SSD

ORGANIZATION FOR THE STUDY OF SEX DIFFERENCES

Founded by the Society for Women's Health Research

2014 Meeting

April 24 – 26

Minneapolis, MN

Sponsored by the University of Minnesota's Department of Neuroscience and the Powell Center for Women's Health

OSSD 2014 PRESIDENT'S WELCOME

Welcome to **OSSD 2014**, the 8th annual meeting of the Organization for the Study of Sex Differences! The meeting has a lot to offer. Co-Chairs, Sally Huber and Sabra Klein have organized a terrific workshop that will lead off the meeting. Under the inspired leadership of Program Chair Gillian Einstein, the OSSD Program Committee has put together a spectacular program with an outstanding line-up of basic and clinical scientists. You will find that the Chair of the Local Committee, Robert Meisel, and his team have created a perfect venue for this meeting.

OSSD's mission is to promote research in, and knowledge of, sex and gender differences in biology and medicine. Our first priority is to hold high-quality annual meetings. We fully expect OSSD 2014 to meet that goal and to generate the same level of inspiring cross-discipline interaction as did previous meetings. Once again, you will meet a terrific crop of up-and-coming researchers in the field of sex differences, who will present their work in the Elizabeth Young New Investigator Symposium and in two poster sessions.

OSSD's official journal, *Biology of Sex Differences* (BSD), celebrated its third anniversary in November. Under the excellent stewardship of editor-in-chief, Art Arnold, and with the assistance of an internationally acclaimed editorial board, BSD has become a terrific place to publish your best work on sex differences in biology and medicine. BSD papers are freely available on the web and several of them have circulated extensively in news media, drawing a lot of attention. Thomson Reuters (ISI) is currently tracking BSD for an official impact factor. Its unofficial impact factor is an encouragingly high 4.3!

Finally, I would like to thank the National Institutes of Health, CIHR's Institute of Gender and Health, the University of Minnesota, and the Mayo Clinic for their generous support of this meeting. I would also like to thank the Society for Women's Health Research for its generous financial as well as administrative support in founding the OSSD and shepherding it through its initial six years.

Geert J. de Vries Ph.D.
President of the OSSD
Professor of Neuroscience
Neuroscience Institute
Georgia State University

OSSD 2014
Program at a Glance

Meridian Foyer
Meridian I Ballroom
Meridian II Ballroom
Meridian III/IV Ballroom
McNamara Alumni Center

Thursday, April 24th

8:00 - 9:00 AM	Registration open	BREAKFAST
9:00 AM - 12:00 PM		**Workshop: "Inflammation 101"**
12:00 - 1:00 PM		FREE TIME
1:00 - 1:15 PM		**Welcome and Introductions** – Geert de Vries
1:15 – 2:15 PM		**Presidential Keynote Address- Jayne Danska:** "Sex Differences in the Gut Microbiome Drive Hormone-Dependent Regulation of Autoimmunity"
2:15 - 3:15 PM		**Elizabeth Young New Investigator Symposium**
3:15 - 3:30 PM		COFFEE BREAK
3:30 - 5:30 PM		**Presidential Symposium: "Sex Differences in the Brain—A Whole Body Perspective"**
5:30- 8:00 PM		**Poster Session 1 and Welcome Reception**

Friday, April 25th

7:30 - 8:30 AM	Registration Open		BREAKFAST	
8:30 – 10:30 AM		Vendor Exhibits Open	**Symposium I: Renovating the Research Pipeline to Include Sex & Gender: How can Research Institutions be Transformational?**	**Symposium II: Sex-Gender Differences in Eating Disorders**
10:30 – 10:45 AM			COFFEE BREAK	
10:45 - 12:45 PM			**Symposium III: Sex Differences in Drug & Device Development**	**Symposium IV: Sex Differences in Neural Development during Adolescence and Affective Disorder Vulnerabilities**
12:45 – 2:00 PM			FREE TIME	
2:00 – 4:00 PM			**Symposium V: Sex Differences in the Microbiome- Urinary Tract Infections**	**Symposium VI: Influence of Sex & Other Individual Differences on Addiction**
4:00 – 6:00 PM			**Poster Session 2**	

Saturday, April 26th

7:30 - 8:30 AM		BREAKFAST	
8:30 – 10:30 AM		**Symposium VII: Mechanisms of Sex-Specific Risk for Cardiovascular Disease**	**Symposium VIII: The Role of Aromatase in Human Sex Differences**
10:30 – 10:45 AM		COFFEE BREAK	
10:45 -12:45 PM		**Symposium IX: Gonadal Regulation of Immune Dysfunction in the Central Nervous System, Vasculature, and Kidney**	**Symposium X: Differences in Gender Development- What Have We Learned from Patients with Disorders of Sex Development?**
12:45 – 2:15 PM		FREE TIME	
2:15 – 4:15 PM		**Plenary Round Table: "Sex Beyond the Gonad"**	
4:15 – 5:15 PM		**Keynote Address-** Jens Pruessner: "Sex and Gender Differences in Stress and Aging"	
5:15 – 6:30 PM		**General OSSD Membership Meeting**	
6:30 – 7:00 PM		**Cocktail Reception**	
7:00 – 9:00 PM		**OSSD Awards Banquet**	

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OSSD 2014 TRAINEE AWARDS AND HONORS

Elizabeth Young New Investigator Symposium

Joel Berletch, Ph.D., University of Washington, WA

Stephanie Correa, Ph.D, University of California San Francisco, CA

Ke Liu, Cincinnati Children's Hospital, OH

NIH-Sponsored Travel Award Winners

George Chen, Ph.D., University of Hong Kong

Pelin Cengiz, M.D., University of Wisconsin, Madison, WI

Sarah Doran, University of Connecticut Health Center, CT

Emily Jacobs, Brigham & Women's Hospital, Harvard Medical School, MA

Natalie Riediger, University of Manitoba, MB

Shayna Williams-Burris, University of California Los Angeles, CA

Lauren Wright, McMaster University, ON

ORGANIZATION FOR THE STUDY OF SEX DIFFERENCES

Founded by the Society for Women's Health Research

Detailed Program

2014 Annual Meeting

Thursday, April 24

8:00am – 9:00 am: Breakfast – Meridian Foyer

9:00 am – 12:00 pm: Workshop: "Inflammation 101" – Meridian III/IV Ballroom

Co-Chairs: Sally Huber & Sabra Klein

Eleanor Fish, Ph.D. – "Sex Differences in the Immune System" *

Sally Huber, Ph.D. – "Effects of Estrogens, Androgens, and Progestogens on Immune Responses"

Shannon Dunn, Ph.D. – "Sex Differences in Autoimmune Disease" *

Jackie Schwarz, Ph.D. – "Diseases of the CNS and Mental Health"

DeLisa Fairweather, Ph.D. – "Inflammation and Cardiovascular Disease"

Kathryn Sandberg, Ph.D. – "Inflammation and Renal Diseases"

Sabra Klein, Ph.D. – "Inflammation and Pulmonary Diseases"

12:00 pm – 1:00 pm: Free Time

1:00 pm – 1:15 pm: Welcome and Introductions – Meridian III/IV Ballroom

1:15 pm – 2:15 pm: Presidential Keynote Address – Meridian III/IV Ballroom

Jayne Danska, Ph.D. – "Sex Differences in the Gut Microbiome Drive Hormone-Dependent Regulation of Autoimmunity" *

2:15 pm – 3:15 pm: Elizabeth Young New Investigator Symposium –

Meridian III/IV Ballroom

Joel Berletch, Ph.D. – "Survey of Escape from X Inactivation in Mouse Tissues"

Stephanie Correa, Ph.D. – "A Subset of Estrogen-responsive Hypothalamic Neurons Promotes Physical Activity and Maintains Energy Balance in Females"

Ke Liu, B.S. – "X Chromosome Dose and Sex Bias in Autoimmune Diseases: Increased 47, XXX in Systemic Lupus Erythematosus and Sjögren's Syndrome"

3:15 pm – 3:30 pm: Coffee Break – Meridian Foyer

3:30 pm – 5:30 pm: Presidential Symposium – Meridian III/IV Ballroom

Sex Differences in the Brain - A Whole Body Perspective

Chair: Geert de Vries, Ph.D.

Nancy Forger, Ph.D. – "What the Sexually Dimorphic Body Tells the Brain"

Tracy Bale, Ph.D. – "Sex Differences in the Placenta and the Role of OGT in Neurodevelopment"

Debbie Clegg, Ph.D. – "Hypothalamic PGC-1α Protects Against High Fat Diet Exposure by Regulating ERα"

5:30 pm – 8:00 pm: Poster Session I and Welcome Reception –

Meridian II Ballroom and Foyer

Friday, April 25

7:30 am – 8:30 am: Breakfast – Meridian Foyer

8:30 am – 10:30 am: Symposium I & II

Symposium I: Renovating the Research Pipeline to Include Sex & Gender: How can Research Institutions be Transformational? – Meridian I Ballroom

Co-Chairs: Joy Johnson, Ph.D. & Janine Clayton, M.D.

Joy Johnson, Ph.D. – "Working to Change Research Funding Institutions"

Janine Clayton, M.D. – "Integrating the Study of Sex Differences in a National Research Agenda"

Shirin Heidari, Ph.D. – "Shifting Minds: Inclusion of Sex/Gender for Better Standards of Science Reporting"

Symposium II: Sex-Gender Differences in Eating Disorders – Meridian III/IV Ballroom

Chair: Debora Romano, M.D.

Debora Romano, M.D. – "Gender Differences in Clinical Complications of Chronic Anorexia"

Christine Tarabbia, M.D. – "The Impact of Sex Hormones on Energetic Homeostasis"

Emilia Manzato, M.D. – "Sex-Gender Differences in Psychiatric Aspects of Anorexia and Bulimia"

10:30 am – 10:45 am: Coffee Break – Meridian Foyer

10:45 am – 12:45 pm Symposium III & IV

Symposium III: Sex Differences in Drug & Device Development – Meridian I Ballroom

Chair: Emmanuel Fadiran, R.Ph., Ph.D.

Pamela Scott, Ph.D. – "Addressing Sex Differences in FDA-Regulated Products: Drugs"

Nada Hanafi, M.Sc. – "Sex Differences in Devices Approved by the FDA"

LT James Coburn, M.Sc. – "Investigating the mechanical causes of increased metal-on-metal hip implant failure rates in women"

Tina Morrison, Ph.D. – “Gender Disparities in Endovascular Treatment Options for Infrarenal Abdominal Aortic Aneurysms”

Symposium IV: Sex Differences in Neural Development during Adolescence and Affective Disorder Vulnerabilities – Meridian III/IV Ballroom

Chair: Janice Juraska, Ph.D.

Janice Juraska, Ph.D. – “Sex Differences & Hormonal Differences Influences during Adolescence on the Cerebral Cortex and its Connectivity”

Susan Andersen, Ph.D. – “The Trajectories of Sex Differences in Adolescent Development and the Emergence of Depression across Species”

Cecile Ladouceur, Ph.D. – “Adolescent Fronto-limbic Development, Puberty and Affective Disorders”

12:45 pm – 2:00 pm: Free Time

2:00 pm – 4:00 pm: Symposium V & VI

Symposium V: Sex Differences in the Microbiome: Urinary Tract Infections – Meridian I Ballroom

Chair: Jeffrey Henderson, M.D., Ph.D.

Thomas J. Hannan, DDM, Ph.D. – “Understanding urinary bladder mucosal immunity during UTI: opportunities for new therapeutic strategies”

Peter J. Mucha & Jeffrey P. Henderson M.D., Ph.D. – “Virulence Network Detection in a Mixed Male and Female Urinary Tract Infection Population”

Amanda Lewis, Ph.D. – “Influence of the Vaginal Microbiota on Genitourinary Health and Disease”

Symposium VI: Influence of Sex & Other Individual Differences on Addiction – Meridian III/IV Ballroom

Chair: Marilyn Carroll, Ph.D.

Dorothy Hatsukami, Ph.D. – “Sex Differences in Treatment for Smoking with Quest Cigarettes vs. Nicotine Patch”

Kelly Klump, Ph.D. – “Sex Differences in Binge Eating and Eating Disorder Phenotypes: Critical Roles for Puberty and Gonadal Hormones”

Jill Becker, Ph.D. – "Sex Differences in the Development of a Preference for Cocaine vs. Tasty Treats: Neurochemistry & Behavior"

Marilyn E. Carroll, Ph.D. – "Sex and Other Individual Differences: Effect on Vulnerability and Treatment for Addiction"

4:00 pm – 6:00 pm: Poster Session II – Meridian II Ballroom

Saturday, April 26

7:30 am – 8:30 am: Breakfast – Meridian Foyer

8:30 am – 10:30 am: Symposium VII & VIII

Symposium VII: Mechanisms of Sex-Specific Risk for Cardiovascular Disease – Meridian I Ballroom

Co-Chairs: Patricia Nguyen, M.D. & Jennifer Tremmel, M.D.

Mansoureh Eghbali, Ph.D. – "The Number of X Chromosomes Influences Protection from Cardiac Ischemia/reperfusion Injury in Mice: One X is Better than Two"

Muthuvel Jayachandran, Ph.D. – "Sex Differences in Blood-borne Extracellular Vesicles in Cardiovascular Health and Disease"

Elena Ladich, M.D. – "Sex Differences in Coronary Plaque"

Symposium VIII: The Role of Aromatase in Human Sex Differences – Meridian III/IV Ballroom

Chair: Anat Biegon, Ph.D.

Anat Biegon, Ph.D. – "Organ-specific Expression and Regulation of Aromatase in Males and Females: Observations and Speculations"

Nelly Alia-Klein, Ph.D. – "Sex-specific Role of Aromatase in Amygdala for Personality in Females and for Learning in Males"

Tom Hildebrandt, Psy.D. – "A Role for Aromatization in the Expression of Eating, Impulsive, and Aggressive Psychopathology"

10:30 am – 10:45 am: Coffee Break – Meridian Foyer

10:45 am - 12:45 pm: Symposium IX & X

Symposium IX: Gonadal Regulation of Immune Dysfunction in the Central Nervous System, Vasculature and Kidney – Meridian I Ballroom

Chair: Kathryn Sandberg, Ph.D.

Shannon Dunn, Ph.D. – "Puberty in Females Enhances the Risk of an Outcome of Multiple Sclerosis in Children and the Development of Central Nervous System Autoimmunity in Mice"

Kathryn Sandberg, Ph.D. – "Sex-specific T cell Modulation of Hypertension"

Carolyn Ecelbarger, Ph.D. – "17-beta Estradiol Regulation of Interleukin-6 in Renal Ischemia and Atherosclerosis"

Symposium X: Differences in Gender Development: What Have We Learned from Patients with DSD? – Meridian III/IV Ballroom

Co-Chairs: Eric Vilain, M.D., Ph.D. & Arthur P. Arnold, Ph.D.

Kenneth Zucker, Ph.D. – "Gender Identity Differentiation in Girls with or without a Disorder of Sex Development"

David Sandberg, Ph.D. – "Clinical Management of DSD: Factors Shaping Gender Development and Health-related Quality of Life"

Heino Meyer-Bahlburg, Dr. rer. nat. – "On the 'Social' in Bio-psycho-social Factors Contributing to Gender Development"

12:45 pm – 2:15 pm: Free Time

2:15 pm – 4:15 pm: Plenary Round Table: Sex Beyond the Gonad

Meridian III/IV Ballroom

Chairs: Marcia Stefanick Ph.D., Margaret McCarthy, Ph.D, Daphna Joel, Ph.D.

Marcia Stefanick, Ph.D. – "Sexual Differentiation: Permanence versus "Plasticity" from Gonads to the Brain"

Margaret M. McCarthy, Ph.D. – "The Brain is a Mosaic: Regional Variation in Sexual Differentiation of the Brain"

Daphna Joel, Ph.D. – "Misconceptions of Sex beyond the Genitalia"

Discussants: Arthur P. Arnold, Ph.D., Geert de Vries, Ph.D.

4:15 pm – 5:15 pm: Keynote Address – Meridian III/IV Ballroom

Jens Pruessner, Ph.D – “Sex & Gender Differences in Stress & Aging” *

5:15 pm – 6:30 pm: General OSSD Membership Meeting – Meridian III/IV Ballroom

6:30 pm – 7:00 pm: Cocktail Reception – McNamara Alumni Center

7:00 pm – 9:00 pm: OSSD Awards Banquet – McNamara Alumni Center

Live music performed by The Gentlemen’s Anti-Temperance League

* Denotes presentations supported by the **CIHR Institute for Gender and Health**

SPEAKER ABSTRACTS – THURSDAY APRIL 24, 2014

PRE-CONFERENCE WORKSHOP: INFLAMMATION 101

Co-Chairs: Sally Huber (The University of Vermont) & Sabra Klein (Johns Hopkins University)

Sex Differences in the Immune System
Eleanor Fish, Ph.D., University of Toronto

Effects of Estrogens, Androgens, and Progestogens on Immune Responses
Sally Huber, Ph.D., The University of Vermont

Sex Differences in Autoimmune Disease
Shannon Dunn, Ph.D., University of Toronto

Diseases of the CNS and Mental Health
Jackie Schwarz, Ph.D., University of Delaware

Inflammation and Cardiovascular Disease
DeLisa Fairweather, Ph.D., Johns Hopkins University

Inflammation and Renal Diseases
Kathryn Sandberg, Ph.D., Georgetown University

Inflammation and Pulmonary Diseases
Sabra Klein, Ph.D., Johns Hopkins University

PRESIDENTIAL KEYNOTE ADDRESS April 24, 2014 1:15 pm – 2:15 pm

Sex Differences in the Gut Microbiome Drive Hormone-Dependent Regulation of Autoimmunity

Jayne S. Danska, Ph.D. (Hospital for Sick Children Research Institute, Toronto, ON Canada)

Many autoimmune diseases including multiple sclerosis (MS), systemic lupus erythematosus (SLE), scleroderma, and rheumatoid arthritis (RA) are far more common in females than in males. Despite enormous strides in identifying genetic risk factors for these diseases, there is little evidence that these differentially affect males and females. The causes of this sex bias may include the effects of genes on the X or Y chromosomes, sex-specific differences in hormone levels, sex differences in patterns of gene expression in many type of cells and sex-biased responses to environmental stimuli. Genome-wide studies have identified common polymorphisms associated with autoimmune disease risk, including causal variants implicated in immune regulation. These analyses have not addressed the impact of two other critical modifiers of autoimmunity: sexual dimorphism and environmental factors. Findings of incomplete disease concordance in monozygotic twins and the recent rise in autoimmune disease incidence in developed countries indicate a causal role of environmental factors in disease. We identified a direct interaction between sex hormones and microbial exposures and show that manipulations of the gut microbial community ("the microbiome") can provoke testosterone-dependent protection from autoimmunity in a genetically high-risk rodent model. In addition we have recent evidence that immune response to the gut microbiome differ in both healthy and autoimmune disease-affected males and females. The lecture will explore the complexity of causes of autoimmune disease, the evidence for sex effects on these mechanisms and then provide a sex-informed framework for future work that can produce "actionable" ways to prevent and treat these diseases. This work was funded by the Canadian Institutes of Health Research, the Juvenile Diabetes Research Foundation, Genome Canada (Ontario Genomics Institute), and the Ontario Ministry of Innovation.

ELIZABETH YOUNG NEW INVESTIGATOR SYMPOSIUM April 24, 2014 2:15 pm – 3:15 pm

Survey of escape from X inactivation in mouse tissues

Joel B. Berletch, Ph.D. (University of Washington, Seattle, WA)

X chromosome inactivation (XCI) silences most genes on one X chromosome in female mammals, but some genes escape XCI. To survey escape gene profiles in vivo and to explore molecular mechanisms that regulate this process we analyzed the allele-specific expression and chromatin structure of X-linked genes in mouse tissues and cells with skewed XCI and distinguishable alleles based on single nucleotide polymorphisms. Using a new method to estimate allelic expression, we demonstrate a continuum between complete silencing and significant expression from the inactive X (Xi). Few genes (2-3%) escape XCI to a significant level and only a minority differs between mouse tissues, suggesting stringent silencing and escape controls. Allelic profiles of DNase I hypersensitivity and RNA polymerase II occupancy of genes on the Xi correlate with escape from XCI. Allelic binding profiles of the DNA binding protein CCCTC-binding factor (CTCF) in different cell types indicate that CTCF binding at the promoter correlates with escape. Importantly, CTCF binding at the boundary between escape and silenced domains may prevent the spreading of active escape chromatin into silenced domains. This work was supported by a fellowship from the National Institutes of Health (HD060402) to J.B., and by a grant (GM046883) from the National Institutes of Health to C.D.

A subset of estrogen-responsive hypothalamic neurons promotes physical activity and maintains energy balance in females

Stephanie M. Correa, Ph.D. (University of California, San Francisco, San Francisco, CA)

Central estrogen signaling via estrogen receptor alpha (ERα) regulates reproduction, food intake, basal thermogenesis, and physical activity in a female-specific manner. Whereas ERα is required in both the arcuate (ARC) and ventromedial (VMH) hypothalamic nuclei for female fertility, ERα regulates basal thermogenesis and food intake in the VMH and ARC, respectively. However, ERα neurons controlling physical activity remain undefined. Here we employ complementary loss- and gain-of-function strategies to target ERα$^+$ VMH neurons. We conditionally ablated *Nkx2-1* (*Ttf-1*), a homeobox transcription factor that is expressed in hypothalamic progenitors and upregulated in adult VMH_{VL} neurons, using *Sf1*-driven Cre recombinase (*Nkx2-1^{Sf1Cre}*). Female *Nkx2-1^{Sf1Cre}* mice are 30% heavier than littermate controls when fed normal chow with increased visceral and subcutaneous adiposity. Male body weight is unaffected. Metabolic analyses revealed that obesity is due to reduced energy expenditure rather than increased food intake; *Nkx2-1^{Sf1Cre}* females exhibit a specific deficit in physical activity. In the *Nkx2-1^{Sf1Cre}* VMH, neurons expressing NKX2-1 and ERα are selectively reduced. Despite decreased ERα, female *Nkx2-1 ^{Sf1Cre}* mice are fertile, a finding that uncouples the roles of hypothalamic ERα in reproduction and metabolism. To demonstrate that *Nkx2-1$^+$* VMH_{VL} neurons regulate locomotion, Cre-dependent DREADDs were used to activate *Nkx2-1Cre$^+$* VMH_{VL} neurons. Activation of *Nkx2-1$^+$* VMH_{VL} neurons resulted in increased locomotion. This burst of physical activity is female-specific and dependent on ERα. Our findings demonstrate that estrogen-responsive *Nkx2-1$^+$* VMH_{VL} neurons constitute an important part of a previously undefined sexually dimorphic locomotor circuit that is used in females to maintain energy homeostasis. This study was funded by grants to HAI (NIDDK R01DK063592, UCSF Diabetes Family Fund, AHA Grant-in-Aid 13GRNT16120004), JLR (NIMH R01 MH081880, R37 MH049428), SMC (NIDDK K01DK098320, AHA Postdoctoral Fellowship 12POST10690005), CCC (NIDDK 1K08DK076721), and AWX (NIDDK R01DK80427).

Title: X Chromosome Dose and Sex Bias in Autoimmune Diseases: Increased 47,XXX in Systemic Lupus Erythematosus and Sjögren's Syndrome

Ke Liu, B.S. (University of Cincinnati, Cincinnati, OH)

Mechanism for female predominance in autoimmunity is unknown. We suspected an X chromosome dose effect and predicted if so, triple X (47,XXX, 1 in ~1,000 live female births) would be increased in female predominant diseases (systemic lupus erythematosus [SLE], primary Sjögren's syndrome [SS], primary biliary cirrhosis [PBC] and rheumatoid arthritis [RA])compared to diseases without female predominance (sarcoidosis, granulomatosis with polyangiitis [GPA]) and healthy controls. We used single nucleotide polymorphism (SNP) arrays to identify 47,XXX and fluorescent *in situ* hybridization, or q-PCR to confirm when possible. 47,XXX was found in 7 of 2,948 SLE and 3 of 1,053 SS female patients, and only 1 in 4,822 female controls (OR=11.46.31, 95% CI: 1.41-93.16 or OR=13.75, 95% CI: 1.43-132.34, respectively). One 47,XXX was present for every ~421 SLE women and ~351 SS women. In addition, we identified one 47,XXX from 1,159 women with PBC. No 47,XXX was identified among 943 women with sarcoidosis, 453 women with RA or 247 with GPA. In conclusion, 47,XXX was present in excess among SLE and SS subjects as predicted by X chromosome dose effect. These estimated prevalence of SLE and SS with 47,XXX being respectively ~2.4 and ~2.8 times higher than in women with 46,XX and ~24 and ~39 times higher than in men with 46,XY. There was no increase of 47,XXX is other female-biased diseases, suggesting multiple pathways to such a bias in autoimmunity.
This work was funded by the USA National Institutes of Health (AI024717, AI031584 , AI062629 , AI063274, AI082714, AI083194, AR042460, AR48204 , AR048940 , AR049084, AR052125, AR053483, AR053734, AR056360, AR058959, AR62277, AR043814, DE015223, DE018209, RR015577, RR020143, GM103510 and HG006828), the Intramural Research Program of the National Institute of Dental and Craniofacial Research, the University of Oklahoma Health Sciences Center and its Clinical and Translational Science OCTSI Summer Scholar Program, the U.S. Department of Veterans Affairs (IMMA9 to RHS and JBH), the U.S. Department of

Defense (PR094002), Alliance for Lupus Research (JBH), Mary Kirkland Scholar (JBH), the Strategic Research Program at Helse Bergen, The Western Norway Regional Health Authority, The Broegelmann Foundation, EvASSESS PHRC (Programme Hospitalier de Recherche Clinique) 2006 from the French Ministry of Health, the Swedish Rheumatism Foundation, Arthritis Australia, an unrestricted grant from Research to Prevent Blindness to the Dean McGee Eye Institute and the Department of Ophthalmology, University of Oklahoma College of Medicine, the Medical Research Council, UK (G0800629), DFG KFO 250 WI1031/6-1 (TW), a Senior Scientific Investigator Award to James Chodosh from Research to Prevent Blindness.

