum testosterone levels and cardiovascular morbidity and mortality in transgender men on gender-affirming testosterone therapy. Electronic bibliographic databases (MEDLINE, Embase, and PsycINFO) from inception to July 30, 2021 were searched. Studies were eligible for inclusion if they included: 1) transgender men ≥18 years old; 2) gender-affirming testosterone therapy (any formulation, dose, route); 3) serum testosterone levels; and 4) cardiovascular-related morbidity and/or mortality (e.g., myocardial infarction, stroke, mortality or surrogate measures of cardiovascular disease [e.g., blood pressure, lipids]). Eligible study designs included randomized controlled trials and observational studies. 7,354 abstracts met inclusion criteria, 153 full text studies assessed for eligibility, 45 of which were deemed eligible. No studies reported on cardiovascular events or mortality, but did report surrogate measures of cardiovascular risk (i.e. body mass index). Meta-analysis is ongoing. Data will be summarized using random effects model on standardized mean differences in surrogate markers of cardiovascular risk stratified by serum testosterone tertiles. Understanding the association between serum testosterone levels and cardiovascular morbidity and mortality in transgender men will inform shared decision-making regarding gender-affirming testosterone therapy.

The Reversal of Antibiotic-Induced Socio-Sexual Behavioral Deficits via Cecal Microbiota Transplantation but Not Androgen Treatment

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Recent evidence suggests a reciprocal relationship between the gut microbiome and sex hormones such as androgens. As such, we asked whether the gut microbiome may influence androgen-dependent socio-sexual behaviors in mice. In experiment 1, we treated C57BL/6 mice with broad-spectrum antibiotics (ABX) to deplete the gut microbiota in either early development [embryonic day 16 – postnatal day (PND 21)] or adulthood (PND 60 – 81). We found that early and adult ABX significantly decreased territorial aggression, while adult ABX also decreased sexual odor preferences, in males but not in females. In experiment 2, we assessed whether testosterone and/or cecal microbiota transplantation (CMT) via oral gavage could restore socio- sexual behavior in adult ABX-treated male mice. Mice were treated with same or other-sex control cecum contents or testosterone for two weeks. Treatment with male CMT restored both olfactory preference and male aggression in adult ABX male mice. Female CMT partially restored olfactory preference but not aggression, while testosterone treatment was insufficient to rescue these behaviors in adult ABX-treated male mice. 16sRNA sequencing of cecal samples revealed depletion of microbiota diversity in ABX-treated mice, which was reversed with CMT. Sex of donor CMT did not affect the relative abundance of the dominant phyla Firmicutes and Bacteroidetes; however, Lactobacillus murinus, reported to improve sociability, was higher in female donors but not in male recipients of female microbiota. Together, we find sex-dependent effects of the gut microbiome's mediation of socio-sexual behaviors that is independent of androgens. We also find evidence that host specificity contributes to the success of microbiota transplantation, and thus the full extent of the role of sex-specific gut microbiota on socio-sexual behavior may not be apparent in these experiments. Funding: This study was funded by a Discovery Grant from the Natural Sciences and Engineering Council of Canada (NSERC) to AS-G.

Construction of Copy Number Variation Map Identifies Small Regions of Overlap and Candidate Genes for Atypical Female Genital Development

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Copy number variations (CNVs) have been implicated in various conditions of differences of sexual development (DSD). Historically, larger CNVs are more often considered as disease- causing or clinically relevant, but recently, relatively smaller CNVs have been associated with DSD. The main objective of this study is to identify small CNVs and smallest regions of overlap (SROs) in patients with atypical female genitalia (AFG). We queried the DECIPHER database for genomic regions containing recurrent duplications and/or deletions in AFG patients. From these data, we constructed a map consisting of SROs and investigated such regions for genes that may be associated with the development of AFG. Our study identified 180 unique SROS. Within these SROs, we investigated 22 genes as candidates. Although none of these genes are known to be associated with AFG, literature review indicated that almost half were potentially involved in the typical development and/or function of the reproductive system, and only one gene was associated with a condition that reported an individual patient with ambiguous genitalia. Our study demonstrates the diagnostic utility of small CNVs, and further investigation will provide better understanding of the genetic etiology of AFG. Data from this investigation may also aid in improving the guidelines for diagnosis and patient management. Funding: This study was funded by the Washington University School of Medicine Dean's Fellowship to AUA.

Role of gonadal hormones and dutasteride treatment on the microbiota-gut- brain axis in a male and female murine model of Parkinson's disease.

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Prevalence and incidence of Parkinson's disease (PD) are higher in men than women suggesting a possible role of sex hormones in neuroprotection. Dutasteride (DUT), a 5α- reductase (5αR) inhibitor used in men, has shown neuroprotective effects in a male PD mouse model. DUT could act indirectly by increasing endogenous levels of female gonadal hormones (FGH) through inhibition of the enzyme 5αR. Our objective was to study the impact of DUT and sex differences in PD. Andropause and menopause were modeled with gonadectomized (GDX) animals. Four groups of mice, male or female, were GDX or sham-operated (SHAM). They received DUT or vehicle for 10 days and MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) to model PD or saline on the 5th day. We previously showed a parkinsonian phenotype in these mice. Now we are measuring microglial inflammatory response in the striatum and investigating gut microbiota profiling. In agreement with our previous results, we expect to observe differences between male and female mice but also between GDX and SHAM mice. We also expect that MPTP will cause differences in all our groups except in SHAM females who should be protected by their FGH. In addition, we expect to see a protective effect of DUT in the SHAM male group. These results will be available for the meeting and will allow us to better characterize the effects of sex and DUT in PD. Funding: CIHR (201711SVB-396851-SVB-CFBA-141783).

Adolescent Hormonal Contraceptive Administration Impacts the Prefrontal Cortex of Female Rats.

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Hormonal contraceptives (HCs) are synthetic hormones that disrupt endogenous hormone levels. They are commonly used during adolescence, which is a period of dramatic neurodevelopment, despite their unknown effects on brain maturation. As prefrontal cortex (PFC) development continues throughout adolescence and can be influenced by hormones, we hypothesized that the PFC may be impacted by adolescent HC exposure. Intact female Sprague-Dawley rats were given daily subcutaneous injections of either vehicle or ethinyl estradiol + levonorgestrel (HC) for 22-23 d beginning on postnatal day 35, thus spanning the duration of adolescence. Using daily vaginal lavage, it was validated that HC treatment disrupted estrous cycling. qPCR analysis showed that HC treatment reduced relative PFC expression of PSD95 (excitatory synapse marker), but not gephryin (inhibitory synapse marker), suggesting that HC administration during adolescence may preferentially diminish excitatory synapses. As HC use is associated with hypothalamic pituitary-adrenal (HPA) axis dysregulation in adults and the PFC is involved in HPA axis negative feedback, ELISA was used to quantify corticosterone (CORT) levels following acute restraint stress. All groups exhibited a similar increase in CORT 30 min after restraint initiation, but HC-treated rats had higher levels of CORT 1 hr after restraint cessation, indicating that HC may impair stress recovery. These data suggest that adolescent HC exposure compromises negative feedback of the HPA axis and excitatory synapses in the PFC, which could have implications for PFC function.

Sex Differences in Contextual Pattern Separation and Functional Connectivity for Fear Memory.

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Sex differences in hippocampus structure and plasticity exist but few studies have examined functional connectivity between the sexes in response to learning. Here, we examined sex differences in the ability for contextual pattern separation and functional connectivity during fear memory. We hypothesized that male rats would have greater ability for separating between two distinct contexts (shock context, no-shock context) compared to females, and males and females would show distinct patterns of coordinated neuronal activation of remote brain area during fear memory retrieval. Two-month-old male and female Sprague-Dawley rats were injected with DNA synthesis markers, iododeoxyuridine (IdU) and chlorodeoxyuridine (CldU) three weeks and four weeks before perfusion, respectively. One week after CldU injection, the rats underwent a fear conditioning context discrimination task. The expression of immediate early gene, zif268, in the hippocampus, frontal cortex, amygdala, and striatum were examined. We found that females, compared to males, showed significantly greater context discrimination. On the last day, the shock context was presented without shock and both sexes displayed similar levels of freezing. Despite the same fear memory, males showed more positive correlations of zif268 activation between the amygdala, hippocampus, striatum and frontal cortex, whereas females showed more negative correlations among these regions. We also noted differences in the activation of new neurons between the sexes with greater activation of 3-week-old adult-born dentate granular cells in females compared to males, which was negatively associated with activation in the lateral amygdala. Furthermore, females showed significantly greater activation in the frontal cortex and in the dorsal CA1 compared to males. These results highlight the importance of studying sex differences in fear memory and the contribution of adult neurogenesis to the neuronal network. Funding: This study was funded by National Sciences and Engineering Research Council of Canada (NSERC) to LAMG, Killam Doctoral Award and Djavad Mowafaghian Center for Brain Health Endowment Award to SY.

Sex Differences in Learning-Induced Protein Degradation in the Dorsal Hippocampus of Male and Female Mice

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Long established dogma holds that long-term memory formation requires *de novo* protein synthesis to produce stable changes in synaptic strength. As such, much attention has focused on the molecular mechanisms that drive transcriptional and translational activity within cells to support learning. However, protein degradation appears equally important to memory, yet considerably less effort has been devoted to understanding this process. The ubiquitin proteasome system (UPS) is the primary mechanism for degrading proteins within cells. Proteins that are no longer needed are tagged with ubiquitin and targeted by the proteasome for degradation. The contributions of UPS activity following learning have largely been studied in the context of fear learning in male rats. However, the extent to which UPS activity differs between male and female mice in non-aversive forms of learning remain unclear. Here, we examined markers of UPS activity in the synaptic and cytoplasmic fractions of dorsal hippocampus (DH) tissue collected 1 h following object training in adult male and female mice. Mice were first handled for 30 s/d for 3 d. Mice were then habituated in an empty testing arena for 5 min/d for 2 d. During training, mice accumulated 30 s of exploration with two identical objects and DH tissue was collected 1 h after completion of training. Object training increased expression of the immediate early gene EGR-1 in both sexes. In males only, object training increased phosphorylation of proteasomal subunit RPT6, chymotrypsin-like activity, and postsynaptic protein PSD95 in the synaptic fraction. In females only, RPT6 phosphorylation was increased in the cytoplasmic fraction. Levels of K48 polyubiquitination were not affected by training in either sex, nor were levels of phosphorylated CaMKII and PKA, both of which regulate proteasome activity. These data suggest that object training drives sex-specific alterations in UPS activity across subcellular compartments. Funding: This work was supported by R01MH107886 awarded to K.M.F. and F31MH118782 to K.S.G.

Maternal Age and Estradiol Influence Cognition in Middle-aged Rats.

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Female-specific characteristics such as pregnancy can influence disease risk. Estrogens, (primarily estrone and estradiol) are closely related to cognitive function and neuroplasticity, particularly in females. Estrogens can improve cognition in postmenopausal women, but their effects vary across studies, in part due to different compositions and doses of estrogens. However, other factors such as pregnancy and motherhood (parity), can have long-term effects on cognition and brain plasticity in both humans and rodents. Past research from our lab indicates that previous parity influences the neuroplastic ability of the hippocampus to respond to estrogens in middle age. Acute estrogens increase cell proliferation in the hippocampus in middle age in multiparous (3 or more litters), but not in nulliparous (never bred or mothered) rats. Furthermore, in humans, a younger age of first pregnancy corresponds with lower global cognitive function. In the present study, we examined whether maternal age (age of first pregnancy) and estradiol treatment differentially affected hippocampal neurogenesis and cognition in middle-age. Female rats were bred at 3 months, 7 months, or were nulliparous. At 13 months, rats received daily injections of 0.03ug estradiol (or sesame oil vehicle) for sixteen days. Rats received a single dose of Bromodeoxyuridine (to label newly dividing cells) on day 2. From day 12-16, rats were trained on the standard reference memory version of the Morris water maze, after which they performed a probe trial and reversal training paradigm. Our findings indicate that younger maternal age coupled with estradiol was associated with impaired reference memory performance compared to controls. However, advanced maternal age showed greater cognitive flexibility with estradiol. This work suggests that maternal age and estradiol can influence cognitive performance in middle age, and upcoming experiments will examine how these factors affect neuroplasticity. Funding: This study was funded by CIHR (PJT-148662) and UBC 4 Year Fellowship to TAP.

The Effects of Hormonal Contraception on Auditory Emotional Memory

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It is well documented that sex hormones regulate regions of the brain that support higher-order cognitive functions and affect brain structure (Griksiene & Ruksenas, 2009; Pletzer et al., 2010). Considering the global usage of oral hormonal contraceptive (OC) pills, it is vitally important to look at the effects that OCs specifically have on executive function. The present study investigated whether OCs influence recall for an emotional auditory episodic memory compared to a neutral one. Women on an OC did not differ significantly from naturally-cycling women (NC) in their recall accuracy (Signal Detection Theory (SDT): d prime) for an emotional or neutral auditory story, however, both groups of women recalled more correct information for the neutral story than the emotional story. Both groups also recalled more gist information than details, regardless of the story condition. Additionally, participants had a more conservative criterion when freely-recalling the emotional story than when recalling the neutral story, and also more conservative when recalling detail information compared to gist (SDT: c). The results of this study indicate that NC women and women on an OC do not significantly differ on memory recall for an auditory story with either emotional or neutral content. The findings also demonstrate the importance of accounting for criterion placements alongside memory accuracy for free recall as they may significantly impact memory performance.
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Female-biased gene regulatory networks in fibrous atherosclerotic plaques point to extracellular matrix producing fibroblast-like Smooth Muscle Cells as key players

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Symptomatic fibrous atherosclerotic plaques are mostly prevalent in women, and have been understudied. Smooth muscle cells (SMCs) and their phenotypic plasticity have been identified as major contributors to fibrous cap composition differences and plaque phenotypes in women, yet how SMCs contribute to the female plaque phenotypes is unknown. Previously identified atherosclerosis sex-biased gene regulatory networks (GRNs) were validated in human atherosclerotic tissue (158 females and 158 males) with female plaques GRNs highlighting SMC biology, and enrichment for CAD GWAS genes. Single-cell RNAseq (26 males and 20 female plaques) showed that the strongest female-biased networks pointed to an extracellular matrix (ECM)-producing fibroblast-like SMC phenotype which strongly expresses both canonical contractile (ACTA2, TAGLN, MYL9) and fibroblast- like (FBLN1, DCN, SFRP2) markers. A sex-stratified siRNA screen in primary fibroblast-like SMCs identified ECM proteins such as BGN as a modulator of the female-biased network. How and why these sex differences occur during atherosclerosis development remains to be determined.
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High prevalence of XCI skewing in female atherosclerotic plaques

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Sex differences in cardiovascular diseases and atherosclerosis have been described, as well as sex-specific mechanisms identified in atherosclerotic plaques such as mosaic loss of the Y chromosome in men. In women, X-chromosome inactivation (XCI) skewing has been reported as common in several tissue substrates. Despite sex differences being widely described, information on the role of XCI in atherosclerotic plaques is lacking. Here we hypothesize that XCI skewing has a role in dictating the severity of atherosclerotic plaques in female patients. XCI skewing was quantified in 189 human plaque and blood DNA samples of women who underwent carotid endarterectomy included in the Athero-Express study. XCI was determined using the HUMARA assay by measuring the band intensities on 8%-page gels of a specific region, containing different number of CAGs between paternal and maternal X chromosomes, of the human androgen receptor gene. Then XCI skewing data were correlated to plaque phenotypes and characteristics. Of 189 patients, 97 plaques (64%) exhibited XCI skewing (≥60%). XCI skewing was higher in blood (n=42/55, 76%) and strongly associated with plaque skewing. XCI skewing was associated with unstable plaque features such as higher calcification (OR: 2.3, 95CI [1.2, 4.6], p = 0.017). Our study shows that XCI skewing is highly prevalent in female atherosclerotic plaques and associated with higher calcification. In our further studies we will look into the association of XCI skewing with clinical data, risk factors and events during follow-up.

Sex Chromosomes and Gonadal Hormones Drive Sex Differences in Hepatic Gene Expression and Response to Statins

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Males and females differ in susceptibility to cardiovascular diseases. Furthermore, women are more likely to experience adverse effects from the most widely prescribed drug for cardiovascular disease prevention, statins. Statin drugs are metabolized in liver and inhibit hepatic cholesterol synthesis. We identified sex differences in the response to statin in the liver transcriptome in the Four Core Genotypes (FCG) mouse model, which was made hypercholesterolemic by genetic apolipoprotein E deficiency. FCG mice allow identification of gonadal and chromosomal sex determinants by comparing four genotypes: XX mice with ovaries or testes and XY mice with ovaries or testes. We detected chromosomal and gonadal effects on hepatic metabolic pathways. Prior to statin treatment, liver mRNA expression patterns showed opposing effects of sex chromosomes and gonadal sex for hundreds of genes. For example, genes with elevated expression in XX compared to XY mice often exhibited reduced expression in mice with ovaries compared to testes. This may represent a means by which chromosomal and gonadal sex components work to balance expression levels. Interestingly, statin treatment disrupted this balance, with particularly prominent dysregulation of genes that modulate the cell cycle. In addition, XX chromosomes or ovaries promoted statin-induced elevations in fatty acid biosynthetic gene expression, whereas the combination of XY chromosomes and testes elevated cholesterol biosynthetic gene expression (a compensatory response to the cholesterol-lowering action of statins). These findings provide molecular insight into sex determinants of hepatic gene expression, and reveal sex-specific responses to one of the most widely prescribed drug classes. **Funding:** These studies were supported by P50 GM115318 (KR), U54 DK120342 (KR), and R21 AR077782 (KR and PZ) from the National Institutes of Health and 20POST35100000 (CBW) from the American Heart Association.

Sexual Dimorphism in Atrial Fibrillation: Investigating the role of atrial fibrosis

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Atrial fibrillation (AF) is characterized by an irregular heart rate and leads to intra-cardiac clot formation and cardio embolic strokes. Women have a disproportionately higher risk of stroke from AF than men. The underlying mechanisms for this sexual dimorphism are understudied. We have previously demonstrated that aged female mice have increased AF events after induction as compared to males. We hypothesized that structural remodeling in the left atrium may lead to enhanced thrombo-embolic risk in the aged females. We used C57BL/6J male and female aged mice (20-22 months old) for this study. Echocardiogram (Vevo 31 imaging system) was performed to measure cardiac output, ejection fraction, stroke volume, and left atrial diameter. Ambulatory electrocardiogram was recorded for 8 hours using implantable telemetry monitors. Carbachol, a cholinergic agonist was injected at a dose of 50µg/kg intraperitoneally for AF induction. All mice were sacrificed and the cardiac tissue was stained with Masson’s Trichrome stain to assess for fibrosis using protocols described previously. Image J was used on 5 sections/ heart to quantify areas of fibrosis. Data was analyzed using two sample t-test on GraphPad PRISM. No significant difference was seen in cardiac output, ejection fraction, stroke volume, or left atrial diameter between aged male and female mice, p>0.5 on echocardiogram. Aged female mice had significantly higher number of AF events (93±11.6, n=8) as compared to aged males (20± 16.6, n=12), p=0.03. They also had significantly increased fibrosis (fibrotic area 2.3±0.19, n=3) compared to males (0.74±0.003, n=8), p=0.02. Our results demonstrate that despite having similar cardiac function (stroke volume, ejection fraction) and left atrial diameter, the cardiac fibrosis is increased in aged females as compared to males. This suggests increased cardiac remodeling leading to conduction abnormalities and arrhythmias in aged females. Ongoing studies in our laboratory are focused on understanding the role of aging and decreased estradiol levels on atrial substrate remodeling.

The Role of Sex and the Gut Microbiome in Mouse Models of Depression

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Major depressive disorder is one of the leading causes of non-fatal disease burden worldwide, with female diagnosis being twofold that of males. Despite this sex-linked disparity, research on the mechanisms of sex differences in depression models is lacking. Here we investigated the role of sex and the gut microbiome on depression-like behaviours using two mouse models of depression. In Experiment 1, C57BL/6 mice were exposed to cecum from male or female olfactory bulbectomized (OBX) mice via oral gavage to evaluate if cecal transfer from the OBX model of depression is sufficient to induce a depressive phenotype (DP). In Experiment 2, we assessed whether findings from Experiment 1 are conserved across mouse strains and models of depression. Specifically, Balb/C mice were exposed to cecum from male or female mice that underwent the chronic unpredictable stress paradigm. For both experiments, a behavioural battery consisted of a sucrose preference test (SPT), splash test (ST), tail suspension test (TST), and forced swim test (FST). In Experiment 1, females demonstrated an overall higher DP on the ST and SPT compared to males. Male recipients of OBX-cecum, regardless of donor sex, showed higher DP than controls, whereas female recipients of OBX-cecum showed a decrease in DP. These results suggest that the gut microbiome contributes to the sex differences in the presentation of depression. Behavioural data for Experiment 2 is currently being analyzed and will assess the generalizability of these findings. Together, this work will provide further understanding of the contribution of the gut brain axis on DP vulnerability and elucidate the underlying mechanisms contributing to sex differences in this disorder. Funding: Funding was provided by a Discovery Grant from the Natural Sciences and Engineering Council of Canada (NSERC) to Dr. Ashlyn Swift-Gallant.

Estrogen Increases NMDA Receptor Expression in Kidney Principal Cells

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N-methyl-D-aspartate (NMDA) receptors are calcium channels gated by glutamate and glycine. In the kidneys, epithelial NMDA receptors induce renal vasodilation. Previous data suggest that estrogen supplementation increases renal vasodilation induced by amino acids in aged rats, however, the mechanism of this increase is not known. Additionally, there is no data showing if there is a sex difference in NMDA expression in kidney. We hypothesized that there is a sex difference in renal NMDA expression and that it is dependent on Estrogen. Methods: We evaluated the renal expression of NMDA receptor type 1 in mouse kidney epithelial cells by immunohistochemistry and immunofluorescence, and in total kidney by Western blot. We also evaluated the expression of NMDA receptors on a continuous cultured epithelial cell line with characteristics of renal collecting duct principal cells (mpkCCD), with and without the presence of estradiol (50nM) in the media for 24 hours. Results: NMDA receptors were found to be present along the nephron, in both cortical and medullar regions, with higher expression on the juxtamedullary and medullary region. Under confocal microscopy, we confirmed the expression of NMDA receptor in AQP2 positive cells. Western blot analysis showed an increased expression of NMDA receptor type 1 in female mouse kidney in comparison with males (3.34± 1.6 vs. 0.95±0.3 AU, p=0.01). Twenty-four-hour mpkCCD cell incubation with estrogen increases NMDA receptor expression (2.3± 1.3 vs. 0.9 ±0.58 AU, p=0.05). Conclusion: There is a sex difference in kidney NMDA receptor expression and that is related to the presence of estrogen. Sex difference in Kidney NMDA may explain renal physiological differences and disease susceptibilities in males and females. Funds: 1K01HL155235 NHLBI NHI and Emory Score Pilot Grant.

Sex differences in lung inflammation in a mouse model of allergic asthma: potential role of the microbiome

Rachel Alford, Patricia Silveyra

Asthma is a chronic inflammatory disease of the airway that leads to compromised lung function and affects more than 300 million patients worldwide. Disparities in sensitivity between men and women to these factors have also been identified; however, it remains unclear whether mediators such as the microbiome contribute to such differences. To understand the mechanisms associated with previously observed sex disparities in allergen-induced asthma, we used a mouse model of house dust mite (HDM) challenge and identified sex differences in respiratory mechanics and activation of the immune response. In the present study, we tested the hypothesis that the lung microbiome undergoes alteration in response to proinflammatory cytokine gene expression in mice challenged with HDM. For this, we analyzed mRNA expression of target pro-inflammatory cytokines and the 16S microbiome in DNA extracted from whole lung tissue of male and female C57BL/6 mice exposed to HDM or phosphate buffered saline (PBS) for 5 weeks (n=3/group). Our results show that females had a significantly higher expression of interleukin (IL)-6, IL-17A, IL-13, and IL-10 in comparison to their male counterparts. Analysis of the lung microbiome also revealed that males had a higher relative abundance of Firmicutes and other bacteria than females at basal levels, but this pattern was reversed with HDM challenge. In addition, the relative ratio of Firmicutes:Bacteroidetes was similar for both sexes in PBS-treated animals, but was almost twice as high in males than in females (4.2 vs 2.24, respectively) in asthmatic mice. Moreover, females treated with HDM displayed higher bacterial diversity and relative abundance of Proteobacteria when compared to controls, an effect that was not observed in males. We conclude that sex specific expression of proinflammatory cytokines is potentially associated with the composition of lung microbial communities in male vs. female asthmatic mice models. Funding: NIH R01HL159764 and R03HL141618

Effects of Pubertal Blockade and Testosterone on Endothelial Function in Transgender Males

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Gender affirming treatment for transgender youth include the use of pubertal blockade via a gonadotropin releasing hormone analogue (GnRHa) to prevent puberty and the initiation of hormone therapy to align secondary sex characteristics with gender identity. Declines in endothelial function are a key initiating event in the development of atherosclerosis and ischemic heart disease. Alterations in sex hormones, including testosterone (T), are associated with adverse changes in endothelial function. However, if the initiation of T adversely affects endothelial function (brachial artery flow mediated dilation [FMD]) in transgender males (TGM) or if this effect differs by pubertal blockade is unknown. Therefore, this prospective pilot study evaluated 1) the change in FMD after 12-months of T; 2) if the effect of T on FMD differed by pubertal blockade in TGM currently receiving GnRHa (n=8, age: 13.8±0.3; yrs; GnRHa duration: 2±1 years; BMI%: 61±10%) or not receiving GnRHa (n=11; age: 14.9±0.3 yrs; 66±10%) at study initiation. In the entire cohort, FMD was unchanged after 12-months of T (time: p=0.95). At baseline, FMD was lower in TGM receiving GnRHa compared with those not receiving GnRHa (7.9±0.9 vs. 10.9±0.9%, p=0.03). The effect of 12-months of T on FMD differed by GnRHa group (group*time: p=0.02). FMD was marginally reduced after 12-months in TGM not receiving GnRHa (11.5±0.7 vs 9.8±0.9%, p=0.05) but increased in TGM receiving GnRHa; however, this change was not statistically significant (7.9±0.9 vs 9.8±1.5%, p=0.16). FMD did not differ by GnRHa group after 12-months of T (p=0.97). These data suggest that the effects of T therapy in TGM may be modulated by the use of GnRHa. Addition studies are needed to evaluate long- term (>12 months) effects of gender affirming therapy. Funding: NIH/NICHD BIRCWH K12 (HD 057022), F32AG071273, K23HL151868, NIH/NCATS Colorado CTSA UL1 TR002535, Ludeman Family Center for Women’s Health Research, Doris Duke Foundation, Endocrine Fellow Foundation

Examining the effects of early-life immune activation and later-life social stress on behavioral and neural outcomes during development

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Environmental stressors and infections during early development can have profound effects on later life behavior. Notably, males are twice as likely as females to be diagnosed with neurodevelopmental disorders, including autism, schizophrenia, and general learning disabilities. The “two-hit hypothesis” suggests that a *combination* of environmental and/or genetic “hits” during early life significantly increases the risk for an individual to be diagnosed with these disorders. This project will utilize a rat model to examine how a combination of well-known environmental factors – maternal immune activation (MIA) and adolescent social isolation (SI) – contribute to the risk of learning deficits and anxiety behaviors. To examine MIA, dams are injected with lipopolysaccharide (LPS; 100ug/kg, i.p.) or saline on embryonic day (E)15. Maternal and fetal cytokine expression following MIA will be reported. Postnatally, the effect of MIA on maternal care will be measured and effects of MIA on the emergence of developmental reflexes in neonatal pups will be examined for sex differences. To examine SI, pups are either isolate- or pair-housed from postnatal days (P)35-49 in adolescence. We believe that, following MIA and SI, adult male offspring will be more likely to exhibit deficits in latent inhibition and novel object recognition, and adult female offspring will exhibit anxiety behaviors in elevated plus maze. These findings will provide us with a better understanding of how early-life environmental factors affect later-life brain and behavioral processes, and how they may be differently dysregulated in males and females. Funding: This study was funded by a R01MH106553 grant to JMS.

The Scientific Body of Knowledge – Whose Body Does It Serve? Factoring women’s health into human brain imaging

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Historically, the biomedical sciences operated under the false premise that females are too variable to study (Prendergast et al., 2014). This oversight disproportionately impacts women, as the presumption of greater variability was a major motivation for excluding female animals from preclinical research (Beery et al., 2011). These reports helped catalyze the NIH’s SABV mandate requiring the inclusion of both sexes in preclinical studies. Here, we parallel their work by spotlighting how far human neuroscience needs to go to address the unmet needs of women’s health. First, we quantified the extent to which human brain imaging overlooks major aspects of the human condition specific to women (e.g., menstrual cycles, hormonal contraceptive use, pregnancy, menopause). After examining >1,000 articles from top brain imaging journals, we found that fewer than 3% considered women’s health factors in their study design – half of which were used as justification to exclude women due to ‘added variability’. Next, using population-level data from UKBiobank (N=21,408, 47% F), we tested the hypothesis that women and men are equally variable across all major trait categories assessed. We found no evidence to support the claim that women are inherently more variable than men by observing no sex differences in variability across 604 cognitive and brain imaging measures. In sum, human brain imaging studies are not designed to serve men and women equally. Moving forward, the neuroimaging community should increase its commitment to advancing knowledge of the brain with a focus on women’s health. Funding: This work was supported by the UCSB Graduate Division (LP) and the National Institute on Aging under award numbers F31AG074634 (LP) and R01AG063843 (EJ, MP)

Linking gametologue expression to sex differences in gene expression and disease

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Humans exhibit sex differences in the prevalence and presentation of many psychiatric, neurodevelopmental, and neurodegenerative conditions. These differences in susceptibility are likely to (in part) reflect evolved sex differences in brain gene expression. Of particular interest are the gametologues: genes that have retained functional copies on the X and Y chromosomes despite having ceased recombination over evolutionary time. Recent work suggests that gametologue pairs vary in X-Y functional equivalence and tissue-specific patterns of co- expression. However, we currently lack an understanding of: 1) how patterns of co-expression between X-Y gametologues compare to their patterns of co-expression with all other genes ('coupled co-expression'); 2) the mechanisms that drive co-expression; or 3) the functional impacts of differential co-expression with X versus Y gametologues. To address these gaps, we used a large, published human gene expression dataset (GTEx V8) to characterize gametologue co-expression and coupled co-expression across >40 tissues. These measures were correlated across tissues and pairs (rho=X; p=X), with larger deviations occuring among the most sexually differentiated tissues (testes, prostate, mammary). X-Y pairs that ceased recombination more recently exhibited more similar promoter and protein sequences. Promoter sequence similarity predicted average co-expression across gametologue pairs (rho=0.681; p=0.021), suggesting that higher co-expression reflects shared regulatory mechanisms. Finally, genes showing higher co-expression with X or Y gametologues were associated with sex- biased conditions (Y>X: autism, schizophrenia, and ADHD in brain tissue; X>Y: autoimmune disease in many tissues; all p adj <0.001). This work improves our understanding of how sex chromosome evolution has influenced the human transcriptome and how these mechanisms may contribute to sex differences in disease.

Sex Differences In Brain Tumor Glutamine Metabolism Reveal Sex-Specific Vulnerabilities To Treatment

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Glioblastoma (GBM) is the most common and aggressive brain tumor in adults. GBM occurs more commonly in males, but female patients survive significantly longer. Understanding the molecular mechanisms that underlie those sex differences could support novel treatment strategies. In this regard, we assessed metabolite abundance in glioblastoma surgical specimens and found that male glioblastomas are enriched for amino acids, including glutamine. Using PET imaging, we found that gliomas in male patients exhibit significantly higher glutamine uptake. These sex differences were well-modeled in murine transformed astrocytes, in which male cells imported and metabolized more glutamine and were more sensitive to inhibition of glutaminase 1 (GLS1) which mediates the conversion from glutamine to glutamate. The sensitivity to GLS1 inhibition in males was driven by their dependence on glutamine-derived glutamate for α-ketoglutarate synthesis and TCA cycle replenishment. Females were resistant to GLS1 inhibition through greater pyruvate carboxylase-mediated TCA cycle replenishment. Together, these data indicate that male and female GBM (i) differ in their metabolite composition and (ii) differ in their dependency on glutamine to replenish their TCA cycle. This reveals novel sex specific metabolic targets for GBM and underlines the importance of considering sex in metabolic targeting approaches.

Arcuate nociceptin/orphanin FQ (N/OFQ) neurons inhibit nearby proopiomelanocortin (POMC) neurons in a sex- and energy status-dependent manner in prepronociceptin (PNOC)-cre mice

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Nociceptin/Orphanin FQ (N/OFQ) is an endogenous opioid hepadecapeptide that binds with high affinity to the nociceptin opioid peptide (NOP) $G_{i/o}$-coupled receptor. The NOP receptor is densely expressed in anorexigenic proopiomelanocortin (POMC) neurons in the hypothalamic arcuate (ARC) nucleus; an essential component within the hypothalamic energy balance circuitry. N/OFQ neurons evoke inhibitory effects on POMC neurons and promote both homeostatic and hedonic feeding. Thus, we tested the hypothesis that under conditions of negative energy balance (i.e., fasting), the inhibitory effect of N/OFQ on anorexigenic POMC neurons would be potentiated, resulting in rebound hyperphagia. Electrophysiological experiments were conducted in hypothalamic slices from PNOC-cre/eGFP POMC double transgenic mice. Recordings from ARC N/OFQ neurons showed that a subpopulation of these cells exhibit a calcium-dependent pulsatile burst firing pattern that is eliminated upon cellular dialysis with the calcium chelator (BAPTA). Additionally, recordings from fasted animals revealed that they are hyperexcitable under conditions of negative energy balance as evidenced by a reduced rheobase, increased basal firing rate, and increased plateau potential frequency. Chemogenetic stimulation of ARC N/OFQ neurons inhibited POMC neurons through activation of the NOP receptor and G protein-gated inwardly rectifying potassium (GIRK) channels, and this effect is enhanced under conditions of negative energy balance. These effects were corroborated by optogenetic stimulation of ARC N/OFQ neurons. Given that estradiol (E_2) uncouples $G_{i/o}$-coupled receptors from GIRK channels, we tested the hypothesis that the extent of inhibition occurs in a sex-dependent manner. The inhibition of POMC neurons caused by chemogenetic stimulation of N/OFQ neurons was comparatively greater in slices from ovariectomized (OVX) PNOC-cre mice than that seen in males, and attenuated in E_2-treated slices from OVX females. Therefore, ARC N/OFQ neurons inhibit POMC neurons, which is potentiated under conditions of negative energy balance in a sex-dependent manner, and likely due to the hyperexcitability of these cells.

Improving antidepressant treatment in the postpartum by manipulating immune signalling

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Women are twice as likely to be diagnosed with depression compared to men, with increased risk during pregnancy and the postpartum. Increased inflammation, and a reduction in neuroplasticity are seen in an animal model of postpartum depression (PPD). Markedly, serotonin reuptake inhibitors (SSRIS) remain the most common treatment option for PPD, despite their low efficacy during this time. Previous research in our lab has found that this low efficacy is associated with increased levels of interleukin-1β (IL-1β) in the hippocampus. Therefore, we investigated whether perturbations to the immune system by blocking the IL-1 receptor (IL-1R) increased SSRI efficacy to reverse depressive-like endophenotypes in a rat model of postpartum depression. In our rodent model of postpartum depression, high corticosterone (CORT) was administered during the postpartum period along with fluoxetine (FLX) and/or anakinra (KIN), an IL-1R antagonist. FLX efficacy was measured using the forced swim test (FST), and maternal care observations. All dams were euthanized at postnatal day 23 to examine inflammation, and markers of neuroplasticity in the hippocampus and prefrontal cortex. Preliminary data shows that dams treated with KIN (with or without FLX) had a reduction in microglia (Iba1 expression). Dams treated with both FLX, and KIN showed reduced immobility in the FST, and increased hippocampal neurogenesis in the dentate gyrus. Further experiments are necessary to evaluate the relationship between neuroinflammation and antidepressant efficacy in PPD.
These findings indicate that IL-1β may serve as a potential target for increasing antidepressant efficacy in people with PPD.
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Effects of Short-Term Ovarian Hormone Suppression on Blood Pressure in Postmenopausal Women

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Prior studies evaluating the role of menopause and changes in ovarian hormones (e.g., estrogen [E2] and follicle stimulating hormone [FSH]) on blood pressure (BP) have demonstrated conflicting findings potentially related to confounding effects of changes in age and cardiovascular disease (CVD) risk factors with menopause. Short-term ovarian hormone suppression (via gonadotropin releasing hormone antagonist [GnRHa]) can be used to isolate the effects of ovarian hormones on BP, independent of the changes with aging and CVD risk factors, and allow for the randomization to receive E2 or placebo (PL) added back in order to isolate the effects of E2. Healthy postmenopausal women (PMW; n=28, mean±SE; age: 58±1 yrs; BMI: 26.8±0.9 kg/m^2) not taking vascular-altering medications completed supine measures of BP (average of triplicate following 20 minutes of rest with an automated cuff (Dynamap]) before and 1) after 3 days of GnRHa (0.25 mg/day ganirelix) alone; 2) after an additional 3 days of GnRHa with randomization to either transdermal E2 (0.075 mg/day) or PL. Supine SBP was reduced from baseline following 3-days of GnRHa (120±2 vs 115±2 mmHg, p=0.003) in all women. Following 3-days of GnRHa with randomization to E2 or PL, SBP was reduced to a similar extent regardless of randomization (p=0.20). SBP was significantly reduced in GnRHa+PL (123±3 vs 112±2 mmHg, p=0.003) but not the GnRH$_{ant}$+E2 (116±4 vs 111±4 mmHg, p=0.18) group. The change in SBP was marginally correlated with the change in FSH (r=0.35, p=0.09) but not E2 (r=0.14, p=0.50). These data suggest that short-term suppression of FSH may reduce supine SBP, regardless of E2-levels. Additional studies are needed to evaluate changes in clinic and 24-hour SBP and the mechanisms underlying the SBP reduction with FSH suppression in PMW. Funding Sources: NIH R01 AG027678, R56HL114073, U54 AG062319, R01 AG049762, F32AG071273, Ludeman Family Center for Women's Health Research and Colorado Clinical and Translational Sciences Institute UL1 TR001082

Oral Contraceptives: Stress responses, Depression, and Motivation in Mice

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Oral contraceptives (OC) are a critical part of women's healthcare and confer vast economic benefits to women worldwide. OCs affect mood, behavior, and the brain in ways that are not fully understood. Some studies report increased depression and anxiety in women using OCs, while others find a protective effect. Studying how OCs contribute to risk and resilience to depression, anxiety, and other stress-related disorders is extremely difficult with human participants due to multiple formulations and individual differences in durations of OC use, genetic predispositions to psychiatric disorders, and prior/current stress exposures. The current project used a mouse model developed in our laboratory to examine the effects of two hormone combinations (0.075 μg/mL ethinyl estradiol (EE) and either 3 μg/mL levonorgestrel (LVNG) or 15 μg/mL drospirenone (DRSP) in 0.25mL of 10% sucrose, daily) on vulnerability to depressive-like and anxiety-like behaviors after subchronic variable stress. Estrous cycles were tracked using vaginal cytology methods. We found that OCs alone did not affect locomotor activity, behavioral despair, or anxiety-like behavior. However, OC exposure did disrupt sucrose preference, without producing a taste aversion to sucrose or global decrease in motivation. We also demonstrate that EE + LVNG blunted acute stress-induced corticosterone increases, which was not seen in mice exposed to EE+ DRSP. We demonstrate that OCs result in a specific decrease in anhedonia-like behaviors without triggering other depression-like phenotypes, and specific types of OC-like exposure blunted the stress response. Ongoing work examines other motivation-related behaviors and circuits and the interaction between motivation and stress in OC-exposed mice. Funding: Oscar Stern award from Eisenberg Family Depression Center University of Michigan (NCT)

Effect of prenatal nicotine and THC on cognitive behaviors in adolescent male and female rats.

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Although there has been a decrease in the prevalence of tobacco smoking in many developed countries, exposure to nicotine/tobacco during pregnancy remains a substantial problem worldwide. Further, given the recent escalation in e-cigarette use among various age groups and legalization of cannabis, it has become essential to better understand the effects of nicotine and cannabinoid co-exposure on neural development and function, especially in consideration of vulnerable populations.
These studies sought to systematically examine the effects of nicotine and THC prenatal exposure on cognitive behaviors in both male and female offspring rats. Dams were exposed to nicotine vape (5 mg/kg) or vape vehicle control, and oral THC (5 mg/kg) or vehicle control (sesame oil). Offspring were then tested in the pre-pulse inhibition test at PND21, novel object recognition task at PND35, and novelty suppressed feeding task at PND37. During adolescence, prenatal vape exposure in males and females resulted in a changed baseline startle reactivity, and significant effects were found in pre-pulse inhibition. Deficits in novel object recognition were also found in males prenatally exposed. Finally, modestly increased anxiety-associated behaviors were found with THC or nicotine exposure in the latency to approach novel food in an open field. Together, these studies demonstrate differential effects of prenatal exposure to e-cigarette nicotine vape and/or edible THC on cognitive processing tasks, with differing effects for males and females.
This work was supported by grants from the NIH National Institute on Drug Abuse (DP1 DA039658 and R01 DA051831 to CDF) and Tobacco-Related Disease Research Program (TRDRP T30FT0967 to VL).