PRESIDENTIAL SYMPOSIUM April 24, 2014 3:30 pm – 5:30 pm

Sex Differences in the Brain - A Whole Body Perspective

Chair: Geert de Vries, Ph.D.

The hundreds of sex differences found in the brain beg the question as to how they develop and what is their function. Factors that cause sex differences in the brain are sex chromosomal gene expression, gonadal hormones, and environmental interactions. A parsimonious explanation is that these factors act directly on the brain. However, the speakers in this symposium will demonstrate that they act on peripheral structures as well. Sex differences may therefore develop because brains reside in fundamentally different bodies. This has consequences for brain function as well. Brains may generate different output autonomously, but if they are wired up to different bodies, similar output will have different consequences. To generate similar behaviors, the nervous system may have to compensate by giving different commands. This interaction between body and brain has to be taken into account for a full understanding of the development as well as function of sex differences in the brain. The three speakers in this symposium will illustrate this point.

What the sexually dimorphic body tells the brain

Nancy G. Forger, Ph.D. (Georgia State University, Atlanta, GA)

Sex differences in behavior are often thought to arise from gonadal steroid hormones acting at receptors in the brain. This was not always the assumption: in early work, investigators looked to peripheral structures for explanations of how steroid hormones affected behavior. Although there is now overwhelming evidence for direct effects of hormones or sex chromosomes on the nervous system, this talk will consider the possibility that the pendulum may have swung too far - we now tend to overlook the periphery when considering how sex differences in brain and behavior arise. I will provide a historical perspective and will consider examples from worms to mammals in arguing that sex differences in musculoskeletal systems, dentition, and other peripheral structures shape differences in behavior. The goal is to stimulate thinking that may lead to a more balanced view of the role of the “body” in sexual differentiation of the brain. Work in the Forger lab is funded by NIH R01 MH068482.

Sex differences in the placenta and the role of OGT in neurodevelopment

Tracy L. Bale, Ph.D. (University of Pennsylvania, Philadelphia, PA)

Neurodevelopmental disorders including autism and schizophrenia show strong sex biases in presentation, onset and treatment, and have been associated with fetal antecedents including maternal stress. The

programming mechanisms through which stress contributes to disease development are not well understood; though likely involve a complex interaction between the maternal environment and effects on the placenta. We have previously identified a sensitive period of early pregnancy where maternal stress produces sex-specific programming effects on offspring stress pathway development. In this mouse model of early prenatal stress (EPS), we have demonstrated robust and sex-specific changes in placental gene expression patterns across gestation. Microarray analyses across gestation examined genes that had sex-specific expression and were affected by maternal stress. One candidate gene, O-GlcNAc transferase (OGT), was further examined for its role in altering neurodevelopment. This X-linked gene was similarly regulated in human placental tissue supporting its translational potential. To more directly link placental OGT with an effect in reprogramming the developing hypothalamus and the EPS phenotype, we examined transgenic mice with a placental-specific targeted reduction in OGT. In these mice, we were able to recapitulate the EPS reduced body weight and increased stress reactivity phenotype. Microarray results from adult PVN identified mitochondria dysfunction, which was confirmed by cytochrome C oxidase assay in tissue from both placental OGT and EPS male mice. Together, these results support a reprogramming of the hypothalamus by EPS acting, in part, through its effects on placental OGT. These results may provide critical insight into the mechanisms contributing to sex-biased disease vulnerability to maternal stress during early pregnancy impacting the developing brain via effects at the placenta that are transmitted to reprogram critical neuroendocrine systems. These studies were funded by NIH grants MH091258, MH087597, and MH099910.

Hypothalamic PGC-1α protects against high fat diet exposure by regulating ERα

Deborah Clegg, Ph.D. (University of Texas Southwestern Medical Center, Dallas, TX)

Consumption of diets high in fat (HFD) increases the prevalence of obesity and results in inflammation in the central nervous system (CNS). Estrogens (E2) directly, or acting through the estrogen receptors (ER), provide anti-inflammatory protective properties. Specifically, ERα has anti-obesity, neuroprotective, and anti-inflammatory activities. We observed male mice exposed to chronic HFD show increased hypothalamic inflammation and this is associated with reductions in ERα as well as markers of mitochondrial activity in the males but not the females. *In vitro*, neuronal cell culture exposed to palmitic acid (PA) show increased markers of inflammation (*tnfα* and *il6*) and reduced mitochondrial respiration, and E2 pre-treatment protects against inflammation and reductions in mitochondrial activity. In hypothalamic neurons, RNAi-mediated knockdown of ERα promotes PA-induced inflammation, whereas over expression of ERα inhibits it. *Erα* expression is regulated by PGC-1α, and PGC-1α is reduced in neurons as well as in astrocytes *in vitro* and *in vivo* following PA-treatment or HFD-feeding in male mice. Our results demonstrate for the first time that *Erα* expression is regulated by HFD or PA-driven reductions in PGC-1α leading to suppression of ERα in the hypothalamus, which facilitates CNS inflammation and reductions in mitochondrial activity.

SPEAKER ABSTRACTS – FRIDAY APRIL 25, 2014

SYMPOSIUM I & II April 25, 2014 8:15 am – 10:15 am

Symposium I: Renovating the Research Pipeline to Include Sex & Gender: How can Research Institutions be Transformational?

Co-Chairs: Joy Johnson, Ph.D. (Canadian Institute of Health Research)
Janine Clayton, M.D. (National Institutes of Health)

Rigorous approaches to science suggest that sex and gender must be considered in research proposal development, data analysis, publishing, and knowledge translation. Serious incorporation of sex and gender considerations in health research will not gain traction without transformation of the entire research system. This transformation requires that funding agencies and journals develop and implement policies and approaches that enhance the uptake of sex and gender considerations by all health researchers. In this panel those involved in this renovation discuss strategies used and progress to date in shaping science through integration of sex and gender.

Working to Change Research Funding Institutions

Joy Johnson, Ph.D. (Scientific Director, Institute of Gender and Health, CIHR)

It is increasingly recognised that scientific evidence often fails to account for sex and gender; consequently it is not always clear whether results can be equally applied to men and women. Funding agencies are starting to develop mechanisms to encourage the uptake of sex/gender considerations in science. Yet, many of these mechanisms have not been formally evaluated. To address this gap, we assessed the impact of introducing a requirement to encourage the uptake of sex/gender in research. Our key goal was to introduce and evaluate a policy approach that would require all researchers applying to CIHR, Canada's National Health Research Funding Agency, to indicate if they have taken sex/gender into account in their research. In this presentation I describe the strategies used to achieve, design, and implement this institutional policy change, and to encourage compliance with the policy and provide an analysis of applicants' responses to the sex/gender items. While we are heartened by the uptake of sex and gender amongst Canadian researchers, there is a great deal of work left to do. We now have excellent baseline data, and know the fields of science that require particular focus. We recognized that the research community requires education and are developing training materials for researchers and peer-reviewers.

Integrating the Study of Sex Differences in a National Research Agenda

Janine Austin Clayton, M.D. (Director, Office of Research on Women's Health and Associate Director for Research on Women's Health, NIH)

Research organizations play a leadership role in setting policy that addresses opportunities and gaps in science. The National Institutes of Health's (NIH's) Office of Research on Women's Health (ORWH) works to enhance, stimulate, and expand efforts to examine the role of sex/gender in health and disease, across the agency. In 2010, ORWH issued a 10-year strategic plan for NIH sex and gender research after conducting an extensive process that gathered and integrated input from the agency's many stakeholders diverse. Putting the

plan into action involves development and use of an action matrix that enables us to track investments strategically and employ resources in the most effective and efficient way possible. To monitor, and showcase, participation in sex/gender research by NIH ICs, ORWH leads the development and issuance of a biennial report that details how NIH Institutes and Centers (ICs) are meeting goals and objectives of the strategic plan for NIH sex and gender research. In FY 2013, ORWH launched a program to leverage current funding by enabling NIH-funded scientists to apply for an administrative supplement that provides extra funds to conduct additional that allow exploration of sex/gender influences not previously considered in their parent grant. ORWH also co-funds with the U.S. Food and Drug Administration the Specialized Centers of Research on Sex Differences program, which supports interdisciplinary collaborations on sex/gender influences in health and bridges basic and clinical approaches. In addition, NIH Institutes and Centers issue funding announcements for research on sex/gender influences on diseases and conditions within their mission

Shifting Minds: Inclusion of Sex/Gender for Better Standards of Science Reporting

Shirin Heidari, Ph.D. (Chair, Gender Policy Committee, European Association of Science Editors)

Editors of scientific journals are the gatekeepers of science and are responsible to ensure that research methodology is sound, data analysis is accurate, reported results are complete, and conclusions are balanced and firmly based on evidence. Ensuring that reported data are disaggregated by sex, and that results and conclusions take into account gender dimensions, should be an integral aspect of editors' responsibilities. In 2013, the Gender Policy Committee of the European Association of Science Editors launched an extensive international survey in order to map existing editorial sex and gender policies. The survey probed not only for current practices, but also for opinions regarding editorial sex and gender policies in scientific journals and publishing houses. In this presentation, Dr Heidari will present the sobering results of this survey, from which it is apparent that the vast majority of journals and publishing houses do not have explicit policies that foster routine gender- and sex-sensitive reporting or policies that address gender biases in the workforce. She will discuss the implications of these findings and provide examples of how appropriate policies can make contributions towards improved scientific rigour and evidence-based practices.

Symposium II: Sex-Gender Differences in Eating Disorders

Chair: Deborah Romano, M.D. (Commission of Women Doctors of the Medical Order of Ferrara, Italy)

Eating disorders are a problem of public health with an incidence of 1-2 % for anorexia nervosa (90% F vs 10% M) and of 1-3 % for bulimia (85 % F vs 15 % M). The female prevalence and gender prejudice induce a clinical underestimation and a late diagnosis in young males, which represent the main prognostic indicator. The different hormonal expression in the sexes influences body weight, basal metabolism, growth, bone metabolism and the psychological profile, according to varied biological and molecular mechanisms causing different clinical patterns. The precocious disregard of the pathology and its becoming chronic are correlated to the onset of complications, reversible or irreversible, which are more serious in males respect to females: principally lack of growth, osteoporosis, hypogonadism, hematologic and cardiovascular disorders. The aim is to focus on the biological, psychiatric and clinical sex differences in eating disorders, to support a correct approach in men and favor a specific medical formation, to begin adequate programs of prevention and gender-differentiated diagnostic and therapeutic protocols.

Gender differences in Clinical Complications of Chronic Anorexia

Deborah Romano, M.D. (*Commission of Women Doctors of the Medical Order of Ferrara, Italy*)

Eating disorders are psychiatric conditions complicated by multiple organ dysfunctions due to malnutrition, binging, purging and excessive compulsive exercise, potentially leading to a variety of severe, life threatening medical consequences; they require multidisciplinary and integrated care strategies. Eating disorders are considered “female –gender-bound-Syndromes”, so men affected with an ED risk may never be identified. Anorexia and Bulimia are disorders characterized generally by sense of denial: for men, beside these denial and resistance aspects there can be awareness that ED’s are more common among females so they can be reluctant to recognize that they suffer from a disorder thought to be a “women ‘s disease”. Moreover, they risk going unrecognized because doctors can fail to identify male ED. In addition, men are screened with tests which have been constructed and validated for female populations. It is consequently more difficult to detect risk subjects among males by using scales or cut-off points used for females. Besides the underestimation of symptoms, males undergo less intensive and prolonged cures than females. The precocious disregard of the pathology and its becoming chronic are correlated to the onset of complications, reversible or irreversible, which are more serious in males respect to females: principally lack of growth, osteoporosis, hypogonadism, hematologic and cardiovascular disorders. The aim is to explore the main medical aspects in ED patients with particular focus on gender differences.

The Impact of Gonadal Hormones on Energetic Homeostasis

Cristina Tarabbia, M.D. (Professor of Gender Medicine, University of Ferrara, Italy)

Homeostatic energetic balance is due to food intake and energy expenditure and it is regulated by particular neurons in hypothalamic centers. Peripheral signals are integrated in the hypothalamus and promote a variety of adaptive responses: neurophysiological, metabolic, hormonal and organic responses. Homeostatic balance also depends on age, gender, ethnicity, sleep-wake rhythm, circadian rhythm and it is influenced by multiple factors: genes, hormones, nutrients, environment and psychological behavior. Gonadal hormones are important to influence the body weight through molecular mechanisms: the distribution of fat mass (subcutaneous or visceral), the modulation of hypothalamic neurons (NPY/AgRp, α-MSH, POMC), of reward circuits and of endocrine peptides (principally ghrelin, leptin, insulin), the expression and the release of neurotransmitters), the control of metabolism and thermogenesis, the regulation of cytokines. Gonadal

hormones give a phenotypic gender dimorphism to the homeostatic energetic balance. Anorexia nervosa is a condition of self-starvation which induces an adaptive response leading to an apparent balance during which vital functions are preserved for a long time. When the weight loss is persistent, severe medical complications are possible and the organic damages could be different between men and women, because of gender differences in body composition, BMI, peak bone mass, growth, osteogenesis, fertility, cardiac repolarization, and composition of bone marrow.

Sex-Gender Differences in Psychiatric Aspects of Anorexia and Bulimia

Emilia Manzato, M.D. (Public University Hospital, Ferrara, Italy)

If we think of the different psychiatric disorders as an expression of the malaise of the historical moment, no mental disorder better expresses the discomfort of our time as Eating Disorders (ED). Eating disorders in males are stimulating a growing clinical interest for the peculiarities of its expressions, and its difficulty of approach and treatment. For these reasons, ED in males represents a true challenge for the clinicians since the first approach. The relation, after some brief historical notes, will focus on the clinical features of ED in males: weight and shape concerns, body dissatisfaction, psychiatric comorbidity and risk factors for an ED.

Symposium III: Sex Differences in Drug & Device Development

Chair: Emmanuel Olutayo Fadiran (Food and Drug Administration, Silver Springs, MD)

The goal of this symposium is to examine sex differences in the safety and efficacy of FDA regulated medical products. The pharmacokinetics and pharmacodynamics of many drugs differ between males and females. Evidence is also accumulating to indicate that use and responses to devices such as cardiac resynchronization therapy, joint implants and exposure to radiation differ between males and females. Dr. Pamela Scott will discuss the results of the FDA report in response to Section 907 of the Food and Drug Administration Safety and Innovation Act of 2012, which required FDA to take a closer look at the inclusion and analysis of demographic subgroups in applications for drugs, biologics and devices, give examples of sex differences for drugs and examine how this information is communicated to the public. Nada Hanafi will review FDA policy regarding inclusion of women in clinical trials that support the approval of devices and the analysis of the device clinical trial data for sex differences in safety and efficacy and discuss examples of sex differences in the safety and efficacy of devices. LT James Coburn will discuss an example of current modeling and simulations research at FDA aimed at determining the anatomical and activity based parameters that contributed to the greatly elevated failure rates seen in women with metal-on-metal implants. Dr. Tina Morrison will discuss the role that anatomy plays in limiting eligibility for women in clinical studies of endovascular repair (EVAR) based on the analysis of nearly 10,000 patients nationwide with a variation in anatomic parameters that drive ineligibility for EVAR in patients with abdominal aortic aneurysm.

Addressing Sex Differences in FDA-Regulated Products: Drugs

Pamela Scott, Ph.D., M.A. (Office of Women's Health, Office of the Commissioner, FDA, Silver Springs, MD)

In August 2013, the Food and Drug Administration (FDA) released a report in response to Section 907 of the Food and Drug Administration Safety and Innovation Act of 2012, which required FDA to take a closer look at the inclusion and analysis of demographic subgroups in applications for drugs, biologics and devices – including by sex, race/ethnicity, and age. For the report, FDA was asked to consider the extent to which demographic subgroups participate in clinical trials, what tools are available to ensure submission of demographic information, the extent to which sponsors conduct subgroup analysis and how information on demographic subgroups is communicated to health care professionals and the American public. FDA's analysis found that the agency's statutes, regulations, and policies generally give product sponsors a solid framework for providing data in their applications on the inclusion and analysis of demographic subgroups. In general, sponsors are describing the demographic profiles of their clinical trial participants, and the majority of applications submitted to FDA include demographic subset analyses. The report also revealed that FDA shares this information with the public in a variety of ways including through product labeling, the FDA website, and summaries of safety and effectiveness for all approved medical products. The report will help inform the development of an action plan that identifies what more we can do regarding demographic subgroups in clinical trials. The results of the FDASIA Section 907 Report will be discussed in this presentation. Additionally, examples of sex differences for drugs as well as how this information is communicated to the public will be examined.

Sex Differences in FDA-Regulated Products: Devices

Nada O Hanafi, M.Sc. (Center for Devices and Radiological Health, FDA, Silver Springs, MD).

There are myriad unique issues in the development and regulation of Medical Devices related to the Health of Women. For example, differences have been observed in medical device trials in terms of safety outcomes as well as treatment effect (effectiveness) outcomes. The mission of the FDA Center for Devices and Radiological Health (CDRH) Health of Women (HoW) Program is to improve the health of women by: increasing availability, consistency and communication of sex-specific information for the safe and effective use of medical devices in women; addressing identified gaps and unmet needs through targeted resources; and fostering the development of innovative strategies, technology and clinical study paradigms. Early accomplishments include guidance policy development, a multi-stakeholder public workshop, and examination of clinical trials submitted and reviewed by FDA. This talk will also highlight select CDRH research snapshots of multi-disciplinary projects exploring research questions important to the health of women.

Investigating the Mechanical Causes of Increased Metal-on-Metal Hip Implant Failure Rates in Women

LT James Coburn, M.Sc. (Center for Devices and Radiological Health, FDA, Silver Springs, MD).

Approximately 400,000 hip joint replacement surgeries are performed each year in the U.S. Many hip implants are modular systems of interchangeable components which a surgeon can combine to suit the clinical need. Historically, devices have an expected lifespan of 10-15 years post-implantation. In recent years, hip implant systems have seen multiple design changes. The contribution each design change has made to the increased rate of failure is yet to be established. Some implants with metal-on-metal bearings and larger head diameters are have been associated with excessive implant wear, high rates of premature failure, and early revision surgery. An analysis of the National Joint Registry of England and Wales showed that failure rates at 5 years were at least 1.4 times those in men of a similar age and implant size. Moreover, certain metal-on-metal implants were 4 times more likely to fail in women than metal-on-polyethylene systems. (Smith 2012). We created a parameterized finite element model and collected data from men and women performing activities of daily living in an IRB approved motion capture study. Traditionally studied movements such as gait have not shown any defined relationship with the sex-specific clinical outcomes. Other activities such as rising from a chair or stumbling are less frequent but more taxing on the implant. By adapting the computational model to use sex-specific boney anatomy and tailored movements, we aim to determine the mechanical factors that most influence the stresses and strains on these hip implants and cause increased failures in women. Recent dramatic increases in hip implant failures have coincided with multiple changes to the design features and target patient demographics. The present study successfully created a statistically-driven, human-based computational model that can help determine the sets of factors that place different patient populations at risk. This study was funded by grants from the FDA's Office of Women's Health and Critical Path Initiative.

Gender Disparities in Endovascular Treatment Options for Infrarenal Abdominal Aortic Aneurysms

Tina M. Morrison, Ph.D. (Office of Device Evaluation, Center for Devices and Radiological Health, Silver Springs, MD).

Of the 200,000 people diagnosed each year with an abdominal aortic aneurysm (AAA), a diseased, bulging, weak section of the aortic wall in the area of the body that supplies blood to the organs in the abdomen, 25% are women. There are three courses of treatment for patients: open surgical repair (where the aneurysm is replaced with a graft material), endovascular repair (where an endovascular graft (EVG) is placed inside of the aneurysm to direct blood flow away from the aneurysm), and observation (where the aneurysm is watched carefully). Endovascular repair (EVAR) is the current standard of care for most patients because the risk of death is lower and the recovery time is shorter compared to open surgical repair. The EVG is typically a tube-like 'fabric' structures supported by a metal frame and is available in a variety of sizes. This therapy can

prevent further growth and possible rupture of the AAA. Despite the large prevalence for women, they comprise only 9-26% (mean of 14%) of clinical studies to investigate EVG performance. We sought to investigate the role that anatomy plays in limiting eligibility for women in clinical studies of EVAR. Using FDA resources and clinical collaborators, we created a database of nearly 10,000 patients nationwide, characterized the variation in anatomic parameters, and identified the anatomic parameters driving ineligibility for EVAR in patients with AAA. We discovered that, despite the dozens of EVG of different sizes available in the U.S., only 50% of women and 70% of men have suitable anatomic criteria for potential treatment with an EVG per the instructions for use. We will discuss the findings of our study and the implications for future EVG design and evaluation. This study was funded by the FDA's Office of Women's Health.

Symposium IV: Sex Differences in Neural Development During Adolescence May Lead to Differential Vulnerabilities for Affective Disorders

Chair: Janice M. Juraska, Ph.D. (University of Illinois, Champaign, IL)

Adolescence often involves emotional turmoil, and the risk for affective disorders increases during this time. This symposium will review the neural reorganization that the cerebral cortex, especially the prefrontal cortex and connected neural regions, undergoes during adolescence and the implications for an increased risk of disorders that varies with sex. Dr. Juraska will show cellular changes in the medial prefrontal cortex that occur during adolescence in a rat model. Sex differences in these modifications, the role of pubertal hormones and disruption from an environmental chemical will be discussed. Dr. Andersen will present a model of depression based on animal work from her laboratory in which the effects of an early stress are differentially manifested in males and females during adolescence. Dr. Ladouceur will review data from studies of humans that show that both the structure and the function of neural areas associated with emotion change during adolescence. This reorganization may differentially predispose the sexes to the risk of affective disorders.

Sex Differences and Hormonal Influences during Adolescence on the Cerebral Cortex and its Connectivity

Janice M. Juraska, Ph.D. (Department of Psychology and Neuroscience Program, University of Illinois at Urbana-Champaign)

The brain and the cerebral cortex in particular are developing during adolescence. This has been seen in human MRI studies as well as in rat models. Perturbations in these changes may be important for syndromes that often start during adolescence such as affective disorders, schizophrenia and substance abuse. Using a rat model, I will show that sex differences in the cellular structure of the cortex are formed and amplified during adolescence, and females exhibit more changes, at least in the rat. One of the cortical areas is the prefrontal cortex which is known to be involved in affective and other disorders in humans. Some of the sex differences that are forming during adolescence are as profoundly basic as the number of neurons themselves. Dendrites and myelination of axonal pathways are also affected. The ovarian hormones secreted during puberty are responsible for many, but not all, of these changes. Indeed, exposure to an endocrine disruptor (bisphenol A, BPA) during adolescence has opposite effects on the number of glia in the prefrontal cortex of males and females. Thus males and females may have differential vulnerabilities during adolescence due to their gonadal hormones and differences in the timing of puberty. This work originally was funded by NSF and more recently by NIAAA, NIMH and NIEHS/EPA.

The Trajectories of Sex differences in Adolescent Development and the Emergence of Expression Across Species

Susan L. Andersen, Ph.D. (Laboratory for Developmental Neuropharmacology, McLean Hospital/Harard Medical School, Belmont, MA)

Depression is more prevalent in males than females – before puberty. Once puberty occurs, this sex difference reverses and females have a higher prevalence of depressive disorder than males. The average age of onset is in the 20's, and often adolescent depression is associated with social anxiety disorder or exposure to early life stress. Humans and rat data support the hypothesis that early life experiences during sensitive periods alter the trajectory of development of neuronal circuits that underlie depression. To produce a species-relevant stress, Sprague-Dawley pups were individually isolated for four hours a day between postnatal days 2-20. At 36 days of age, subjects were tested for depressive-like behavior using the triadic paradigm. Male rats who experienced maternal separation exhibit significant helplessness under controllable conditions; in contrast, females maintain control, but show deficits in motivation. Reduced preferences for sucrose solutions were evident in adolescent and adult subjects who underwent early separation, suggesting anhedonia. Human structural MRI identified regions vulnerable to developmental life stress. Parallel observations were made in animal studies, although we focused primarily on the prefrontal cortex for in-depth analyses. Maternal separation reduced D1 dopamine receptors on cortical projections to the accumbens, which likely explains increased anhedonia. Second, parvalbumin-immunoreactive neurons (a GABA neuron) are reduced in the cortex, consistent with GABA decreases in human depressed adolescents. Together, a number of parallels in behavioral endophenotypes and brain regions associated with depression across species can be made that will facilitate translational research. This study was funded by NARSAD (to HCB), the Simches and the Rosenberg Families.

Adolescent Fronto-Limbic Development, Puberty, and Affective Disorders

Cecile D. Ladouceur, Ph.D. (Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA.)

Onset of puberty represents a period of vulnerability that, in the context of adverse events, can contribute to the developmental trajectory toward affective disorders—particularly in girls. Neuroimaging findings indicate that altered functioning of fronto-limbic circruitry supporting emotion processing and regulation plays an important role in the pathophysiology of affective disorders. While it is known, mainly from work in animals, that sex hormones influence activity in subcortical regions implicated in processing emotionally salient information (e.g., threat, reward), what is not clear is how puberty specifically affects the development of fronto-limbic circuitry. Healthy adolescent girls (9-16 years old, 36% early-mid puberty, 64% late puberty) at high- and low-familial risk for affective disorders, by virtue of having a parent diagnosed with bipolar disorder or depression, completed an emotional processing task during a functional magnetic resonance imaging (fMRI) scan and self-report measure of pubertal maturation. Regression analyses were performed (fearful vs. neutral faces), with age as a covariate and bilateral amygdala, ventral striatum, and ventromedial prefrontal cortex as a priori regions-of-interest. Results suggested that girls at higher risk showed greater amygdalar response ($p<.05$ corrected) whereas girls at lower risk for affective disorders showed greater striatal and prefrontal cortical response ($p<.05$corrected) as a function of increasing pubertal maturation. These findings suggest that increased reactivity to emotional stimuli may represent a marker of vulnerability in youth with a family history of affective disorders. Longitudinal studies are needed to examine how these findings relate to future onset of psychopathology in these at-risk youth. This study was funded by and NIMH grant (K01MH083001) to CDL.