CRF Infusion into the Medial Septum Modulates Hippocampal Cholinergic Transmission in Male and Female Rats

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The cholinergic septohippocampal pathway (SHP) modulates hippocampal-dependent mnemonic processes, including spatial and contextual memory. Previously, studies have shown that rats exhibit impaired spatial memory after 100ng infusion of corticotropin releasing factor (CRF) into the medial septum (MS). A clear sex difference emerged at the low 3ng dose of CRF, with only males exhibiting impaired spatial memory. However, the mechanism remains unclear but could involve changes in hippocampal acetylcholine (ACh) due to localization of CRF 1 receptors on cholinergic neurons in the MS. We tested whether CRF in the MS alters hippocampal cholinergic transmission via in vivo electrochemical recordings with choline sensitive microelectrodes using rats. Depolarization pulses with potassium chloride were applied to stimulate local ACh release. In both sexes, MS CRF (100ng) infusion reduced the amplitudes of depolarization-evoked cholinergic transients by 46% and resting cholinergic levels tended to decline over the monitored 30-minute period following CRF infusion. Preliminary data in male rats revealed no suppression of signal amplitudes with a low (3ng) dose CRF infusion, suggesting the effects of MS CRF on hippocampal cholinergic transmission are dose dependent. Ongoing studies are examining the effects of low dose CRF in female rats to investigate the above-mentioned sex differences in spatial memory performance. Thus far our findings indicate that MS CRF exerts an inhibitory effect on hippocampal cholinergic transmission in a dose-dependent manner. Future studies to delineate the mechanisms by which CRF regulates cholinergic SHP circuitry may aid in the development of novel treatments for stress related psychiatric disorders. Funding: This study is funded by NSF grant #1929829.

Reproductive Status Shapes Working Memory Circuitry in Midlife Women

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Sex steroid hormones are neuromodulators of learning and memory. Yet, human neuroimaging studies of the aging brain typically focus on neurocognitive changes brought on by chronological aging, leaving much less known about effects of endocrine aging. Here, midlife adults (n=129) underwent neuropsychological evaluations, MRI, and serological hormone assessments. Reproductive stage was determined via STRAW-10 guidelines. Participants completed a verbal working memory (WM) paradigm during fMRI to characterize effects of reproductive stage and sex hormone concentrations on WM circuitry. Behaviorally, across women, lower 17 beta- estradiol was associated with longer response times when WM demands were taxed (e.g., 2-back condition [t(43)=-2.76, p<0.01, r=-0.39]; lure trials [t(43)=-3.40, p<0.01, r=-0.46]; but not the 0- back condition [t(42)=-0.83, p=0.41, r =-0.13]). Similarly, increased FSH, an indicator of advanced reproductive stage, was associated with longer WM-related response times (e.g., 2- back [t(43)=2.55, p<0.05, r=0.36]; lure trials [t(43)= 2.03, p<0.05, r=0.30]). In an exploratory analysis at p<0.001unc (k=10), postmenopausal women exhibited greater task-evoked activity in right middle frontal gyrus (MFG; cluster coordinates: 24, 14, 34) relative to premenopausal women and perimenopausal women (22, 12, 34). FSH was positively associated with activity in a near-identical cluster in MFG (24,12, 38). These findings suggest that advancing reproductive stage is associated with increased WM-related prefrontal cortex activity, which may be a compensatory mechanism that sustains task accuracy or as an indicator of neural inefficiency. These findings begin to address a knowledge gap in women’s brain health. Funding: This work is supported by NIH R01 AG063843.

Egr1 is a sex-specific, estrous cycle-dependent regulator of neuronal chromatin organization and behavioral plasticity

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Women have twice the risk for anxiety and depression disorders compared to men, though the biological basis for this phenomenon is understudied. Clinical data indicate fluctuating ovarian hormones are critical contributors to this sex-biased risk. We previously demonstrated that the estrous cycle in female mice affects their anxiety-like behavior. We also showed, for the first time, estrous cycle-dependent changes in neuronal chromatin organization and co-occurring changes in gene expression in the ventral hippocampus, a brain region involved in emotion regulation in rodents. Analysis of this data identified Egr1, the product of an estrogen-responsive immediate early gene and a downstream regulator in estrogen signaling, as a candidate regulator of these effects. Here, we test the hypothesis that ventral hippocampal Egr1 mediates estrous cycle-driven changes in chromatin, gene expression, and behavioral dynamics. By examining the behavior of female mice across the four estrous cycle phases, we found that high-estrogenic proestrus mice have low anxiety indices relative to mice at the other low-estrogenic phases. A significant correlation between serum estrogen and a low-anxiety behavioral index, as well as the ability of acute estrogen treatment to decrease anxiety indices in low estrogenic cycling mice, further supports that this effect is driven by estrogen. Interestingly, we also observed cycling levels of Egr1 mRNA expression across the estrous cycle. To mechanistically link Egr1 and the observed behavioral phenotype, we overexpressed Egr1 in ventral hippocampal neurons of intact-male and OVX-female mice and performed a series of anxiety- and depression-related behavioral tests. Results from these experiments implicate Egr1 in driving cyclical anxiety- and depression-related behavioral phenotypes in females. We observed no behavioral changes in male animals, indicating the effects of Egr1 overexpression were sex-specific. Our results link Egr1 to estrous cycle-dependent gene regulation and behavioral plasticity and establish a foundation for developing sex-specific treatments for anxiety and depression. Funding: This research was supported by the National Institutes of Health (R01MH123523) and the Brain & Behavior Research Foundation (NARSAD Young Investigator Grant #22811) grants awarded to M.K.

Neural Sexual Dimorphisms and Estrous Cycling in Four Core Genotypes-like Rats

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We recently knocked out testis-determining function on the rat Y chromosome, producing fertile XY^{Δ} gonadal females. We also inserted a *Sry* transgene onto an autosome to produce XX(*Sry*TG+) gonadal males. This rat model, similar to Four Core Genotypes mice, allows comparison of XX and XY rats with the same type of gonad, either testes or ovaries. We compared the estrous cycling of XX and XY^{Δ} gonadal females. In XX and XY groups with testes or ovaries, we also examined two CNS sexual dimorphisms, the Spinal Nucleus of the Bulbocavernosus (SNB), and the Sexually Dimorphic Nucleus of the Preoptic Area (SDN-POA), which reflect the differential effects of gonadal hormones. XX and XY^{Δ} gonadal females had estrous cycles of similar length. XX and XY gonadal males had a comparable number and size of Nissl-stained SNB motoneurons, and both groups had significantly more and larger motoneurons than those of XX and XY gonadal females. Preliminary measurements of the SDN-POA similarly show that gonadal male groups, irrespective of their sex chromosome complement, had comparable volumes and number of calbindin-immunoreactive neurons, larger than in the XX or XY^{Δ} gonadal female groups. These results confirm the long-standing conclusion that sexual differentiation of these CNS regions is controlled by differential effects of gonadal hormones in the two sexes. These two nuclei are considered a sensitive bioassay for the effects of gonadal steroids, both perinatally and in adulthood. Thus, XX and XY rats with the same type of gonad appear to have comparable levels of gonadal hormones.

Sex differences in alcohol dependence: a role for steroid sex hormones and related proteins

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Sex and gender are associated with differences in alcohol consumption and risk for alcohol dependence (AD). Compared to men, women consume less alcohol and drink less frequently, but are more likely to drink while experiencing unpleasant emotions and are more susceptible to long-term health problems. Biological mechanisms underlying these sex differences remain poorly understood but may be related to sex hormones and proteins. Using the large population-based UK Biobank, we examined phenotypic associations between alcohol dependence (AD) and sex-related hormones/proteins, as well as their genetic correlation. AD cases (N=2900) were defined by having any ICD-9 or ICD-10 codes of AD, alcohol withdrawal, or alcohol-induced medical issues. Controls (N=448,918) were defined based on the absence of those ICD codes, as well as an AUDIT score < 8. Serum levels of total testosterone (TT), total estradiol (TE2), sex hormone binding globulin (SHBG), and albumin were measured by immunoassays. For each hormone/protein, we compared levels between AD and controls using linear regression, stratified by sex and adjusted for age and BMI, as well as menopausal status and use of hormone-influencing medications in females. We also assessed whether these associations were moderated by current consumption frequency, and whether SHBG mediated relationships between AD and TT or TE2. AD males had significantly higher TT ($p<0.001$), TE2 ($p<0.001$), and SHBG levels ($p<0.001$) but lower albumin levels ($p<0.0001$). After adjusting for menopause, AD females also had higher TT ($p<0.001$) and SHBG ($p=0.002$) but lower albumin levels ($p<0.001$). These trends were the same in both pre- and postmenopausal females. Although hormone levels were associated with current drinking frequency, their association with AD remained significant after accounting for current drinking. We also found that SHBG positively mediated the relationship between TT and AD in both sexes and between TE2 and AD in males ($p<0.05$).

Rapid exercise-induced stress resilience in females is independent of running distance

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Common stress-related disorders affect women more than men. Physical activity can enable stress resilience in both sexes, but little research has been done to characterize exercise-induced stress resilience in females. We have observed that female rats are more responsive to the stress-buffering effects of exercise than are males. In females, 3 weeks of voluntary wheel running prior to stress exposure prevents the behavioral effects of inescapable stress, while males require 6 weeks of running. Female rats run greater nightly distances than males, but it is currently unclear if running distance contributes to the rapid stress resilience from exercise in females. The goal of the current study was to determine if 3 weeks of voluntary wheel running produces stress resilience in female rats that run similar distances as males. Adult, female Sprague Dawley rats either remained sedentary, were allowed unlimited daily access to running wheels, or were allowed only limited access to wheels during the first 3 h of the active cycle. Females in the unlimited running group ran up to 1.5 times more than males, while females in the limited group ran distances similar to males. After 3 weeks of sedentary or running conditions, rats were exposed to no stress or inescapable stress. Three weeks of both unlimited and limited running prevented stress-induced social avoidance and exaggerated fear learning, and the degree of stress protection afforded by 3 weeks of wheel running was similar between groups. These results suggest that running distance does not drive the rapid acquisition of exercise-induced stress resilience in females. Further research is needed to identify the mechanisms underlying the accelerated stress resilience produced by exercise in females.

Sex differences in rodent model of context-dependent reward prediction

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When faced with ambiguous stimuli, organisms often rely on the background circumstances —the context— to interpret these stimuli and generate situation-appropriate predictions. Impaired context processing is a core deficit in several psychiatric disorders (schizophrenia, autism, PTSD). While sex differences in these disorders is well-established, potential sex-differences in context processing remains uncertain. To investigate potential sex differences in context processing, we trained male and female rats in a context-dependent Pavlovian discrimination task in which the validity of two brief auditory cues (X and Y) to signal reward depends on the contextual visual background (Cx1: X+, Y- ; Cx2: X-, Y+). To control for nonspecific effects, we trained two additional groups in tasks that do not require contextual processing: simple discrimination (only one cue rewarded regardless of context: Cx1: X+, Y- ; Cx2: X+, Y-) and no discrimination (both cues rewarded probabilistically regardless of context). After initial learning, the effect of an acute restrain test on performance was assessed for all rats. Critically, we observed significant sex differences only in the context-dependent discrimination task. While a majority (72%) of males successfully learned the task, only a minority (43%) of females learned the task after 70 daily sessions. Acute stress disrupted performance only in the context-dependent discrimination task and this effect was more pronounced in males. Therefore, it appears that female rats are slower to learn a context-dependent discrimination task, but once acquired, contextual processing is more robust in females and less sensitive to stress. These sex differences in contextual processing could contribute to the sex biases in psychiatric disorders.

Impact of X Chromosome Genes on Preadipocyte and Mature Adipocyte Function

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The physiological and functional properties of adipose tissue differ between females and males, which leads to sex differences in obesity and metabolic syndrome risk. While the gonadal hormones contribute to these physiological sex differences, we have demonstrated that the presence of XX or XY sex chromosomes further impacts adipose development and function in a sex-dependent manner. We have used mouse models to dissociate gonadal sex from chromosomal sex and have detected independent roles for the sex chromosomes in the regulation of adipose tissue function. In particular, we have determined that XX chromosome complement can influence adipose tissue through the expression of specific X chromosome genes that escape X inactivation. This leads to higher expression of X escape genes in XX (female) compared to XY (male) tissues. We identified two X escape genes, *Kdm5c* and *Kdm6a*, that impact adiposity by altering function of preadipocytes and mature adipocytes, respectively. Mice with preadipocyte-specific reduction in *Kdm5c* dosage had lower body weight and adiposity compared to wildtype females after 12 wks of high-fat diet, while mature adipocyte-specific *Kdm5c* reduction had no impact on adiposity. Furthermore, siRNA knockdown of *Kdm5c* in 3T3-L1 preadipocytes reduced cellular proliferation and differentiation. Conversely, mice with mature adipocyte-specific *Kdm6a* reduction had lower body weight and adiposity compared to wildtype females after 12 wks of high-fat diet. Furthermore, siRNA knockdown of *Kdm6a* in differentiated 3T3-L1 adipocytes altered mature adipocyte functions, including lipid droplet size and mitochondrial activity. *Kdm5c* and *Kdm6a* encode histone demethylase enzymes that regulate gene expression via chromatin modifications. We hypothesize that differential gene dosage of these histone modifying activities in XX compared to XY cells confers sex differences in expression levels of genes associated with adipocyte function and susceptibility to obesity.

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Reproductive Senescence Alters Mitochondrial Function Post-Stroke

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Adult female rats (5-7 mos) typically sustain much smaller infarcts after middle cerebral artery occlusion (MCAo) compared to middle-aged rats (10-12 mos). This may be due to loss of estrogen, which may also impair mitochondrial function, as mitochondria express both ERα and ERβ and respond to estrogen treatment. This study aims to assess whether female reproductive senescence (a stage of low endogenous ovarian hormones) alters mitochondrial function at baseline and after stroke. Reproductively senescent (RS) and mature adult (MA) female rats were subjected to MCAo via endothelin-1 (ET-1) or sham surgery. Animals were terminated at 5d post-stroke, the ischemic hemisphere was removed, and mitochondria were isolated and subjected to Seahorse XFe96 analysis. Mitochondria from RS shams showed slightly elevated basal respiration compared to MA shams, though this difference was abolished after drug injections. However, mitochondria from RS stroke animals showed greater oxygen consumption compared to MA stroke animals in all phases of the assay. These results suggest that subtle basal differences in mitochondrial respiration between reproductively senescent and reproductively competent females is exacerbated during conditions of hypoxia/aglycemia, such as stroke. These data may help to explain why RS animals exhibit poorer stroke outcomes, as RS mitochondria are increasing oxygen consumption an oxygen-deficient environment. Studies are underway to assess whether ovarian senescence directly plays a role in this difference. Supported by RFAG042189 to FS and 1F31NS118970-01A to TEB.

Chromatin footprints in the ventral hippocampus in a double-hit model of heterotypic acute stress.

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Stress induces allostatic responses, whose limits depend on both genetic background and the nature of the challenges. While acute stress generally assembles immediate adaptive responses, maladaptive coping may occur depending on the intensity of the stressors, individual differences, and previous adverse experiences. We investigated genome-wide chromatin accessibility in the ventral hippocampus in a double-hit model of acute stress in adult male and female mice in which heterotypic stressors, acute restraint stress (ARS) and forced swim stress (FSS), were applied within a 7-day interval period from each other. ATAC-sequencing was used to determine regions of chromatin that were accessible for transcription. We found that, depending on the type and number of acute stressors, genes were related to discrete sequences that encoded networks involved in development, immune function, cell starvation, translation, cytoskeleton remodeling, and DNA modification, and were differentially accessible in males and females. Chromatin accessibility was significantly affected by double-hit stress at the binding sites of the androgen (AR), glucocorticoid (GR), and mineralocorticoid receptor (MR), with double-hit mice displaying a profile that differed from either stressor alone and between males and females. Transcription-start sites (TSSs) adjacent to stress-related chromatin accessible regions encoded for genes implicated in several neuropsychiatric disorders, suggesting that the epigenetic action of stress involved broad regions of the DNA that flanked the AR, MR, and GR binding elements. Funding: Gary R Helman Fellowship to Dr. Jordan Marrocco. Hope for Depression Research Foundation

Allelic skewing as a possible explanation for the female bias in Sjögren's syndrome

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Many autoimmune diseases exhibit a strikingly increased prevalence in females, with primary Sjögren's syndrome (pSS) being the most female-predominant example. To understand the female bias in pSS, we performed transcriptomic profiling of minor salivary gland-derived mesenchymal stromal cells (MSCs) from primary Sjögren's syndrome (pSS) patients and control subjects. In control female MSCs, X-linked genes were expressed from both parental and maternal X chromosomes with a median paternal ratio of ~0.5. However, in pSS female MSCs, X-linked genes exhibited preferential expression from one of the two X chromosomes. pSS MSCs showed decrease in *XIST* levels, reorganization of H3K27me3^{+} foci in the nucleus, and mislocation of protein products encoded by the skewed genes, which was recapitulated by *XIST* disruption in control MSCs. Our data highlight the importance of restoring X-chromosomal allelic balance in the clinical management of pSS. Given that the maintenance of XX allelic balance does not apply to XY males, it is possible that females are uniquely susceptible to dysfunction in this pathway; therefore, our findings provide a possible explanation for the female bias in pSS. Funding The authors are supported by NIH grant UL1TR002373 and KL2TR002374 (S.S.M), R01 DK109508 (J.G.), and K01AR073340 (Y.L.).

Genetic deletion of the α7 nAChR reduces hippocampal granule and pyramidal cells in both sexes but specifically impairs pattern separation in males

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Juvenile and adult neurogenesis within the dentate gyrus is thought to play an important role in cognitive processes such as pattern separation. The α7 nicotinic acetylcholine receptor (α7 nAChR) subtype is highly expressed within the hippocampus though it's role in dentate gyrus neurogenesis and impact on cognitive function is not fully understood. To better characterize this, we performed unbiased stereology to quantify hippocampal granule cells, pyramidal cells, and total volume and used touchscreen operant chambers to test pattern separation in a global α7 nAChR knockout mouse line. Our results indicate the α7 nAChR knockout impairs male performance in the touchscreen task, but does not impact females. The knockout resulted in an approximately 30% reduction in granule cells and a 30% reduction in pyramidal cells in both sexes, but no change in total hippocampal volume. These results indicate a vital role of α7 nAChR function for cellular formation in the hippocampus. The sex- dependent difference in behavioral but not stereological results suggests a divergence in the structure- function relationship in males vs. females. Females may be more resilient to the structural deficits or may more effectively use alternative hippocampus-independent strategies to perform the task. These findings argue that the α7 nAChR plays a critical role in hippocampal development and highlights the importance of considering sex as a biological variable when connecting brain structure and function. Funding provided by the NIEHS Intramural Research Program

Sex-Specific Effects of Inhibiting Glutamatergic Cells in the Hippocampus on Stress- Induced Cognitive Bias

Amanda B. Namchuk 1, 2 , Travis E. Hodges 1 , Liisa A. M. Galea 1: 1. Dept. of Psychology, Univ. of British Columbia 2. Graduate Program in Neuroscience, Univ. of British ColumbiaMajor depressive disorder (MDD) is a debilitating illness affecting 20% of the population.

Females are twice as likely to develop MDD compared to males and experience more severe cognitive symptoms such as negative cognitive bias. Negative cognitive bias is the interpretation of ambiguous situations/stimuli as negative. MDD, including negative cognitive bias, is associated with decreased network connectivity between limbic regions. We developed a model of negative cognitive bias by training rats to discriminate between shocked context A and non-shocked context B. After 16 d of training, rats are tested in ambiguous context C (mix of contexts A and B), with higher freezing indicating negative cognitive bias. Chronic unpredictable stress (CUS) increases depressive-like endophenotypes, including negative cognitive bias, in both sexes. Pilot data from our lab indicate that despite similar negative cognitive bias levels, we find sex-specific patterns of functional connectivity following CUS-induced negative cognitive bias, with mostly positive correlations in females and negative correlations in males in circuits involving the ventral hippocampus (vHPC). To investigate this mechanism, we are utilizing designer receptors exclusively activated by designer drugs (DREADDs) that target glutamatergic neurons in the vHPC. Based on our pilot data, we expect that silencing vHPC glutamatergic neurons prior to testing in the ambiguous context will ameliorate CUS-induced negative cognitive bias in male, but not female, rats. This research will help to elucidate the sex-specific mechanisms by which stress precipitates negative cognitive bias. Funding: Canadian Institutes for Health Research (MOP-142308) to LAMG

Pharmacologic, but not physiological, increases in plasma fibroblast growth factor 21 (FGF21) influences sucrose and protein intake in females.