Symposium V: Sex Differences in the Microbiome: Urinary Tract Infections

Chair: Jeffery Henderson, Ph.D. (Washington University Medical School, St. Louis, MO)

Urinary tract infections exhibit well-described epidemiologic sex differences that have guided existing antibiotic treatment guidelines. As marked increases in antibiotic resistance coincide with an aging, more UTI-susceptible population, new approaches to UTI prevention and treatment are finding renewed interest. This session will cover efforts to understand sex differences in UTI pathophysiology and to translate this knowledge to future therapeutic strategies.

Understanding Urinary Bladder Mucosal Immunity During UTI: Opportunities for New Therapeutic Strategies

Thomas J. Hannan, D.V.M., Ph.D. (Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO)

Urinary tract infections (UTI) are common and highly recurrent. Although UTI can occur in all stages of life in both sexes, males are affected primarily in early childhood and with old age, whereas women are at greatest risk during childbearing years. Uropathogenic *E. coli* (UPEC) are the causative agent in approximately 85% of community-acquired UTI and the emergence of multi-drug resistant UPEC worldwide highlights the need for new therapeutic strategies. Using a murine model of urinary bladder infection (cystitis), we have identified a host-pathogen checkpoint early in experimental UPEC infection that determines the outcome of infection, i.e. whether chronic bacterial cystitis ensues. This model suggests that immunomodulatory therapy may provide clinical benefit, as treatment of mice with dexamethasone during acute UTI improved UTI outcome by reducing the development of chronic cystitis, which in turn predisposes to recurrent infection. Translating these findings to a study of women with acute UTI, we discovered that elevation of serum cytokine biomarkers engaged in myeloid cell responses were predictive of future UTI recurrence. Concordantly, moderation of myeloid responses during experimental UTI in mice and specifically disruption of bladder epithelial transmigration of neutrophils by inhibiting COX-2 protected against chronic and recurrent cystitis. Thus, COX-2 expression during acute UTI is a critical molecular trigger determining disease outcome and drugs targeting COX-2 could prevent recurrent UTI. Overall, these studies have generated a new model for recurrent cystitis that will inform future translational studies targeting specific populations susceptible to UTI. This study was funded by National Institutes of Health grants U01 AI095542 (MC & SJH), DK51406 (SJH), and K08 AI083746 (TJH), an Office of Research on Women's Health SCOR Grant P50 DK64540 (SJH & AES), and a Mucosal Immunology Studies Team consortium U01 AI095776 pilot grant (TJH).

Virulence Network Detection in a Mixed Male and Female Urinary Tract Infection Population

Jeffrey P. Henderson M.D., Ph.D. (Center for Women's Infectious Diseases Research, Division of Infectious Diseases, Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO)

Urinary tract infection (UTI) is a highly prevalent bacterial infection both inside and outside hospitals. Although female UTIs predominate in early adulthood, male UTI incidence increases with age and is treated differently in accordance with most professional guidelines. Sex differences in UTI are interpreted predominantly in terms of anatomical and hormonal differences. Here, we investigate whether there exist sex differences in the virulence strategies used by clinical E.coli isolates. We applied mathematical and statistical clustering algorithms to the distribution of bacterial virulence factors (VFs). VFs are non-conserved, often mobile, genetic elements that have individually been associated with disease. This analysis revealed the existence of previously-

unappreciated VF groupings among urinary pathogens that are significantly associated with both patient sex and antibiotic resistance. These findings are consistent with pathogenic niche specialization in male and female patients. In addition to providing unexpected clues to disease pathogenesis and epidemiology, these findings also suggest new routes toward virulence-targeting therapies. This work was funded by Burroughs-Welcome Fund Career Awards for Clinical Scientists (JH), NIGMS R21 (PM), James S. McDonnell Foundation Scholar Award in Studying Complex Systems (PM), Center for Women's Infectious Diseases Pilot Grant (KC), and NIH BIRCWH Fellowship (JM).

Influence of the Vaginal Microbiota on Genitourinary Health and Disease

Amanda L. Lewis, Ph.D. (Departments of Molecular Microbiology and OB/Gyn, Washington University School of Medicine, St. Louis, MO)

Urogenital physiology is an inherent sex difference. The urogenital microbiota might be argued as intrinsic to the physiology and proper functioning of urogenital systems. Mounting evidence shows that in women, patterns of bacterial vaginal colonization can be linked with a wide variety of health outcomes, both good and bad. This talk will focus on a pattern of bacterial vaginal colonization known as bacterial vaginosis (BV), a very common, but little understood "imbalance" of the vaginal microbiota. BV is characterized by a lack of "healthy" bacteria (lactobacilli), and an overgrowth of a polymicrobial population of potentially "unhealthy" bacteria. The condition has been associated with increased risks of adverse pregnancy outcomes, sexually transmitted infections, and urinary tract infections. However, a basic understanding of BV has been hampered by a lack of relevant experimental models to test hypotheses and address the many controversies in the field. One of the most important questions is, "which of the many species of BV-associated bacteria are required and/or sufficient to cause the clinical features or adverse health events associated with the condition?" We have recently developed several experimental models that capture clinical features and health complications associated with BV. This talk will focus on a single bacterial species known as *Gardnerella vaginalis* and will present evidence that this bacterium is sufficient to cause clinical features of BV and trigger other urogenital health complications associated with BV in mouse models of urogenital infection. This work is funded by March of Dimes Foundation, National Institute of Diabetes and Digestive and Kidney Diseases, Burroughs Welcome Fund.

Symposium VI: Influence of Sex & Other Individual Differences on Addiction

Chair: Marilyn Carroll, Ph.D. (University of Minnesota, Minneapolis, MN)

The goal of this symposium is to examine sex and gonadal hormones, and other individual differences in addictive behaviors such as cigarette smoking, binge eating, overindulgence in palatable foods, and other eating disorder phenotypes. Evidence suggests an interchangeable relationship between drugs of abuse such as cocaine, nicotine, and palatable dietary substances. There are sex differences and female gonadal hormonal influences in these behaviors. Treatments such as nondrug rewards and reduced nicotine content in cigarettes and pharmacological treatments differ by sex. Hormonal manipulations such as progesterone also alter rewarded by drug and preferred dietary substances. Dr. Hatsukami will discuss sex differences in treatment for smoking using reduced nicotine content cigarettes vs. the nicotine patch. Dr. Klump will discuss critical roles for puberty and gonadal hormones for Binge eating and other eating disorder phenotypes, Dr. Becker will discuss the behavior and neurobiology of sex differences in preference for cocaine vs. palatable foods, and Dr. Carroll will describe how sex and hormonal status influence the vulnerability to and development of drug addiction as well as its treatment using animal models.

Sex differences in treatment for smoking with Quest cigarettes vs. nicotine patch

Dorothy K. Hatsukami, Ph.D. (University of Minnesota, Masonic Comprehensive Cancer Center and Tobacco Research Programs, Minneapolis, MN)

Background. Studies have shown that very low nicotine content (VLNC) cigarettes can lead to a reduction in the number of cigarettes smoked, toxicant exposure, withdrawal symptoms and dependence. One area that has been relatively unexplored is what factors might moderate the effects of VLNC cigarettes. One important factor may be sex of the smokers. Females are observed to be more sensitive to the sensory aspects of smoking, whereas males are more sensitive to nicotine. This presentation focuses on sex differences in responses to VLNC cigarettes and nicotine replacement therapy (NRT). Method. Smokers were (58% female) were randomized to a) 0.05-0.09 mg nicotine yield cigarettes; b) 21 mg nicotine patch and 3) 0.05-0.09 nicotine yield cigarettes with 21 mg nicotine patch. Results. The combination of VLNC cigarettes and nicotine patch was more effective in reducing use of VLNC cigarettes and withdrawal symptoms among males than females, whereas females were equally responsive to VLNC cigarettes with and without the nicotine patch. Females were more likely to quit smoking than males when conditions incorporated the VLNC cigarettes; however, males were more likely to quit smoking in the nicotine patch alone condition than females. Conclusion. Sex of the smoker may be an important determinant for effects of VLNC cigarettes and nicotine patch. Implications of this study will be discussed.

Sex Differences in Binge Eating and Eating Disorder Phenotypes: Critical Roles for Puberty and Gonadal Hormones

Kelly L. Klump, Ph.D. (Michigan State University, Department of Psychology, East Lansing, MI)

The focus of this presentation is to describe the role of puberty and gonadal hormones in sex-differentiated patterns of risk for binge eating and related eating disorder phenotypes. Prior studies of etiology and sex differences have focused almost exclusively on psychosocial causes with a lack of emphasis on developmental or biological risk factors. Although these types of risk factors are clearly important, recent data highlight dramatic shifts in genetic and biological risk factors for eating disorders across puberty. These genetic/biological shifts are highly sexually differentiated, suggesting that they may contribute to both the etiology of eating disorders and the dramatic sex differences in their prevalence (i.e., female to male ratios of 4:1 to 10:1). The current presentation will review these data from both human and animal studies and suggest

directions for future research aimed at further elucidating biological and genetic contributions to sex differences in eating disorder phenotypes. This work is funded by NIH grants MH70542, MH92337, DA5147, AA9376.

Sex differences in the development of a preference for cocaine vs. tasty treats: neurochemistry and behavior.

Jill B. Becker, Ph.D. (The University of Michigan, Molecular and Behavioral Neuroscience Institute, Neuroscience Program, Ann Arbor, MI)

Cocaine dependence is characterized by compulsive drug taking that supersedes other recreational, occupational or social pursuits. We have developed a behavioral paradigm in which rats vulnerable to addiction can be identified within the larger population based on their preference for cocaine over palatable food rewards. To validate the paradigm as a preclinical model of addiction, we examined changes in motivation for cocaine and recidivism to drug seeking in cocaine-preferring and pellet-preferring rats. We also examined behavior in males and females to identify sex differences in this "addicted" phenotype. Preferences were identified during self-administration on a fixed-ratio schedule with cocaine-only, pellet-only and choice sessions and motivation for each reward was probed using a progressive-ratio schedule. We find that cocaine-preferring rats increased their drug intake at the expense of pellets, displayed increased motivation for cocaine, attenuated motivation for pellets and an attenuated cocaine-induced dopamine release in dialysate, compared with animals that preferred palatable food pellets. Females were more likely to develop a cocaine preferences than were males. The unbiased selection criteria also revealed that vulnerability factors could be distinguished from generalized sex differences in behavior, which has implications for the neurobiology of addiction. This work is funded by R21-DA-032856 and R01-DA-012677 to JBB.

Sex and Other Individual Differences: Effect on Vulnerability and Treatment for Addiction

Marilyn E. Carroll, Ph.D. (University of Minnesota, Department of Psychiatry, Minneapolis, MN)

The focus of this presentation is to discuss sex differences in all phases of addiction, from initiation to escalation of drug use to withdrawal, abstinence, and eventually relapse. Sex differences in treatments for drug abuse will also be considered. Animal research and limited human research indicates that females initiate drug abuse more readily than males, they have more severe problems with drug abuse than males, yet they seem to respond better to treatment than males. Extensive work in rats indicates that endogenous or administered estrogen (EST) (in ovariectomized females) facilitates drug seeking, while progesterone (PROG) reduces it to levels seen in males. Recent animal studies indicate that PROG and its metabolite, allopregnanolone, are effective at reducing drug seeking in rats, and limited studies in nonhuman primates and humans concur. In recent studies with rhesus monkeys cocaine self-administration has been compared during the follicular phase of the menstrual cycle when the PROG/EST ratio is higher than during the luteal phase when this ratio is lower. The effects of PROG were also compared in the presence of another potential treatment that independently reduces drug seeking, access to a nondrug reward - a sweet substance, and combined effects were compared to individual effects. This work is funded by NIDA grants R01 DA002486, R01 DA003240, SCOR P50 DA033942 (MEC) F31 DA036248 (NEZ), and T32 DA007097(JRS).

SYMPOSIUM VII & VIII April 26, 2014 8:30 am – 10:30 am

Symposium VII: Mechanisms of Sex-Specific Risk for Cardiovascular Disease

Co-Chairs: Patricia Nguyen, M.D. (Stanford University School of Medicine, CA)
Jennifer Tremmel, M.D. (Stanford University School of Medicine, CA)

Multiple sex differences have been identified in the manifestation of coronary artery disease; however, the underlying sex-based mechanisms of risk remain unclear. A range of sex-based investigations including chromosomal influences, the role of extracellular vesicles, and coronary plaque formation will be discussed, with a focus on how these discoveries ultimately might affect our care of women and men with heart disease. How we better study and evaluate sex differences in cardiovascular disease will take collaboration with basic, translational, and clinical scientists.

The number of X chromosomes influences protection from cardiac ischemia / reperfusion injury in mice: One X is better than two.

Mansoureh Eghbali, Ph.D. (University of California, Los Angeles, CA)

Sex differences in coronary heart disease have been attributed to sex hormones, whereas the potential role of the sex chromosomes has been ignored so far. Here we investigated the role of the sex chromosomes in causing sex differences in myocardial ischemia reperfusion injury. We used two unique mouse models, the "four core genotypes" (FCG) (XX mice with ovaries (XXF) or testes (XXM) and XY mice with ovaries (XYF) or testes (XYM)) and XY* (gonadal male or female mice with one or two X chromosomes). All mice were gonadectomized. Although gonadectomized FCG mice have similar heart function at baseline, XX mice have much larger myocardial infarct size, both *in vivo* and *ex vivo,* compared to XY mice, irrespective of their gonadal sex. The higher susceptibility of XX mice to I/R injury was associated with lower mitochondrial calcium retention capacity for triggering mithochondrial permibility transition pore opening. In XY* mice, mice with two X chromosomes had larger infarct size than mice with one X chromosome suggesting that the higher susceptibility of XX mice to I/R injury compared to XY is due to the number of X chromosomes rather than the absence of a Y chromosome. Several X genes that escape X inactivation *(Eif2s3x, Kdm6a, and Kdm5c)* showed higher expression in XX than XY hearts. In conclusion, XX mice have higher vulnerability to ischemia/reperfusion injury compared to XY mice, which is due to the number of X chromosomes rather than the absence of the Y chromosome. Support for this work is through NIH grant HL089876.

Sex Differences in Blood-borne Extracellular Vesicles in Cardiovascular Health and Disease

Muthuvel Jayachandran, Ph.D. (Mayo Clinic, Rochester, MN)

The incidence of atherosclerotic and thrombotic associated events such as ischemic heart disease, stroke, and pulmonary embolism increases with age and differs between men and women. Heterogeneous populations of biologically active microvesicles (MV,100-1000nm) derived from plasma membrane contribute to a variety of disease processes including cardiovascular disease. Populations of these vesicles differ between middle age-

matched men and women in the general population such that men have significantly higher numbers of procoagulant MV and those derived from platelets, monocytes, leukocytes and endothelial cells compared to women. MV expressing adhesion molecules ICAM- and VCAM-1 are also greater in healthy men compared to women. However, in men and women with angiographically-defined coronary artery disease, procoagulant MV and those derived from platelets but not leukocytes, monocytes or endothelial cells are greater in women compared to men. Circulating MV populations reflect sex differences in cellular activation contributing to development of cardiovascular disease. Funded in part by grants from the American Heart Association (12GRNT12050147), and Mayo Clinic NIH Specialized Center of Research on Sex-differences (P50AG044170).

Sex Differences in Coronary Plaque

Elena Ladich, M.D. (CVPath Institute Inc., Gaithersburg, MD)

There are apparent differences in the development of atherosclerosis and thrombosis in coronary arteries between women and men. The coronary plaque morphologies primarily responsible for thrombosis are plaque rupture and plaque erosion, with plaque rupture being the most common cause of acute myocardial infarction in men, while women less than 50 years of age more frequently have coronary thrombosis from plaque erosion. Estrogen has been implicated in playing a main role in these gender differences in plaque morphologies, through its effects on vascular smooth muscle cells and lipid metabolism. Given that the most powerful risk factor associated with erosion is shown to be smoking in women and that smoking results in an endothelium-dependent coronary vasomotion endothelial function loss and vasospasm may be the main pathogenesis of plaque erosion. Besides acute coronary thrombosis, differences are also seen in the morphology of stable plaque between women and men. Overall, it appears that atherosclerotic progression in women lags 10-15 years behind men where the number of patients dying of stable coronary disease are approximately 3 times higher in men than women. Perhaps, different strategies may be required for the treatment of CAD in women vs men.

Symposium VIII: The Role of Aromatase in Human Sex Differences

Chair: Anat Biegnon, Ph.D. (Stony Brook University, NY)

Aromatase, the enzyme catalyzing the last step of estrogen biosynthesis, is expressed throughout the animal kingdom. However, the human aromatase gene has unique properties in terms of location, non-coding regions and regulation. Since estrogen are made from androgens, changes in aromatase activity influence not only estrogen levels but also the ratio of androgens to estrogens, making aromatase a prime modulator of sex differences in target organs for both hormones, including bone, adipose tissue, male and female reproductive organs and the brain. The first presentation will address the structure and organ-specific regulation of the human aromatase gene and their involvement in male and female physiology and pathology. The second presentation, by Dr. Nelly-Alia Klein will describe new findings on sex and brain-region specific correlates of aromatase availability with aspects of cognition and personality. The third presentation, by Dr. Tom Hildebrandt, will address emerging concepts on the role of aromatase in aggression and obesity. Funded by NIH 1R21EB012707 grant to Anat Biegon and 1R21DA032858 to Tom Hildebrandt and Anat Biegon (MPI).

Organ-specific Expression and Regulation of Aromatase in Males and Females: Observations and Speculations

Anat Biegon Ph.D. (Stony Brook University, NY)

Aromatase, the enzyme catalyzing the last step of estrogen biosynthesis, is a single gene product. The human aromatase gene (Cyp19A1) is located on chromosome 15. Both gain of function and loss of function mutations have been described, which have sexually dimorphic manifestations. Thus, aromatase excess syndrome (AES) is characterized by increased extraglandular aromatization of steroids and presented with heterosexual precocity in males and isosexual precocity in females, while aromatase deficiency can cause pseudohermaphrodism and virilization in females and osteoporosis in males. The gene contains 10 promoter regions, which are differentially expressed in various organs and tissues and differentially regulated by hormones and drugs. Examples include the I.4 promoter, found in skin and adipose tissue and regulated by cytokines and glucocorticoids and the I.3 and PII promoters, mainly found in breast, endometrial and ovarian cancer and regulated by prostaglandins and gonadotrophins. The brain promoter I.f may also be regulated by glucocorticoids and nicotine was found to inhbit aromatase in the brain and peripheral tissues. The latter observations may contribute to sexually dimorphic responses to stress and nicotine use. Funded by NIH 1R21EB012707 grant to AB.

Sex-specific role of aromatase in amygdala for personality in females and for learning in males

Nelly Alia-Klein Ph.D. (Mount Sinai School of Medicine, New York, NY)

Estrogen biosynthesis depends on the enzyme aromatase, which irreversibly converts androgens such as androstenedione and testosterone synthesized in both the ovary and testes, to the estrogens estrone and estradiol, respectively. Brain aromatase was found to play a role in behavior across species, yet nothing is known about the role brain aromatase may have in human cognition and personality. Visualization of aromatase expression in humans is made possible in-vivo with a Positron Emission Tomography (PET) scan with [^{11}C]vorozole as recently documented. Here we hypothesized that aromatase activity in amygdala and thalamus will have a sex specific role in memory recall and personality measures of self-control. Sixteen healthy participants (50% females) were recruited from the community for a PET scan with [^{11}C]vorozole to obtain the brain distribution of aromatase activity. Participants preformed a memory test (California Verbal

Learning Test; CVLT) and completed the Multidimensional Personality Questionnaire (MPQ) prior to the PET scan. To obtain quantitative data from each brain region where aromatase is most active, regions of interest were placed over the amygdala and thalamus bilaterally. Memory performance (CVLT) and MPQ measures were tested for correlations with aromatase activity in thalamus and amygdala. There were no differences between the sexes on aromatase activity across brain regions. However, results showed sexual dimorphism in the association between the brain regions and memory and personality. On performance of the CVLT, aromatase in thalamus was negatively correlated with recall on the memory trials from trial 1-5 in females (R=-.73, p<.05) but not in males (R=.13, p=ns). Instead, in men aromatase activity in amygdala was correlated with memory recall on trials 1-5 (R=-.76, p<.05) but not in females (R=-.11,p=ns). Other measures of CVLT performance were similarly correlated with aromatase activity in male but not female amygdala (totally recall, short delay, long delay, p<0.05). On the MPQ, thalamic aromatase activity in males but not in females positively correlated with personality measures of self-control (R=.94, p<.001). In females, the amygdala positively correlated with personality measures of constraint (R=.87, p<.01) but not in males (R.19, p=ns). These preliminary results suggest that for females, memory is associated with thalamus and personality with amygdala aromatase activity; while in males, memory is associated with amygdala and personality with thalamus aromatase activity. Funding provided by the US Department of Energy, life sciences.

A Role for Aromatization in the Expression of Eating, Impulsive, and Aggressive Psychopathology

Tom Hildebrandt, Psy.D. (Ichan School of Medicine at Mount Sinai, New York, NY)

Gonadal hormones play a developmental and acute role in the expression of a wide range of appetitive and rewarding behaviors including eating, sex, drug use, and aggression. Significant sex differences exist in the prevalence of these behaviors and suggest a role for estrogen in the central nervous system. We describe a theoretical model which positions aromatization as a key regulatory factor in the sex-specific expression of these behaviors, highlighting findings studying the role of gonadal hormones among individuals with eating disorders, obesity, and addiction. We report preliminary data designed to test this theory using the PET ligand (11)C-Vorozol to study aromatase activity in the brain among obese men and women and longitudinal data with adults that abuse synthetic androgens. Males abusing androgens demonstrated variable changes in aromatase levels as a function of their synthetic androgen abuse, however these changes in aromatase were significantly correlated (*r* = -.67) with changes in behavioral measures of impulsive aggression, behavioral disinhibition, and self-reported changes in affect. Levels of (11)C-Vorozol in the amygdala were positively related to body mass index and the same relationship was found in a sample of obese men, suggesting that site specific aromatase levels may have a unique relationship with appetitive behaviors. The results may have implications for understanding sex-specific variability in eating and addictive disorders. This study was funded by National Institutes of Drug Abuse grant DA032858 awarded to Drs. Hildebrandt and Biegon.

Symposium IX: Gonadal regulation of immune dysfunction in the central nervous system, vasculature and kidney

Chair: Kathryn Sandberg Ph.D.(Georgetown University, Washington D.C.)

This symposium will explore gonadal regulation of immune dysfunction in the central nervous system, vasculature and kidney and its consequences for multiple sclerosis and systemic lupus erythematosus, hypertension, renal disease, and atherosclerosis. Dr. Shannon Dunn (University of Toronto) will present her findings on the influence of puberty in females on central nervous system autoimmunity. Dr. Kathryn Sandberg will address the impact of biological sex on T cell modulation of hypertension and associated end organ damage. Lastly, Dr. Carolyn Ecelbarger (Georgetown University) will focus on 17beta-estradiol regulation of immune function in renal ischemia and atherosclerosis.

Puberty in females enhances the risk of an outcome of multiple sclerosis in children and the development of central nervous system autoimmunity in mice

Shannon E. Dunn, Ph.D. (University of Toronto, Department of Immunology, Toronto, ON)

Background: For reasons that remain unclear, three times more women develop MS than men. This female preponderance is evident only after 12 years of age, implicating pubertal factors in MS risk.
Objective: To investigate the influence of puberty in females on CNS autoimmunity.
Methods: We examined the relationship between age of menarche on MS outcomes in 116 female children (<16 years) who presented with incident acquired demyelinating syndromes (ADS) and were followed prospectively in the national Canadian Pediatric Demyelinating Disease study from 2004-2013. Furthermore, we directly investigated the effects of puberty on susceptibility to experimental autoimmune encephalomyelitis (EAE) in two groups of female mice that differed only in their pubertal status. Results: In ADS children, a later age of menarche was associated with a decreased risk of subsequent MS diagnosis. This relationship persisted after accounting for patient age at ADS presentation and the presence of ≥1 T2 lesions on brain MRI (hazard ratio=0.64) and additional factors that associate with MS outcomes in ADS children including low vitamin D levels. Furthermore, female mice that had transitioned through puberty were more susceptible to EAE than age-matched, pre-pubertal mice. Conclusion: Puberty in females enhances CNS autoimmune mechanisms that lead to MS and EAE.

Sex-specific T cell regulation of angiotensin II-dependent hypertension

Kathryn Sandberg, Ph.D. (Georgetown University, Washington D.C.)

Background: Recent studies indicate T cells modulate arterial pressure. *Hypothesis*: Since robust sex differences exist in the immune system and in hypertension, we hypothesized that sex differences in T cell function contribute to sex differences in hypertension. *Methods*: We measured mean arterial pressure (MAP) by telemetry and investigated T cell action by flow cytometry, immunohistochemistry, real-time PCR and ELISA in male (M) and female (F) wild type (WT) and in recombination-activating-gene-1-deficient ($Rag1^{-/-}$) mice before and after adoptive transfer of male and female T cells followed 4 weeks later with 2 weeks of angiotensin II (Ang II) infusion. *Results*: Sex-differences in Ang II-induced hypertension in WT mice were lost in $Rag1^{-/-}$ mice [peak MAP (mmHg): WT-F, 136±4.9 vs. WT-M, 153±1.7; $p<0.02$; $Rag1^{-/-}$-F, 135±2.1 vs. $Rag1^{-/-}$-M, 141±3.8]. Peak MAP was 13 mmHg higher after adoptive transfer of male ($CD3^{M}gRag1^{-/-}$-M) vs. female ($CD3^{F}gRag1^{-/-}$-M) T-cells. $CD3^{M}gRag1^{-/-}$-M mice exhibited higher splenic frequencies of pro-inflammatory

interleukin-17A (2.4-fold) and tumor-necrosis factor-a (2.2-fold)-producing T cells and lower plasma levels (13-fold) and renal mRNA expression (2.4-fold) of interleukin-10 while $CD3^{F}gRag1^{-/-}$-M mice displayed a higher activation state in general and T-helper 1-biased renal inflammation. Greater T cell infiltration into perivascular adipose tissue and kidney associated with increased pressor responses to Ang II if the T cell donor was male but not female and these sex differences in T cell subset expansion and tissue infiltration were maintained for 7-8 weeks within the male host. *Conclusions***:** Sex differences in the adaptive immune response and role of pro- and anti-inflammatory cytokine signaling contributes to sex differences in arterial pressure in a model of Ang II-dependent hypertension. Funding was provided by NIH grants to HJ (AG-037832) and KS (AG/HL-19291, AG-039779 & AG-16902) and by a grant to KS and JGU from the MedStar Health Research Institute-Georgetown University partnership.

17-beta Estradiol Regulation of Interleukin-6 in Renal Ischemia and Atherosclerosis

Carolyn M. Ecelbarger, Ph.D. (Georgetown University, Department of Medicine, Washington D.C.)

Renal artery stenosis (RAS), a manifestation of general aging, results in ischemia due primarily to plaque formation in the renal artery. RAS increases the risk for adverse cardiovascular outcomes, including renal disease and atherosclerosis, nearly 2-fold. These events are not completely understood, but likely involve the production and release of local and systemic inflammatory mediators. Endogenous ovarian hormones such as 17β estradiol (E_2), and potentially related E_2-receptor modulators have the potential to provide protection against the progression and severity of cardiovascular disease; however the mechanisms are greatly in need of further study. Partial renal artery ligation (RAL) in the mouse produces a model of renal ischemia (stenosis) associated with systemic and local inflammation. Using RAL in the apolipoprotein E (ApoE) knockout mouse, a model highly susceptible to atherosclerosis, we examine the protective actions of 17β estradiol (E_2) replacement after ovariectomy on markers of renal pathology, function, metabolic parameters, and artery disease. We discuss the merits of three candidate mechanisms of E_2 action: 1) reduction in blood pressure with attenuation of renin angiotensin II activity; 2) enhancement in T regulatory cell activity, and 3) stimulation of the expression of proteins involved in vascular remodeling/repair and maintenance. In particular we examine the modulation the expression of growth factors, e.g., transforming-growth factor (TGFβ1) and the vascular endothelial growth factor (VEGF). We discuss interleukin-6, which has both pro- and anti-inflammatory actions, and we discuss the potential interplay of metabolic modulators such as insulin sensitivity and circulating triglycerides in this model system. Funding provided by Marriott Fellowship (to L. L.), DK082507 (to C.M.E), APS STEP-UP Award (to C.H).

Symposium X: Differences in Gender Development: What Have We Learned from Patients with DSD?

Co-Chairs: Eric Vilain M.D./Ph.D. (University of California, Los Angeles, CA)
Arthur P. Arnold Ph.D. (University of California, Los Angeles, CA)

Development of gender role and gender identity are one of the most sexually dimorphic traits in humans. Yet the developmental mechanisms leading to male or female gender remain poorly understood. This session will discuss how the investigation of individuals with Disorders of Sex Development (DSD- variations in chromosomal, gonadal, or anatomic makeup) has enlightened our understanding of gender.

Gender Identity Differentiation in Girls with or without a Disorder of Sex Development

Kenneth J. Zucker, Ph.D. (University of Toronto, Toronto, ON)

This study provides psychosexual assessment data on girls (age range, 3-12 years) with gender identity disorder (GID) (with no known disorder of sex development [DSD]), girls with a confirmed DSD diagnosis (e.g., genetic females with congenital adrenal hyperplasia and girls with various other DSDs that involve androgen resistance or under-virilization, such as partial androgen insensitivity syndrome, 5-alpha-reductase 2 deficiency, mixed gonadal dysgenesis, penile agenesis, etc.), and unaffected female controls. Despite no evidence of a DSD that involves unambiguous prenatal androgenization, girls with GID, on average, were more behaviorally masculinized that the DSD girls, all of whom had clear evidence of at least some prenatal hormonal androgenization, who, in turn, were more behaviorally masculinized than the unaffected female controls. The similarities and differences between girls with GID and girls with DSDs that involve at least some prenatal androgenization lend support to a model of psychosexual differentiation that involves an interplay of both biological and psychosocial factors. Funding provided by the Hospital for Sick Children Research Institute.

Clinical Management of DSD: Factors Shaping Gender Development and Health-Related Quality of Life

David E. Sandberg, Ph.D. (University of Michigan, Ann Arbor, MI)

Prior to the mid-2000s, the focus of psychological development studies in persons with disorders of sex development (DSD) reflected an extension of experimental research in non-humans examining the influence of early atypical sex hormone exposure on the development of behaviors exhibiting sex-related variation. Accordingly, psychosexual differentiation (ie, formation and stability of gender identity, gender role, and sexual orientation) had been the most extensively studied outcome in DSD clinical populations. The 2006 *Consensus Statement on Management of Intersex Disorders* called for a rebalancing of clinical research agendas that included attention to the quality of social relationships and health-related quality of life, and acknowledgement that social factors can modify psychological outcomes. This presentation describes an “interdisciplinary” approach to DSD health care that is comprehensive, integrated, and patient/family-centered. The proposed model balances the traditional focus on gender-related aspects of DSD with a perspective that conceptualizes DSD as a chronic condition, akin to other pediatric conditions, where coping and adjustment are influenced as much by the social environment, including the delivery of health care services, as by the specific medical condition. A “noncategorical” and developmentally tailored psychosocial assessment protocol and its relationship to preventative psychoeducational counseling will be described. This research is funded by R01 HD068138 from the *Eunice Kennedy Shriver* National Institute of Child Health & Human Development and the Patient Centered Outcomes Research Institute to DES.

On the 'social' in bio-psycho-social factors contributing to gender development

Heino F. L. Meyer-Bahlburg, Dr. rer. nat. (Columbia University, New York, NY)

Reproduction is sexual in all mammalian species. In line with this fact, ontogenetic sexual differentiation usually results in the development of unambiguously male or female individuals, and societies categorize individuals according to a primarily binary gender system. Newborns are assigned to the gender corresponding to their genital sex and reared to conform to the societal expectations for the respective gender role. Partial or inconsistent sexual differentiation results in inter-sexuality, i.e., newborns with a sex-ambiguous reproductive tract that lies somewhere on a continuum between male and female poles and who show increased variability of gender-related behavior. Such somatic and behavioral sex/gender ambiguities carry a risk of social stigma. At the very least, intersex newborns require a deliberate decision on their assignment to one of the two categories in the societal binary gender system, and societies vary in the biases that operate in such gender assignments. The children=s social environment may attempt to >normalize= their body and behavior with procedures such as surgery and gender-typing that may have their own potentially adverse side effects. Moreover, the intersex individuals= experience of various sex/gender incongruencies may result in gender dysphoria and patient-initiated gender change, while also providing clues as to factors that contribute to gender development. Today=s era of the anthropocene, however, is associated with a beginning devolution of the binary gender system and increasing societal gender fluidity that are likely to facilitate the accommodation of sex/gender-atypical individuals in the future. Funding provided by the NIMH Center Grant P-30-MH045320-25 "HIV Center for Clinical and Behavioral Studies" (PI, R. R. Remien).

Plenary Round Table: Re-conceptualizing sex beyond the gonads and genitalia

Marcia L. Stefanick Ph.D. (Department of Medicine, Stanford University School of Medicine)
Margaret M. McCarthy Ph.D. (Pharmacology Department, University of Maryland School of Medicine),
Daphna Joel Ph.D. (School of Psychological Sciences and Sagol School of Neuroscience, Tel Aviv University)

Our current thinking on the effects of "sex" on the body and brain is dominated by our interpretation of the effects of "sex" on the reproductive organs. "Sex" is often viewed as a divergent system that exerts an overriding effect on the form of other systems (e.g., the brain) leading to "sexual dimorphism" (i.e., two forms, male versus female) of these other systems. In recent years, several lines of research have challenged this perspective. We believe that the convergence of these lines of research calls for a reconceptualization of sex, and aim to devote this round table to discussing the relevant evidence as well as the merits of advancing a new view of sex beyond the gonads and genitalia. Marcia Stefanick will talk about permanence versus "plasticity" in the sexual differentiation of tissues from the gonads to the brain. Margaret M. McCarthy will discuss the possibility that regional variation in sexual differentiation of the brain leads to brains that are a mosaic. Daphna Joel will discuss prevalent misconceptions of sex beyond the genitalia, and present data from human brain and behavior demonstrating that gender and brain are better conceptualized in terms of mosaic rather than as belonging to two distinct categories (men/women, male brain/female brain). Following the responses of Geert de Vries and Arthur P. Arnold we will conduct an open discussion with the audience.

Sex and gender differences in stress and aging

Jens C Pruessner, Ph.D. (McGill Centre for Studies in Aging, McGill University, Montreal, QC)

Stress plays an important role in health and disease, and appears to be a strong factor in amplifying age-related physical and cognitive decline. Sex and gender are important factors that modify endocrine and physiological stress reactivity to a psychosocial challenge task. Typically, men display a larger cortisol response compared to women when exposed to a standardized psychosocial stressor. Past studies suggest that gonadal hormones, namely estrogens, partially contribute to the observed discrepancy between men and women. However, some studies suggest that the motivated performance that is typically embedded in the psychosocial stressor might also be perceived as more relevant by men when compared to women, and that this contributes to the larger stress responses in men in these tasks. Indeed, when manipulating the stress task to emphasize the social aspects of the situation, women do tend to show larger stress responses than men. This sex and gender differential gets even more complex when taking the menstrual cycle phase into account, with women showing different stress reactivities throughout their menstrual cycle when exposed to a psychosocial stressor in the presence of age-matched men, which is not observed in the presence of women.
Upon entering menopause, women then undergo a change in their availability of gonadal hormones with important consequences for their endocrine stress response, cognitive function, and brain integrity. The ongoing debate about the benefits and risks of hormone replacement therapy might benefit from the results from the endocrine stress literature, and the demonstrated effects of stress on brain integrity and cognition. Part of the studies reported in this presentation were funded by operating grants from FRSQ, NSERC and CIHR to JCP.

POSTER ABSTRACTS

1. Gender differences in reasons for smoking among daily and nondaily smokers

Author List: Alicia Allen, Ph.D., [1] Taneisha S. Scheuermann, Ph.D., [2] Nicole Nollen, Ph.D., [2] Hee Yun Lee, Ph.D., [3] Dorothy Hatsukami, Ph.D., [4] and Jasjit Ahluwalia, Ph.D.[5]

Author Affiliations: [1] Department of Family Medicine & Community Health, Medical School, University of Minnesota, [2] Department of Preventive Medicine and Public Health, University of Kansas Medical Center, [3] School of Social Work, University of Minnesota, [4] Department of Psychiatry, Medical School, University of Minnesota, [5] Department of Internal Medicine, Medical School, University of Minnesota

Abstract: Background: While the prevalence of smoking has declined overall, nondaily smoking (NDS) is on the rise. Motivations for smoking vary by gender among daily smokers (DS), but little is known about gender differences in motivations for smoking among NDS. Therefore, we explored the reasons for smoking among both DS and NDS by gender. We hypothesized that, in both groups of smokers, women have greater reasons for smoking than men. Methods: This project is a secondary data analysis utilizing data from a cross-sectional online survey. Eligible participants were either DS (smoked ≥ 1 cigarette/day on ≥ 25 days of last 30 days) or NDS (≥ 1 cigarette/day on 4-24 days of last 30 days). The Modified Reasons for Smoking Survey was used to assess seven subscales: addictive smoking, pleasure to smoke, relaxation, social smoking, stimulation, habit and handling. Statistical analyses included linear regression models, adjusting for age, cigarettes/day, education and employment when necessary. Results: The DS group contained 1175 participants (60% women) and the NDS group contained 1201 participants (56% women). Among the NDS group, women scored significantly lower than men in all subscales except for the relaxation subscale on which women scored significantly higher than men (3.1±0.0 vs. 3.0±0.0, p=0.02). Among the DS group, women scored significantly higher on addictive smoking (3.6±0.0 vs. 3.3±0.1, p<0.01) and relaxation (4.0±0.0 vs. 3.5±0.0, p=0.02), whereas men scored significantly higher on social smoking (2.8±0.0 vs. 3.0±0.1, p<0.01). Conclusions: Overall, within the NDS group, women had lower reasons for smoking whereas, while within in the DS group, women had higher reasons for smoking. Additional research is needed to explore how the gender difference in reasons for smoking may impact smoking cessation efforts in nondaily smokers. Funding for this research was provided by Pfizer's Global Research Awards for Nicotine Dependence (to J. S. Ahluwalia). Additional support provided by the National Institute for Minority Health Disparities (NCMHD/NIH - 1P60MD003422) and University of Minnesota's Masonic Cancer Center and The Building Interdisciplinary Research Careers in Women's Health Program.

2. Angiotensin Converting Enzyme Inhibitors (ACEI) and Doxorubicin (DOX) Pharmacokinetics in Women undergoing Chemotherapy for Breast Cancer

Author List: Anne Blaes[1], M.D., Ryan Shanley, M.S., Heather Beckwith[1], M.D., Daniel Duprez, M.D., Ph.D., David Potter[1], M.D., David Yee[1], M.D., Kinjal Sanghavi[3], Pamala Jacobson[3], Pharm.D.

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Abstract: Background: DOX chemotherapy can cause cardiac complications in breast cancer survivors. ACE-I may protect against these complications. Objective: We performed a pharmacokinetics study to determine whether DOX exposure is altered in breast cancer patients receiving DOX concurrently with ACEI. Methods: Women with locally advanced breast cancer prescribed DOX and cyclophosphamide every 14 days were randomized to receive enalapril 10 mg daily or no enalapril during DOX chemotherapy. Blood samples for pharmacokinetics (DOX and doxorubicinol levels) were drawn at baseline, 0.5, 1.0, 2.0, 4.0, 24.0 and 48.0 hours after infusion with and without enalapril. Correlative laboratories were also obtained. Pharmacokinetic

data was analyzed using non compartmental methods and DOX and doxorubicinol area under the curve (AUC) 0 to infinity, Cmax and half-life were estimated. Paired t-tests, two tailed, were used to determine whether DOX and its metabolite were altered with the use of enalapril ($P<0.05$). Results: Nine women (median age 41 years) with no cardiac history received 60mg/m2 DOX every two weeks for four cycles. Mean (SD) AUC0- ∞ for DOX and doxorubicinol with enalapril exposure was 1235 (168.1) hr*ng/ml and 954.6 (219.6) hr*ng/ml, respectively. AUC0- ∞ for DOX and doxobubicinol without enalapril was 1238 (193.2) hr*ng/ml and 984.4 (219.9) hr*ng/ml, respectively. There appears to be no interaction between DOX ($p=0.97$) or doxorubicinol ($p=0.78$) and enalapril (Figure 1). Enalapril was well tolerated (33% grade 1 dizziness). Conclusion: ACEI, enalapril, does not appear to alter the pharmacokinetics of DOX chemotherapy. Ongoing efforts to determine the effectiveness of ACEI as a cardioprotective agent in women receiving DOX chemotherapy should be continued. Funding was provided by The Masonic Cancer Center, University of Minnesota, NIH# K12-HD055887

3. Sex differences in urinary extracellular vesicles from healthy humans

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Abstract: There are unexplained sex-differences in the presentation of renal and urological diseases. Cells of the kidney and urinary tract shed distinct populations of biologically active extracellular vesicles (EVs) into the urine that may contribute to or reflect early pathophysiology. This study characterized the cellular origin of EVs > 0.2 microns (exosomes and microvesicles) in urine of healthy men and women. EVs in cell-free random urine samples obtained from age-matched potential kidney donors (49 males and 46 females; 20-70 years old) were analyzed by digital flow cytometry using fluorophore conjugated cell-specific antibodies and annexin-V. EV counts were expressed as cell specific vesicles/μL urine and also normalized to urine creatinine concentration. The mean ± SD: age (45±14years), body mass index (28±4; 27±5 kg/m^2), and glomerular filtration rate (100±16; 106±20 mL/min/1.73m^2) were similar in men and women. However, urine creatinine (marker of muscle mass) was higher in men as expected (147±86; 104±61 mg/dL, $p<0.05$). The concentration of urinary EVs expressing phosphatidylserine, and markers of parietal epithelial cells, proximal tubular cuboidal epithelium, and collecting tubular principal cells were higher in women ($P<0.05$ for all). In contrast, the concentration of urinary EVs expressing markers of exosomes, podocytes, the simple squamous epithelium of the thin loop of Henle, and kidney epithelium were higher in men ($P<0.05$ for all). Levels of urinary EVs expressing markers characteristic of the urinary tract did not differ between the sexes. Results were similar after normalizing EV concentrations to urine creatinine. Thus, this study demonstrates sex differences in nephron segment-specific release of EVs into urine of healthy persons. Sex-specific reference ranges for EVs from healthy persons may be useful in development of diagnostic tests for renal disease. This study was partially funded by American Heart Association (12GRNT12050147), NIH/NIDDK (R01 DK90358 and U54 DK100227), and Mayo Clinic NIH Specialized Center of Research on Sex-differences (P50AG044170).

4. Effect of Menopausal Hormone Therapy on Salivary Extracellular Vesicles in Recently Menopausal Women

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Abstract: Saliva aids in digestion and contributes to oral health. It contains extracellular vesicles (EV), such as exosomes (<0.1 micron in size) and microvesicles (MV) (<1 micron in size). EVs are shed physiologically, but changes in number or cellular origin of EVs shed under pathologic conditions could serve as biomarkers for

diagnostic and prognostic evaluations. The purpose of this study was to develop a method to characterize salivary EVs and compare the population of EVs between recently menopausal women of the Kronos Early Estrogen Prevention Study (KEEPS) undergoing menopausal hormone therapy (oral conjugated equine estrogen, oCEE or transdermal 17- β estradiol, tE2) or placebo (PL) treatment. EVs were analyzed from pooled samples as well as by treatment group, by digital flow cytometry and using cell-specific fluorescent staining. Recovery and characterization of EVs was maximal after staining of cells free saliva obtained after 3000g centrifugation for 15 minutes. Using this method, total EVs in the sample were greatest in the oCEE group (640.46 MV/μL) compared to tE2 (253.09 MV/μL) of PL (153.94 MV/μL). No statistically significant differences where observed regarding origin of these salivary EVs when stained for leukocytes, granulocytes, monocytes, mast cells, endothelial cells, and tissue factor. This study optimized a method for recovery of EVs from saliva and showed that menopausal hormone therapy affects the overall number of EVs present in the saliva of recently menopausal women. The pathophysiologic role of these EVs as well as comparisons of numbers to healthy, age-matched men remains to be determined. This work was supported by the Kronos Longevity Research Institute, Grant AG44170, and the Mayo Foundation.

5. Impact of Menopausal Hormone Therapy and Assay Methods on Salivary Sex Hormones in Recently Menopausal Women

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Abstract: Saliva is used to monitor sex steroids because it is easy to collect and analysis by enzyme immunofluorescent assays (ELISA) is inexpensive. However, high sensitivity liquid chromatography-tandem mass spectrometry (HLC-MS/MS) is the “gold standard” for measuring hormones in the blood. This study compared hormone levels in saliva by these two methods. Saliva was collected after an overnight fast from women enrolled in the Kronos Early Estrogen Prevention Study (KEEPS). KEEPS was a multi-center, randomized, placebo controlled trial to determine enrolling women between the ages of 42-58 years and within 6 mo-3 yrs. of their last menses. They were randomized to oral conjugated equine estrogen (oCEE, 0.45 mg/day), transdermal 17β-estradiol [tE2, 50μg/day both with intermittent progesterone (200mg/day for 12 days/ month)] or placebo pills and patches (PL) for 48 months. Thirty-nine saliva samples (13 per treatment group) were analyzed. Salivary concentrations of estrone were comparable among groups using ELISA (averaging about 20 pg/mL); however, with HLC-MS/MS, E1 varied by treatment group such that oCEE>tE2=PL. Likewise, salivary levels of 17 β-estradiol were comparable using ELISA and in the E2 group were comparable with HLS-MS/MS (2.6 pg/mL) but greater than E2 measured in either the oCEE or PL groups (0.76±0.57 and 0.96.±0.92 pg/mL, respectively. These results indicate that concentrations of estrogens monitored in saliva vary by assay method and that choice of methodology will affect the ability to compare treatment effects across studies. This work was supported by the Kronos Longevity Research Institute, Grant AG44170, and the Mayo Foundation.

6. Methodological Differences Account for Inconsistencies in Reported Free VEGF Concentrations in Pregnant Rats

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Abstract: The hypothesis that excess soluble fms-like tyrosine kinase-1 (sFLT-1) released by the placenta causes preeclampsia has been the subject of intensive research for over a decade. Rat models are often used to study this hypothesis, as invasive placental experiments cannot be performed in women. sFLT-1 acts as an anti-angiogenic factor by binding pro-angiogenic vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) as a non-signaling decoy. Free VEGF is undetectable in plasma during human pregnancy. However, studies examining pregnant rats have reported both low (8-29 pg/mL) and high (597-1030 pg/mL) free VEGF. These discrepancies cast uncertainty over the use of rat models to study angiogenic factors in pregnancy and preeclampsia. We hypothesized that methodological factors likely explain these discrepancies. Plasma VEGF in non-pregnant, day 7 pregnant and day 19 pregnant rats was measured using rat and mouse VEGF ELISAs (R&D Systems). The rat ELISA detected VEGF in plasma from non-pregnant rats, but not in plasma from day 19 pregnant rats. The mouse ELISA detected higher VEGF concentrations than the rat ELISA in every sample tested. This discrepancy was greater in day 19 pregnant rats (Median: 2,273 vs. 0 pg/mL) than in non-pregnant (97 vs. 20 pg/mL) and day 7 pregnant (66 vs. 2 pg/mL) rats. Recovery of recombinant rat VEGF (rrVEGF) spiked into plasma from non-pregnant and day 7 pregnant rats was high for the rat ELISA (82-105%), but low for the mouse ELISA (17-22%). The rat ELISA did not recover rrVEGF in plasma from day 19 pregnant rats, suggesting that this ELISA measures free VEGF. The use of the rat vs. mouse ELISA likely explains the differences in reported VEGF concentrations in pregnant rats. While the rat ELISA appears to measure free VEGF, plasma concentrations are close to or below the assay sensitivity limit. As almost all previous studies of pregnant rats used the mouse VEGF ELISA, this data should be interpreted cautiously. This project was supported by Award Number P-50 AG44170 (V.D. Garovic, V.M. Miller) from the National Institute on Aging. Tracey Weissgerber was supported by the Office of Women's Health Research (Building Interdisciplinary Careers in Women's Health award K12HD065987).

7. History of childhood abuse victimization and risk of common non-hunger eating behaviors in women

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Abstract: A history of childhood abuse victimization is associated with obesity in adult women through mechanisms that likely involve eating behaviors, but these have not been well described. We estimated the association between childhood abuse victimization and risk of common non-hunger eating behaviors. Methods: We used data from female participants in the Growing Up Today Study (GUTS), a longitudinal cohort of young people aged 9-14 at 1996 enrolment, who have been followed with regular questionnaires assessing disordered eating and other health behaviors. Self-reported exposure to childhood physical, sexual, and emotional abuse was ascertained when participants were aged 20-25. We used modified Poisson regression to estimate risk ratios for overeating (defined as eating an extremely large amount of food in a short amount of time) and binge eating (overeating accompanied by feelings of loss of control) in adolescence or adulthood (age 11+) as a function of abuse exposure in childhood (age <11). Results: Of the roughly 5200 women included, 10% reported severe physical abuse, 10% reported sexual abuse, and 6% reported two or more types of severe abuse in childhood. Thirty percent of the sample reported monthly overeating, 23% reported weekly overeating, 21% reported monthly binge eating, and 10% reported weekly binge eating during at least one year of follow-up. Severe physical abuse was associated with a 40% (95%CI: 1.25, 1.59) increased risk of monthly overeating, a 54% increased risk of weekly overeating (95% CI: 1.34, 1.76), a 56% increased risk of monthly binge eating (95% CI: 1.35, 1.81), and a 90% increased risk of weekly binge eating (95% CI: 1.52, 2.35). Similar associations were seen for sexual and emotional abuse. Joint exposure to multiple types of abuse did not appear to increase risk further. Conclusion: Childhood abuse is associated with increased risk of

a range of common non-hunger eating behaviors that may warrant intervention. This study was funded by NIH grant 2K12-HD0055887-06 (Minnesota BIRCWH Program).

8. Female mice liberated for inclusion in research

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Abstract: The underrepresentation of female mice in neuroscience and biomedical research is based on the assumption that females are intrinsically more variable than males and must be tested at each of four stages of the estrous cycle to generate reliable data. Neither belief is empirically based. In a meta-analysis of 293 articles, behavioral, morphological, physiological, and molecular traits were monitored in male mice and females tested without regard to estrous cycle stage; variability was not significantly greater in females than males for any endpoint and was substantially greater in males for several traits. Group housing of mice increased variability in both males and females by 37%. Utilization of female mice in neuroscience research does not require monitoring of the estrous cycle. The prevalence of sex differences at all levels of biological organization, and limitations in generalizing findings obtained with males to females, argue for the routine inclusion of female rodents in most research protocols. This study was funded by the Social Sciences Division of the University of Chicago.