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The liver-derived protein hormone FGF21 is secreted in response to high sucrose and/ or low dietary protein intake. In turn, its pharmacological administration reduces sucrose intake and increases protein consumption in male mice and humans. Because sex is a key biological variable affecting feeding behavior and macronutrient selection, due to sex-dependent demands of reproduction, the aim of this study was to interrogate the sex-dependent pharmacologic and physiological effects of FGF21 on macronutrient preference in C57BL/6J mice. First, in agreement with our previous finding in males, we report that FGF21-treated females reduced sucrose intake and increased chow intake compared to saline-treated controls [P (treatment x diet)< 0.0001]. Also consistent with our previous findings in males, FGF21-treated females increased the percentage of kcal consumed from protein, while decreasing the consumption of kcal from either carbohydrate or fat, in a series of two-diet choice experiments [P<0.05 and 0.01, respectively]. Next we examined the effect of endogenous FGF21 on the consumption of sucrose and dietary protein in females. When protein-restricted (PR) mice were offered 30% sucrose and 36% high protein diets (HPD), we observed a sex-dependent response. PR males, but not females, increased HPD intake compared to protein-replete controls [p<0.001]. Accordingly, we observed a sex-dependent increase in plasma FGF21 from mice consuming low-protein diets [5.43 vs. 9.31 ng/ml; P(sex x diet)< 0.05]; females exhibit a less-robust response. Thus, our results suggest that pharmacologic FGF21 treatment inhibits sucrose intake and increases protein consumption in females, but diet-induced FGF21 is not sufficient to elicit these behavioral responses in females. Funded by NIH R01DK121035

The VPAC2 Receptor Mediates Resilience to Stress in Female, but Not Male Mice

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Pituitary adenylate cyclase activating peptide (PACAP) is a neuropeptide that has previously been implicated in the pathophysiology of post-traumatic stress disorder (PTSD). Although PACAP binds to the vasoactive intestinal peptide receptor 2 (VPAC2) it is still unknown whether VPAC2 plays a role in the psychopathology of stress-induced psychiatric disease. Here, we hypothesized that administration of the pharmacological antagonist of VPAC2, Bay 55-9837, prevent a wide variety of stress-induced fear, behavioral despair, and anxiety-like behaviors. A single injection of saline, (R,S) ketamine, or Bay 55-9837 was administered before or after contextual fear conditioning (CFC) stress in male and female 129S6/SvEv mice (n = 5-18 mice per group). Drug efficacy was assayed using the forced swim test (FST), elevated plus maze (EPM), open field (OF), novelty-suppressed feeding (NSF), contextual fear discrimination (CFD), and Piezo sleep boxes. Brain-wide VPAC2 expression was assayed using immunohistochemistry. Activating VPAC2 prior to stress attenuated learned fear, reduced behavioral despair, suppressed hyponeophagia, and facilitated CFD in female, but not male mice. Prophylactic Bay 55-9837 administration protected against stress-induced changes in sleep/wake cycles in female mice. Administration of Bay 55-9837 after stress reduced behavioral despair and hyponeophagia in both sexes. CFD learning in female mice upregulated VPAC2 expression in hippocampal CA3 and the agranular insular cortex. Our data indicate that agonism of the VPAC2 receptor using the peptide agonist Bay 55-9837 suppresses fear behavior and facilitates CFD learning in female, but not male mice. Overall, these results suggest that VPAC2 receptor activation critically modulates fear learning and retrieval in a sex- specific manner. Overall, our results suggest that VPAC2 may be a novel, female-specific target for preventing and treating stress-induced psychiatric disorders. Funding: This work was supported by an NIMH F31MH121023 to BKC, an NIMH T32MH126036 to AS, an NIA K99AG059952 to HCH, and an NICHD R01HD101402 to CAD.

Hormone levels, gonadal histology, body growth in Four Core Genotypes-like rats dissociating hormonal and sex chromosomal effects on sexual differentiation

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The Four Core Genotypes mouse model dissociates sex-biasing effects of gonadal hormones and sex chromosomes in any tissue. To allow measurement of sex chromosome effects in rats, we previously produced FCG-like rats with a knockout of the testis-determining function of the Y chromosome, and 4 different lines of rats with an autosomal transgene of the testis-determining gene Sry. The model allows comparison of 2 kinds of gonadal females (XX and XY Δ), and 3 kinds of gonadal males [XX(SryTG+), XY WT, and XY(SryTG+)], to compare effects of XX vs. XY sex chromosomes in either an ovarian or testicular hormonal environment. Here we compared serum levels of testosterone, anogenital distance (AGD) as a bioassay for prenatal androgen levels, adult gonadal histology, and body and organ growth in all 5 groups in 4 transgenic lines and in XY gonadal females. AGDs, measured at about postnatal day (PND) 6, were comparable in rats with the same type of gonad in most lines. Adult serum testosterone levels in gonadal males, at PND60, were comparable in male groups XY WT, XX(SryTG+), and XY(SryTG+) in 2 transgenic lines. Body and kidney and spleen weights at PND60 were comparable in XX and XY rats with the same type of gonad except in one transgenic line. XX(SryTG+) testes lacked sperm and were smaller than XY testes but expressed typical testis markers. The results suggest prenatal and postnatal androgen levels are comparable in two lines of transgenic XX and XY gonadal males, supporting the comparison of these groups as a test of sex chromosome effects. Supported by NIH grants R01OD030496 and R21OD026560

Locomotor Activity and Exploratory Behavior in Mice Lacking Androgen Receptor

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Androgen receptor (AR)-mediated androgen actions are necessary for the display of both sexual and aggressive behaviors in male mice, yet to what extent AR is involved in the development and expression of locomotor and exploratory behavior has yet to be established. Here, male mice carrying the testicular feminization mutation (Tfm) in the *Ar* gene (n=17), wild-type male (n=22) and wild-type female (n=21) mice were tested in an open field test to assess the role of the AR in these functions. Behavioral testing was recorded by a video camera mounted above the arena (L69 cm × W52 cm), and subsequently analyzed by computer-assisted tracking. In 15-min sessions, Tfm mice tended to spend less time in the peripheral zone and more time in the center zone compared to wild-type mice. As such, percentage of session time spent in the center was higher for Tfm mice than wild-type controls (p=0.013). On the other hand, no significant differences in time spent in the center or periphery, or the percentage were observed between wild-type male and female groups. In addition, Tfm mice traveled a shorter distance than wild-type females (p=0.003). Although no sex differences were observed for distance travelled, wild-type females displayed significantly more crossings into the center zones than the two male groups (p=0.008). These findings indicate that the behaviors tested in the open field paradigm are not sexually dimorphic, but rather the presence of AR, not testosterone, has a critical role in open field test behaviors.

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BPA Effects on Human Endocrine System and Reproduction

Andrea Pezzullo

It is known that many chemicals, which come in contact with the human body either through consumption or simply contact, negatively affects human body functions. These chemicals interfere with metabolism and hormonal balance (the endocrine and thyroid system in particular). Typically, the most common long-term result of chemical exposure is associated with fertility function, gamete production and development, while the beginning symptoms and the progression process may vary significantly. In the conduction of this research, there is extended literature analyzed in order to assess the effects of BPA as the most common chemical found in plastics, including multiple plastic materials used in household. In general, the BPA is a high-production chemical widely used in industry worldwide. Based on most recently published studies, BPA has an effect on the endocrine system leading to hormonal disruptions, such as high infertility and egg as well as sperm development issues. The studies done with a different dose of BPA have identified different changes based on the expose dose (2.5, 25 and 250 micrograms/kg of body weight). Some studies have looked at increasing concentrations of BPA in food and drink containers, medical tools and a high assortment of everyday products. The findings are important to increase awareness about the presence of BPA in commonly used plastics and will provide women and men with a better understanding and the need for health monitoring. This can also help to reduce the exposure, to improve future risk assessment and initiate policy actions.

Gender Differences in Sudden Cardiac Death Prevalence and Predictors

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Sudden cardiac death (SCD) while more common in male individuals, occurs also in females. However, females often have fewer predictors prior to the event. As most cases are made up of males, there is less data for females to determine risk factors and predictors. This study was conducted using the analysis of current information regarding male and female SCD prevalence and predictors. A literature search was performed through PubMed and various other databases using keywords, “sudden cardiac death” “gender” “channelopathies” and “risk factors”. It has been identified that males have more predictors prior to SCD events than females and the prevalence of SCD is higher in males. Females often are asymptomatic with one study showing approximately 13% to be symptomatic prior to SCD. Some of these predictors such as left ventricular hypertrophy or inverse T-waves may be indicators for females but other conditions like asymptomatic ventricular dysrhythmias are more common for males. A common cause of death amongst both groups is ischemic heart disease. Genetics also plays a role when dealing with male or female SCD occurrence. For instance, it was discovered that the long QT type 1 gene and CPVT gene are more consistent with young males whereas the long QT type 2 gene is more prominent in females. While all these predictors may be consistent with either gender, the prevalence of SCD in females is still much lower than in males. A United States study discovered that sports related SCD had an incident rate of 0.60 million athlete years for females whereas for males the rate reached approximately 5.01 million athlete years (O’Riordan 2021). It is questioned that perhaps this major difference in variation is due to different hormonal influence, blood pressure, among other factors like genetics. In addition, females that experience SCD tend to be older than their male counterparts. Overall, females experience SCD half as often as males.

Effects of sex and oxytocin treatment on anxiety-like behavior after cagemate separation

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Social isolation and relationship dissolution are common experiences that induce stress and anxiety. However, oxytocin (OT) can buffer these responses. While increases in anxiety-like behavior following cagemate separation has been shown in rats, OT decreased these behaviors. However, less is known about the role sex or housing type plays in separation-related effects. Thus, the present study determined sex differences in stress responses following brief separation in opposite- vs. same-sex cagemate pairs, and whether intranasal OT treatment would block these stress-related responses. Adult male and female rats bred in-house were housed for 14 days across a perforated divider that allowed contact but prevented mating. On the day of testing, rats received either 10ul of saline or 10ug OT per nare 30 minutes prior to a 5-minute separation period. Anxiety-like behavior was then assessed in the elevated plus maze (EPM). Tail blood was collected ~15 min post-separation for analysis of corticosterone (CORT) levels. Significant main effects of sex for open arm time, closed arm time, percent open entries, and percent closed entries in the EPM showed that females exhibited less anxiety-like behavior than males. A significant main effect of treatment for closed arm time, and strong trends for the other measures, showed that OT decreased anxiety-like behavior relative to vehicle. No sex by treatment interactions, or effects of housing condition were found. Analysis of serum CORT levels is currently underway, and correlations between stress hormone concentrations and behavior will be analyzed. While these data show predicted results for reduced anxiety-like behavior in females, and following OT, this treatment appears to be effective in both sexes. Further, the current social housing and isolation model did not alter anxiety-like behavior.

Resilience and its Neural Correlates in Irritable Bowel Syndrome: Influence of Sex

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Irritable bowel syndrome (IBS) is a female-predominant disorder of gut-brain interactions. Compared to healthy controls (HCs), patients with IBS have lower psychological resilience (ability to recover and adapt positively to stress), which is associated with greater IBS symptom severity and poorer health-related quality of life (HRQOL). We investigated the influence of sex on resilience and its neural correlates in IBS. Participants comprised 405 individuals (66 male controls; 62 male IBS; 104 female controls; 173 female IBS). Connor-Davidson Resilience Scale (CDRISC) and Brief Resilience Scale (BRS) scores were significantly lower in IBS than in HCs ($p<.05$). While there were no significant sex differences in HCs, CDRSIC scores (total and persistence, control-meaning, and meaning domains), but not BRS scores, were significantly lower in male IBS than in female IBS ($p<.05$). Partial least squares correlation analysis of resting-state connectivity data revealed CDRSIC total scores as associated with integration among regions in emotion regulation, central executive, and default mode networks to a greater extent in HCs than in IBS, and in female IBS than in male IBS, which may better support emotional regulation and cognitive functions that aid in responding to stress. These results suggest that some psychological resources that aid in recovery may be especially affected in male IBS. Sex should be considered in the development of therapies aimed at improving resilience in patients with IBS.

Less age-related hippocampal volume decline among female APOE e4 non-carriers

Ursula G. Saelzler[1], Erin E. Sundermann[1], Erin Joinatis[2], Carey Gleason[2], Matthew S. Panizzon[1] [1]University of California San Diego [2]University of Wisconsin Madison

The presence of the APOE e4 allele has been shown to impact women more than men, imparting greater Alzheimer's disease (AD)-risk and steeper cognitive decline. We examined whether this pattern extends to age-related hippocampal volume (HV) decline, and whether age of menopause, a proxy of lifetime estradiol exposure, moderates this relationship. Data from 809 structural MRI scans obtained from 338 adults (225 female) aged 60 and older participating in the Wisconsin Registry for Alzheimer's Prevention were included. Participants were scanned between 1 and 8 times for a mean follow-up duration of 3.24 years (SD=3.50). A linear mixed-effects model was applied, predicting HV from time since baseline, baseline age, MRI head coil, intracranial volume, body mass index, sex, APOE e4 status (carrier versus non-carrier) and a sex x APOE e4 x time interaction (including all lower-order 2-way interactions). All four groups showed significant HV decline (ps < 0.005), but female APOE e4 non-carriers demonstrated less decline in HV (b = -12.98) than all other groups (ps < 0.03), which did not differ from each other (b = -29.16, b = -31.02 and b = -30.39). An analogous model conducted in women only, and substituting age of menopause for sex, indicated that neither the main effect of age of menopause nor its interactions with time and/or APOE e4 status significantly predicted HV (ps > 0.27). Restricting inclusion to naturally post-menopausal women did not alter results. These results extend prior findings of a stronger APOE e4 effect on AD-related outcomes in women to age-related HV; however, the pattern suggests that APOE e4 non-carrier status is protective in women only. The APOE e4 effect on HV in women was not moderated by age at menopause.

Genetic correlations of alcohol consumption and alcohol use disorder against sex hormones in females and males

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Behaviors of Alcohol Consumption (AC) (quantity, frequency, and context) and aspects of Alcohol Use Disorder (AUD) (risk, symptoms, and prognosis) differ by biological sex. Prior research implicates a role for sex hormonal regulation of physiology across multiple tissues including brain development and function. Here we compared the genetic determinants of AC and AUD to those of Estradiol (E2), Testosterone (T), and Sex Steroid Hormone Binding Globulin (SHBG). In UK Biobank data, we ran genome-wide association studies (GWAS) on blood concentrations of E2, T, and SHBG with stratification by sex and by menopause status in females. We then used these GWAS results along with publicly available GWAS for AC (drinks per week) and AUD (case-control) to estimate the genetic correlations (rg) between these traits by linkage disequilibrium score regression. In males, we observed positive correlations of T with both AC (rg: 0.066; 95% CI: 0.012 ... 0.119) and AUD (rg: 0.105; 95% CI: 0.004 ... 0.206) and a positive correlation of SHBG with AUD (rg: 0.140; 95% CI: 0.031 ... 0.248). In females, we observed positive correlations of T with AC in both pre- (rg: 0.128; 95% CI: 0.013 ... 0.242) and peri-menopause (rg: 0.146; 95% CI: 0.048 ... 0.244), but we observed a negative correlation of T with AUD only in peri-menopause (rg: -0.310; 95% CI: -0.537 ... -0.083) and no correlation with SHBG. We did not observe significant non-zero correlations between E2 and either AC or AUD for either sex. Our observations suggest that some of the genetic factors that regulate E2, T, and SHBG also associate with AC and AUD and that there are differences in these genetic correlations by sex and by menopause status in females. Limitations of this work include missingness in measurements of E2 and T. Our future work includes evaluating the potential for mediation between SHBG and these sex hormones in their genetic correlations with AC and AUD.

Declining Contribution of Environmental Factors Underlies the Gradual Decrease in Mean Age at Menarche Across the 20th Century

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Age of Menarche is an essential factor in the study of women's health, marking the start of the reproductive period. Over the last century there has been a gradual decrease in the age at menarche, a trend that has been across multiple geographical, political, and economic settings. The mechanisms behind this change are not fully understood. Using a sample of female twins from the Swedish Twin Registry we sought to establish the degree to which age at menarche changed over the past century, and whether changes manifest at the level of genetic and environmental influences. Data were utilized from 3,894 monozygotic twin pairs, 4,094 dizygotic twin pairs, and 6,056 unpaired women. Birth years ranged from 1896 to 1985 (Median = 1951), while age at menarche ranged from 8 to 18 years old. From 1900 to 1980 the average age at menarche declined from 14.3 years to 12.8 years. This change coincided with a 20% reduction in the overall variance in age at menarche. Genetic contributions to the observed variance increased slightly over the examined time-period, while environmental contributions decreased substantially, declining by more than 50% over an 80-year period. As a result of these changes the heritability of age at menarche increased from .42 at 1900 to .61 at 1980. These results suggest that trends towards earlier ages at menarche in populations are driven by a reduction in the magnitude of environmental influences. Additional research is needed to identify the specific factors contributing to this effect and the potential impacts of this reduction in age of menarche on women's health across the lifespan.

Females at low-estrogen stages of the cycle during mild traumatic brain injury present worse TBI outcomes than females at high-estrogen levels

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There is increasing evidence that females present worse mild traumatic brain injury (mTBI) outcomes. Yet, females have been historically excluded from clinical trials and research studies, what might have led to ineffective treatments and inadequate care. Recent studies suggest that the moment of the menstrual cycle can directly affect TBI outcomes. Yet, the mechanism causing worse outcomes in females after mTBI remains unknown. Estrogen controls the modulation of factors involved in TBI outcomes, such as brain energy demand and metabolism. This study hypothesizes that estrogen levels during different moment of the estrous cycle determine the severity of TBI outcomes. We used a repeated closed-head mTBI mouse model that reproduces many features found in concussion patients. mTBI was induced in females in high-estrogen stage (proestrus) and low-estrogen stage of the cycle (diestrus). Mortality rate and recovery time after the injury were recorded. Three weeks after the injury, cognition decline was evaluated using novel object recognition. Low-estrogen females presented a 60% mortality rate compared to a 10% mortality rate of high-estrogen levels. Furthermore, low-estrogen females presented longer recovery times. We did not observe differences between groups in the novel object recognition test. We concluded that low-estrogen females present worse immediate outcomes affecting mortality and recovery, but not in cognitive tests three months after the injury. This study sets the stone to analyze the impact of sex hormone levels during the estrous cycle following TBI.

Progesterone-Oxytocin Interactions in Motivation for Cocaine.

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There are substantial sex differences in drug abuse, and a key feature of cocaine addiction is pathologically high economic demand for drug. The hypothalamic neuropeptide oxytocin (OXT) is heavily implicated in the modern treatment of substance abuse disorders. Using a within-session threshold behavioral economics (BE) procedure, we quantified demand elasticity (a, inverse motivation) and free consumption (Q_0, hedonic setpoint) in female rats. We examined Fos reactivity in OXT neurons during BE. We tested the effects of OXT (0.1 mg/kg, 0.3mg/kg IP) to reduce demand in both sexes. We confirm our prior findings that progesterone (P_4) attenuates cocaine-demand in female rats and that chronic cocaine self-administration disrupts estrus cyclicity. In intact rats, we found that OXT's efficacy to decrease motivation for cocaine is greater during the high-demand phase of the estrous cycle (diestrus, low P_4) as compared to low demand phases (proestrus, high P_4). Following each individual injection, OXT at either 0.1mg/kg or 0.3mg/kg restored estrous cycling in intact females with prior cocaine experience for a period of one week and was continuously effective with up to 4 weeks of injections. Centrally, Fos reactivity in OXT+ neurons (OXT/Fos+) was greater during proestrus compared diestrus, and significantly correlated to motivation (a) and increased circulating levels of P_4. Interesting, we also show that P4's demand attenuating effects are reversed by Atosiban (1.0 mg/kg, IP), an OXT antagonist. Thus, OXT may represent a viable treatment option, not only for acute suppression of drug demand, but also for its long-term effects to reverse the endocrine disrupting effects of cocaine.

Faster decline in memory amongst women with four or more children

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Multiparty has been associated with worse cognitive outcomes later in life; however, most studies have examined this association cross-sectionally. It is unclear if multiparity truly drives faster cognitive decline, or if long-standing cognitive differences amongst women with different parity exist. The present study leveraged longitudinal data from the Wisconsin Registry for Alzheimer's Prevention to test if the rate of cognitive changes differs between women with 0, 1-3, or 4+ children. Four hundred and twenty-one cognitively normal, post-menopausal women with self-reported reproductive history data, and *APOE*e4 status were included. Participants completed between 1 and 5 annual visits (Mean=2.70, SD=1.34) of cognitive testing. Analyses focused on performance from the Logical Memory (LM) Delayed Recall task. Linear mixed-effects models were applied to the data to predict LM scores from number of children, time, baseline age, age of menopause, practice effects, and APOE-e4 status. A second analogous model included a time by number of children interaction. The model including the interaction term fit significantly better than the model without ($c^2 = 6.51$, $p = 0.04$), and an examination of the interaction indicated that women with 4+ children, compared to those with 1-3 children had a significantly greater decline in LM scores over time (b=- 0.95, p=0.006). No significant main effects of the number of children or APOE status were observed on either model. Overall, our study provides evidence supporting that multiparity affects memory decline later in life. Additional work is needed to determine the degree to which socioeconomic factors mediate this association.