9. Sexual dimorphism may explain sex differences in susceptibility to motion sickness

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Abstract: Motion sickness plagues millions of people every year. One common feature of motion sickness is that women are more susceptible than men, a phenomenon typically blamed on sex or gender roles, or on sex differences in hormones. These explanations are not parsimonious with traditional theories of motion sickness etiology; for example, the claim that motion sickness is caused by a mismatch of information gathered from the different perceptual modalities. An alternative theory relates motion sickness to the control of bodily movement. Recent research has shown that the predisposition toward motion sickness is related to characteristic patterns of subtle body movement in the control of posture; these differences exist before people are exposed to motion stimuli that are associated with motion sickness. This effect motivates research into factors that can influence individual differences in body sway, such as biomechanical characteristics. Men and women are sexually dimorphic, meaning that they tend to have different physical structures. For example, men and women tend to differ in height, and in the vertical distribution of mass. If these physical differences affect body sway, then they might underlie the well-known sex difference in susceptibility while being consistent with the postural instability theory of motion sickness etiology. We collected anthropometric data from 114 healthy young adults (44 men, 70 women) before exposing each to a potentially nauseogenic visual stimulus. Consistent with previous research, motion sickness incidence was greater in women (37%) than in men (7%). In addition, motion sickness incidence showed significant negative correlations with the height of the body's center of mass (-.184), with overall height when controlling for weight (-.199), and with the height of the center of mass when controlling for weight (-.201). These results support a qualitatively new understanding of sex differences in susceptibility to motion sickness. This study was funded by the University of Minnesota.

10. Sex differences in the relationship between facial ratios and personality characteristics in women and men

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Abstract: Testosterone exposure during puberty influences craniofacial growth and substantially contributes to sexual dimorphisms in human facial morphology. Higher levels of pubertal testosterone, more typical of males, drive growth in the maxilla and mandible (upper and lower jaw bones). Thus, facial morphology may represent a somatic marker for pubertal testosterone. The current study examined the hypothesis that personality and affective traits putatively linked to testosterone, such as sensation seeking, risk-taking and anxiety would be significantly related to facial morphology in women and men. Female (n=79) and male (n=51) participants completed self-report measures of sensation seeking, risk-taking, and overall anxiety. Two ratios of facial measurements (facial width-to-height ratios), one from the upper, and one from the lower, face were measured from digital pictures of participants. Analysis revealed that lower facial ratios but not upper facial ratios were sexually dimorphic with females possessing significantly higher values for lower facial ratios than males. These data support the recent hypothesis that measurements from the lower face may more reliably represent sex differences in facial morphology. Analysis also revealed a significant positive correlation between lower-facial ratios and anxiety in females but not males. That is, females with larger, more female-typical, lower-facial ratios scored higher on measures of overall anxiety. A significant negative correlation was also found between lower-facial ratios and sensation seeking in males but not females. Males with smaller, more male-typical, lower-facial ratios displayed higher scores for sensation seeking. No significant correlations were found between lower facial ratios and risk taking in females or males. Together, these findings suggest that pubertal testosterone differentially affects personality and affective traits in women and men and may contribute to their development. This study was funded by Brescia University College.

11. Factors associated with change in activities of daily living dependence in older long-stay nursing home residents

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Abstract: Background: Understanding change in Activities of Daily Living (ADL) dependence in long-stay nursing home residents may help identify when to intervene to delay its progression.
Objective: To describe change in ADL dependence among long-stay nursing home residents and identify associated factors. Methods: Longitudinal analysis of nursing home Minimum Data Set data linked to the 2004 National Nursing Home Survey using a sample of 7,735 residents, age ≥65 years without terminal illness living in 1097 nursing homes for at least 6 months. Linear mixed models estimated change in ADL dependence over 18 months. Results: Most residents were non-Hispanic white (88.9%) females (75.4%) with a mean age of 84.8±8.0 years and mean length-of-stay of 3.3 years living in urban (52.24%) for-profit (59.2%) nursing homes. Only 60% of the sample remained at 18 months. On average, ADL dependence increased 1.5 points (12.4%) over 18 months. Age, gender, and length-of-stay did not predict ADL change. Baseline cognitive impairment levels were associated with increasing rates of ADL change. Residents with no baseline cognitive impairment had no change in ADL dependence, those with the mean level (2.4) of impairment increased 1.8 points (11.1%), and those with the highest level of impairment (6.0) increased 3.2 points (15.8%). Conclusions: Older women comprise 75% of long-stay nursing home residents, who experience increasing ADL dependence throughout their residency. The greatest ADL change occurred with higher levels of cognitive impairment. Interventions to maintain ADL function should be developed for residents with cognitive impairment and be provided throughout a resident's entire stay. This project was supported by Grant Number K12HD055887 from the Building Interdisciplinary Research Careers in Women's Health Program of the National Institutes of Child

Health and Human Development to the Deborah E. Powell Center for Women's Health at the University of Minnesota and by grant number 1R03AG037127-01A1 from the National Institute on Aging.

12. Gonadal steroid hormone-independent sex differences in motor performance

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Abstract: Gonadal hormones influence motor function in both sexes. However, it remains unclear if there are hormone-independent sex differences in motor performance. To test for this possibility, we compared castrated (CAST) male mice and ovariectomized (OVX) female mice on three different motor tasks that measure striatal and cerebellar motor function: a raised balance beam task, an accelerated and fixed rotarod test, and the Digigait treadmill gait analysis system. OVX females outperformed CAST males in every task. In the raised balance beam task, OVX females crossed the raised beam more quickly during the 3 training days and also on each of the six different beams on the test day. During the accelerated rotarod task, CAST males did not differ from OVX females, but the fixed rotarod task determined that OVX females had longer latencies before falling off the rotarod than CAST males at both 20 rpm and 35 rpm. Utilizing the Digigait system, numerous changes in various gait measurements were also observed at both 10 cm/s and 25 cm/s. These include differences in stride, stance, stride length and paw area, all which point to deficits in CAST males when compared to OVX females. The observed sex differences in motor function that were independent of gonadal steroid hormones indicate motor deficits in CAST males when compared to OVX females. Interestingly, many measurements of motor function were improved when comparing intact males to CAST males. These behavioral data indicate that there are indeed sex differences in motor function independent of gonadal hormones following gonadectomy during adulthood. Furthermore, gonadal hormones actively improve locomotor performance in males. How these sex differences arise, and elucidating the mechanisms by which hormones improve locomotor activity in males require further study. This work was supported by grant NIH NS077661 to PGM and TJE.

13. Increased accumbal glutamate release during sex behavior in female hamsters

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Abstract: Motivated behaviors are essential in the survival and success of all animals. These behaviors are largely driven by reward-based learning circuitry in which the nucleus accumbens (NAc) receives both dopaminergic and glutamatergic innervation from areas like prefrontal cortex, amygdala, ventral tegmental area, and hippocampus. While the roles of dopaminergic neurotransmission and plasticity as well as glutamatergic plasticity mediating these behaviors have been well studied in the NAc, we know remarkably little about patterns of glutamate release occurring during naturally motivated behaviors. The goal of this study was to begin to elucidate the pattern of glutamate transmission in the NAc during naturally motivated behavior, using sex behavior as a model system. To do this, we implanted sexually naïve female Syrian hamsters with glutamate enzymatic probes in the NAc, and after collecting a baseline recording, introduced a stimulus male and measured the resulting glutamate release in real time. Our preliminary results demonstrate increased glutamate release during bouts of male sex behavior. Intriguingly, the immobile sexual behavior response, termed lordosis, in female hamsters was accompanied by accumbal glutamate release that corresponded to the male's copulatory actions. Due to the large literature on glutamate transmission in many paradigms of drugs of abuse, we also examined the pattern of glutamate release to an initial cocaine administration, finding that it mimics the amplitude of response seen in sex behavior. These preliminary findings suggest commonalities between glutamate transmission in motivated behavior and drug abuse, providing a promising avenue to investigate interactions between these behaviors. This study was funded by NSF IOS 1256799 to

R.L.M and also by the National Institute on Drug Abuse of the National Institutes of Health under Award Number T32DA007234.

14. Aggressive experience increases PSD-95 in the nucleus accumbens of female hamsters via the Fragile X Mental Retardation Protein Signaling Pathway

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Abstract: We recently discovered that aggressive experience increases dendritic spine density on medium spiny neurons in the nucleus accumbens (NAc) of female hamsters, suggesting that, like in males, the NAc may be a key brain region for modulating plasticity associated with aggressive experience in females. How aggressive experience produces changes in dendritic spine formation is, however, unknown. One possible mechanism underlying this synaptic plasticity is the Fragile X Mental Retardation Protein (FMRP) signaling pathway. Indeed, *in vitro* studies have demonstrated that binding at G-protein coupled receptors leads to activation of FMRP and dendritic protein synthesis consistent with spine formation. We therefore hypothesized that aggressive experience activates metabotropic glutamate receptor 5 (mGluR5), resulting in a decreased phosphorylation of FMRP and increased expression of spine scaffolding proteins, such as PSD-95, in the NAc. To test this hypothesis, adult female hamsters were randomly assigned to one of two behavioral conditions: experienced subjects received five consecutive days of aggressive experience, whereas naïve control subjects remained in their home cage. Thirty minutes prior to each aggressive or control experience, females received an i.p. injection of MPEP, an mGluR5 antagonist, or vehicle control. Following the last aggressive experience, subjects were sacrificed and bilateral tissue punches were taken from the NAc. PCR analysis revealed significant increases in PSD95 mRNA in the NAc of experienced subjects compared with naïve control subjects. This increase was blocked by systemic administration of MPEP. In addition, Western blot analysis revealed a significant decrease in the phosphorylation of FMRP protein in the NAc of experienced subjects compared with naïve controls. Together, these data suggest that the FMRP pathway is involved in regulating synaptic plasticity in the NAc following aggressive experience in female hamsters. Research presented in this abstract was supported by NIH R01DA013680 to R.L.M. and also by the National Institute on Drug Abuse of the National Institutes of Health under Award Number T32DA007234. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Health.

15. Do sex differences in the neuronal activity of arginine vasopressin containing neurons mediate sex differences in the expression of aggressive behavior in Syrian hamsters?

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Abstract: Arginine vasopressin (AVP) plays a critical role in controlling aggression in Syrian hamsters. In males, AVP acts within the anterior hypothalamus (AH) to stimulate aggression. However, in females, AVP within the AH inhibits aggression. The supraoptic nucleus (SON) and nucleus circularis (NC), which contain AVP cell bodies that project to the AH, are activated in males after a single agonistic encounter. The purpose of the present study was to investigate whether sex differences exist in the activation of AVP cell bodies in the SON and NC as the result of agonistic encounters. Male and female Syrian hamsters were allowed to engage in a single agonistic encounter or were moved to an unoccupied, dirty cage as a control. All animals were sacrificed after testing and tissue was processed for AVP and c-Fos (an immediate early gene that indicates neuronal activation) immunofluorescence. Confocal microscopy was used to determine the magnitude of AVP and c-Fos colocalization. In the SON, there were significantly higher levels of AVP cell activation in males compared to male controls but not in females compared to female controls. In the NC, there were significantly

higher levels of AVP cell activation in females compared to female controls but not in males compared to male controls. These data suggest that sex differences in AVP cell activation in the SON and NC contribute to sex differences in the expression of aggression. This work is supported by NSF Grant IOS-0923301.

16. Mast cells contribute to early life programming of sex differences in brain and behavior

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In the neonatal preoptic area (POA), the proinflammatory molecule, prostaglandin E2 (PGE2) is a crucial component of sex-specific brain development. PGE2 induces a two-fold increased density of dendritic spines on male POA dendrites compared to females and masculinizes adult copulatory behavior (Nat. Neurosci. 7:643, 2004). We have reported that microglia, the primary immunocompetent cell in the brain, are twice as numerous in the POA of males than females and have a more activated phenotype; these microglia contribute to prostaglandin production, masculinization of dendritic spine density in the POA, and adult male copulatory behavior (J. Neurosci. 33:2761, 2013). Recently, we have extended our studies to include mast cells, another innate immune cell population in the brain, to determine whether they also contribute to this masculinization process. Males have twice the number of mast cells in the neonatal POA compared to females. These mast cells are immunopositive for histamine, serotonin, and GNRH. We have found that pharmacological activation of mast cells early in life produces a male-typical activated microglial morphology and dendritic spine marker in the POA. Moreover, neonatal stimulation of mast cell activation in females leads to masculinized adult copulatory behavior, thus mast cells are a critical node in sex-specific development of the POA. We have also discovered that neonatal males also have significantly more mast cells than females located in the perivascular region near the hippocampus, thalamus, and amygdala. Currently, we are finding that mast cell activation early in life programs behaviors that depend upon these brain regions, including juvenile social play and anxiety behaviors. Together, these data show that immune signaling is a crucial and unappreciated factor shaping brain development and sex-specific physiological and behavioral outcomes throughout the lifespan. This study was funded by R01MH52716 (MMM) and F32NS076327 (KML).

17. Allopregnanolone modulates sexually dimorphic contextual fear via the bed nucleus of the stria terminalis in rats

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Abstract: In humans, sex differences in anxiety disorders are widely reported, suggesting a role for hormones and their metabolites in the regulation of fear. In rats, Pavlovian fear conditioning is sexually dimorphic with males exhibiting higher levels of contextual fear compared to females. The sexually dimorphic bed nucleus of the stria terminalis (BNST) is a critical brain region for the expression of contextual fear and a potential site for its hormonal regulation. Allopregnanolone (ALLO), a progesterone metabolite, is present at higher concentrations in females compared to males, is a potent modulator of $GABA_A$ receptors (GABARs), and has anxiolytic properties, suggesting a role for this neurosteroid in the observed sex difference in conditioned fear. We hypothesized that ALLO in the BNST suppresses the expression of contextual but not cued fear in intact adult male and female rats. All rats were trained with five tone-shock pairings and tested over two subsequent days. On day 1, contextual fear was tested by exposure to the shock context for 10 minutes. On day 2, cued fear was tested in a novel context with four tone presentations. To determine its role in conditioned fear of males, ALLO was infused into the BNST prior to context and tone tests. In females, ALLO's role was studied by inhibiting local synthesis and GABAR binding using separate BNST infusions of the 5α-reductase inhibitor,

finasteride (FIN), and the competitive neurosteroid antagonist, 17-PA, respectively. In males, intra-BNST ALLO significantly reduced freezing for contextual fear only. In females, intra-BNST FIN significantly increased freezing for only contextual fear in rats expected to have low ALLO levels (diestrous phase of the estrous cycle). Intra-BNST 17-PA significantly increased only contextual fear in females regardless of cycle phase. Taken together, these results provide evidence that ALLO's anxiolytic actions are mediated by the BNST and may contribute to sex differences in contextual fear. This study was supported by a HEEP Fellowship (GMA), NIH (R01MH065961 to SM), and the College of Liberal Arts, Texas A&M University (SM and NN).

18. Altered resting state brain connectivity in male and female patients with urologic chronic pelvic pain syndrome (UCPPS)

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Abstract: Urologic chronic pelvic pain syndrome (UCPPS) refers to pain syndromes associated with the male and female pelvises. Traditionally, the female version was called interstitial cystitis/painful bladder syndrome, or IC/PBS, and the male version was called chronic prostatitis/chronic pelvic pain syndrome, or CP/CPPS. The pathophsyiology of UCPPS is thought to involve a central disturbance in the processing of pain and viscerosensory signals. It is unknown to what extent male and female patients with UCPPS have similar pathophysiology. We aimed to identify differences in brain activity and connectivity between male and female UCPPS patients and healthy controls in order to advance clinical phenotyping and treatment efforts for UCPPS. We examined oscillation dynamics and functional connectivity of intrinsic brain activity during a 10-minute resting fMRI scan as part of the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network project. Men and women with UCPPS both appear to have alterations within a cortico-cerebellar network which includes motor regions known to be involved in pelvic muscle control. These findings suggest that men and women with UCPPS have a neuromotor component to their pathology. Funding for the MAPP Research Network was obtained under a cooperative agreement from National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH) (DK82370, DK82342, DK82315, DK82344, DK82325, DK82345, DK82333, and DK82316.). In addition, this work was supported in part by: R01 DK04835 and K01 DK085133.

19. METAGEM study (a gender medicine post-hoc analysis): Background and methods

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Abstract: Gender is a social construct, expressed in terms of masculinity and femininity. It is defined by the way people perceive themselves and how they expect others to behave. Gender is largely determined by culture. Gender medicine is the field of medicine that studies the biological and physiological differences between the human sexes and how that affects differences in disease. The progress of research has confirmed that men and women differ not only sexually but also in relationship to factors such as liver enzymes, sex hormones and to variables determined by the environment, education, culture and psychology of the individual [Soldin and Mattison, 2009; Regitz-Zagrosek and Seeland 2012]. The Italian Drug Agency has recognized the importance of gender-specific analysis when evaluating new drug efficacy [AIFA: Farmaci e Genere - Avviso alle Aziende Farmaceutiche 30/01/2013].The gender-medicine METAGEM project aims to test whether gender differences subsist in clinical outcomes, therapeutic approach and safety parameters. Areas of interest were defined regarding Dermatology, Central nervous system, Infectivology, Rheumatology, and Transplantation; data collected in ten observational studies conducted between 2002 and 2013 in Italy in routine clinical practice were considered. The number of enrolled patients range between 238 to 1746 considering Rheumatology and Dermatology areas respectively. A post-hoc subgroup analysis is performed by study. The subgroup analysis provides useful exploratory findings the validity of which has to be discussed in the light of current scientific knowledge and the findings from similar studies. The group of male is compared to that of female patients by statistical tests. The papers and congress communications which will arise from METAGEM project will make the scientific community more aware of the importance of a gender-dedicated approach in the care of patients. The project is fully supported by Novartis Italia SpA.

20. Sex related differences in body temperature and sickness behaviour in CD1 mice.

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Abstract: Puberty is an important developmental event that occurs within adolescence and is characterized by the cumulating reproductive competence and remodelling of the brain and immune system. Puberty is also a vulnerable period to stress and the exposure to stressors during this critical period of development can alter brain development, neuroendocrine activity, and immune system development. There is evidence that exposure to an immune challenge during the six-week sensitive period results in enduring behavioral alterations in adult female mice. However, the effect of pubertal immune challenge in male mice is unknown. The purpose of this series of experiments is to investigate first whether there are age and sex differences in sickness behaviour and body temperature changes following an immune challenge. Male and female mice were treated either with saline or lipopolysaccharide (LPS) at 6 weeks (pubertal) or 10 weeks (adult) of age. Sickness behavior was monitored and changes in body temperature were recorded using a telemetry system. Results showed that male mice display more sickness behavior and greater fluctuations in body temperature following LPS treatment than do female mice, regardless of age. Moreover, adult male mice display more sickness behavior and changes in body temperature following LPS treatment compared to pubertal male mice. The second aim of these studies was to examine the effects of circulating gonadal hormones on the age and sex differences in LPS-induced immune response. Male and female mice were gonadectomised one week prior to the saline or LPS treatment. Result showed that the removal of gonads eliminated age and sex differences in LPS-induced sickness behavior and changes in body temperature such that pubertal mice were now behaving more like adult mice. These findings suggest that circulating gonadal hormones play an important role in rendering puberty a period vulnerable and sensitive to stress and immune challenge. This work was funded by the University of Ottawa.

21. Effects of sex chromosome complement on neuronal morphology and depression-like behavior in mice

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Abstract: Depression is more often diagnosed in females than males, a sex difference that is not well understood. Although morphological and physiological differences between the sexes have typically been attributed to the effects of sex hormones, recent studies indicate that additional mechanisms such as sex chromosome complement are likely involved in some dimorphic traits. These two mechanisms, genetic vs. hormonal sex, can be tested simultaneously in four core genotype (FCG) mice, a mouse model which consists of male and female mice with either the XX or XY sex chromosome complement. Using FCG mice, we identified various effects of genetic and/or hormonal sex on neuronal morphology, gene transcription, and behavior. In line with the typical age of onset of depression being in late adolescence in humans, affective behaviors were tested in juvenile mice. In a tail-suspension test, XY mice spent significantly greater amounts of time immobile than XX mice, implying increased depression-like behavior in XY mice. In contrast, anxiety levels were comparable between the four groups of mice when tested on an elevated plus maze. In these juveniles, the body and brain weight were similar between the genotypes; the testis weight, however, was significantly higher in XY than XX males. In addition, morphological analysis of cultured hippocampal neurons indicated that XX neurons grew longer neurites than XY neurons. Depolarization of membrane potential resulted in a genotype-dependent activation of synaptic plasticity genes. Arc, for instance, was similarly expressed between the four genotypes prior to depolarization; upon stimulation, it was expressed more highly in XX female cells than XY female cells. Together, these results suggest that sex chromosome complement plays an important role in brain development and behavior; it may also account for the sex differences in disease vulnerability. This study was funded by a NIH grant to JX (MH083949).

22. The sex chromosome complement causes sex differences in diet-induced obesity

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Abstract: Obesity is a rising epidemic, tightly correlated with cardiovascular disease, the leading cause of death worldwide. There are prominent sex differences in fat accumulation and distribution. For example, men tend to accumulate fat above the waist, while women tend to accumulate fat below the waist. The molecular mechanisms by which these sex differences manifest are still unknown. Historically, sex differences in obesity have been largely attributed to ovarian and testicular hormones, and the role of genetic sex (XX *vs.* XY) has not been evaluated in mice with intact gonads. Using the four core genotype mouse model, we are able to distinguish genetic from hormonal causes of sex difference. We found that male and female mice with two X chromosomes have increased body weight, fat mass, and fatty liver compared to XY male and female mice. By contrast, sexual dimorphism in serum cholesterol and triglyceride levels is determined by gonadal sex. This demonstrates that genetic sex—even on the background of normal levels of gonadal hormones—contributes to the well-known sex differences in obesity and metabolic disorders. We hypothesize that genes escaping X-inactivation result in different levels of gene dosage between XX and XY cells, thus creating sexual dimorphism in fat accumulation. The results from these studies will help clarify the genetic mechanisms of sex differences in metabolism, and are crucial for understanding and improving treatment of metabolic disorders in women (and men). This study was funded by U.S. Public Health Service R01 DK083561 and Ruth L. Kirschstein National Research Service Award GM007185 to JCL.

23. Programming of a sex-specific high fat diet-resistance phenotype : epigenetic mechanisms

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Abstract: Both paternal and maternal malnutrition, obesity and type 2 diabetes (T2D) at conception and during gestation/lactation increase susceptibility to the development of obesity and T2D in the offspring. In contrast, when obese and diabetic mothers are fed a control diet (CD) during gestation/lactation, there is a pronounced shift (17% to 43%) from susceptibility to resistance to a high fat diet (HFD), but only in the female offspring [1,2]. Exposure of the father to carbon tetrachloride [3], or cocaine [4], leads to similar processes of sex-specific increased resistance to the deleterious effects of the same chemical in male offspring. To further identify the transcriptomic and epigenetic signatures of the programmed increased resistance to a HFD we compared obesity prone (OP) and obesity resistant (OR) female offspring and OP males born to either lean or obese mothers [1]. We measured gene expression by Affymetrix Exon microarrays and by RT-qPCR, global DNA methylation by LUMA, locus-specific DNA methylation by bisulphite-pyrosequencing and global and locus specific histone marks by western blotting and ChIP-PCR. The leptin receptor *Lepr* and the leptin gene *Lep* were the top-ranking genes in liver and muscle microarrays respectively. This revealed the prominent role of leptin-leptin receptor cytokine pathways in programmed resistance, with liver *Lepr* expression decreased in males compared to females. Locus-specific epigenetic changes correlated with gene expression of *Lepr* and *Socs2.* Several epigenetic marks and chromatin-modifying enzymes that best characterized the separation between resistance or susceptibility, the sexes and/or leanness or obesity of the mother and hepatosteatosis under a HFD were also identified. Thus feeding obese mothers a CD during gestation may lead to a hitherto unsuspected epigenetic programming of increased sex-specific resistance to a HFD in the offspring, calling for new perspectives in terms of evolution. These studies were funded by the Fondation Cœur et Artères (FCA N° 05-T4), and Contrat Cadre d'Aide au Projet d'Innovation Stratégique Industrielle "IT-Diab" OSEO-ISI (18/12/2008). The Institut Benjamin Delessert, and the Agence Nationale pour la Recherche (ANR 06-PNRA-022-01).

24. The Impact of Pre-Pregnancy Body Mass Index on Autonomic Function and Circadian Rhythms during Pregnancy: Preliminary Results

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Abstract: Elevated body mass index (BMI) has been identified as a risk factor for depression and for cardiovascular disease (CVD). Decreases in autonomic response and circadian interruptions may partially reflect BMI's contribution to this risk. As pregnancy is a period of increased susceptibility to the development of depressive mood and other CVD obstetric complications including preeclampsia, we assessed the impact of pre-pregnancy BMI on autonomic and circadian function during pregnancy. Our sample consisted of 24 women (28-34 weeks gestation). Maternal pre-pregnancy BMI was calculated by averaging reported pre-pregnancy weight, and reported weight gain since conception subtracted from current weight. Women were grouped by normal BMI (<25) and high BMI (≥25). Biological rhythms were assessed using the self-report Biological Rhythm Interview of Assessment in Neuropsychiatry (BRIAN) and autonomic functioning was examined through 24 hour electrocardiography, focusing on heart rate variability (HRV). Statistical analysis included Welch's t-tests with the false discovery rate correction. Significant differences were observed between normal and overweight women in HRV(ASDNN5:t(21)=2.38, p=0.035; SDANN5:t(21)=2.43, p=0.035; SDNN:t(21)=2.61, p=0.031), where high BMI was associated with decreased HRV. BRIAN scores also differed between groups(t(21)=2.93, p=0.023), with the overweight women having increased scores, suggesting

increased circadian disruption. The activity (t(21)=4.08, p=0.004) and social(t(16)=2.97, p=0.023) subscales of the BRIAN significantly contributed to the group differences, while sleep and eating did not. Women who have high BMI prior to pregnancy manifest altered autonomic and circadian functioning. This may predispose women to the development of depressed mood and risk for CVD obstetric complications, such as preeclampsia. This research is supported by the ISIS Cardiovascular Network, funded by the Society for Women's Health Research, Washington, DC.

25. Does Moderate *In Utero* Alcohol Exposure Result in Inflammation in the Developing Brain?

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Abstract: Activation of the developing immune system can have significant consequences for neural development and long-term neural function. Recent evidence indicates that alcohol exposure can activate the peripheral immune system and microglia, the immune cells in the brain. The purpose of this experiment was to determine whether moderate alcohol exposure early in pregnancy would cause pro-inflammatory immune activation in the fetal brain. Pregnant Sprague-Dawley rats were assigned to the following treatment groups: no gavage, gavage with water, or gavage with 50% ethanol twice a day (8 AM and 12 PM) from embryonic days 10 to 16, the equivalent of the human first trimester. Analysis of blood alcohol concentrations produced a consistent 0.08% blood alcohol concentration for approximately 8 hours each day. On E17, the hippocampus-cortex of both male and female rat pups was collected for the analysis of pro-inflammatory cytokines and chemokines using real-time PCR. We found that alcohol exposure increased the expression of cytokines in the tumor necrosis factor superfamily, including TNF-alpha, TNF-SF13, and TNF-SF13B in females but not males. Interestingly, CCL2 and CCL20 revealed opposing expression trends in males and females such that levels decreased in males and increased in females following alcohol treatment. We have also collected the placenta, maternal brain, maternal serum and maternal liver and spleen to analyze the expression of maternal or placental inflammation as this may also be a source of inflammation in response to alcohol and might thereby influence fetal brain development. In an on-going experiment, we repeated the experimental paradigm and raised pups to adulthood to measure cytokines and chemokines following a mild lipopolysaccharide challenge. We found no effect of moderate alcohol exposure on maternal behavior in these rats and/or the weight gain of the dams in this study. We predict that pups exposed to moderate prenatal alcohol may express a stronger neuroimmune or peripheral immune response to this challenge in adulthood and given our preliminary data, there may be a sex difference in this adult immune response. Future experiments will investigate potential behavioral or cognitive deficits brought on by mild prenatal ethanol exposure in both males and females. This study was funded by 5P20GM103653-02 to JMS.

26. Effect of LPS exposure on c-Fos expression in the mouse brain.

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Abstract: Puberty is a critical period of development during which sexual maturity is reached. It is also an important period during which the brain is remodelled and reorganized, making it a sensitive and vulnerable period to environmental stress. Pubertal exposure to an immune challenge results in an enduring decrease in behavioral responsiveness to estradiol as measured both in reproductive and non-reproductive behaviors. The objective of the present study was to examine whether exposure to an immune challenge activates different populations of neurons in pubertal compared to adult mice and whether there are any sex differences in LPS-induced neuronal activation. To examine this question, 80 male and female mice were injected either with saline or with the bacterial endotoxin, lipopolysaccharide (LPS), at six (puberty) or ten (adult) weeks of age. Two hours later, mice were euthanized, perfused and the brains were extracted. Brain tissue was sliced and

processed using immunocytochemistry to examine the expression of c- Fos, an immediate early gene marking brain activity. C-Fos expression was examined in the arcuate, the ventromedial and the paraventricular nuclei of the hypothalamus, the hippocampus and the medial and basolateral amygdala. Adult female mice treated with LPS displayed significantly more c-Fos expression than adult female mice treated with saline. This difference is also present in the males, but to a lesser extent. Interestingly, LPS treatment failed to increase c-expression in pubertal mice in the arcuate nucleus. These results suggest that the pubertal brain responds differently to stress than does the adult brain. These findings also suggest that reduced neuronal activation following an immune challenge during puberty may indeed be harmful and lead to long-lasting changes in brain functioning and decreased behavioral responsiveness to estradiol in adulthood. This work was funded by the University of Ottawa.

27. Global sex and age differences in hepatic and renal gene expression and the epigenome in a rat model system

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Abstract: The susceptibility of individuals at the extremes of the population age-distribution (the very young and the very old) and differences between sexes are often not adequately assessed. By understanding the genes expressed in each sex at the various life stages, the assessment of health risk versus benefit can be more rationally determined. Comprehensive analysis of the transcriptome, including miRNA, and the epigenome in the liver and kidney of Fisher 344 rats from 2 weeks to 2 years of age revealed substantial differences at various life-stages and between the sexes. In the liver and kidney, the expression of nearly 4000 genes was found to significantly vary with sex and/or age. Many of these genes are involved in xenobiotic metabolism and transport, processes that impact drug efficacy and safety. Substantial sex and age differences were also found in the epigenome of the liver and kidney. Examination of miRNA expression in the liver showed that nearly 200 miRNAs varied with sex and/or age. Notably, a group of 42 miRNAs was expressed at a relatively high level at 2 weeks of age in both sexes followed by low or no detectable expression at older ages. Analysis of potential target mRNAs for liver miRNAs suggested roles in disease susceptibility involving fibrosis as well as regulation of xenobiotic metabolism-related genes. A similar number of kidney miRNAs displayed sex- and age-related changes which may be linked to gene regulation. The DNA methylation profile of male kidneys was dramatically different from females and correlated with onset of fibrosis. These differences in functional genomic and epigenomic profiles may be related to sex- and age-specific susceptibilities to adverse drug reactions or disease states. Understanding these differences should improve personalized medicine both in terms of disease prevention and management, and safer use of drugs. This study was partially supported by the FDA Office of Women's Health.

28. Gender-specific association of prodynorphin sequence variation with alcohol dependence

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Abstract: In a previous study we demonstrated that prodynorphin (*PDYN*) haplotypes including the rs2281285 single nucleotide polymorphism (SNP) are associated with alcohol dependence (p=8E-4). A recent study demonstrated that the major allele of another *PDYN* variant (rs1022563), which is in linkage disequilibrium with rs2281285 and rs6132153 SNPs in Europeans, is associated with increased risk of heroin dependence in females, suggesting sex differences in the effect of *PDYN* variants on substance dependence. Therefore we examined gender-specific associations of a *PDYN* haplotype, as well as rs2281285 and rs6132153 SNP genotypes, with alcohol dependence in European American (exploratory) and German (replication) samples. The *PDYN* haplotype was significantly associated with alcohol dependence in males (p=2E-4) but not females (p=0.70). We observed evidence of a SNP-gender interaction with an increased effect of the minor rs2281285 G allele on alcohol dependence risk in males only in the discovery sample (OR=1.49, p=0.002), with similar results in the replication sample (OR=1.35, p=0.09). We also observe a trend for a similar association with the minor rs6132153 G allele, suggesting a higher risk of dependence in males compared to females (OR=1.26 vs. 1.15). Our findings support gender-specific genetic effects of *PDYN* SNPs on substance dependence risk, yet indicate that these gender-specific effects may vary depending on population and the substance of abuse. This study was supported by grants from the NIH K12 HD65987 (SJW), St. Marys Hospital Sponsorship Award (VMK), Samuel C. Johnson Genomics of Addiction Program (VMK, JMB), NIH/NIAAA P20 AA17830Z (VMK, JMB), the Swedish Council for Working Life and Social Research (GB), and Swedish Science Research Council and Swedish Research Council FORMAS(GB). Controls were recruited and genotyped as part of the GWAS of Venous Thrombosis study (NIH/NHGRI grant HG04735, PI J.A. Heit).

29. Novel mouse model of Klinefelter syndrome shows XXY vs. XY differences in metabolism and bone density mediated by both gonadal hormones and non-gonadal effects of the second X chromosome.

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Abstract: The karyotype XXY, which causes Klinefelter syndrome (KS), is the most common sex chromosome trisomy in humans. KS is characterized by low testosterone and infertility, speech and language delays, cognitive deficits in executive function, and health problems including increased rates of obesity, metabolic syndrome, diabetes, and osteoporosis. In humans with KS, low testosterone levels are always confounded with sex chromosome complement, making it difficult to attribute KS symptoms to genetic vs. hormonal mechanisms. We hypothesized that many of the syndromic features of KS are caused by direct genetic effects rather than low testosterone. To test this hypothesis we used a new mouse model for the study of KS, the Sex Chromosome Trisomy model (SCT), which makes it possible to dissect these contributing factors by separating gonadal sex from sex chromosome complement. The SCT model allows for within-litter comparison of XX, XY, XXY, and XYY mice, each genotype possessing either testes or ovaries. We tested several metabolic phenotypes as well as bone density. We found that the SCT mouse model recapitulates aspects of the metabolic phenotype seen in KS; XXY mice had increased body weight, increased percent body fat, and increased fat pad weights compared to XY mice. The results provide strong evidence that these traits are the result of direct sex chromosome effects, because they do not require the presence of testes and occur in both gonadal males and females. We also show that the sex chromosome effects persist after adult gonadectomy and testosterone replacement therapy. Additionally, XXY male mice had lower bone density compared to XY males. This difference was not found in females or in gonadectomized male mice, suggesting that XXY vs. XY differences in testicular secretions (such as testosterone) account for the differences in bone density. Funding for this study was provided by R01 DK83561, P01 HL90553, R01 NS043196, T32 HD007228.

30. The depletion of sex steroid hormones during the menopausal transition is related to altered prefrontal cortex signaling during working memory

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Abstract: Historically, basic neuroscience research on sex steroids focused on their role in reproduction, but a rapidly growing body of work from nonhuman primates has established estradiol's influence on synaptic organization within the dorsolateral prefrontal cortex (DLPFC). Consistent with these findings, recent work from our group (Jacobs *J. Neurosci*. 2011) demonstrated significant estradiol-dependent effects on DLPFC fMRI BOLD and working memory performance in young women. Given estradiol's regulation of memory circuitry, the loss of ovarian estrogens during menopause likely plays a significant role in shaping age-related neural changes in mid-life. To investigate this, healthy mid-life men and women (N=114; age range 46-53) who are part of a prospective birth cohort study (now entering its 5th decade) were enrolled in a follow-up fMRI study. Detailed menstrual cycle histories in conjunction with fasting serum samples collected on the morning of the scan day were used to determine pre/peri/post-menopausal status of women per STRAW guidelines. Participants performed a visual working memory task during fMRI scanning. Results show robust changes in DLPFC function over the menopausal transition, despite minimal variance in chronological age. Women exhibited exaggerated DLPFC activity as estradiol levels declined and FSH levels increased. These results are consistent with our previous work in young women, showing exaggerated DLPFC activity under low versus high estradiol conditions (despite comparable performance), a putative marker of neural efficiency. We see the same inefficient DLPFC response in mid-life as women lose ovarian estrogens. These data underscore the importance of studying adults early in the aging process in order to understand sex-specific mechanisms that shape cognitive aging trajectories and, ultimately, disease-risk. Planned analyses will also examine prenatal risk factors, including *in utero* exposure to inflammatory cytokines and adrenal stress hormones, that may accelerate age-related deficits in DLPFC function. This study was funded by NIMH MH090291 (PI: JG)

31. Fetal and Maternal Microchimerism in Ischemic Stroke

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Abstract: Ischemic stroke is a sexually dimorphic disease. Events that occur during pregnancy can have lasting implications on both the mother and fetus for decades after parturition. In particular, feto-maternal microchimerism, the bidirectional exchange and persistence of cells between a pregnant female and her fetus, has been shown to play a role in various disease pathologies but has not been well studied in the brain. We hypothesized that microchimeric cells (MCs) home to sites of injury in the maternal brain as part of the immune response to stroke and display a stem cell phenotype with potential to aid in repair. C57Bl/6 mice were bred with GFP transgenic mice using specific mating paradigms to generate fetal and maternal microchimeras. Young 10-12 week old maternal-microchimeric mice and 12-14 week old parous fetal-microchimeric mice were subject to a 90 min MCAO or sham surgery and sacrificed at 72hr or 30 day post-reperfusion (n=10). Blood, bone marrow (BM), and brain tissue were analyzed by co-labeling with anti-GFP antibody using immunohistochemistry, flow cytometry, and cytospin preparations. DNA was extracted from maternal microchimeric brain samples to further characterize MCs. At 72hrs after stroke, clusters of MCs were present

in the ischemic area. Flow cytometry of maternal blood and BM identified a rare cell population (0.001-0.01%) of $GFP^+CD45^+CD34^+$, indicative of umbilical cord stem cells. Engrafted MCs with stem-like phenotypes were also found in cytospin preparations of blood and BM. At 30 day after stroke, MCs in the brain displayed mature endothelial morphology. This was validated by PCR, confirming the presence of MCs in the ischemic brain. We have shown that MCs display stem cell phenotypes, are actively recruited to the ischemic brain, and selectively aggregate at sites of injury. Our data show that MCs are mobilized in BM and blood early after stroke and later give rise to endothelial cells, indicating a potential angiogenic role in promoting revascularization. Further characterization of the functional contribution and mechanisms that underlie these responses may prove useful in assessing female responses to brain injury. This study was supported by NIH/NINDS R01 NS077769 to LDM.

32. Sex Differences in Vitamin D binding protein are associated with increased myocarditis in men and male mice.

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Abstract: Vitamin D (VitD) deficiency has been hypothesized to exacerbate many inflammatory-based diseases including autoimmune disease, cancer, and heart disease. Vitamin D binding protein (DBP) is the primary transporter of VitD and its metabolites in the sera. DBP may increase autoimmune inflammation via its known macrophage activating functions. Macrophages are the primary infiltrate in the autoimmune heart disease, myocarditis. Like other cardiovascular diseases, the incidence of myocarditis is higher in men. In this study, we examined whether sex differences exist in DBP levels in myocarditis patients and in a coxsackievirus B3 (CVB3)-induced model of myocarditis in BALB/c mice. We found that men with myocarditis had significantly higher levels of DBP in the sera ($p = 0.03$). We verified that DBP mRNA was significantly increased in the heart of male mice with myocarditis, but not in female mice ($p = 0.04$). Male mice also had significantly increased cardiac mRNA levels of Cyp2R1 ($p = 0.03$), which is one of the enzymes that converts VitD to its active form resulting in release of DBP. Our results suggest that DPB is protective in females, but not males. Gonadectomy and hormone replacement experiments showed that testosterone is responsible for these DBP increases in the heart ($p = 0.03$). Our findings suggest that testosterone increases Cyp2R1 ($p = 0.03$), influences DBP expression ($p = 0.03$) and may be responsible for the higher incidence of myocarditis in males. To our knowledge, we are the first to report a sex difference in DBP levels in the sera of myocarditis patients. This work was supported by National Institutes of Health Grant HL087033.

33. Neonatal administration of 17α-hydroxyprogesterone impairs cognitive flexibility performance in both male and female rats

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Abstract: The incidence of premature birth has been increasing at an alarming rate over the past several decades. Synthetic progestins, including 17alpha-hydroxyprogesterone (17P), are currently being prescribed for the prevention of premature delivery, despite a lack of information regarding the potential long-term effects of these drugs on the offspring. Previous evidence shows that 17P can be transferred from maternal to fetal circuits, and that the developing brain is highly sensitive to progestins. Receptors for progesterone (PR) are

expressed during critical developmental periods in the Ventral Tegmental Area (VTA) and prefrontal cortex (PFC), which represent the origin and target of the mesocortical dopaminergic pathway, a circuit critical for executive function and cognitive flexibility. PR, a powerful transcription factor is expressed in cells of the VTA during fetal and neonatal life, many of which are dopaminergic cells that project to the PFC. In the present study, male and female rat pups were given daily injections of 17P (0.5mg/kg s.c.) or sesame oil from the day of birth (P1) to P14. In adulthood, the animals were tested on the Attentional Set-Shift Task, which assesses cognitive flexibility, the ability to change behavioral strategies in light of changes in reward contingencies. Results demonstrate that 17P treated animals displayed an impaired ability to perform extradimensional set-shifts stemming from an increased tendency towards perseveration. This impairment was present in both males and females, yet results suggest subtle sex differences in responses to 17P treatment. This collectively suggests that 17P administration may be affecting the development of the mesocortical circuit, perhaps by acting at nuclear progesterone receptors during the neonatal period. This study was funded by NSF grant IOB0447492 and a March of Dimes National Research Grant to CKW.

34. Steroid co-regulators facilitate the effects of female sex steroids on the HPA axis.

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Abstract: Early life stress precipitates dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, and this effect is most pronounced in females. Female adolescent rats with a history of chronic stress exposure demonstrate a delayed resolution of the plasma corticosterone response to an acute stressor and this delay is accompanied by attenuated glucocorticoid receptor (GR) translocation compared to control adolescent female rats. Expression patterns of GR co-regulators in the hippocampus are sexually dimorphic at baseline and become more sexually dimorphic following chronic stress. These data suggest that divergent function of the GR system between male and female adolescent rats may be attributable to alternate expression or function of GR co-regulators. In order to determine whether ovarian steroids directly altered expression of GR or GR co-regulators, we tested the role of ovarian steroids both *in vitro* and *in vivo*. Although there are numerous GR co-regulators, we focused on FKBP5 and PPID which function to inhibit and facilitate GR translocation, respectively. Both estradiol and progesterone altered expression of GR co-regulators *in vitro* and the effects interacted with the concentration of corticosterone applied to immortal hippocampal cells. In order to assess our hypothesis *in vivo*, we assessed variation in gene expression over the estrous cycle and the effects of ovariectomy on gene expression in adolescent female rats. Ovariectomy reduced expression of GR, FKBP5, and PPID in the hippocampus of adolescent female rats. In order to determine the effects of removal of ovarian hormones on the binding of GR to FKBP5 and PPID, co-immunoprecipitation was used to examine GR binding to these co-regulators. These data demonstrate that ovarian hormones alter the expression and function of GR and its co-regulators. Interactions among ovarian hormones and GR may account for the divergent effects of chronic adolescent stress on the HPA axis of males and females. This work was funded by startup funds provided by Emory University (G.N.) and Emory's Child and Adolescent Mood Program.

35. The role of estrogen in the proliferation and growth of hepatocellular carcinoma, a male-dominant malignancy

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Abstract: Hepatocellular carcinoma (HCC) occurs much more frequently in men than in women. It is commonly agreed that the difference in sex hormones between men and women play a role in this male

dominant malignancy. However, the underlying mechanism is far from clear. Estrogen, the major female hormone, mainly metabolized in liver and its metabolites may exert various effects on cell proliferation. Unfortunately, whether and how estrogen contributes to the development of HCC remains largely unknown. In this study, we sought to investigate if the estrogen metabolizing enzyme cytochrome P450 1A2 (CYP1A2) mediated the inhibitory effect of estrogen on liver cancer cells. We examined the expression of estrogen receptors and metabolizing enzymes in HCC tissues and liver cell lines by real-time RT-PCR. Target genes were introduced to liver cancer cell line Hep3B to explore the relevant pathways. Cell proliferation was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide assay. Results showed that estrogen suppressed the proliferation of liver cancer cells by inducing apoptosis in a dose- and time-dependent manner and it could additively interact with carcinogen diethylnitrosamine, proinflammatory cytokine TNF-α or cancer drug sorafenib to inhibit the cancer cell growth. The expression of CYP1A2 was greatly repressed in HCC but topoisomerase or methylation inhibitors could upregulate its expression in liver cells. The inhibitory effect of estrogen on liver cancer cells was significantly enhanced by overexpressed CYP1A2 which simultaneously promoted the conversion of estrogen to the cytotoxic metabolite 2-methoxyestradiol. Collectively, CYP1A2 mediates estrogen-induced suppression on liver cancer cells via promoting estrogen metabolisms. Our findings have provided significant insights into molecular mechanisms underlying the gender disparity in HCC development. This study was partly funded by a direct grant from CUHK.

36. Gender differences in colorectal cancer: are estrogens really protective?

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Abstract: Although colorectal cancer (CRC) is not a hormone-dependent tumour, epidemiological and clinical data highlight gender differences that suggest a protective role of estrogens. However, findings are controversial: some authors postulate that reproductive history is not consistent on the incidence of CRC and further studies show that a long-term lifetime hormonal exposure would lead to a greater risk of developing CRC. The aim is to focus on the role of estrogens in CRC, to support a correct hormonal use in prevention and therapy. In line with this aim, the CDM undertook a review of literature. In colon cells, the ratio hydroxysteroid dehydrogenase1 (17β-HSD-1) / hydroxysteroid dehydrogenase2 (17β-HSD-2) and the ratio steroid sulphatase (STS) /estrone sulphotransferase (EST) change during lifetime and implies changes in Estradiol (E2) local concentration. Furthermore, the distribution of ERβ, ERα, ERα splice-variants and the genomic/non genomic responses change in reproductive or postmenopausal age. In prevention, the colon protection or susceptibility to hormonal therapies, modulators of ERα/ERβ responses and nutraceutics depend on the dinamic cell phenotype during lifetime. Neoplastic colon tissue shows an increased concentration of E2, driven by the prevalence of 17β-HSD-1 and STS activity, a loss of ERβ expression, a shift towards ERα-dominant genotype and non-genomic responses. The estrogenic effect on tumour cell is mitogenic, partially contrasted by ERβB and ERα splice-variants mediated actions. In citostatic treatment of CRC, the use of HRT, fitoestrogens and ERβ agonists would improve prognosis only in tumours of low grading and low staging, with limited loss of ERβ. The use of ERα-antagonists, STS-inhibitors, 17B-HSD-1-inhibitors and 17B-HSD-2 activators could decelerate malignant progression. Further studies are needed: although the colon tissue express progesteron receptors (PR) and androgen receptors (AR), the complex role of steroids in CRC is not clear. The author(s) declare no financial support or receipt for the study.

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37. A Novel Model of Premenstrual Affective Disorders in the Cycling Rat

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Abstract: It is estimated that up to 35% of women experiences symptoms during the premenstrual period that have a moderate to severe impact on daily life. These symptoms often include changes in affect, producing cycle-dependent anxiety and depression. Animal research suggests that disturbances in affect during the premenstrual period may result from decreasing levels of allopregnanolone, a metabolite of progesterone. Unfortunately, current rodent models of premenstrual disorders lack strong face validity, as these models often require ovariectomy, and utilize supraphysiological doses of gonadal steroids. In the Long Evans rat, licking/grooming (LG) received from dams during the first week of life differentially programs the female hypothalamo-gonadal-pituitary axis, affecting progesterone levels. Female offspring of Low LG show a greater difference in progesterone between proestrus and metestrus than High LG offspring. We hypothesized that Low LG offspring would show significant variability in depressive-like and anxious behavior across the estrous cycle, and that this variability was driven by changes in allopregnanolone levels. Maternal behavior was observed in Long Evans rats, and dams were classified as Low and High LG mothers. Adult female offspring were tested on the forced swim test and the elevated plus maze. On both tests, Low LG offspring showed increased depressive-like and anxious behavior at metestrus relative to proestrus. There were no differences in High LG female offspring. Futhermore, an ELISA assay of corticosterone levels immediately after the forced swim test showed that Low LG metestrus animals also had higher levels of plasma corticosterone. Additionally, we will show the effects of finasteride, a 5alpha-reductase inhibitor that blocks the conversion of progesterone to allopregnanolone, on behavior on the elevated plus maze. We predict that finasteride will increase anxious behavior in both Low and High LG offspring, and will remove estrous cycle-related differences in anxious behavior in Low LG animals. Findings from this research links early life experience to affective disorders and will provide a means of investigating estrous cycle-related changes in affect without hormonal manipulation, which will more effectively model premenstrual mood disorders. This study was funded by the State University of New York at Binghamton.

38. Sex and social status differences in neuroendocrine function of naked mole-rats

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Abstract: Naked mole-rats are a eusocial mammalian species; they live in large colonies with a single breeding female, called the queen, and 1-3 breeding males. Breeders are socially dominant though non-reproductive subordinates can transition to dominant breeding status if they are removed from their colony and housed with an opposite sex conspecific. Interestingly, subordinates exhibit little to no sex differences in their overall body size, behavioral profile, and external genitalia, and we have previously demonstrated that social status is more important than sex for controlling the morphology of reproductive brain regions. However, traditional sex differences in circulating gonadal steroid hormones and reproductive behaviors are seen in breeders. To assess a potential role for gonadal steroid hormones in sex and status differences in brain and behavior, we measured the expression of steroid hormone receptor mRNA in the brains of male and female animals as they transitioned in social and reproductive status. We removed male and female subordinates from their colony and paired them with an opposite sex animal for either 1 day, 1 week, 1 month, or until they became breeders (i.e., produced a litter). We compared all groups to in-colony subordinates. Hypothalamic tissue was collected and species-specific primers were used with qPCR to measure mRNA of androgen receptor (AR), estrogen receptor (ER) alpha, progesterone receptor (PR), and aromatase. No differences were seen in the 1 day and 1 month groups. At 1 week post-removal, males had reduced ER alpha mRNA and females had increased aromatase mRNA. Striking effects were seen in breeders where females had a 3-fold increase in aromatase and 5-fold increase in ER alpha and PR whereas breeding males had reduced PR and increased AR. These data demonstrate sex and status differences in neuroendocrine function and suggest that distinct but overlapping mechanisms mediate the transition to breeding status and reproduction, per se. This

study was funded by a NSERC Discovery Grant and Connaught Foundation New Investigator award to MMH and a NSERC CGS D award to ASG.

39. Interactions between estradiol and group 1 mGluR signaling influence dendritic spine density in the female rat nucleus accumbens

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Abstract: Estrogen receptors (ERs) can be localized to the cell membrane where their activity can result in alterations in neurotransmission, neural structure, and behavior. These effects are often mediated by group 1 mGluRs associated with surface ERs. Previous research in our lab has found that 17β-estradiol (17βE) influences spine density in the female rat nucleus accumbens (NAc). In the NAc core (NAcC), 17βE decreases dendritic spine density in an mGluR5 dependent mechanism. In contrast, in the NAc shell (NAcSh), 17βE increases spines via mGluR1. Given that these contrasting effects are a result of 17βE-mediated activation of disparate group 1 mGluRs, we wanted to determine if independent activation of individual group 1 mGluRs would bidirectionally change dendritic spine density in the NAc. Ovariectomized female rats were given a systemic injection of an mGluR5 positive allosteric modulator (PAM), CDPPB, at either 5 or 10 mg/kg and sacrificed 24 hours later. Neurons in the NAc were ballistically labeled with DiI, and spine densities were determined. At both doses, CDPPB decreased dendritic spine density throughout the NAc, paralleling the effect of 17βE in the NAcC. Experiments are currently underway to determine if an mGluR1 PAM will increase spine density throughout the NAc, similar to the increase within the NAcSh after 17βE administration. These data would suggest that within the NAc different group 1 mGluRs are functionally coupled to opposing signaling pathways that result in bidirectional effects on dendritic spine density. Furthermore, 17βE produces opposing effects on spine density within subregions of the NAc by activating distinct group 1 mGluRs within the NAcC and NAcSh. The ability of 17βE to differentially regulate plasticity within subregions of the NAc may have consequences for the functional output of this brain region. This work was supported by NIH R01DA035008 to PGM and RLM and the NIH National Institute of Drug Abuse under awards number T32DA007234.