Role of Sex-Associated DNA Methylation Changes in Irritable Bowel Syndrome Pathophysiology

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Irritable bowel syndrome (IBS) is a female-predominant disorder of gut-brain interactions that is associated with alterations in stress response and immune pathways. Epigenetic mechanisms underlie dysregulations of several centrally mediated pathways which show sex differences in IBS. However, its role in sex-specific pathophysiologic mechanisms in IBS is not known. Our aims were to compare DNA methylation changes between sexes within IBS and healthy controls (HCs) and to identify gene ontology (GO) terms related to sex-specific changes in DNA methylation associated with IBS. Rome+ IBS patients and HCs underwent sigmoidoscopy with biopsies and DNA was extracted. DNA methylation was measured using Illumina HM450 array. FDR<0.05 was considered significant. 102 IBS (mean age 33.2 yrs, 66% F, 35 IBS-D, 36 IBS-C, 31 IBS-M) and 36 HCs (mean age 33.7 yrs, 56% F) were analyzed. Average DNA methylation was similar between sexes within IBS (p=0.4) and HCs (p=0.19). Significant differences in methylation were found between sexes within IBS (346 CpGs) and in HCs (151 CpGs). IBS specific sex-associated DNA methylation changes included stress response gene in glucocorticoid pathway FKBP Prolyl Isomerase 1B (*FKBP1B*) and Serotonin Receptor 7 (*HTR7*), which were hypermethylated in IBS females vs males (FDR<0.05). IBS-specific differentially methylated regions in females vs males included hypermethylation of solute carrier family 6 member 4 (*SLC6A4*) and hypomethylation of NLR Family Pyrin Domain Containing 2 (*NLRP2*) genes involved in pain hypersensitivity. In conclusion, sex-specific DNA methylation changes in the colonic mucosa of IBS patients in neuronal and stress related pathways are relevant in IBS pathogenesis. Epigenetic mechanisms provide important insights into sex-specific pathophysiology of IBS. Funding: NIH R21 DK104078-01A1 (07/01/15-06/30/17)

Loss of ovarian function in a mouse model of Alzheimer's disease modulates forebrain metabolic profiles: Exercise produces an adaptive protective response

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Two-thirds of individuals with Alzheimer's Disease (AD) are post-menopausal women. This vulnerability may be caused by loss of ovarian hormones during the perimenopausal transition which greatly impacts cognitive function and energy metabolism. As a result, we asked whether chronic exercise following the ovarian senescence might prevent changes in cognition and forebrain metabolite levels in the CVN-AD mouse model (*APPSwDI*$^{+/+}$*/mNos2*$^{-/-}$) that shows progressive, age-related, histological, and cognitive hallmarks of dementia. Gradual loss of ovarian function was induced at 12 wks of age (WOA) for ½ of the mice with 4-vinylcyclohexene diepoxide (VCD), a treatment that accelerates the natural process of follicular atresia, producing a menopause-like transition. Exercise, both voluntary wheel running and forced treadmill training, began at 24 WOA with control mice remaining sedentary. Novel object recognition tests at 36 WOA revealed that exercise improved short-term memory of both cycling and VCD-treated CVN-AD mice. VCD-treatment induced numerous metabolic changes in forebrain of CVN-AD mice including a decrease in activity in the non-oxidative phase of the pentose phosphate pathway. This latter effect was ameliorated by exercise. These results suggest that estrogens play a crucial role in regulating energy compounds in the brain and that exercise may work through a shared metabolic pathway to compensate for the loss when induced within the window of estrogen depletion.

Cannabis Use and Emotion Dysregulation Contribute to Sex Differences in Fear Extinction

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Cannabis is the most used drug among college students. When used chronically, cannabis has been linked to deficits in fear discrimination, fear extinction, and deficient emotion regulation. Little is known about sex-dependent effects of cannabis use on fear conditioning processes. The purpose of the present study is to examine how cannabis use may contribute to sex differences in conditioned fear extinction. Participants completed the Emotion Dysregulation Scale, which evaluates the ability to flexibly manage and monitor intense emotional states. They also completed the Kreek McHugh Schluger Kellogg scale to measure overall degree of self-exposure to cannabis (frequency, duration, and amount). Total scores were calculated and divided into high vs. low groups based on a median split. During fear acquisition, a neutral conditioned stimulus (CS) was paired with a threatening unconditioned stimulus (US) and a different CS was never paired with a US. Ten minutes later, during fear extinction, both CSs were presented repeatedly without the US. Compared to men, women who reported high cannabis use showed heightened fear expression throughout extinction training, and women who reported low emotion dysregulation and high cannabis use showed greater fear discrimination during the final block of extinction. These findings suggest that sex differences in fear extinction are in part related to emotion dysregulation and cannabis use. More research is needed to understand whether women's emotion dysregulation predisposes them to consume cannabis or is a consequence of cannabis use. NIMH grant: 1R15MH125303-01

Impact of Midlife Metabolic Health on Memory Function: Role of Sex and Reproductive Aging

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Menopause and estradiol decline is associated with altered memory circuitry function and decreased glucose metabolism, resulting in a period of vulnerability in some women. Here, we assessed the impact of metabolic health, in relation to sex and reproductive aging, on memory performance and cellular aging in early midlife. 103 participants (48M:55F) underwent metabolic and memory assessments at ages 40-50 years old. At ages 45-55, the same participants underwent follow-up memory assessments. Generalized estimating equations, stratified by sex and with the inclusion of an interaction term, were used to assess associations between metabolic function and memory outcomes. We found that at ages 40-50, there was no significant relationship between metabolic health and memory (p>0.05). However, women with prediabetic/diabetic levels of HbA1c (³5.7) at ages 40-50 performed significantly worse on memory tasks 5 years later compared to those with lower HbA1c levels (b=-6.32, p<0.01) and differed significantly from men (p<0.01). Examining the impact of menopause, we found that women with higher HbA1c levels who transitioned to postmenopause during the follow-up period had the worst memory performance 5 years later (b=-1.22, p<0.01) compared with those who remained in pre/perimenopause (p=0.05). Finally, we found that poor metabolic health was also related to accelerated cellular aging in the form of shorter telomere length (β=-0.41, p<0.05). These results suggest that midlife metabolic health is related to cellular aging and has a greater longitudinal impact on memory performance in women compared to men as women transition through menopause.
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Sex-differences in KLF5 transcriptional regulation in glioblastoma

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Sex differences are evident in the incidence, therapeutic response and survival in patients with various cancers including GBM. We generated a murine GBM model of male and female astrocytes with dual loss of NF1 and P53 that yielded a sex-biased transformation of the astrocytes with male cells being significantly more tumorigenic and therapeutically resistant compared to female cells. We examined the inhibition of a key transcription factor, KLF5 on tumorigenic phenotypes and used barcoded transposon calling cards to determine genomic localization of KLF5 and the differentially induced gene expression in male and female GBM cells. Targeting KLF5 significantly reduced proliferation, migration, clonogenic stem-cell frequency, tumorigenic protein expression and survival, but increased cell death and apoptosis in male and female GBM cells. Interestingly, male, but not female, GBM cells exhibited an increased migratory phenotype after radiation that inhibition of KLF5 significantly reduced. Transposon calling cards mapped unique KLF5 genomic localization and significantly differential gene expression profiles in male versus female GBM cells. The top genes induced by KLF5 in male cells were primarily affiliated with poorer prognosis and reduced survival, whereas in female cells they were affiliated with better prognosis and improved survival in patients with GBM. Our findings provide a promising exploratory avenue for KLF5 as a therapeutic target in male patients with GBM and warrants further investigation to delineate the precise molecular mechanisms driving sex-differences in transcriptional regulation that may lead to tumorigenic gene expression in male patients with GBM. This work is supported by the National Cancer Institute R01 CA174737-06 and P01CA245705.

Sex-specific effects of arsenic exposure on the risk of influenza infections

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Epidemiological studies consistently report that arsenic increases susceptibility to respiratory infections, including the H1N1 influenza A virus (IAV). We previously observed that IAV-infected female mice exposed to arsenic experienced greater morbidity and mortality compared to males; however, the sex-specific mechanisms that contribute to these observed differences remain unknown. We hypothesize that sex steroid hormones contribute to the arsenic-induced exacerbated immune response against IAV in females. To address this hypothesis, ovariectomized (OVX) mice, with or without intranasal IAV infection, were exposed to 0,10, or 100ppb of sodium (meta)arsenite in drinking water. Viral titers using median tissue culture infectious dose, hormone levels as assessed by ELISA, and cytokine measurements using a multiplex Luminex assay were performed. While there is a reduction in body temperature in OVX mice post-infection compared to the control group, the absence of sex hormones did not significantly alter the arsenic-related disease severity. Cytokine analysis revealed statistically significant differences in the expression of Chitinase-3-like-1 in the infected group, an inflammatory marker involved in viral clearance and influenza mediated pathology. Though not significant, higher levels of LDLR, TIMP-1, and BAFF cytokines were also observed in the OVX groups. Flow cytometry analysis of immune cells in the lung and mediastinal lymph nodes is in progress. We will also determine whether arsenic alters the expression of immune-related X-linked genes, including TLR7 and TLR8. This research will further elucidate the mechanism by which arsenic alters cytokine levels and cell function differently in females and help explain the observed sex differences during IAV infection. Funding: 5T32HL007534-37 (SS), T32ES07141 (KR, EI)

Oral Contraceptive Use Suppresses Environmental Influences on Age at Natural Menopause

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Menopause represents the end of a woman's reproductive period and is marked by a dramatic decline in sex steroid hormone production. Age at natural menopause (ANM) is a critical indictor of women's health and has been associated with a wide variety of diseases. The factors regulating the genetic and environmental determinants of ANM have not been broadly examined. We hypothesized that the mild endogenous sex hormone suppression and stable synthetic hormone levels resulting from oral contraceptive (OC) use would impact ANM, and modify the degree to which genetic and environmental factors contribute to ANM. Data were obtained from naturally post-menopausal women born between 1905 and 1958 (median = 1941) who were members of the Swedish Twin Registry. Information on ANM and history of OC use were available from 665 monozygotic and 920 dizyogotic twin pairs, as well as 1,283 unpaired women. Women who reported any past OC use had a mean ANM roughly 6 months earlier than non-users. In genetically informative analyses, the heritability of ANM in OC users was .88, compared to .50 in non-users. This difference was driven by a suppression of unique environmental influences on ANM in the OC users. Restricting the sample to those with widespread access to OC during their reproductive period (i.e., those born between 1945 and 1958), eliminated the effect of OC use on the mean ANM but did not alter the effect on genetic and environmental determinants of ANM. These finding provide novel insights into how OC may impact ANM, a critical aspect of women's health. Additional studies are needed to determine if and how duration of OC use and age at initiation impact the observed effects.

Translation of Cancer Sex Differences into Clinical Practice

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Significant and clinically important sex differences exist in cancer incidence, response to treatment, and survival. While appreciation and understanding of sex differences are advancing, it remains unclear how to incorporate sex differences into clinical practice. Based on published data, we hypothesized that sex differences in cancer phenotypes would resemble sex differences in height, and be highly overlapping while varying continuously between female- and male- biased extremes or poles. We further hypothesized that determining an individual patient's balance of male- and female- biased cancer mechanisms could inform targeted treatment planning. To test this hypothesis, we applied a Bayesian Nearest Neighbor (BNN) analysis to whole cancer transcriptomes from adult and pediatric patients suffering from 31 different cancer types to establish individual Transcriptomic Sex Indices (TSI). TSI values represent a precise partitioning of individual patient transcriptomes into female- and male- biased components. Most cancers exhibited strong polarization involving inflammation/immunity and cell cycle regulation at the female and male extremes, respectively. Importantly, females and males with overlapping TSI values possessed sex-based differences in underlying gene and pathway profiles. We conclude that patient sex, cancer type, and TSI values can augment the precision of cancer therapy.

Microglial Responses to High Fat Diet Vary by Brain Region and Sex

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Microglia are the resident immune cells in the central nervous system (CNS). Microglia activate and release cytokines in response to immune challenges, mediating the innate response. Microglia also maintain brain homeostasis through phagocytosing cellular debris and apoptotic cells, pruning synapses, and secreting trophic factors. Microglia play a central role in several pathologies, such as neurodegenerative and psychiatric disorders. Current research demonstrates that consumption of a high fat diet alters microglial activity in the CNS, contributing to the progression and consequences of metabolic diseases (e.g. obesity and type 2 diabetes). Studies to date have failed to explore sex differences in the effects of high fat diet/metabolic disease on microglia across several brain areas concurrently. This is vital given the heterogeneity of microglial distribution, morphology, and function across brain regions and in various pathological states. Male and female C57Bl6/J mice were fed either a low fat (LF; 10% fat) control or high fat (HF; 60% fat) diet from 2-6 months of age. HF diet resulted in significantly increased weight gain and glucose intolerance compared to LF diet to a similar degree in males and females. Immunofluorescence was performed to quantify markers of microglia (Iba1) and phagocytosis (CD68) across several brain areas, including subregions of the cortex, hippocampus, striatum, substantia nigra, amygdala, and hypothalamus, as well as white matter areas, such as the corpus callosum. Across several brain regions, HF diet increased microglial activity in males, but decreased it in females. Additionally, metabolic outcomes were associated with microglial markers in a sex-specific manner. Taken together, these findings demonstrate the contrast of microglial responses in males and females following chronic consumption of a high fat diet, which may contribute to the different rates of neurodegenerative and psychiatric diseases observed in men and women.
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Navigation strategy tied to sex steroid hormones

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Accumulating evidence suggests that distinct aspects of successful navigation change with advanced age. Further, rodent studies have established sex hormones' role in supporting spatial learning and memory, especially in strategy selection. Female rodents preferentially select more place-based strategies during the proestrus phase of their cycle (elevated estradiol) than during estrus (low estradiol), when their preference shifts towards response-based strategies. However, the influence of endocrine aging and sex hormones on navigation performance in women is underexplored. Midlife is a period when women transition to menopause, ovarian hormone production declines up to ~90%, and structural and functional changes within the brain's memory and navigation circuitries may first emerge. This represents a critical gap in our understanding of the aging brain and the factors that influence navigational deficits. We tested the hypothesis that deficits in spatial navigation covary with sex hormones in midlife, healthy women (n=64, *age 43-61*) who completed three immersive virtual navigation tasks: 1) *path integration,* which relies on proprioceptive and vestibular self-motion cues 2) *spatial knowledge*, the process of forming and using a cognitive map to navigate in a new environment; and 3) *navigational strategy*, which refers to the nature of the route individuals select to navigate to a goal location in a known environment. Preliminary findings reveal better spatial memory and heightened preference for shortcuts is positively associated with higher concentrations of estradiol. Elevated FSH, a marker of advanced reproductive stage, is marginally associated with reduced shortcuts. Sex hormones had no discernable influence on path integration or acquisition of spatial knowledge. Based on these findings, it appears that the decline in ovarian sex hormone production in midlife influences navigation strategy in women.
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Dissecting the role of hypothalamic estrogen receptor alpha in the regulation of body temperature in mice

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Estrogen Receptor alpha (ER) signaling in the hypothalamus modulates heat generation and heat dissipation, leading to changes in temperature balance. ERa signaling specifically in the ventromedial nucleus of the hypothalamus (VMH) is thought to regulate heat generation but not heat dissipation, suggesting that specific hypothalamic regions can alter distinct components of temperature balance. We recently discovered sex-specific expression of the estrogen-responsive gene, reprimo (*Rprm*), in the VMH. *Rprm* knockdown using small interfering RNAs increased heat generation and core temperature in female but not male mice. However, it is unknown if *Rprm* and ER function in the same cells to alter body temperature. To test the hypothesis that ER within *Rprm*-expressing neurons alters heat generation and core temperature, we engineered a new knock-in mouse line that encodes Cre recombinase downstream of *Rprm* (Rprm-Cre) and crossed it to the conditional knockout allele of *Esr1*, the gene that encodes ER. We find that Esr1 f/f; Rprm-Cre mice lack ER in *Rprm*-expressing cells within the VMH and other endocrine tissues. *Esr1 f/f; Rprm-Cre* mice exhibit decreased core temperature in the dark phase when mice are active. The effect is selective, as we do not observe changes in physical activity or body weight. Furthermore, mice lacking ER in *Rprm* expressing cells showed higher lipid content in heat-generating brown adipose tissue, suggesting a reduced capacity for heat generation. This conditional knockout mouse model suggests a critical role for *Rprm*-expressing cells, within the VMH or elsewhere, in mediating the effects of estrogens on heat generation in female mice.

Non-Genomic Estrogen Effects on Mitochondria in Human Bronchial Epithelium

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A strong sex bias in asthma is highlighted by epidemiological studies showing increased asthma prevalence and severity in females after puberty onset, suggesting that estrogen plays an important role in this phenomenon. Estrogen exposure can regulate the function of airway epithelial cells, a cell type critical for asthma pathogenesis. Furthermore, mitochondria are relevant to airway diseases and become dysfunctional in response to increased inflammation and oxidative stress. While the effects of estrogen are classically thought to involve genomic mechanisms via its nuclear receptors, there is also robust evidence for rapid, non-genomic mechanisms of estrogen signaling through either estrogen receptor (ER) alpha (ERa), ERb, or G-protein coupled estrogen receptor (GPER). Here, we sought to determine whether acute 17b-estradiol exposure or ERa, ERb, or GPER agonism detrimentally affects mitochondrial structure (MitoTracker imaging) and function (Seahorse mitochondrial stress tests) through non-genomic pathways (immunoblotting) in normal human bronchial epithelial cells. Our findings link acute estrogen exposure with altered mitochondrial structure/function in the normal airway epithelium, which may involve mitochondrial fission protein DRP1, MAPK, and CREB signaling. These studies are supported through the Mayo Clinic Specialized Center of Research Excellence and Women's Health Research Center, NIH U54 AG044170 (Bartman and Chiarella), AHA 20POST35210002 (Bartman), and NIH K08 AI141765 (Chiarella).

Sex and hormonal contraceptive use contributes to differences in fear acquisition and extinction learning

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Risk for developing posttraumatic stress disorder (PTSD) is multi-determined; but in part depends on sex, with women having approximately twice the risk as men. Fear learning processes underlie the pathogenesis and maintenance of PTSD, and fear extinction processes model exposure therapy – a key treatment for PTSD. Hormonal contraceptive usage is a critical area of research as 85% of women in the United States will use hormonal contraceptives in their lifetime. However, there is a dearth of preclinical research examining sex-related variables in fear conditioning and even less taking hormonal contraceptive use into account. We measured changes in acoustic startle response (defensive reflex) during a fear discrimination learning task in naturally cycling and hormonal contraceptive-using females and males. During fear acquisition, a neutral conditioned stimulus (CS+, danger cue) was paired with a threatening unconditioned stimulus (US) and a different neutral stimulus (CS-, safety cue) was never paired with a US. Ten minutes later, during fear extinction, both danger and safety cues were presented repeatedly without the US. Males failed to show the same discrimination between the danger and safety cues that naturally cycling and hormonal contraceptive-using females showed at the end of acquisition. Naturally cycling females differed from females using hormonal contraceptives and males, with only naturally cycling females acquiring extinction learning in the final block of extinction. These findings underscore the importance of considering sex differences and hormonal contraceptive use in fear learning and could provide insight into woman's increased risk of developing PTSD.
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Differential effects of nucleus accumbens deep brain stimulation on cocaine reinstatement in female and male rats.

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There are currently no FDA approved treatments to reduce cocaine craving and relapse. Recent basic and clinical studies have shown that deep brain stimulation (DBS) in limbic regions involved in drug craving, particularly the nucleus accumbens (NAc), can reduce drug seeking behavior. Our previous work indicated that DBS of the NAc shell attenuated cocaine reinstatement in male rats, but in a significant oversight, this effect was not assessed in female rats. Mounting evidence indicates female rats are more susceptible to reinforcing effects of cocaine and are therefore more vulnerable to relapse. Here, we sought to determine the effect of NAc shell DBS on cocaine seeking behavior in male and female rats, and whether this effect is dependent on estrous cycle phase in females. Rats were allowed to lever press for i.v. cocaine 2 hours daily for 21 days. Lever responding was extinguished in daily sessions during which cocaine was replaced with saline. Cocaine seeking was measured as lever presses in reinstatement sessions immediately preceded by acute cocaine delivery (10mg/kg/i.p.). All rats received both sham (0 µA) stimulation and 150 µA stimulation of the NAc shell throughout two counterbalanced reinstatement sessions; female rats were assessed in either estrus or non-estrus phases for both reinstatement sessions. Preliminary data suggest, contrary to the effect in male rats, DBS of the NAc shell in female rats fails to attenuate, and in non-estrus phases, may even enhance cocaine seeking behavior. Our initial results indicate clear sex differences in the effects of DBS on cocaine seeking in rats. Funded by R01 DA33641.

The sex chromosomes regulate sex-biased networks throughout cardiac development.