40. Estrogen receptor interactions with metabotropic glutamate receptors underlie estradiol-regulation of dendritic spines

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Abstract: Estradiol (17βE) influences structural plasticity across the female nervous system. In several brain regions such as the hippocampus, hypothalamus, and prefrontal cortex, 17βE increases dendritic spine densities. Conversely, 17βE was recently shown to decrease dendritic spine density in the core of the nucleus accumbens (NAcC). The mechanism by which 17βE regulates spine density remains elusive. Work from our lab and others have demonstrated that membrane-localized estrogen receptors are functionally coupled to group I metabotropic glutamate receptors (mGluR1 and mGluR5). Activation of group I mGluRs themselves have been shown to directly influence dendritic spine density, thus providing a putative mechanism for 17βE regulation of dendritic spines. Consistent with previous findings, 17βE decreased dendritic spine density in the NAcC of female rats. These actions of 17βE were eliminated by pre-treatment with an mGluR5 antagonist. Conversely, 17βE treatment increased dendritic spine density in both the shell region of the NAc (NAcSh) and, consistent with previous findings, the CA1 region of the hippocampus. Neither of these structural alterations were affected by mGluR5 antagonism. We are currently testing whether the 17βE-mediated increase in dendritic spine densities within these two brain regions is dependent upon mGluR1. This work was funded by the National Institutes of Health DA035008 and National Science Foundation IOS-114616 and Grant No. 00006595.

41. Estradiol facilitation of cocaine-induced locomotor sensitization in female rats requires activation of mGluR5

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Abstract: In comparison to men, women exhibit enhanced responsiveness to the stimulating and addictive properties of cocaine. A growing body of evidence implicates the steroid hormone estradiol in mediating this sex difference, yet the mechanisms underlying estradiol enhancement of behavioral responses to cocaine in females are not known. Recently, we have found that estrogen receptor alpha (ERα) functionally couples with the metabotropic glutamate receptor 5 (mGluR5) to mediate the effects of estradiol on both cellular activation as well as dendritic spine plasticity in brain regions involved in cocaine-induced behavioral sensitization. Thus, we sought to determine whether mGluR5 activation is required for the facilitative effects of estradiol on locomotor responses to cocaine in females. To test this hypothesis, ovariectomized (OVX) female rats were tested for locomotor activity on the first and fifth days of daily systemic injections of cocaine. For the two days prior to each locomotor test, animals were injected with the mGluR5 antagonist MPEP (or vehicle) and estradiol (or oil). MPEP treatment blocked the facilitative effects of estradiol on cocaine-induced locomotor sensitization, without affecting acute responses to cocaine or the inhibitory actions of estradiol on weight gain. Considered together, these data indicate that mGluR5 activation is critical for the actions of estradiol on cocaine-induced behavioral sensitization in females. Further research is needed to determine whether ERα/mGluR5 interactions directly within areas implicated in the regulation of locomotor sensitization (e.g., the nucleus accumbens) mediate this effect. This work was funded by NIH grants DA035008 to PGM and RLM, DA035008-S1 to PGM and LAM, and core funding NS062158.

42. Title: Detrimental Effect of Post-Stroke Isolation is mediated by Mitochondrial P53 Translocation in Both Sexes

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Abstract: Background**:** Social isolation (SI) is an important contributing risk factor for increased stroke injury and delayed functional recovery. Although previous studies strongly suggests that isolation prior to stroke increases injury in experimental models, little is known regarding the effects of social isolation after injury has occurred. As most patients who are isolated do not come to medical attention until after the injury occurs, the ability to manipulate post-stroke housing environments has broader translational significance. In this study, we aimed to investigate the effects of post-stroke social isolation. Methods: C57Bl6 mice were housed as pairs (male and ovariectomized female) for 2 weeks, are subjected to right middle cerebral artery occlusion (MCAO) and then transferred back to their assigned housing conditions. Initially, we tested for the effects of SI on infarct volumes and neurological defict scores. A separate cohort of mice was used for mitochondrial p53, caspase 3 and BCl2 protein analysis. Using pifithrin-μ (PFT-μ), a pharmacological mitochondrial p53inhibitor we then explored for role of P53 in post-stroke SI mice. Results: Post-stroke SI significantly exuberated infarct size compared to pair housed (PH) mice in males (Cortex: 61.6±4.8 vs 38.4±5.1; $p<0.05$); (Striatum: 79.2±6.1 vs 60.4±5.7; $p<0.05$); (Total: 58.1±4.6 vs 34.4±5.1; $p<0.05$) and such effect is more pronounced in females (Cortex: 64.3±5.1 vs 37.1±3.1; $p<0.01$); (Striatum: 82.1±4.6 vs 56.5±4.6; $p<0.01$); (Total: 51.4±3.4 vs 30.3±3.8; $p<0.01$). PH mice also expressed improved neurological deficit scores compared to SI mice. These detrimental effects of SI are observed in parallel to increased mitochondrial p53 translocation analyzed by western blots (n=6/grp; $p<0.05$). Consistently, pharmacological inhibition of mitochondrial p53 expression using PFT-μ abolished the effects of SI and reduced TUNEL positive cells, suggesting that p53 plays a critical role in

housing effects. Conclusions: We found that post-stroke SI is associated with worse stroke outcome in both males and females, in parallel to increased mitochondrial p53 expression. P53 is a critical stress sensor activates in response to stress and ischemic insult. Inhibition of mitochondrial P53 by PFT-μ abolished SI effects on stroke volume in parallel to reducing apoptotic cell death. These findings suggest that mitochondrial P53 inhibition might offer novel therapeutic strategy to minimize the detrimental effects of SI in stroke patients.

43. Bisphenol A (BPA) exacerbates acute myocarditis in female BALB/c by activating mast cells and the inflammasome

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Abstract: Myocarditis is an inflammatory heart disease that is the leading cause of heart failure in young adults. Sex hormones play a vital role in development of myocarditis with testosterone driving disease in males. Whereas estrogen, via Estrogen Receptor α (ERα), mediates cardioprotection in females. Since myocarditis is influenced by sex hormones, it is highly probable that endocrine disruptors (EDs); which interfere with natural hormones, will play a part in the progression of the disease. The human population is exposed to Bisphenol A (BPA), a known ED that binds the ER, from plastics, such as water bottles and plastic food containers. BPA has been found to act through ERβ in cardiomyoctes to increase cardiac arrhythmias. Thus, BPA could increase myocarditis through deleterious actions of the ERβ rather than beneficial effects via ERα. To our knowledge no one has examined the role of EDs like BPA on myocarditis. We found that clinically relevant doses (25 μg/L and 250 μg/L) of BPA increased acute myocarditis compared to control water. We found BPA significantly decreased ERα, while ERβ was significantly increased. We found that mast cells (cKit) are largely responsible for the increase in inflammation along with CD4 Thelper cells. IL-1β, IFN-γ, TLR4, Caspase-1, Mmp9, and ST2 were all increased with BPA treatment which all lead to increased inflammation and disease. We believe that BPA is influencing the inflammasome and mast cells leading to exacerbation of disease in females. This work was funded by NIH R01 HL111938, AHA 12GRNT12050000, and NIEHS training grant ES07141.

44. Sex differences in Transforming Growth Factor B Activated Kinase-1 signaling following experimental stroke

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Abstract: Evidence suggests post-stroke inflammation and outcome is influenced by sex, yet the mechanisms behind these disparities remain largely unknown. Studies have shown that ischemic damage following experimental stroke is reduced in young female mice when compared to males, an effect which can be diminished by ovariectomy (OVX). TGF-B activated kinase (TAK-1) is a mediator of inflammatory signaling and has been shown decrease following cerebral ischemia and inhibition of TAK-1 reduces infarct size following experimental stroke. TAB2 binds to TAK-1 following stimulation, allowing activation of the p38/MAPK and JNK inflammatory pathways. Post-stroke regulation of TAK levels and activation may contribute to the ischemic resistance seen in females. We hypothesized that female mice would have a greater decrease in TAK1 and p-TAK1 than males following experimental stroke. Male, intact female and ovariectomized female (OVX) mice were subjected to 90-minute middle cerebral artery occlusion (MCAO) and sacrificed at 24 hours post-stroke. Protein levels of TAK-1, TAB2, pTAK and JNK were examined by Western Blot. TAK and pTAK levels were reduced following stroke in female, male and OVX mice. Baseline levels of both TAK1 and pTAK were lower in female mice and the loss of TAK, pTAK and TAB2 following stroke was more dramatic. JNK levels were lower in female mice than in male mice at baseline ($p<.05$) and following stroke ($p<.01$), while the

OVX females exhibited intermediate JNK levels. Female mice exhibit lower baseline JNK levels and do not demonstrate a stroke-induced increase in JNK, indicating protection against stroke-induced inflammation. The dramatic loss of TAB2 in females results in less binding and activation of TAK, reducing inflammation and conferring neuro-protection. OVX mice showed an intermediate phenotype, indicating that the neuro-protective estrogen may play a role in TAK regulation following stroke. Work described was supported by grants from the NIH/NINDS (1R01N0055215).

45. Crosstalk between ERα and TrkB in mediating neuroprotection after neonatal brain injury

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Abstract: Male neonate brains are more susceptible to the effects of hypoxia-ischemia (HI) resulting in more long-term cognitive deficits as compared to females with comparable brain injury. Sex differences in expression and actions of neurotrophins may account for sexually differentiated consequences of HI. Our recent findings reveal that tyrosine kinase B receptor (TrkB) agonist, 7,8-dihydroxyflavone (7,8-DHF), exerts a profound neuroprotective effect in the hippocampi of female but not male neonates. We hypothesized that differential TrkB phosphorylation is associated with increased hippocampal ERα expression post-HI and the crosstalk between ERα and TrkB is mediated by Src-family kinases. ERα+/+ and ERα-/- P9 pups were subjected to Vannucci's neonatal HI model. Pups received PBS or 7,8- DHF (5 mg/kg, ip) at 10 min, 24 h and 48 h post-HI. On day 3 post-HI, the brains were either fixed for immunohistological staining or the hippocampi dissected out for immunoblotting or RTPCR. To assess the effects of sex, treatment and HI on immunoblotting band densities and RTPCR, 3- way ANOVA was used. In ERα+/+ mice, HI and 7,8-DHF preferentially increased phosphorylated TrkB (pTrkB) in female vs male hippocampi 3 d post-HI ($p < 0.0001$). Immunoblotting and qRTPCR show that there is 2.3 fold increase in ERαmRNA and ERα protein expression in the hippocampus of female mice compared to the male mice at 3 d post-HI ($p= 0.002$). In ERα-/- mice, pTrkB was significantly decreased. Thus, HI and 7,8-DHF failed to induce increased pTrkB in ERα-/- female mice. HI and 7,8-DHF also increased pSrc in female ERα+/+ hippocampi and that the increase is more robust in females. HI and 7,8-DHF result in sexually differential phosphorylation of TrkB that is abolished in ERα-/- mice. Identifying mechanisms resulting in cross talk between ERα and TrkB post-HI carry a significant potential to understand male susceptibility to HI and develop therapeutic targets. This study was funded by NIH - Waisman IDDRC P30HD003352 (Cengiz P), ICTR KL2 National Center for Advancing Translational Sciences, grant UL1TR000427 (Cengiz P), University of Wisconsin- Madison Department of Pediatrics Research & Development Grant (Cengiz P).

46. Anxiety-like behavior dissociates from microglial activation in female, but not male, rats

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Abstract: A causal relationship exists between increased monocyte recruitment and subsequent microglial activation and the manifestation of anxiety-like behaviors in rodent models. Similarly, the induction of diffuse microembolic (ME) lesions in male rats is sufficient to generate anxiety- and depressive-like behaviors two weeks following the insult- a time that corresponds with increased hippocampal gene expression of inflammatory markers. However, as observed in models of global ischemia, the consequences of cerebral injury as determined in male rodents cannot be generalized to females and the behavioral and inflammatory effects of ME infarction in females has not been established. Female sex steroids offer neuroprotection in models of stroke via anti-inflammatory mechanisms and have been shown to attenuate the augmentative

effects of stress on neuroinflammation. Given the relative neuronal protection from injury in ovary-intact females, female rats exposed to ME injury may be behavioral protected as compared to males. Adult male and female rats were exposed to SHAM or ME surgery and fourteen days later, behavior and expression of IBA1+ microglia and sphingosine-1 phosphate ($S1P_1$), a signaling sphingolipid involved in regulating vascular tone via estrogen control of nitrous oxide, were measured. Indeed, male rats manifest anxiety-like behavior at two weeks as evidenced by decreased time in the open arms of an elevated plus maze and an increased number of stretch attend postures. Though female rats exposed to ME showed no difference in behavior and a less marked response of $S1P_1$ to injury, both male and female rats show a comparable response of IBA1+ activated microglial cells following ME. The data presented demonstrate that although both males and females exhibit increased microglial activation following microembolic stroke, anxiety-like behavior is increased in males only, illustrating a dissociation between neuroinflammation and behavior in females. This study was funded by the American Heart Association and the National Alliance for Research in Schizophrenia and Affective Disorders.

47. Understanding the Mechanics of Cooperation between Same-Sex Individuals

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Abstract: Measuring cooperation between two individuals in any species is notoriously difficult. Yet, cooperation and alliances are associated with status and concomitant health and well-being in social species, including humans' closest living genetic relatives, chimpanzees and bonobos. Few objective measures of cooperation between humans exist. A recent study using number of co-authored publications within a department at 50 universities showed that high status men (full professors) were significantly more likely than high status women to co-author publications with low status same-sex individuals (assistant professors), but no sex differences were obtained in number of co-authored publications between high status individuals of the same sex (Benenson, Markovits, & Wrangham, 2014). If low status but skilled women cooperate less than their male counterparts with high status same-sex individuals, they confront a barrier to attaining success, with many ramifications. In this study, using a fully automated computer program, we asked undergraduate women and men to respond to a bogus leadership questionnaire, then randomly identified them as either leaders or followers. Next, participants learned a new non-gendered task (how to tie a small knot) by watching a video of how to execute the task repeatedly until they mastered the task. Finally, we next asked participants to evaluate in real time through written comments a video of a same-sex individual from the same college who was experiencing difficulty executing the task. Participants stopped the video and recorded their comments during their evaluations. Sex differences appeared in the leadership condition only: Women made significantly more comments than men, and virtually every comment by both sexes was negative. We provide an evolutionary perspective to suggest that women are more detail-oriented than men which can provide a barrier to collaboration between adults, but is critical to ensuring survival of offspring. This study was funded by a grant from the Social Sciences and Humanities Research Council of Canada to Henry Markovits and Joyce Benenson.

48. Exploring Sex and Gender Differences in Sleep to Improve Women's Sleep Health

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Abstract: Large knowledge gaps and lack of awareness continues to exist among the research community regarding sleep in women. There is a great need for researchers and clinicians to consider sex and gender differences in research and in the clinic, particularly with respect to the unique biology of women during life transitions. The Society for Women's Health Research (SWHR) routinely holds interdisciplinary roundtable meetings to address sex and gender differences in diseases or conditions that disproportionately affect women. The purpose of these roundtable meetings is to bring together the experts to summarize the state of the art of sex and gender differences in various diseases, highlight knowledge gaps and develop research and clinical recommendations. Restless Leg Syndrome, Sleep Disordered Breathing and Insomnia exhibit female predominance. Gender differences also exist in the way women and men report symptoms in sleep apnea; men report snoring, gasping and sleepiness and women tend to report fatigue, insomnia and depression. However, sex and gender differences in many other areas of sleep biology including epidemiology, sleep regulation, sleep quality, diagnosis and treatment need further exploration. Focusing on sex and gender differences in sleep research has potential to transform disease research to accelerate improved care for both men and women including better diagnosis, treatment and ultimately prevention of sleep disorders and related co-morbid conditions. On October 24-25, 2013 SWHR hosted a closed scientific roundtable entitled "Sex and Gender Differences in Sleep" and convened an interdisciplinary expert panel of well-established sleep researchers and clinicians to discuss sex and gender differences relevant to basic and clinical research, including sleep therapies. The identification of knowledge gaps and subsequent discussions at the roundtable led to the development of research and policy related recommendations by the expert panel. This poster describes SWHR's roundtable model and the outcomes of the sleep roundtable meeting. This work was funded in part by a grant from Jazz Pharmaceuticals, Inc.

49. Do sex differences exist in juvenile rat play behavior?

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Abstract: Among many species of mammals, juvenile social play is sexually dimorphic, with males playing more than females. In rats, while some studies show that males play more, there is considerable inconsistency across studies. Three possibilities were explored that may account for this inconsistency: (1) strain differences, (2) testing paradigms, and (3) rearing conditions. Some strains show more robust sex differences than others and how play is tested and scored can also influence whether sex differences are detected. However, rearing conditions may be particularly critical. Recent findings from our laboratory indicate that the development of play behavior is particularly sensitive to the influence of peers in the early juvenile period. How these factors interact need to be fully assessed to ensure that sex differences are maximized for studies of the mechanisms underlying such differences. The work was funded in part by the Natural Sciences and Engineering Research Council of Canada and the National Science Center in Poland.

50. Sex differences in behavioral states across pre-juvenile and juvenile development

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Abstract: Our lab has previously shown that under certain conditions, social play emerges earlier in female rats compared to male rats. We hypothesized that this may be due to sex differences in the development of behavioral states such as anxiety and/or explorative behavior. In this study, we examine developmental changes in anxiety-like and explorative behaviors by testing male and female Wistar rats in the elevated plus maze (EPM) and the open-field test (OFT) at ages when play emerges (postnatal days (P) 18, 20, 22 and 24) and when play peaks (P35 and P37). We predicted that anxiety-like behavior would decrease and explorative

behaviors increase as play behavior increases across these ages. To account for the sex difference in play onset, we further predicted that females would show less anxiety-like behavior and greater exploration compared to males around play onset (~P18). Both male and female rats spent more time in the open arms and less freezing in the EPM as they aged, with the lowest levels of anxiety-like behaviors occurring at P35 and P37. Notably, females spent significantly more time in the open arms of the EPM than did males at P18. Likewise, P18 females exhibited greater levels of explorative behaviors in the OFT than did P18 males: increased numbers of ambulatory episodes, total distance moved, and ambulatory counts. Males spent significantly more time resting in the OFT than did females at P18. Sex differences in the OFT were lost at P20, P22 and P24, but returned at P35 and P37. These data may explain our previous finding of earlier play onset in female rats and suggests that behavioral states impact sex differences in social development. Determining the systems and circuits that influence social development can provide clues on how to treat disorders of social behavior such as autism spectrum disorders. This research was supported by NIHM R01 MH47538 (to G.J.D.).

51. Susceptibility to affective-like behavior in HIV-1 transgenic rats is sex-specific irrespective of stress history

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Abstract: Sex differences in susceptibility to mood disorders begin to emerge during adolescence, and the prevalence of mood disorders in HIV+ adolescents is particularly high, with as many as 85% diagnosed with an Axis 1 DSM IV disorder one study (Pao et al 2000). Previous work has demonstrated that the sex difference routinely observed in depression can vary depending on comorbid disease and assessment of depression in HIV-infected individuals is complicated by the high incidence of chronic stress in this population. Therefore, the goal of the current study was to determine if previously reported sex differences in the effects of chronic stress on behavior were present in the HIV-1 transgenic rat. Female HIV-1 tg rats demonstrated reduced central tendency in the open field compared to female wild type (wt) littermates regardless of exposure to chronic adolescent stress. Conversely, male HIV-1 tg rats showed no differences in central tendency relative to wt littermates, regardless of stress exposure. Due to the behavioral disruptions seen in females, we expanded the assessment of behavioral implications of HIV-1 in females. Female HIV-1 tg rats exhibited decreased social interaction and increased immobility in the forced swim test relative to female wt rats, suggesting a depressive-like behavioral phenotype precipitated by expression of HIV-1 proteins. To assess the impact of stress and genotype on endocrine disruption, adrenal weights were compared among all male and female cohorts. Stress and genotype increased adrenal weight in both males and females. These data demonstrate that although expression of HIV-1 proteins precipitates anxiety-like and depressive-like behavior in females, males are behaviorally resistant to the effects of HIV-1 proteins and stress does not interact with HIV-1 to alter susceptibility in either males or females. Moreover, both males and females remain vulnerable to potential endocrine effects of stress and expression of HIV-1 proteins. Funding for this project was provided by a Creative and Novel Ideas in HIV Research Award to GNN.

52. Global and neural overexpression of androgen receptors differentially influence copulatory and social behaviors

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Abstract: Androgens masculinize the central nervous system (CNS) and behavior in part by acting on androgen receptors (AR). It is presently unclear to what extent these actions are mediated by AR in non-neural

and/or neural tissues. To further address this question, we have developed a loxP-based transgenic mouse, which overexpresses AR only when activated by Cre. We used this transgene to overexpress AR globally in all tissues using a CMV-Cre driver. We also used these mice to overexpress AR only in neural tissue, using a Nestin-Cre driver. We examined aggressive behaviors, copulatory behaviors as well as anogenital (AG) investigation in these mice in response to both male and female intruders. Preliminary results suggest an effect of AR on aggressive behaviors, such that either global or neural overexpression of AR leads to a decrease in chasing of intruders. As expected, wildtype males show an increase in AG investigation of female intruders compared to male intruders. In contrast, CMV-AR and Nestin-AR males investigate male and female intruders to the same extent. Regardless of genotype, females AG investigate male and female intruders equivalently. However, CMV-AR females show an increase in investigation of both types of intruders compared to wildtype females. Lastly, copulation was affected by neural, but not global overexpression of AR: Nestin-AR mice exhibited a decrease in mounting behaviors compared to both CMV-AR and wildtype animals. Notably, Nestin-AR males do not show a difference in mounting behaviors between male and female intruders, whereas wildtype and CMV-AR males show an increase in mounting with a female intruder. Results suggest a neural influence of AR on copulation and aggressive behaviors, however neural overexpression of AR alone is not sufficient to affect AG investigation. This study was funded by a NSERC Discovery Grant award to DAM and a NSERC CGS D award to ASG.

53. Social competition enhances amygdalar neurogenesis in the eusocial naked mole-rat: a female-specific effect

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Abstract: Naked mole-rats live in large polyandrous colonies characterized by the strictest social and reproductive hierarchy among mammals. Colonies average 70-80 individuals and breeding is typically restricted to one dominant female (the queen) and 1-3 males, with all other colony members being socially subordinate and reproductively suppressed. Though profound neural and endocrine alterations accompany the change, male and female subordinates are capable of transitioning to breeding status following the death/removal of breeders, or pair-housing with an opposite-sex conspecific. Nevertheless, competition over breeding status is sex-specific, with female-female, but not male-male, aggression observed following breeder death/removal. While the effects of social competition on neural structure and function are well-documented in diverse male-dominated species, they have been largely overlooked in matrilineal species like the naked mole-rat. The current study investigated how removal from the colony and subsequent pair-housing influences adult neurogenesis in the naked mole-rat basolateral amygdala (BLA), a brain region implicated in danger appraisal, aggression, and fear conditioning. We examined doublecortin (DCX: a marker for immature neurons) immunoreactivity in colony-housed subordinates, and subordinate animals that had been removed from the colony and paired with a same- or opposite-sex conspecific for one month. Removal from the colony upregulated DCX immunoreactivity in the BLA for same-sex paired females exclusively. Further, same-sex paired females had more DCX immunoreactivity than both same-sex paired males and opposite-sex paired animals. Same-sex paired females also exhibited significantly more aggression during the final week of pairing than both opposite-sex paired animals and same-sex paired males, reinforcing the notion that competition for status in this species is confined to females. Results suggest that neurogenesis in the BLA is stimulated by aggressive competition and/or social threat, and highlight the importance of including females in research on aggression and intrasexual competition. This study was funded by a Natural Sciences and Engineering Research Council of Canada Discovery Grant to MMH and an Ontario Graduate Scholarship to DEP.

54. The effect of parity on olfactory acuity and spine density in the piriform cortex

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Abstract: Cognitive decline, a result of aging, can be attenuated in females that have given birth. Multiparous females preserve spatial memory ability with age, past the time that they are actively caring for young. We hypothesized that other behaviors and brain areas important to parity may also be affected. Olfaction is a necessary component of the maternal ability to identify offspring and it shares connections with memory and emotion centers in the brain. Three groups of female F344 rats: young virgins, retired breeders and middle-aged virgins were studied. Olfactory ability was tested using an olfactory acuity task and an olfactory habituation/dis-habituation task. The anxiety measures used were a latency to approach a novel object task, open field and plasma corticosterone levels. No difference was found in the latency to approach the object task and the olfactory acuity task. Rearing ($p < .05$) and wall climbing ($p<.05$) behaviors were significantly different between the aged virgin and young virgin groups and corticosterone were lower in retired breeders versus aged females ($p < .05$). For the habituation/dis-habituation task all groups are able to do the task successfully, but on the test trial, retired breeders did not spend as much time with the novel scent as the other two groups. These results suggest that the retired breeders are impaired in distinguishing old and new odors. In addition there were no changes in spine density of the semi-lunar cells in layer II/III of the piriform cortex were found. Semi-lunar cells were targeted because of the numerous inputs from the olfactory bulb. Though parity has been shown to mitigate the spatial memory decline that accompanies age, the preservation benefits do not seem to extend to olfactory acuity behaviors and the semi-lunar cells of the piriform cortex. This work was funded by RISE Grant GM 060665 CUNY Collaborative Grant and RCMI grant RR03037.