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There is a surprising amount of transcriptional and epigenomic variability between males and females in all somatic cells, including the heart. We have previously shown sex-biased gene expression even in stages of cardiogenesis before gonad formation. This suggests that the sex chromosome constitution contributes to sex biases, especially considering that genes on the sex chromosomes encode transcriptional and epigenetic factors with downstream autosomal gene targets. We hypothesize that both sex chromosome and hormonal effects contribute independently and jointly to sex-biased expression and epigenetics throughout cardiogenesis, with consequences for the sex differences in the adult heart at the molecular and functional levels. To identify the independent effects of sex chromosomes on cardiac development, with a focus on transcription and epigenetic factors, we exploit the Four Core Genotypes (FCG) mouse model. We isolated RNA from embryonic, neonatal and adult hearts from FCG mice and performed RNA-seq. Interestingly, we found differential expression exclusively dependent on the difference in sex chromosome composition throughout cardiogenesis, with the strongest effects at the adult stage. We also identified sex-specific expression jointly regulated by sex chromosome composition and sex hormone effect. Among the sex-biased factors, we focused on the sex chromosome-dependent transcription and epigenetic factors. Systems level analysis of sex chromosome effects on regulatory factors in the adult heart identified XX versus XY subnetworks embedded in the overall cardiogenic regulatory network. These subnetworks are independent of sex hormone effects and their master regulatory nodes are candidates for explaining the sex differences in cardiovascular health and disease.

Sex, age, and bone compartment dependent consequences of Fragile X mental retardation 1 deletion in mice.

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Mutations of the chromosome X-encoded fragile X mental retardation1 (FMR1) gene lead to Fragile X syndrome, intellectual disabilities and craniofacial defects, features reproduced in FMR1 KO mice. Others showed that 4-months (mo) male FMR1-deficient mice exhibit a mild femoral bone phenotype, with high cortical thickness (CtTh) and relative bone volume. Yet, FMR1 role in young and aged mice and in males vs females is unknown. We now report the bone phenotype of 2mo and 9mo male $FMR1^{y/+}$ and $FMR1^{y/-}$ and female $FMR1^{+/+}$ and $FMR1^{-/-}$ mice. Bone mineral density is 4-5% higher in male and 9-13% higher in 2mo female FMR1 KOs vs wild type (WT) mice and remains 4-8% higher in 9mo $FMR1^{y/-}$ and $FMR1^{-/-}$ mice. Yet, tissue mineral density (TMD) in distal femur is 3% high in males and 8% in females, but only high (+1.2%) in females in femoral cortical bone at the mid-diaphysis at 2mo and unchanged in 9mo FMR1 KOs for either sex or site. Further, femur cortical relative bone area (BA/TA) and CtTh are 2-5% higher for either sex vs WT mice at 2mo. But periosteal surface is 2% higher in males and marrow area (MA) is 8% lower in females, suggesting that structural changes stem from distinct mechanisms in the 2 surfaces of the cortical bone in male vs female mice. At 9mo, BA/TA and CtTh are 10-17% higher in males but 15-20% lower in females and MA is 38% higher only in female KO mice. Trabecular bone measurements in distal femur are higher only in female KOs at 2 (30-140%) or 9mo (30-80%) vs WT mice. In summary, we have uncovered sex, age, and bone compartment dependent skeletal effects of FMR1 deletion. Future studies are needed to determine the cellular and molecular basis of these divergent consequences of the absence of the FMR1 gene.

Exploring the associations between gender-related variables and physical activity levels in women with atrial fibrillation.

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The Canadian 24-Hour Movement guidelines recommend adults engage in ≥150 min/wk of moderate-to-vigorous physical activity (PA). Females with atrial fibrillation (AF) report low PA levels. Yet, there is limited research exploring gender-related factors (i.e. socially constructed roles, behaviours and self-expressions) influence and PA behaviours. To explore the associations between gender-related variables and meeting PA guidelines in women with AF. Women with AF completed questionnaires assessing gender-related variables and PA levels (IPAQ questionnaire). Chi-square analyses were used to test the associations between gender-related variables and PA status (i.e. inactive vs. active). Of 208 participants who self-identified as women (65±13y), 48.6% did not achieve PA recommendations. No significant associations between PA status and ethnicity (British: 48.1% [n=50] inactive vs 38.5% [n=27] active, p=0.10), marital status (married: 45.3% [n=63] inactive vs 35.3% [n=49] active, p=0.80), employment status (retired: 50.0% [n=72] inactive vs 29.9% [n=43] active, p=0.13), or income (50-75K: 48.8% [n=20] inactive vs 36.6% [n=15] active, p=0.46) were observed. Significant associations between PA status and household demographics (living with one person: 56.0% [n=14] inactive vs 12.0% [n=3] active, p<0.001) and education levels (university degree: 46.3% [n=25] inactive vs 35.2% [n=17] active, p=0.02) were observed. Most women with AF did not meet the PA guidelines. Household and education levels were significantly associated with PA status. Women with AF may need additional support to be regularly active.

Sex-Specific Impact of Excessive Daytime Sleepiness on Mortality Risk in Obstructive Sleep Apnea

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Excessive daytime sleepiness (EDS) is the hallmark symptom of obstructive sleep apnea (OSA), and is associated with impaired functioning and increased risk of adverse outcomes. However, it is unclear whether the prognostic impact of EDS differs as a function of sex. With this study we aimed to evaluate the association between EDS and all-cause mortality in a large sample of male and female patients with OSA. Adult patients who were newly diagnosed with OSA following polysomnography at the Mayo Clinic Sleep Center, and who completed the Epworth Sleepiness Scale for assessment of perceived sleepiness were included in this study (N=14,745; 61% males; median [interquartile range] age, 61 [51, 70] years). EDS was defined as a score >10 at the Epworth Sleepiness Scale. After a median 6.2 (4.5, 8.1) years of follow up, risk of death was 1.27-times (95% CI 1.07-1.49) greater in women with EDS than in those without, after adjusting for age, body mass index, race and smoking history. The association was not attenuated after further controlling for sleep characteristics and comorbidities at baseline. In fully adjusted model, the hazard ratio for mortality in sleepy OSA women vs their non-sleepy counterpart was 1.26 (95% CI 1.05-1.47). Importantly, further adjustment for OSA therapy did not diminish the strength of the observed associations. In men, EDS was not associated with mortality in any models. Our results show that the prognostic implications of EDS in OSA are sex-dependent, with hypersomnolence being independently associated with greater vulnerability to premature death only in female patients.

The Second X Chromosome Mediates Stroke Sensitivity in The Aged

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Introduction: In addition to hormonal effect, the intrinsic genetic factors also contribute to stroke sensitivity. Our previous studies suggested sex chromosomal effect plays an important role in the sex differences in aged mice; however, which chromosomal complement (X vs. Y) is involved has been elusive. In this study, we utilized the XY* mice model in which mice of four genotypes were generated including XX, XO, XY, and XXY, to investigate the effects of X and Y chromosome on stroke sensitivity in aged animals. We hypothesized that it is the second X chromosome that leads to the sexual dimorphic stroke phenotypes in the aged.
Methods: Aged XY* mice were subjected to a 60-min middle cerebral artery occlusion (MCAO). Infarct volumes and behavior deficits were quantified 3 days after MCAO. To examine the neuroinflammation after stroke, microglial activation was analyzed with flow cytometry, and plasma levels of inflammatory mediators were determined by ELISA. Another cohort of XY* mice were gonadectomized (GDX) at 3 weeks old, and subjected to MCAO at 8-12 weeks of age to exclude the hormonal activational effect. X chromosome escapee genes, *Kdm5c* and *Kdm6a,* was examined in microglia by IHC in XY* aged mice, and by RNAseq in wild type C57BL/6 mice.
Results: Aged XX mice had significantly larger infarct size than XO mice, in the striatum ($p = 0.0451$) and the ipsilateral hemisphere ($p = 0.0413$); increased infarction was also seen in XXY vs. XY mice striatum ($p = 0.0027$). Neurological deficit scores were higher in XX vs. XO aged mice. Mice with two copies of X chromosome had significantly higher plasma levels of the pro-inflammatory cytokines TNFα and IL-1β than the mice with one X. Microglial expression of *kdm5c* and *Kdm6a* was significantly higher in mice with two vs. one copy of X chromosome by RNAseq and IHC. GDX XY* mice showed similar stroke outcome pattern as aged XY* mice.
Conclusion: The second X chromosome contributes to stroke sensitivity in aged mice, a chromosomal effect that is independent of hormonal activational effect. The X chromosomal effect might be implicated with the neuroinflammation after stroke and the X chromosome escapee genes *kdm5c* and *Kdm6a.* Keywords: Aging; *Kdm5c; Kdm6a;* Sex differences; Stroke; X chromosome; XY* mice. Funding: This work was supported by funding from NIH Grants R01 NS093042/NS108779 to Fudong Liu.

Diurnal fluctuations in testosterone tied to variation in intrinsic functional connectivity in a densely sampled male

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Sex hormones are powerful neuromodulators of learning and memory, influencing the brain at the level of microscopic intracellular events to macroscopic brain organization. A central feature of the mammalian endocrine system is that hormone secretion varies over time. Human neuroimaging studies that densely sample individuals over timescales of days, weeks, and months provide unique insight into the role our endocrine system plays in regulating the dynamic properties of the human brain. For example, we recently established estrogen's ability to drive widespread patterns of connectivity and enhance the global efficiency of large-scale brain networks in a densely-sampled female (Pritschet et al., 2020). In men, sex hormone production follows a sinusoidal pattern with a peak in testosterone between 6-7am and nadir between 7-8pm. To capture these diurnal changes in hormone production, a male participant underwent brain imaging and venipuncture every 12-24h for 30 consecutive days, resulting in 40 sessions. Results confirmed diurnal fluctuations in testosterone and cortisol, with decreases of ~63% and ~92%, respectively. Standardized regression revealed predominantly positive associations between whole-brain patterns of coherence and testosterone concentrations. Nodes in the Dorsal Attention and Salience/Ventral Attention Networks displayed the strongest associations with testosterone concentrations. Findings from this study enhance our understanding of testosterone as a rapid neuromodulatory hormone and provide evidence that diurnal changes in steroid hormone production impact large-scale brain networks.

Antagonism of astrocytic glutamate transporter 1 in the dorsal hippocampus blocks the memory-enhancing effects of 17-beta estradiol in ovariectomized mice

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Infusion of 17b-estradiol (E_2) into the dorsal hippocampus (DH) enhances memory consolidation, an effect that depends upon rapid phosphorylation of extracellular signal-regulated kinase (ERK) and Akt, presumably in glutamatergic neurons. Astrocytic glutamate transporter 1 (GLT-1) is responsible for glutamate uptake from the synaptic cleft, thereby modulating glutamatergic neurotransmission. However, little is known about the contribution of DH astrocytes to the memory-enhancing effects of E_2. The main aims of this study were to establish whether DH GLT-1 activity is necessary for E_2-mediated memory enhancement, and to determine the extent to which E_2 triggers rapid ERK and Akt phosphorylation in DH astrocytes. Female mice underwent bilateral ovariectomy (OVX) and implantation of infusion cannulae into the DH or into the DH and dorsal third ventricle (ICV). Immediately after training in object recognition and object placement memory tasks, mice were infused into the DH with vehicle or a dose of DHK that does not impair memory, and ICV with vehicle or E_2. Astrocytes were later isolated using Magnetic Activated Cell Sorting, and levels of pERK and pAkt were measured by Western blot. The memory-enhancing effects of E_2 in both tasks were blocked by DH DHK infusion. DHK also prevented E_2 from increasing levels of p42ERK and pAkt in DH astrocytes. Our results suggest that DH GLT-1 activity is necessary for E_2 to enhance memory and rapidly activate ERK and Akt signaling in OVX mice. These findings suggest a key role for DH astrocytic GLT-1 in E_2-mediated regulation of memory consolidation. Supported by R01MH107886 to KMF, 1F31MH118822-01A1 to LRT, National Scholarship Programme of the Slovak Republic, SAIA, n.o., and Tatra bank foundation grant to MP.

Sex Differences in Arterial Hemodynamics in Patients with Degenerative Thoracic Aortic Aneurysms

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Women with thoracic aortic aneurysm (TAA) experience worse outcomes as compared to men. Further, we have previously shown that female sex is associated with faster TAA growth, which is unique to those with degenerative forms of TAA (dTAA). We sought to evaluate potential differences in arterial health parameters that could explain sex differences in TAA growth and outcomes. We included 80 participants (33% women) with unoperated dTAA. Aortic stiffness, central blood pressure, and measures of steady and pulsatile arterial load were estimated with validated techniques that combine applanation tonometry and transthoracic echocardiography. Men and women did not significantly differ in age (67.2±9.5 vs 67.2±9.8 years, *P*=0.33), hypertension (HTN) (68.5% vs 65.4%, *P*=0.78), mean arterial pressure (MAP) (91±10 vs 91±10 mmHg, *P*=0.79) and TAA size (46.6±3.9 vs 46.4±5.7 mm, *P*=0.87). Women had higher aortic characteristic impedance (Z_c) (mean difference [MD]: 37.35±16.07 dyne x s/cm^{5,} *P*=0.03) and reflected pressure wave amplitude (Pb) (MD: 4.34±2.05 mmHg, *P*=0.04), and lower total arterial compliance (TAC) (MD: -0.65±0.18 mL/mmHg, *P*=0.001) than men. In multivariable linear regression analysis adjusted for age, HTN, MAP, diabetes, smoking history and body mass index, Z_c and Pb remained significantly higher (respectively 17.18±6.36 dyne x s/cm^5, *P*=0.009 and 1.74±0.77 mmHg, *P*=0.03), while TAC (-0.29±0.09 mL/mmHg, *P*=0.002) was significantly lower in women compared to men. In conclusion, despite comparable TAA sizes and MAP, women with dTAA may be affected by more severe alterations in arterial health and pulsatile hemodynamics, which could explain sex differences in dTAA growth and outcomes. This remains amenable to testing in future prospective studies.

Characterizing sex specific changes in astrocytic mitochondria in an AAV-alpha-synuclein model of early Parkinson's disease

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Parkinson's disease (PD) primarily degenerates dopaminergic neurons in the nigrostriatal pathway and human male and females progress on separate timelines and present with separate symptoms. While dysfunctions in astrocytes and brain mitochondria have separately been tied to PD and sex hormones are known to affect astrocyte and mitochondrial function, the subset of astrocytic mitochondria has not been evaluated for sex differences in health or in the context of PD. Here, we study astrocytic mitochondrial dysfunction in the dorsal lateral striatum (DLS) of a mouse model of early PD. To create this model, we performed unilateral stereotaxic injection of adeno-associated virus (AAV) overexpressing human alpha-synuclein (α-syn) in the substantia nigra pars compacta (SNc). At 5 months post injection, equivalent loss of SNc tyrosine hydroxylase expression occurred in both sexes of control and α-syn mice. Interestingly, male and female α-syn mice displayed significant, inverse changes in dopamine and major metabolite concentration in the DLS compared to control counterparts. Kinetics of Ca^{2+} signaling in DLS astrocytic mitochondria did occur with α-syn injection. Specific changes in spontaneous Ca^{2+} event kinetics occurred in all α-syn mice, but changes were more prominent in female mice. We also assessed sex differences in other mitochondrial characteristics: morphology, protein expression, and respiration. Together these data suggest that significant pathological alterations in astrocytic mitochondria can occur in an early model of PD and underlying dysfunctions could be sex dependent.

Sex Differences in Novel Transgenic Mice with Constitutively Upregulated Acetylcholine Receptors: Implications for Parkinson's Disease

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Parkinson's disease (PD) incidence rates predict a worldwide pandemic that will affect over 12 million people by 2040, underscoring an urgent need for neuroprotective drugs. Unfortunately, no neuroprotective drugs are currently available, and most proposed neuroprotective drugs failed clinical trials because PD is produced by a range of insults not replicated in any one animal model. For this reason, we focus on hyperactivated endoplasmic reticulum (ER) stress, a convergent apoptotic mechanism for multiple PD-related toxicities. Nicotine reduces PD risk, however, nicotine concentrations in tobacco users cannot activate neuronal nicotinic acetylcholine receptors (nAChRs), making this an unlikely mechanism for neuroprotection of dopaminergic (DA) neurons. We have previously shown that nanomolar concentrations of nicotinic ligand, cytisine, rapidly chaperone β2-subunit-containing (β2*) nAChRs out of the ER. This directly reduces the ER stress response, which is critical for neuroprotection. To test this hypothesis, we created a novel transgenic mouse line named β2-mutant, with enhanced ER export of β2* nAChRs. Surprisingly, β2-mutant mice demonstrate significant increases in Sec24D ER exit sites (ERES) within substantia nigra pars compacta (SNc) DA neurons of only female, but not male mice. We also induced parkinsonism in mice by unilateral injection of 6-OHDA in the dorsolateral striatum. Interestingly the β2-mutantions reduced apomorphine rotations only in female mice. Our data suggests the β2-mutations exert neuroprotection in female mice only.

The impact of testosterone on Coxsackievirus B3 pathogenesis

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Enteroviruses are some of the most common viruses infecting humans worldwide, with an estimated economic burden of seven billion dollars annually in the United States. Among enteroviruses, Coxsackievirus B3 (CVB3) is frequently isolated and a primary cause of viral myocarditis and pleurodynia. Moreover, CVB3 infection exhibits a strong sex bias, with males showing increased susceptibility to disease, which is incompletely understood. Using an oral inoculation mouse model to study CVB3, we previously found that male mice supported robust intestinal CVB3 replication and succumbed to CVB3-induced disease, whereas female mice did not. Further, castration rescued male mice from CVB3-induced lethality, suggesting that testosterone plays an important factor following oral inoculation. To further characterize testosterone's role, we examined CVB3 pathogenesis in testosterone-depleted and testosterone-treated mice. We found that testosterone-treated mice had higher fecal CVB3 titers than testosterone-depleted mice. Further, CVB3 was more lethal in testosterone-treated male mice than in testosterone-depleted male mice. We also observed that testosterone impacts the cytokine and adaptive immune response to CVB3. Overall, these data indicate that testosterone promotes CVB3 replication in the intestine and alters the immune response to enhance pathogenesis.

Hormonal Mechanisms of Sex Differences In Migraine

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A prominent sex difference exists for migraine such that roughly 18% of adult women report experiencing migraine compared to only 6% of adult men. We hypothesized that hormonal mechanisms may contribute to the observed sex difference in migraine and utilized applications of the classical twin design to investigate this theory. Data were utilized from 51,872 participants from the Swedish Twin Registry. First, we fit a sex-limitation model to determine if quantitative and/or qualitative genetic differences exist for migraine risk. Next, we used a multilevel logistic regression model to compare the prevalence of migraine in individuals from opposite-sex and same-sex twin pairs to determine whether differences in the prenatal hormone environment contribute to migraine risk. Finally, we used multivariate biometrical models to determine the degree of genetic and environmental overlap between migraine and early menarche. Women had a significantly higher rate of migraine relative to men (17.6% vs. 5.5%). Results from an ADE sex-limitation model indicate that migraine is equally heritable in men and women, with a broad sense heritability of 0.45, (95% CI = 0.40–0.50). A reduced AE model provided evidence for differences in the genes underlying migraine across men and women. The logistic regression analysis revealed a significant increase in migraine risk for females with a male co-twin relative to females with a female co-twin (OR = 1.51, 95% CI = 1.26–1.81). Our multivariate biometric modeling found that a significant genetic correlation exists between early menarche and migraine. These results suggest that hormonal mechanisms play a substantial role in contributing to the observed sex difference in migraine risk.

Age-, sex-disparities in dysregulated hepatic one-carbon metabolism (OCM) in patients with nonalcoholic fatty liver disease (NAFLD)

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Elevated tissue homocysteine (Hcy) caused by dysregulated OCM contributes to the pathogenesis of NAFLD in mice models, while Hcy-lowering OCM cofactors protect from NAFLD (manuscript under review). With small negative trials published, the therapeutic potential of Hcy-lowering supplements in NAFLD patients is unknown. Since sex hormones regulate OCM enzymes expression, OCM may be differentially dysregulated in patients with NAFLD by age and sex. We evaluated the associations between hepatic gene probes relevant to homocysteine metabolism (PON1, PON2, PON3, PEMT, BHMT, MTHFR, and CBS) and histologic severity in patients with NAFLD using existing human hepatic microarray data (NIH RC2-AA019399, PI: AMD) for personalizing a Hcy-lowering approach. Multiple ordinal logistic regression models were used to analyze the associations, considering a p-value of 0.05 to declare the significance. Lower PON3 and BHMT expressions were associated with worse steatosis, and lower PEMT and CBS expressions were associated with more severe fibrosis. In sub-analyses by age 50 and sex, lower CBS and PON1 expressions were associated with more severe steatosis and fibrosis among young women; lower PEMT expression was associated with more advanced fibrosis among young men; lower PEMT and CBS expressions were associated with more advanced fibrosis among older women. In summary, decreased gene expressions of enzymes, increasing tissue Hcy or its highly reactive thiolactone-metabolite, were associated with more severe fibrosis and steatosis in patients with NAFLD in age-/sex-specific manners.

Estrous cycle modulation of fear extinction and relapse

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Impaired inhibition of learned fear is a feature of stress-related psychiatric disorders such as depression, generalized anxiety disorder and post-traumatic stress disorder. Extinction-based exposure therapy is the behavioral therapy of choice for these disorders but has limited long term efficacy due to vulnerability of fear memories to relapse. Women are up to 60% more likely to experience anxiety disorders and up to twice as likely to experience PTSD compared to men. Although sex differences exist, they are not fully considered in neuroscience. Females can have enhanced extinction compared to males, and strength of extinction in females varies depending on phase of the estrous cycle during which extinction is learned. However, whether estrous phase during fear extinction impacts later relapse has not been well characterized. The goal of this experiment was to determine if sex and phase during extinction modulates fear relapse. Adult, male and female Long-Evans rats were exposed to auditory conditioning in Context A, followed 24 h later by extinction in Context B. The next day, rats were re-exposed to the conditioned stimulus in either the same context, (Context B), or a different context (Context C) to assess renewal. Spontaneous recovery of fear was assessed 1 wk later. Males and females learned conditioning and extinction similarly. Compared to males and females exposed to extinction during metestrus or diestrus, females exposed to extinction during proestrus or estrus were protected from fear relapse. These data suggest that learning fear extinction under high levels of ovarian hormones is protective against fear relapse. These results could have important clinical implications for use of exposure therapy in women.

An International Comparison of Postoperative Pain Management in Patients Undergoing Orthopedic Surgery

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A retrospective review of US and Romanian patients was performed to determine if a substantial discrepancy in opioid use would result in differences in subjective pain control. 244 Romanian patients and 184 US patients underwent one of six pre-determined orthopedic procedures in a six-month period beginning May 2019. Opioid and nonopioid analgesic medication use and subjective pain scores during the first 24 hours after surgery were analyzed. In the US, women reported higher pain than men (p=.0181), while in Romania, women and men did not report statistically different pain scores (p=.1686). There was no significant difference in the amount of opioids given to US patients with regard to sex (p=.4258) or age (p=.0975). Rather, in the United States, the effect of opioids depends on injury type (p=.0215), but not on age (p=.5812), or sex (p=.4701). For the Romanian patients, there was no significant difference between men and women in the amount of acetaminophen (p=.8287), diclofenac (p=.4089), or metamizole (p=.7087) given. Higher pain scores in American females despite equivalent amounts of narcotic to their male counterparts, and the absence of a difference in Romanian patients suggests that the current American postoperative pain regimen may be tailored to the needs of male patients. Further research needs to be performed to determine the safest, most efficacious pain regimen suitable for all patients. Funding: No grant or external funding was received for this project.

Assessing the Extent to Which Mobile Health Applications Integrate Sex and Gender: A Multiple Sclerosis Example

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Nearly 100,000 people live with Multiple Sclerosis (MS) in Canada, the majority of whom are female adults. Sex and gender differences in MS experiences have been reported with respect to menstruation, pregnancy, breastfeeding, menopause, and health maintenance. The overarching purpose of this work was to illuminate the extent to which apps for the management of MS took sex and gender into consideration. The researchers searched the App Store using the search term “Multiple Sclerosis” for mobile health applications (apps) that are designed to help people who have MS manage their symptoms. Apps that were not used for MS management or not in English were excluded. Eligible apps were downloaded using an iPhone 13. The researchers evaluated the apps by creating a profile and exploring the app to determine if any content was available that was specific to a user’s sex and gender. In total, the search returned 80 apps. Of those, six (N = 6) met the eligibility criteria. All apps (N = 6) asked for the user’s sex when creating a profile. Two of the apps (n = 2) forced the user to select a sex in binary terms. Additionally, two other apps (n = 2) provided educational content about how MS may impact female adults. None of the apps provided tailored content according to one’s sex or gender or any functions such as a menstruation or pregnancy tracking tool. No apps provided any content related to non-binary identities. Although female adults experience MS more than other sexes, this is not reflected in apps which could impact the effectiveness and usefulness of the apps. The impact of innovations for health could be stifled if app developers are not considering the unique needs of users according to their sex and gender.

Sex Differences Among U.S. Active Duty Service Members With Posttraumatic Stress Disorder and Comorbid Disorders

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Psychological comorbidity, the co-occurrence of mental health disorders, is more often the rule than the exception among both military service members and civilians with posttraumatic stress disorder (PTSD). Extensive research shows prevalence estimates for specific psychological disorders differ by sex, however, little is known about whether these patterns persist in the presence of a comorbid PTSD diagnosis. This study examined sex differences in prevalence estimates for conditions comorbid with PTSD using medical records for 523,626 active duty Sailors and Marines who entered the U.S. military over an eight-year period. Using a chi-square test of independence, we detected statistically significant sex differences for specific comorbid conditions in the sub-sample of 9,447 service members with a PTSD diagnosis. Specifically, women were more likely than men to have PTSD with comorbid adjustment ($\chi^2 = 40.33$, p=.01), depressive ($\chi^2 = 130.92$, p=.01), eating ($\chi^2 = 185.50$, p=.01), and personality disorders ($\chi^2 = 294.80$, p=.01). Men had a greater likelihood than women to have a diagnosis of PTSD with comorbid alcohol use ($\chi^2 = 46.88$, p=.01), drug use ($\chi^2 = 14.82$, p=.01), and insomnia disorders ($\chi^2 = 82.45$, p=.01). No significant sex differences emerged for comorbid bipolar, obsessive compulsive, phobia, psychotic, or somatoform disorders (p’s $\geq$.05). Results indicate that there are sex differences among many conditions comorbid with PTSD, which could have implications for treatment development and delivery. This work was supported by the Defense Health Agency Restoral Funding under work unit no. N1809.

X-linked histone demethylase dosage in pre-adipocytes influences sex differences in energy expenditure and cold tolerance

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Males and females differ in body fat content, basal metabolic rate, and metabolic fuel utilization. Sex differences in adiposity have been studied extensively in the context of gonadal hormones from ovaries and testes. Less is known about the mechanisms that underlie sex differences in energy expenditure and brown adipose tissue thermogenesis. Our previous work demonstrated that adiposity is influenced by global X-chromosome dosage (XX vs. XY) independent of gonadal hormones. Further, we demonstrated correlations between adiposity in mice and humans and levels of the X-linked histone demethylase, *Kdm5c*, which escapes X chromosome inactivation. In the current study, we reduced *Kdm5c* gene dosage in white and brown adipocyte precursors, which led to reduced white adipose tissue mass, but maintenance of brown adipose tissue mass. Reduced *Kdm5c* gene dosage in adipose tissue was associated with increased basal metabolic rate, increased mitochondrial content, reduced weight gain on a high-fat diet, and improved tolerance to acute cold exposure. We hypothesize that KDM5C histone demethylase activity regulates the expression of key metabolic genes that control energy balance and thermogenesis. These findings have important implications for sex differences in obesity, as female mice and humans have higher *Kdm5c* expression levels than males. Funding: This study is funded by NIH SCORE NIDDK U54 DK120342. The student researcher is funded by NIH NRSA F31 DK127735.

Sex differences in the effects of GLP-1-estradiol to mitigate detrimental effects of a high-fat high-sugar diet

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Consuming a diet high in saturated fats and refined carbohydrates, such as the western diet (WD), not only increases the risk of obesity, but can also negatively impact cognition. However, most studies investigating the effects of diet on cognition thus far have only included male subjects despite sex differences in several processes involved in metabolic health. Glucagon-like peptide-1 (GLP-1)-based treatments have emerged as effective obesity drugs that reduce appetite and body weight and improve blood glucose regulation. Sex hormones, such as 17β-estradiol (E), increase GLP-1's satiating efficacy, and also has positive effects on body weight and neurological health. A combined molecule of these hormones, GLP-1-E (GE), reduces body weight and improves blood glucose regulation more potently than each hormone administered alone or in unconjugated form. Due to the beneficial effects of these hormones on metabolic and neurological health, in addition to the expression of both receptor types in the hippocampus (HPC), we hypothesized that GE may be beneficial for cognition, and acts in a neuroprotective manner in individuals consuming WD. Adult male and female Sprague Dawley rats were given WD or standard diet for 2 months. After 1 month of diet exposure, rats received daily subcutaneous injections of GE or control for 1 month. GE significantly reduced body weight in both sex and diet groups compared to controls, although a larger percentage of weight loss was observed in males. Females on WD had a significantly higher percentual body weight increase than males after diet exposure alone. Furthermore, GE was only sufficient to improve blood glucose in females. Effects of GE on an HPC-dependent task are currently being analyzed. These findings suggest that although high-fat, high-sugar diets negatively impact body weight and blood glucose regulation in both males and females, GE's efficacy to offset each metabolic parameter varies by sex. Funding: CIHR (to LAMG PJT 148662), Swedish Research Council (to JR 2021-00220).

Testosterone and Aromatase levels are linked to COVID-19 disease severity

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Males have a higher fatality rate than females when infected with severe acute respiratory syndrome coronavirus 2 (COVID-19). We investigated the role of gonadal hormones as potential mediators of these sex difference. Blood samples were collected after consent in adult patients that were hospitalized with a positive COVID-19 PCR at Memorial Hermann Hospital under IRB approved protocols. Control samples were collected from non-hospitalized volunteers who had a negative PCR for COVID-19. ELISA was run on plasma samples of the control (n= 67) and COVID-19 patients (n=332) for testosterone and aromatase. Average patient age was 54 years. COVID-19 severity was categorized as severe (CRP levels >75 mg/L upon admission, intubated and/or later died due to COVID-19) or mild (CRP <75 mg/L upon admission, oxygen supplementation with normal/high flow nasal cannula). When compared to controls (2.81±0.49 ng/mL, n=21) testosterone levels decreased significantly in both males with mild disease (1.68±0.15 ng/mL, n=75, $p<0.0049$) and with severe disease (1.02±0.13 ng/mL, n=80, $p<0.001$). There was no significant difference in testosterone levels in control, mild, and severe females. Aromatase concentration were elevated in males with mild (7.14±0.39 ng/mL, n=98, $p<0.001$) and severe disease (8.31±0.47 ng/mL, n=85, $p<0.0001$) compared to male controls (4.65±0.21 ng/mL, n=44). Similarly, severely affected females had higher aromatase concentrations (8.49±0.82 ng/mL, n=54, $p<0.0001$) compared to females with mild disease (6.26±0.28 ng/mL, n=83), which remained higher than controls females (4.65±0.21 ng/mL, n=44). Our study shows that males with severe COVID-19 have decreased testosterone and higher aromatase levels as compared to controls. This suggests increased aromatization of testosterone which is associated to disease severity in males and not in females.

In Utero Response to Prenatal Infection of a Novel Rat-Adapted Zika Virus

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Zika Virus (ZIKV) is a flavivirus that, in cases of in utero fetal infection, is known to cause microcephaly and other neurological and developmental disorders. Like humans, rats exhibit immunosuppression during pregnancy which allows the Zika Virus to infect fetuses in utero. Building on our previously developed model of prenatal ZIKV infection, we are currently using a novel rat-adapted virus that has been passaged on rat cochlear microglial cells, which is expected to produce a stronger infection in rats than a human-derived virus. Pregnant rats were infected at gestational day 18 as previously established by our model. They were inoculated either with the virus, a diluent control, a UV-inactivated virus (iZIKV), or a non-neutralizing antibody combined with the virus. This antibody was included to increase infection in vivo through antibody-dependent enhancement (ADE). This process can drive entry of the virus into cells that express an Fc receptor, a receptor located on immune cells and involved in antibody recognition. We collected tissue from fetuses 24, 48, and 72 hours following maternal infection. Peripheral and brain tissue were collected to assess the response to viral infection in utero to determine timeline of fetal infection. These findings will validate our model as etiologically relevant for ZIKV infection and will allow us to identify which brain areas are more affected by the virus. Previous experiments found that prenatal ZIKV infection led to a long-term motor deficit that was more prominent in the infected females. Surprisingly, we have found no sex differences in the effects of the virus during the postnatal period, but it is possible that any differences may be isolated to the time directly following infection, when the animals are still in utero. Funding: NIH grant R01MH106553

Phenotypic sex and sex chromosome complement impact DNA methylome in a tissue-specific manner between mouse liver and brain

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Methylation of cytosines plays important roles in regulating transcription and chromatin structure. Moreover, sex-specific DNA methylation has been associated with neurodegenerative diseases. We have previously demonstrated that both phenotypic sex and sex chromosome contribute to sex-biased DNA methylome in liver. Here we hypothesize that sex-biased methylation is tissue-specific and hence, phenotypic sex and sex-chromosome complement will impact the DNA methylation differently in the brain. To test our hypothesis, we identify sex-associated differentially methylated regions (sDMRs) in both liver and brain for CpG dinucleotide and non-CpG sites using whole-genome bisulfite sequencing (WGBS). We decoupled the effects of phenotypic sex and sex chromosome complement by three comparisons: sex reversed XY female mice (XY.F) vs XY males (XY.M), XY.F vs XX female mice (XX.F), and XX.F vs XY.M. We find that phenotypic sex has a larger influence on CpG methylation on autosomes in liver as compared to the brain, the sex-chromosome complement impacts CpG methylation on the X chromosome in both liver and brain. Most tissue-shared CpG sDMRs reside on the X chromosome. We also explore all 15 non-CpG trinucleotides (CHG or CHH) and found that CAC has the highest abundance of sex-biased methylation in the brain but not in the liver. Interestingly, X-linked CAC sDMRs show predominantly higher methylation levels in XY in both comparisons XX.F vs XY.M and XX.F vs XY.F. Furthermore, the majority (243/249) of X-linked CAC sDMR-proximal genes in comparisons XX.F vs XY.M and XX.F vs XY.F also have CpG sDMRs in the vicinity, which is not the case for autosomal CAC sDMRs (43/391), indicating high spatial correlation and potential interplays between CAC and CpG X-linked sDMRs. We conclude that both phenotypic sex and sex-chromosome complement shape DNA methylome in brain, but with patterns different from that in liver. Funding: This study was funded by Natural Sciences and Engineering Research Council of Canada to AKN. QKWZ is supported by Japan Science and Technology Agency Support for Pioneering Research Initiated by the Next Generation (JST SPRING).

The Influence of the Y Chromosome on Autosomal DNA Methylation in the Mouse

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Sexual dimorphism refers to the differences between males and females of a given species. These differences range from gross phenotypes to gene expression and DNA methylation levels. The main factors that contribute to sexually dimorphic DNA methylation in mammals are the sex-phenotype and sex-chromosome complement. Here, we focused on the impact of the Y chromosome with the goal to determine how the presence of the Y chromosome influenced DNA methylation by 1) identifying Y-chromosome dependent differentially methylated regions (yDMRs) and 2) determining if the impact of the Y on methylation was tissue-specific. To isolate the effect of the Y chromosome on DNA methylation, we used mice with different combinations of sex and sex-chromosome complement (XX females, XY males, sex-reversed XY females and XO females) and conducted whole genome bisulfite sequencing analysis (WGBS) of DNA methylation in their brains and livers. Next, we used a targeted approach and designed pyrosequencing methylation assays for selected “reporter” yDMRs. Assays were first validated and then used for analysis of tissue-specificity of DNA methylation patterns. Using the cut-off of 20% difference and adjusted p-value < $1x10^{-5}$, we identified yDMRs in liver and brain of adult mice and mostly on repeat elements of autosomes. We find that yDMRs may have higher or lower DNA methylation in XY mice compared to XX or XO mice. We also find that yDMRs are present in all the tissues tested, i.e. there is no evidence of tissue-specific effects. We conclude that a modest proportion of sex-biased DMRs in mouse liver and brain are associated with the presence of the Y chromosome. The majority of the yDMRs are located within LINE and LTR sequences. Furthermore, we find no evidence of tissue-specificity of yDMRs, which suggests that the Y-linked gene(s) responsible for influencing autosomal DNA methylation is expressed in all the tissues tested or, alternatively, yDMRs arise early in development. Funding: This study was funded by Natural Sciences and Engineering Research Council of Canada to AKN.

APOEe4 genotype differentially influences hippocampal neurogenesis in middle age depending on sex

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Females have a greater lifetime risk and show more severe symptoms and progression of Alzheimer’s disease (AD) compared to males. This sex difference is exacerbated with possession of APOEe4 alleles, the greatest genetic risk factor for sporadic AD. Indeed, females with APOEe4 show an earlier age of disease onset and greater neuropathology, including faster hippocampal atrophy compared to males. Neurogenesis in the hippocampus has been shown to decline as AD advances in humans, but much of the research has either not included both sexes or failed to analyze for sex differences. As such, we investigated whether sex and APOEe4 genotype interact to influence hippocampal neurogenesis in middle-aged rats.
Age-matched wildtype (WT) and humanized (h) APOEe4e4 knock-in male and female rats were euthanized at middle age (13-14 months old) to examine markers of hippocampal neurogenesis (neural stem cells and immature neurons).
hAPOEe4 rats had fewer immature neurons than WT rats in males, whereas hAPOEe4 rats had more immature neurons than WT rats in females. Furthermore, the reduction in the number of immature neurons in male hAPOEe4 rats was mirrored by a reduction in the number of neural stem cells. This correlation was not seen in females, suggesting a sex difference in the progression of neural stem cells to immature neurons. Further experiments are needed to determine how function of these new neurons may differ depending on sex and APOEe4 genotype. These findings indicate that sex and APOEe4 genotype need to be considered in aging and Alzheimer’s disease research.

Sex Differences in Immune Crosstalk between Allo-immunity and Ischemia-Reperfusion Injury in Liver Transplantation

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Recipient sensitization to donor HLA remains an important clinical problem in transplantation, leading to increased wait times to find a compatible donor and increased risk of acute and chronic antibody-mediated rejection (AMR). Ischemia-reperfusion injury (IRI) is another major risk factor for allograft rejection, involving complex interactions between innate and adaptive immune systems, raising the question whether the activity of donor-specific alloreactive cells present at the time of transplant potentiates IRI, and/or if IRI enhances the generation of donor alloreactive cells which then mediate graft rejection and lower long-term graft outcome. We evaluated the production of HLA class I/II antibodies pre- and post-transplant (early, <3 mo; late, 4-24 mo) in liver transplant (LT) recipients with or without biopsy-proven IRI (n=78; IRI-=37, IRI+=41) using single antigen bead-based HLA antibody testing and intermediate resolution HLA typing. LT recipients who were pre-sensitized to HLA were mostly protected from IRI, especially if pre-sensitized to donor-specific antigens (DSA). There was also a significant decrease in the number of IRI- patients producing HLA antibodies early post-transplant, which further decreased late post-transplant. Non-sensitized IRI+ patients either had an early memory response that persisted through late testing or produced de novo antibodies late post-transplant. IRI+ patients had a significantly higher incidence of post-transplant DSA at both acute and chronic AMR timepoints. Although more females were pre-sensitized, IRI+ recipients experienced increased post-transplant alloimmunity irrespective of gender. However, female Hispanic LT recipients with NASH and IRI had the highest incidence of post-transplant alloimmune responses and worsened outcomes. An increased frequency of pre-transplant baseline testing and post-transplant monitoring of liver transplant recipients should be considered.
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Gender Differences in Patients with Ventricular Arrhythmias Undergoing Catheter Ablation

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Significant differences in the clinical presentation and outcomes of cardiovascular diseases in men and woman have been described in the last few decades, including the management of cardiac arrhythmias. Recently, catheter ablation for ventricular arrhythmia (VA) has been shown to be highly effective at reducing ventricular tachycardia (VT) and premature ventricular contractions (PVC) burden, including reduction of VA episodes. Much of the published data has identified risk factors for VA recurrence but have not included enough women for meaningful comparison of gender-based differences. The goal of this study was to examine gender differences in patients with ventricular arrhythmias who have undergone VA ablation. We conducted a retrospective analysis on a cohort of 115 patients receiving VA ablation at a tertiary hospital (54 pts for VT and 63 pts for PVC, 76 males and 39 females) to identify gender differences in patient characteristics at time of ablation. Females were younger (52.2 vs 63.9 years) and had higher LVEF (53.7% vs 43.1%) as compared to men. History of pre-procedure implantable cardioverter-defibrillator (ICD), ischemic heart disease and procedures, such as CABG or stent placement, were higher in men. Also, more males were diagnosed with hypertension and dyslipidemia. VT patients had lower LVEF and a higher proportion of males with worse pre-procedure characteristics than PVC patients. Overall, study results show female patients had less severe comorbidities, younger age and better LVEF. These findings can reflect differences in VA ablation outcomes as well as the selection of treatments for patients with VA.