55. Toll-like receptor 2 and sex differences in SCN vasopressin expression

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Abstract: Toll-like receptors are key players in the innate and adaptive immune system in vertebrates. These receptors register the presence of molecular patterns that signal the presence of infectious agents or aberrant physiological processes, and their activation leads to an immune response. By and large, their role in normal brain development and physiology remains elusive. In our facilities, TLR2-/- mice show high levels of anxious behaviors (unpublished observations). We therefore investigated whether TLR2-/- mice display aberrant levels of vasopressin, a neuropeptide implicated in anxiety-related behaviors as well as in innate immune responses. Sections from brains of wildtype (WT) and TRL2-/- male and female mice were processed immunohistochemically for the presence of vasopressin. Vasopressin-immunoreactivity was measured using gray-level thresholding in hypothalamic nuclei and both hypothalamic and extrahypothalamic vasopressin projection areas. Expectedly, projection areas from the bed nucleus of the stria terminalis (BNST) showed a large sex difference in fiber density (males > females) but no effects of genotype. Unexpectedly, mice displayed an overall sex difference in vasopressin immunoreactivity in the suprachiasmatic nucleus (SCN), with females displaying an increase in immunoreactivity compared with males. In addition, TLR2-/- mice displayed a higher density of vasopressin immunoreactivity in the SCN compared with their WT counterparts. There was no significant interaction of sex and genotype, although the power of the comparison was too small to exclude such an interaction. No sex or genotype effects were observed in anti-vasopressin staining for the PVN or SON. Interestingly, vasopressin is one of most pronounced neuropeptide outputs of the SCN, and its expression shows a distinct circadian rhythm, which may underlie circadian rhythms in the immune system. It is unknown whether TLR2 signaling affects those rhythms. To our knowledge, this is the first study to show a sex difference in SCN vasopressin immunoreactivity in mice. This research was supported by NIDCH RO1 DC004562 (to J.D.L.) and NIMH RO1 MH47538 (to G.J.D.)

56. Androgen can affect photic response of the Suprachiasmatic nucleus via actions on neural androgen receptors in male mice

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Abstract: Testosterone plays a powerful role in determining male-typical behaviours, yet we have very limited knowledge of how androgen receptors (ARs) influence neural function. The suprachiasmatic nucleus (SCN) of the hypothalamus is the locus of a master circadian (daily) clock that is critical in the temporal organization of circadian activity. Light entrains (synchronizes) circadian rhythms by modulating neural activity of the SCN, and several lines of evidence suggest androgens can alter these responses. Using transgenic mice that over-express ARs only in neurons, the current study investigated the influence of neural AR on the function of the SCN by measuring FOS immunoreactivity after a phase-shifting light pulse. Mutant mice were compared to wild-type siblings and mice of both genotypes were assigned to one of three hormone conditions. Gonadally intact male mice were compared to castrated males treated with dihydrotestosterone, and castrated males treated with vehicle. To test neural activation following exposure to light, animals were exposed to a 30-min light pulse (800 lux) between ZT13.5-14 and then sacrificed after a 60 minute interval. Control animals were not exposed to light. An interaction was observed between androgen status and light condition such that higher levels of circulating androgens were associated with a lesser response to light. Genotype was also found to affect the FOS response to light such that AR overexpressing mice demonstrated a smaller increase in FOS immunoreactivity in response to a light pulse than did wildtypes, indicating that neural AR can modulate the function of the SCN. Together, this culminated in mutant males treated with dihydrotestosterone demonstrating no significant increase in FOS after exposure to light. The current study supports a role for neural AR in regulating circadian function. This work was supported by NSERC RGPIN 312458-11 (DAM).

57. Sex differences in the relationship between anxiety and error-related brain activity: A meta-analysis

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Abstract: Anxious individuals characterized by repetitive, uncontrollable worries (i.e., anxious thoughts) show exaggerated brain responses to errors indicating they invest greater neural resources to bounce back after mistakes. Recently, we were the first to show that the relationship between higher anxiety and exaggerated error-related brain activity is several times greater in female than male undergraduates, consistent with other behavioral research indicating that anxiety may disproportionately impact women's functioning. The purpose of the present investigation was to significantly extend our previous finding by testing for this sex difference in a meta-analytic review of available data. Six separate studies comprised of undergraduate (k = 2) and patient (k = 4) samples were obtained from literature search and contact with researchers. As expected, the relationship between anxiety and error-related brain activity for women (N = 256) was moderate and significant (r = -.31, p < .001) but was non-significant for men (N = 152; r = -.10, p = .27). The homogeneity test indicated significant moderation by sex (Q = 4.40, p = .04). These results confirm that, indeed, a sex difference exists in the relationship between anxiety and error-related brain activity across a range of different populations (i.e. students and patients). Although only a small number of studies were available for analysis, the findings are consistent with our previous work and suggest that female-specific factors may be responsible for the association between anxiety and error-related brain activity. Future research will attempt to identify female-specific factors that underlie this relationship, with specific focus on ovarian hormones. In short, exaggerated error-related brain activity may only serve as a marker of anxiety in women. Better understanding of this sex-dependent mechanism of anxiety may lead to improved assessment and treatment of anxiety-related disorders

in women. This study was funded in part by a National Institute of Health Building Interdisciplinary Research Careers in Women's Health (BIRCWH) K12 grant to JSM.

58. Sex-based quality of life differences in a women predominant disease (lupus).

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Abstract: Background: Systemic Lupus Erythematosus (SLE) is 9 times more common in women than men. Men with SLE have severe disease (activity/damage) than women. Quality of life (QOL) measured by generic tools (SF-36) in SLE patients did not report significant differences by sex. In one of two QOL studies using a disease specific tool (LupusQoL), worse QOL was reported among women with SLE. Herein, we describe sex differences in disease specific QOL assessment scores in a large International Study of Outcome in Lupus (SOUL). Methods: Cross sectional data from 1,701 SLE patients (USA, Canada, Argentina, Mexico, Philippines, Turkey, Italy & China) on age, sex, LupusPRO, disease activity [measured by SLEDAI (physician global assessment, PGA & Total score)], damage (measured by SLICC-ACR/SDI) were analyzed. LupusPRO, a well-validated disease-specific QOL tool, has two constructs: health related QOL (HRQOL) and non-HRQOL. Student's t tests were used to compare QOL, disease activity and disease damage and p-value of $\leq$0.05 was considered significant on 2-tailed tests. Results: Complete QOL data were available for 1696 participants. Mean age was similar by sex. QOL was lower in Lupus Medications and Emotional Health domains among men as compared to women. Women had significantly worse QOL on Procreation domain compared to men. PGA was significantly higher among men than women, indicating greater disease activity. Total SLEDAI and SDI were higher among men, though not statistically different. Conclusions: In the largest study of ethnically and geographically diverse SLE patients, sex differences in QOL were observed on 3 lupus specific domains; two not represented in generic QOL tools. The differences in QOL domains affected may reflect sex based differences in disease phenotype and/or specific life roles important in this age group. This information will help physicians to screen and educate SLE patients in these specific areas.

59. Sex-dependent Differences in Autophagy Following Ischemic Stroke

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Abstract: Background: Autophagy is a catabolic process where the cell consumes pieces of itself as a part of normal cellular growth and homeostasis. In response to cellular stress, including ischemia, levels of autophagy rise, enabling cell survival. Sex differences in autophagy have been found in cell culture models of neuronal nutrient starvation. However, the role of sex in the autophagic response to stroke has not been investigated *in vivo*. The objective of this study was to examine if sex differences are present in autophagy following

experimental stroke. Methods: In experiment 1, stroke was induced by middle cerebral artery occlusion (MCAO) for 90 minutes in male, gonadally-intact female, ovariectomized (OVX) females with estrogen replacement and OVX females with oil replacement WT C57BL/6 mice. Autophagic activity was assessed at 6 and 24 hours following MCAO by western blot. In experiment 2, an autophagy inhibitor or vehicle was administered via intracerebroventricular injection 30 minutes after stroke induction. Mice were sacrificed 72 hrs later, and brain tissue was analyzed for infarct size. Results: At 6h post- MCAO, females and OVX females with estrogen showed increased levels of Atg7, an autophagy inducing protein, relative to sham, while at 24h OVX females with oil had decreased levels. Beclin 1 levels were unchanged relative to both sex and surgery at 6h. At 24h, Beclin 1 was significantly decreased in stroke mice as compared to sham, however no sex difference were seen. P-ULK1, a kinase upstream of Beclin 1, also showed no sex differences at 6h or 24h. Inhibition of autophagy with 3-methyladenine (3-MA, Sigma-Aldrich) led to a significant decrease in infarct size in males (35% to 10% $p<0.05$) and OVX females with oil (30% to 20%), while drug-treated intact (25% to 30%) and estrogen replaced females (15% to 25%) had larger infarcts. Conclusions: Levels of stroke-induced autophagy differed between the sexes. Inhibition of autophagy led to neuroprotection in males but increased stroke damage in females. Changes in autophagy may contribute to differential outcomes seen after stroke. This study was supported by funding from NIH-RO1 NS055215.

60. Sex differences in overlapping chronic pain conditions and opioid treatment in a tertiary pain clinic.

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Abstract: Sex differences have been reported repeatedly in clinical pain studies with women representing the majority of chronic non-cancer pain (CNCP) patients. However, none of these studies has reported sex differences in overlapping CNCP conditions, or in treatments and their sequela. Hence, we undertook a retrospective chart review of 254 patients with CNCP attending a specialized pain clinic with multiple sub-practices in a large Canadian city over a one-year period. Practices were chosen because they compromised three types of patients: (1) those under opioid treatment (UOT), (2) those with problematic opioid use (POU) and (3) those not under opioid treatment (NOT). Across the three practices, 174 were women and 80 were men. 72% of women reported overlapping CNCP conditions, while only 28% of men did so. Sex differences were found in the types of CNCP conditions overlapping together. In women, chronic pelvic pain (CPP) was the most common pain condition to co-occur, while in men, Fibromyalgia was the most common. Eighty percent of women with CPP were within their reproductive years, more than half of those women were UOT and approximately two thirds of them suffered from POU. As for men with CPP, only 19% of them were UOT, 1% of them were suffering POU and 50% of them were above the age of 55. Kappa opioids were the most common opioids prescribed for both sexes, equal percentages of women and men developed POU but men had a higher likelihood of having a history of drug abuse than did women. Taken together all of these suggest that sex differences are significant in CNCP patients and taking them into consideration when devising treatment plans might provide more effective treatment. Dr. Hassan's work was supported by training fellowships from IMPART (Intersections of Mental Health Perspectives in Addictions Research Training), H. David Archibald Queen Elizabeth II-graduate student scholarship training fellowship and the Carol Mitchell & Richard Venn Graduate Student Fellowship in Women's Mental Health from Women's College Hospital.

61. Women and men have equal lengths of stay after intra-arterial thrombectomy for acute anterior circulation strokes.

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Abstract: There is strong evidence for gender differences in acute ischemic stroke, including worse outcomes in women overall. Interestingly, women respond better then men to IV tPA, with overall outcomes in men and women who are treated with IV tPA the same. However it is less clear what gender differences exist in men and women who undergo acute intra-arterial treatment. We hypothesize that mortality of men and women are similar after intra-arterial treatment for an acute large vessel ischemic stroke. We performed a retrospective analysis of patients who presented to our institution with acute strokes due to MCA or ICA occlusion and underwent thrombectomy. 186 men and 190 women between 1/2009 and 10/2013 were identified. Our data show that men were younger than women (65.6 vs. 70.6; p = 0.0005). There was equal number of in hospital deaths (17.6% vs. 20.0%; NS) and equal lengths of stay (7.6 vs 7.3; NS). This suggests that despite older age, women and men do equally as well after mechanical thrombectomy for acute anterior circulation stroke. We are further exploring explanations for why treatment with IV or IA tPA equalize the playing field for outcomes between men and women after acute stroke. Describing these predictors will help in selecting and treating patients for IA treatment in the future.

62. Body mass index, body image disturbance and medical complications in a small sample of males with severe Anorexia Nervosa at the long-term follow up

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Abstract: Eating Disorders (ED) are psychiatric conditions complicated by multiple organ dysfunctions, due to malnutrition, binging, purging and/or excessive exercise, potentially leading to severe, life threatening medical consequences. ED can be considered a female-gender-bound syndrome since the female to male ratio is estimated 10:1. Recent studies have nevertheless underlined that ED is present and even more severe in men, so stimulating a growing interest towards the peculiarity of its clinical expression and difficulty of diagnosis in the male gender. Actually, the different phenotype in men, the stigma of ED as a female disease may lead to under recognize the disease whose early diagnosis is essential for a good prognosis. The authors report psychiatric and medical data concerning a small size sample of men affected by severe Anorexia Nervosa treated in the ED Unit of Ferrara, at diagnosis and at a long term follow up (7-10 ys). Psychiatric data concern particularly the Body Image disturbance (BID), evaluated through Body Attitudes Questionnaire (BAQ) and through a structured interview focused on body dissatisfaction and body checking. These data were collected at basis, outcome and follow up. In addition BMI trends and it’s impact on the main indicators of somatic damage (bone mineral density (BMD), laboratory tests, testosterone levels) were evaluated at diagnosis and follow up. The results, beyond indicating the strong positive relationship between BMI and BID, indicate how even severe somatic complications related to low BMI, may be totally reversible if are adequately diagnosed and treated.

63. Progression of cardiometabolic disease in a Canadian First Nation cohort: the importance of sex

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Abstract: The burden of diabetes in the Canadian First Nation population has been well-documented. However, little is known about the progression of diabetes and cardiometabolic disease in this population related to changes in risk factors, and the role of sex, mostly due to a lack of longitudinal data. The purpose of the study was to identify predictors of weight loss, change in apolipoproteinB (apoB), and change in homocysteine. Study data were from two diabetes screening studies in Sandy Bay First Nation in Manitoba,

Canada, collected in 2002/2003 and 2011/2012. A cohort was created from participants in both screening studies (*n*=171). Fasting blood samples, anthropometric, and health status and demographic data were collected. At baseline (i.e., 2002/2003), 25% (*n* = 43) of the cohort members had diabetes. At follow-up (i.e., 2011/2012), an additional 20% (n = 35) developed diabetes. Among all those with diabetes at follow-up, 35% of men and 19% of women lost >10 kg between the two study periods. Both men and women lost weight in response to decreases in insulin, while men also lost weight due to uncontrolled glucose. Women experienced significantly greater increases in apoB compared to men as well as significantly greater increases in apoB in response to increases in fasting glucose, independent of changes in other risk factors. For men, weight change was positively associated with apoB at follow-up, independent of changes in other risk factors. In conclusion, progression of cardiometabolic disease in a First Nation cohort is dependent on sex. These sex differences may partially explain the greater cardiovascular risk associated with diabetes for women as compared to men, observed in other populations. The apparent lack of association of weight change with apoB among women may have implications for weight loss interventions for prevention of diabetes and related complications.
This study was funded by the Canadian Institutes of Health Research and the Manitoba Health Research Council (SGB). NDR is the recipient of a CIHR Frederick Banting and Charles Best Canada Graduate Scholarship Doctoral Award (2009-2012), an MHRC Studentship (2012-2014), a Manitoba Network Environment for Aboriginal Health Research Award (2011-2013), as well as top-up funding from the University of Manitoba, Faculty of Medicine, Faculty of Graduate Studies, and Department of Community Health Sciences.

64. Sex differences in Girk signaling in layer 5/6 pyramidal neurons of the mouse prefrontal cortex

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Abstract: Studies in both humans and rodents have demonstrated sexually-dimorphic behavioral responses to cocaine and cocaine-related cues including increased behavioral activation, place preference, and reinstatement of drug-seeking. This sex disparity has been linked to intrinsic differences in inhibitory G protein-coupled receptor (GPCR) function in the mesocorticolimbic system, including differential D_2R activation in the mPFC. Along with D_2R, $GABA_BR$-Girk signaling also plays a key role in regulating mPFC glutamatergic output. Thus, we hypothesized that sex differences do exist in mPFC Layer 5/6 pyramidal neurons in $GABA_BR$-Girk signaling and that these differences may play a role in the sex differences found in response to cocaine. Somatodendritic currents induced by the $GABA_BR$ agonist baclofen were measured using the whole-cell voltage-clamp technique in Layer 5/6 pyramidal neurons in acutely-isolated coronal slices from adolescent male and female C57BL/6 mice. Baclofen triggered robust outward currents in both male and female mice with characteristics consistent with the activation of a Girk channel. $I_{Baclofen}$ was significantly larger in wild-type male mice compared to age-matched female counterparts. Importantly this sex dependent difference in current was abolished in mice lacking either Girk1 or Girk2 subunits. A study of the expression of the different components of the $GABA_BR$-Girk signaling by qRT-PCR and inmunoblot shows a different pattern of distribution (cell plasma membrane vs. internal stores) between males and females and suggests a differential trafficking dependent on protein phosphorylation. Taken together, these data suggest that female mice display enhanced excitability of mPFC Layer 5/6 pyramidal neuron and this could be underlying some of the differences found between males and females in their response to cocaine and other psychostimulants. This study was funded by NIH grants RO1 DA011806 and MH061933 (KW) and training grant T32 DA007097 (MH).

65. Sexually dimorphic expression of *Sfswap* protein in the developing mouse cortex and hippocampus

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Abstract: The cortex and hippocampus are important for the control of cognitive and social behaviours, many of which are sexually dimorphic. Since increasing evidence points to defects in regulation of splicing implicated in a variety of neurological diseases and mental illnesses served by these brain regions, we speculate similar molecular mechanisms might be involved in sexual differentiation of the cortex and hippocampus. Splicing factor suppressor of white apricot (*Sfswap*) is one of the novel splicing factors highly expressed throughout the mouse brain, including the cortex and the hippocampus. Thus, we hypothesized that *Sfswap* expression in the developing mouse cortex/hippocampus was sexually dimorphic, which might be critical for establishing differential neural structures and behaviors between the sexes. To test our hypothesis, we used reverse transcription with quantitative polymerase chain reaction (RT-qPCR) and immunoblotting to measure mRNA and protein levels of *Sfswap* in the cortex/hippocampus of male and female mice collected on the day of birth (PN0), and 7 (PN7), 14 (PN14), and 21 (PN21) days after birth. We found no sex difference in *Sfswap* mRNA levels, but they decreased as age advanced. On the other hand, *Sfswap* protein was detected in the developing mouse cortex/hippocampus with an expected molecular size of 104 kDa. As compared to females, *Sfswap* protein decreases in the male cortex/hippocampus on PN7, and this difference is persist on both PN14 and PN21. Our data supports the hypothesis that *Sfswap* expression is sexually dimorphic in the developing mouse cortex/hippocampus, occurring at the protein, but not mRNA, level. Discordance between relative mRNA and protein data suggests differential regulation of *Sfswap* expression by transcription and translation. Our findings indicate that sexually dimorphic *Sfswap* expression might help formulate sex differences in brain structures and behaviors. This work was supported by National Institutes of Health (SC3-GM102051to HWT).

66. Sex chromosome complement regulates expression of mood-related genes

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Abstract: Studies on mood disorders suggest dysfunctions in brain corticolimbic circuits, including altered gamma-aminobutyric acid (GABA) and modulatory (serotonin and dopamine) neurotransmission. Interestingly, sexual dimorphisms in these systems are also reported, and may underlie the heightened female vulnerability to mood disorders. As genetic sex determines gonadal sex, the role of sex chromosomes cannot be investigated individually in humans. Thus, we used the Four Core Genotypes (FCG) mice, in which genetic sex and gonadal sex are artificially decoupled, to examine expression of 13 GABA-related genes and 14 serotonin-/dopamine-related genes. Results were analyzed by 3-way ANOVA (genetic sex x gonadal sex x circulating testosterone). A global perspective of gene expression changes was provided by heatmap representation and gene co-expression networks. The main factor influencing expression of GABA-, serotonin-, dopamine-related gene expression in the frontal cortex was sex chromosome complement, with XY mice consistently having lower gene expression compared to XX mice (*Calb1*, *Gat1*, *Trkb*, *Htr2c*, *Adcy5*; $p<0.02$ for all comparisons); these results suggest an unexpected pro-disease effect in XY versus XX mice. This effect was partially opposed by gonadal sex and circulating testosterone; overall however, gonadal sex and circulating testosterone exhibited less pronounced, more complex control over gene expression. Across factors, male conditions were associated with a tightly co-expressed set of signal transduction genes. Since GABA, serotonin, and dopamine changes are also observed in other brain disorders, these findings have broader implications for understanding sexual dimorphism in adult psychopathology. This study was funded by R01 MH093723 (ES) and R01 MH077159 (ES).

67. Survey of escape from X inactivation in mouse tissues

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Abstract: X chromosome inactivation (XCI) silences most genes on one X chromosome in female mammals, but some genes escape XCI. To survey escape gene profiles in vivo and to explore molecular mechanisms that regulate this process we analyzed the allele-specific expression and chromatin structure of X-linked genes in mouse tissues and cells with skewed XCI and distinguishable alleles based on single nucleotide polymorphisms. Using a new method to estimate allelic expression, we demonstrate a continuum between complete silencing and significant expression from the inactive X (Xi). Few genes (2-3%) escape XCI to a significant level and only a minority differs between mouse tissues, suggesting stringent silencing and escape controls. Allelic profiles of DNase I hypersensitivity and RNA polymerase II occupancy of genes on the Xi correlate with escape from XCI. Allelic binding profiles of the DNA binding protein CCCTC-binding factor (CTCF) in different cell types indicate that CTCF binding at the promoter correlates with escape. Importantly, CTCF binding at the boundary between escape and silenced domains may prevent the spreading of active escape chromatin into silenced domains. This project was funded by an R01 NIH grant to CMD.

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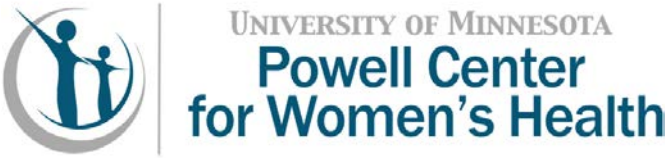

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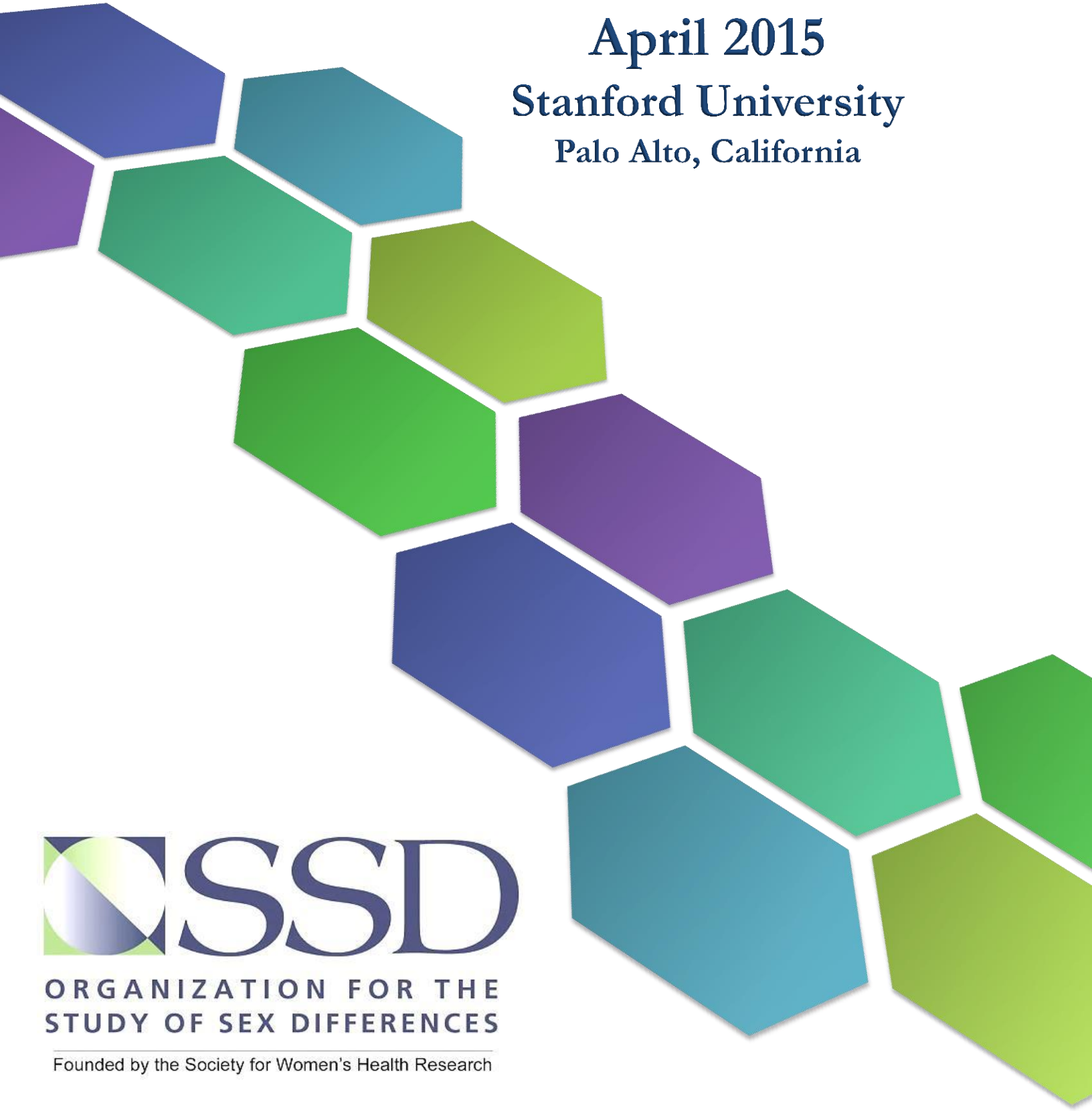

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