The diagnostic potential of female-specific plaque methylation patterns in cell-free DNA

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There is an urgent and unmet need for early, non-invasive diagnosis of atherosclerosis in females that can identify high-risk patients with potentially symptomatic plaques. Recent breakthroughs in the use of circulating fragments of cell-free DNA (cfDNA), have provided major advances in early cancer detection. Furthermore, it has been shown that, using deconvolution of tissue-specific methylation patterns, the contribution of different tissue origins to cfDNA can be quantified. In the current study, we aim to identify sex differences in plaque methylation patterns and define a female-specific epigenetic signature. We hypothesize that an increased contribution of plaque-derived cfDNA, as a proxy for the accumulation of atherosclerotic plaque, can be measured in female cardiovascular disease patients. DNA methylation patterns were obtained for plaque samples from 148 female and 344 male patients that underwent carotid endarterectomy (CEA) from the Athero-Express Biobank using the Infinium HumanMethylation450 Beadchip Array. cfDNA methylation from 18 female patients, spanning three cardiovascular clinical cohorts, was measured using the NEBNext Enzymatic Methyl-seq kit. We were able to identify a female-specific plaque methylation signature encompassing 200 CpG sides, of which a portion was found to be involved in atherosclerotic processes. Furthermore, we built a comprehensive human methylation atlas using methylation data on 25 different tissue and cell types, including plaque, which is used for the deconvolution of cfDNA. Our plaque-centric methylation atlas together with our well-established biobanks, offer the unique opportunity to study sex differences in DNA methylation patterns and use that to investigate cfDNA composition in patients spanning various types of cardiovascular disease. We believe this comprehensive methylation framework would help unravel the potential of cfDNA as an innovative sex-specific biomarker for cardiovascular disease.
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Effects of Exposure to Violence on Telomere Length Operate through Different Pathways in Young African American Adult Females and Males

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OBJECTIVE: Exposure to violence (ETV), an environmental stressor, impacts human health and can have long-term biological effects, including shorter telomere length (TL). The relationship between TL, environmental stressors (ETV and policing), and depression have been studied, but not yet extensively in young African American adults (YAAA). This study examines relationships between environmental stressors and depression on TL, measured by a quantitative PCR assay, in YAAA in Washington DC. METHOD: We analyzed 98 saliva samples of 18 to 25-year-old AA men (48) and women (50). Participants who reported either ETV or no ETV on several measures of ETV were selected. Correlations were calculated between TL, depression, and ETV measures. Stepwise regressions examined the effects of ETV variables substance use, and depression on TL, controlling for age, gender, receiving welfare or public assistance, age at first substance use, and BMI. RESULTS: In males TL is negatively associated with depression; police interactions; physical violence; community violence; threat of violence and witnessing violence. Females' attitude towards police was positively correlated with TL. In stepwise regressions for men, police interaction (β = .657) and the threat of violence (β = .260) had strong effects on depression, which has a large negative effect (β = -.464) on TL. For women community violence (β = .224), and ages at first alcohol (β = .278) and tobacco (β = .567) all increase the age of first marijuana use, which in turn was associated with longer TL (β = .352). CONCLUSION: The regression shows the effects of ETV on TL differ for males and females and operate through different pathways. For men, police interaction and threats of violence affect TL through depression, and community violence affected TL through age of first marijuana use for women. The findings point to important sex differences in the psychosocial internalization of environmental stressors among YAAA.

In mouse models of Alzheimer's disease and mixed dementia, females are more severely affected by high fat diet than males

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Diabetes and prediabetes are risk factors for all-cause dementia. 1 in 10 people in the U.S. currently have diabetes and 1 in 3 have prediabetes. Investigating how mid-life prediabetes contributes to dementia may emphasize prediabetes as a risk factor. Here we study the impact of prediabetes on two common dementia types: Alzheimer's disease (AD) and mixed dementia (MxD) which is the overlap of AD and vascular pathology. Given that there are sex differences in dementia prevalence, we ask if prediabetes affects males and females differently. We hypothesize that females will face greater pathology due to prediabetes and that this will worsen AD and vascular pathology in AD and MxD mouse models. Using B6129SF2/J mice as wild type controls, we modeled AD using the 3xTg-AD model of AD and MxD by performing a unilateral common carotid artery occlusion to create chronic cerebral hypoperfusion. To model prediabetes, we fed mice a high fat diet (60% fat, controls received 10% fat). We found that high fat diet caused greater metabolic effects in females and this was worsened by 3xTg-AD genotype in females only. We measured microgliosis, astrogliosis and amyloid-β burden (IHC and qPCR) and tau levels (western blot). We found that females had greater hippocampal astrogliosis and cortical amyloid-β positive cells than males. Our findings show increased sensitivity of females to the adverse metabolic effects of high fat diet and pathological effects of AD and MxD.

Sex Differences in Stroke Outcome Correspond to Rapid and Severe Changes in Gut Permeability in Adult Sprague-Dawley Rats

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Sex differences in experimental stroke are well documented, such that adult males show worse outcomes compared to adult females. Based on recent evidence that gut dysbiosis may be an early response to stroke, the present study tested the hypothesis that in the acute phase, stroke will result in greater gut dysbiosis and greater permeability of the gut blood barrier in males as compared to females. Adult male and female Sprague Dawley rats were subject to endothelin-1-induced middle cerebral artery occlusion (MCAo). Fecal samples, blood draws and sensory motor tests were collected/conducted pre and 2d post MCAo. Fecal samples were subject to 16s sequencing. Gut permeability was assessed by histology and by functional assays using oral dextran gavage. We confirmed stroke-induced sex differences, including increased mortality and more severe sensory motor deficit in males as compared to age-matched female rats. Fecal 16s sequencing showed greater bacterial diversity in females prior to stroke while 2 days after stroke, these measures were similar between the sexes. In contrast, MCAo-induced gut permeability was much worse in males as compared to females. Male rats had a decreased villus length to crypt length ratio, higher levels of biochemical gut permeability markers in serum, and higher levels of fluorescent-labeled dextrans following oral gavage in serum. Additionally, males had higher serum levels of proinflammatory cytokines IL-17A, MCP-1, IL-5 and EGF compared to females after stroke. Collectively, these results indicate that the worse stroke outcomes seen in males is associated with increased gut permeability in this group. These data suggest that therapeutics that target the gut epithelium to reinforce the gut-blood barrier post stroke may be an effective intervention especially in vulnerable populations such as the elderly or those with co-morbid conditions who have already been shown to have more permeable barriers.
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Exploring Sex-Differences and a Role for the 5-HT System in Early Life Stress-Induced Compulsive Ethanol Consumption in Adulthood

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Stress-vulnerable communities including women, sexual/gender minorities and those with adverse childhood experiences are understudied and more motivated to drink to 'self-medicate their negative affect. The dorsal raphe nucleus (DRN)-serotonin (5-HT) system is sexually dimorphic, vulnerable to developmental stress, important in stress-reward regulation and the ideal target to treat compulsive (despite negative consequences) drinking. We hypothesized that DRN 5-HT neurotransmission underlies the sex-dependent effects of stress on ethanol (EtOH) intake and compulsive drinking. Male and female Tph2-iCre rats or wild-type Sprague-Dawley littermates underwent social isolation stress (SIS) or group housing in adolescence, intraDRN viral delivery of Cre-dependent excitatory Gq-DREADDs (AAV-hSyn-DIO-hM3Dq-mCherry) and tamoxifen induction. They were exposed to 20% EtOH in a 3-week intermittent-access two-bottle choice (IA2BC) model. Next, transferred to a 10-day oral EtOH self-administration (SA) model and 3 days of punished SA (50% of EtOH deliveries + 0.24mA footshock) to test compulsive drinking. Clozapine-N-oxide was administered 30 mins prior to each punishment session to activate Gq-DREADDs. Results showed a sex x stress interaction in IA2BC EtOH intake. SA drinking revealed a time x stress interaction in males (higher drinking in SIS). No effect of SIS but females had higher SA EtOH intake than males and more shock-resistant (compulsive) drinking. Chemogenetic activation of DRN 5-HT neurons blunted punishment suppression of SA. Results suggest females are more sensitive to early life stress-induced rise in EtOH intake and adult compulsive drinking. Prior studies in our lab support stress-induced suppression of the DRN 5-HT system and chemogenetic activation of 5-HT appears to buffer these stress effects. In conclusion, we show the important effects of sex and stress on drinking and motivation for EtOH and a novel role of the 5-HT system in compulsive drinking. The authors have no conflicts of interest. Funding: NIDA T32 DA007237, R01 DA045771 and P30 DA013429

Genetic deletion of mineralocorticoid receptors in CA2 impairs the precision of behavioral circadian rhythmicity in a sex-dependent manner

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Mineralocorticoid receptors (MRs) are found throughout the body and the brain, but are especially concentrated in the hippocampus, particularly in area CA2. Conditional knockout (KO) models have shown MRs to be important for maintaining physiological responses to stress at the hypothalamic-pituitary-adrenal (HPA) axis and regulating circadian rhythmicity, but in addition, KO of MRs in CA2 has been found to disrupt CA2 neuron phenotype and CA2-dependent behaviors. In order to further investigate these overlapping functional roles, we studied two conditional MR KO mice lines with a broad neuronal deletion (Nestin-Cre) and a CA2-targeted deletion (Amigo2-Cre) using an automated home cage monitoring system to track spontaneous behavior over a 2.5 day period. We found baseline differences in activity between males and females, primarily driven by increased activity of females in the dark phase, as well as subtle circadian rhythm disruptions in the conditional knockouts. Cre-negative control mice show an increase in activity just prior to light onset, likely driven by a precise expectancy about when the light phase transition will occur. The conditional MR KO lines showed overall normal circadian rythmicity but failed to show this precise anticipation of light onset, with the effect being more pronounced in the Nestin-Cre line. These findings suggest that MRs expressed throughout the brain and specifically in CA2 may play an important role in precise regulation of circadian rhythms in a way that is dependent upon sex of the animal.
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Sex Differences in Norepinephrine and Dopamine Release in the Hippocampus During Contextual Fear Conditioning

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Dopamine (DA) and norepinephrine (NE) are potent neuromodulators implicated in a wide variety of emotional and cognitive functions. Their role in contextual learning and fear conditioning is only beginning to be understood. In the present study we utilized in vivo fiber photometry with GRAB-DA and GRAB-NE sensors to assess DA and NE release in hippocampal CA1 region of adult C57BL/6J mice during a pre-exposure-dependent contextual fear conditioning paradigm. This approach allows us to independently assess DA and NE dynamics during contextual encoding, during the immediate-shock phase the context-shock association is formed and during the contextual retrieval phase when the context-shock association is retrieved to drive expression of freezing. Release of both NE and DA was greatly increased in response to the shock; however, females showed a larger increase in NE relative to males. Detailed analysis of behavioral transition, such as the transition to active exploration during the pre-exposure and in the transition to freezing during the context test showed a complex sex-dependent pattern. We did not observe any sex differences in behavior, indicating that the observed sex differences in DA/NE dynamics do not produce noticeable behavioral differences. Overall, these findings provide important insight into the sex-dependent dynamics of DA and NE release in the hippocampus during contextual fear conditioning.
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Characterizing Sex Differences in Automated Home Cage Systems

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Automated home cage monitoring systems can provide novel insight into subtle, yet meaningful behavioral measures not previously characterized in animal models and have the potential for higher throughput behavioral studies without confounds associated with human intervention. There is often an underrepresentation of female subjects in behavioral studies, leading to results that are difficult to interpret when sex differences interact with the experimental manipulation. Therefore, this project sought to characterize locomotor activity, cognitive function, and feeding and drinking behaviors in male and female C57Bl/6 mice using two automated home cage systems. Results of the Noldus PhenoTyper system, where mice are single-housed, showed greater activity in the dark phase in females across the lifespan. In the cognition wall task, an assay of discrimination and reversal learning, females showed increased task engagement and received more reward pellets during discrimination learning, although overall reversal learning was similar between males and females. In the TSE IntelliCage, where mice are group-housed, we observed sex differences in social networks as assessed by the relative temporal order of access to the water ports. Additionally, females spent more time than males in corner visits during open access to water, but this difference was not seen during learning and avoidance tasks. Establishing baseline behaviors of males and females is important to better interpret genotypic differences in health and disease models, allowing for informed differences in clinical treatment for men and women. Funding provided by the NIEHS Intramural Research Program

Investigating neurogenesis dynamics in the hippocampus of males and females in an animal model of late onset sporadic Alzheimer's Disease.

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The greatest risk factors for Alzheimer's disease (AD) are advancing age, possession of one or two APOEe4 alleles, and female sex. Furthermore, human females with APOEε4 alleles demonstrate a higher risk of AD, greater cognitive decline, and higher levels of phosphorylated tau compared to males and compared to females without APOEε4 alleles. The hippocampus is a site of adult neurogenesis and is compromised early in AD, which is critical because these new neurons play a role in pattern separation, which is compromised early in AD. Previous work in our laboratory has shown sex differences in neurogenesis in young adult rodents as males have a faster rate of maturation and more neural stem cells in the dorsal hippocampus compared to females. Therefore, we will examine sex and genotype differences in the dynamics of neurogenesis in humanized (h) hAPOEε3 vs hAPOEε4 adult mice. Adult mice were administered bromodeoxyuridine (BrdU), a thymidine analogue, that is incorporated into actively dividing cells, 24 hours before the experimental endpoint to study early proliferative events between sexes and genotypes. Fluorescent immunohistochemistry was used to stain brain sections against BrdU and Sox2, a neural stem cell marker. Preliminary data in three3 month old mice showed that there was no sex difference in the percentage of BrdU/Sox2-ir cells in hAPOEε3 mice. However, hAPOEε4 females had higher percentage of BrdUY/Sox2-ir cells than hAPOEε4 males. This suggests early sex differences in the dynamics of neurogenesis in hAPOEε4 genotype mice which may have implications for disease risk. Further research is underway in older animals administering BrdU 2hr, 24hr, 2 weeks and 4 weeks before experimental endpoint to discern the rate and attrition of new neuron formation on a sex and genotype basis. Funding: Funding was provided by Alzheimer'sby Alzheimer's Society of Canada to LAMG. SAB was funded by the Canadian Institutes for Health Research (CIHR).

The effects of advanced maternal age on neuroplasticity in the hippocampus

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The age at which women first experience pregnancy has increased in the past few decades, with 18% of births occurring in women older than 35 years old. Studies suggest that advanced maternal age affects maternal cardiovascular health and can adversely affect offspring health, however there is less research on the effect of advanced maternal age on maternal brain health. Both parity and aging affect neurogenesis in the hippocampus (HPC). Doublecortin (DCX) is an endogenous marker for immature neurons and its expression decreases with age. Perineuronal nets (PNNs) are extracellular matrix structures considered to be markers for neuronal plasticity. DCX and PNN expression were quantified in the HPC of young (3 to 4 month) and aged (9 to 10 month) female Sprague-Dawley rats. Female brains were collected in late gestation (Gestation Day 20) or in age-matched nulliparous rats. Fluorescence immunohistochemistry was used to label and quantify DCX and PNNs in the dorsal and ventral HPC. Preliminary data demonstrate a significant decrease in DCX-expressing neurons in all HPC regions of older animals compared to young animals, with pregnancy reducing neurogenesis in the younger group. Intriguingly we found PNNs were increased with pregnancy, regardless of age, throughout the HPC. Although there was little expression of PNNs in the dentate gyrus, older dams had a higher expression of PNNs than all other groups. These data suggest that maternal age plays a role in the influence of pregnancy to alter plasticity in the hippocampus. Given that plasticity plays a role in brain health, our work sheds light on the potential effects of maternal age on the female brain. Funding: Canadian Institutes of Health Research (CIHR) support to LAMG (PJT-148662); CIHR to SD (FS154313)

Developmental Programming of Sex Differences in Mast Cells: Role of Androgen Receptors

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Mast cells (MC) are important modulators of the immune and nervous system function in the brain and peripheral organs and play critical roles in host defense and inflammation. A striking sex bias exists in many MC-associated inflammatory diseases with females often at increased risk. We demonstrated that neonatal and adult male mice exhibit reduced IgE- and stress-induced MC histamine levels and corresponding reduction in the clinical severe MC-mediated anaphylaxis, compared with females. Further, we showed that compared with female MCs, male MCs have a reduced capacity to store and release MC histamine, serotonin, and proteases, and that perinatal androgens are important in organizing the masculinized MC phenotype. Here, we utilized a mouse model in which the androgen receptor (AR) is reduced by Cre-LoxP technology (induced TFM; iTfm), we tested the hypothesis that androgen receptors (AR) drive sex differences in MC histamine storage and release and severity of anaphylaxis. Compared with WT males, peritoneal tissue MCs (pMCs) and bone marrow-derived mast cells (BMMCs) derived from iTFm males exhibited a feminized phenotype with greater cellular histamine content and IgE-mediated histamine release that was similar to female mast cells (n=5-6; P<0.01,1 Way ANOVA). Compared with WT males, iTFm male mice exhibited greater IgE-mediated histamine release and more severe hypothermia responses and comparable with females. Together, these studies demonstrate that ARs are important in mediating sex differences in MC phenotype and function and that iTFm mice are a valid model to explore AR-mediated sex differences in MC function and disease pathogenesis. This research was supported by NIH Grants R21 AI140413 and R01 HD072968 (to AJM).

Androgen Action Within the Vomeronasal Organ Contribute to the Sexual Differentiation of the Brain and Behaviour

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The vomeronasal organ (VNO), situated in the nasal cavity, is responsible for pheromone processing and plays a critical role in mediating socio-sexual behaviors in mice. Testosterone (T) masculinizes and defeminizes the brain and socio-sexual behavior during early development by acting directly on androgen receptors (AR) or indirectly via estrogen receptors (ER). In Experiment 1, we asked whether androgens can act via the VNO in early development to affect the display of socio-sexual behaviors in adulthood by administering a microinjection of T locally to the VNO on the day of birth (PND1) in mice. In Experiment 2, we asked whether T acts on AR or ER by injecting the VNO with a vehicle, estradiol or the non-aromatizable androgen, dihydrotestosterone (DHT) on PND1. Both experiments utilized a behavioural battery including a buried food test, olfactory preference test, a resident intruder paradigm, and a test of sexual behavior. In Experiment 1, we found that a single microinjection of T on PND1 was sufficient to alter olfactory investigation and increase territorial aggression in males but did not affect female behavior. In Experiment 2, we found that a single microinjection of DHT on PND1 was sufficient to increase male territorial aggression with increases in boxing, biting, and attacking behavior towards males. FOS analysis, a marker of neural activity, after exposure to opposite sex odors did not yield any differences between groups in the nucleus accumbens, bed nucleus of the stria terminalis or the medial preoptic area. Our findings suggest that androgens act via the VNO in early critical periods in development to affect adult socio-sexual behaviours. Funding: This study was funded by a Discovery Grant from the Natural Sciences and Engineering Council of Canada (NSERC) to Dr. Swift-Gallant.

Winning increases aggression in postpartum female rats

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Winning boosts motivation and secures resources. In addition to these intuitive effects, winning affects hormones, strengthens performance, and even increases the probability of future victories, and this phenomenon is called the Winner Effect. Although some data suggest that this pattern holds in women, men are ~3x more likely to be included in such studies, and the Winner Effect has never been studied in nonhuman females. This noteworthy gap in the field is largely explained by low or absent aggression by female rodents. However, females aggressively defend their home territory during the postpartum period. To test the Winner Effect in postpartum rats, subjects were impregnated and randomly assigned to be Winners or Naïve. Winners had three winning experiences: male intruders were placed into Winners' home cages for ten-minute sessions on Postpartum days (PPD) 4, 7, & 10. Winners and Naïve subjects were then tested with an inexperienced intruder for ten minutes on PPD 13. All aggression tests were video recorded to measure behaviors. Winners and Naïve subjects were then sacrificed and brains were harvested for histological analyses. Previous winning experience increased aggression in postpartum rats: compared to naïve postpartum rats, winners lunged at intruders more ($p = .05$) pinned them down more ($p = .04$), and, to some extent, bit intruders more often ($p > .05$). Ongoing analyses are investigating gene expression in the brains of winners and naïve postpartum females and the intruders' behaviors in response to Winners and Naïve females. This novel protocol that will allow investigations into effects of winning on the female brain to uncover overlapping and distinct neural responses to competition among the sexes.

Sex differences in the effects of prior-social isolation stress on stroke severity and outcomes in middle-aged rats.

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Background: Patients often experience social isolation after stroke due to persistent disability and/or post-stroke changes in mood and affect, which delays recovery. Considering the long periods of social distancing imposed by the current pandemic, it is likely that prior social isolation stress (SIS) might also significantly affect stroke outcome. **Aim:** We hypothesized that prior-SIS will increase stroke severity in middle-aged rats due to increased neuroinflammation. **Method:** Middle-aged male and female rats were single or double housed for 5 weeks and then subjected to middle cerebral artery occlusion. Rats were terminated 5 days post-stroke, and infarct volume, survival, and sensorimotor performance was assessed. Serum was analyzed for inflammatory cytokines and the bacterial metabolite lipopolysaccharide (LPS). **Results/Conclusions:** SIS showed a trending increase in stroke-induced mortality ($p=0.058$) in females but not males. Infarct volume ($p = 0.0181$) in surviving SIS females was increased compared to SIS males. No significant difference in sensorimotor deficit was observed. Females demonstrated increased circulating levels of LPS ($p=0.0023$) and the pro-inflammatory factor, RANTES, ($p=0.0037$). Additionally, there was an interaction effect of sex X housing on the pro-inflammatory factor MIP-1a. Increased LPS after stroke suggests greater gut permeability, and coupled with elevated cytokine expression, could impact blood brain barrier permeability, resulting in more severe chronic stroke outcomes in females. Our data indicate a sex-specific effect of SIS on the gut-immune response to stroke.