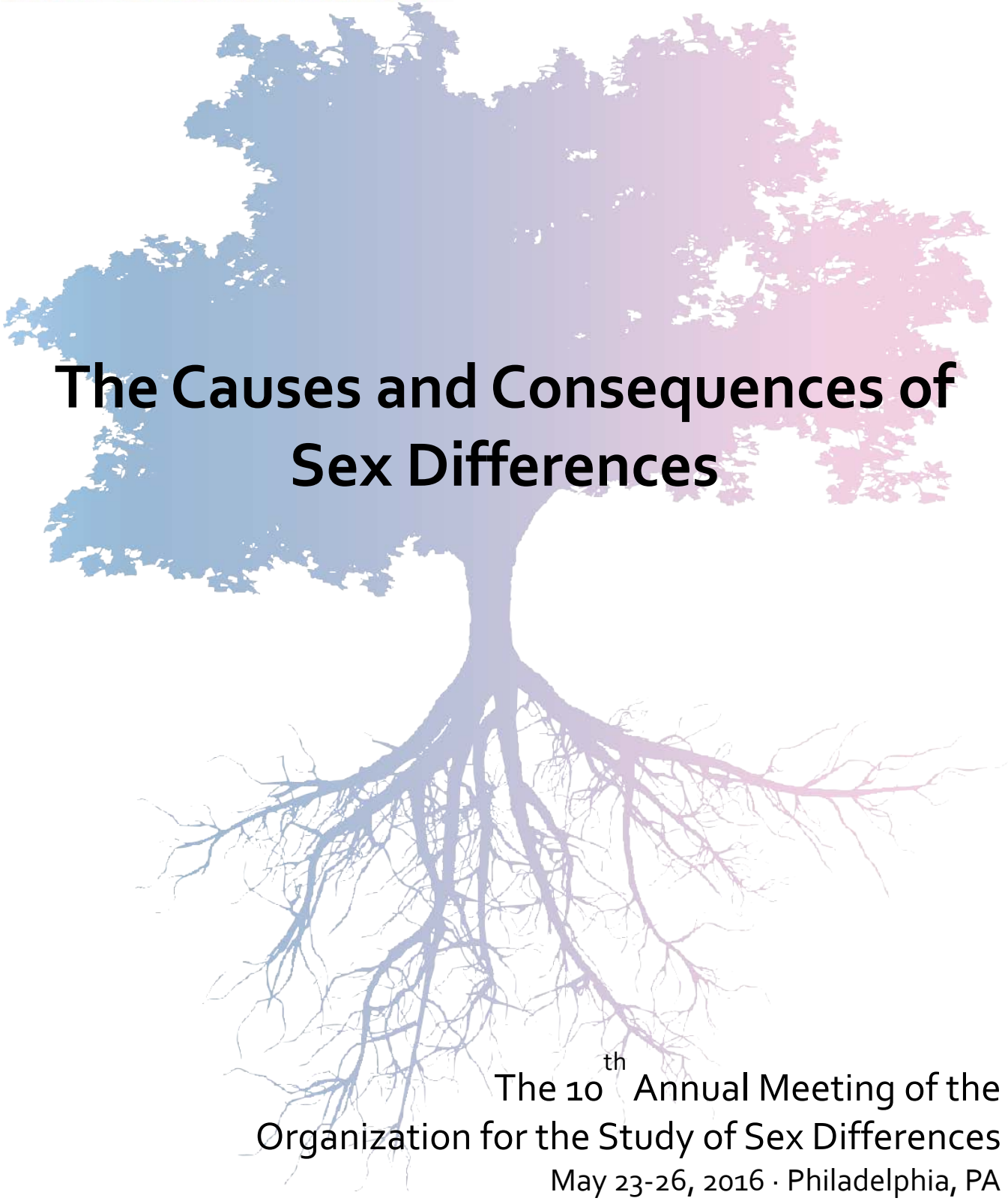
SSD

ORGANIZATION FOR THE STUDY OF SEX DIFFERENCES



The Causes and Consequences of Sex Differences

The 10th Annual Meeting of the
Organization for the Study of Sex Differences

May 23-26, 2016 · Philadelphia, PA

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OSSD 2016 Program Table of Contents

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Schedule at a Glance

MONDAY MAY 23, 2016	
5:00 - 7:30 **BRB Auditorium**	**OPENING PLENARY & RECEPTION** Sex differences in the molecular basis of depression Eric J. Nestler, M.D., Ph.D.
TUESDAY MAY 24, 2016	
7:30 - 9:00 **side Woodlands Ballroom**	Breakfast
9:00 – 12:00 **Woodlands Ballroom**	**WORKSHOP:** VARIABILITY IN RESPONSE TO DRUGS AND DEVICES: INFLUENCE OF SEX
10:30 - 10:45	Coffee Break
10:45 - 12:00 **Woodlands Ballroom**	Sex Differences and the FDA Review Process: Perspectives from FDA Reviewers
12:00 **Woodlands Ballroom**	Lunch
12:20 - 1:50 **Woodlands Ballroom**	**LUNCH SYMPOSIUM:** THINKING ABOUT GENDER IN PRECLINICAL MODELS: EXAMPLES OF MODELS AND BEST PRACTICES
1:50 - 2:05	Coffee Break
2:05 - 3:40 **Woodlands Ballroom**	**PRESIDENTIAL SYMPOSIUM:** GETTING BASIC SCIENTISTS TO THINK ABOUT SEX
3:40 – 3:55	Coffee Break
3:55 - 5:00 **Woodlands Ballroom**	**KEYNOTE ADDRESS:** Sex differences in multiple sclerosis: Past steps, future paths Rhonda R. Voskuhl, M.D. University of California, Los Angeles
5:00 - 7:30 **Regents/St. Mark's**	WELCOME RECEPTION and POSTER SESSION I

WEDNESDAY MAY 25, 2016		
7:00 - 8:20 Outside Woodlands Ballroom	Breakfast	
8:20 – 10:10	Sessions I and II	
WOODLANDS A SESSION I: SEX DIFFERENCES IN DEVELOPMENTAL ORIGINS OF METABOLIC DISEASE	WOODLANDS B SESSION II: SEX DIFFERENCES IN SENSORIMOTOR CONTROL	
10:10 - 10:30	Coffee Break	
10:30 – 12:20	Sessions III and IV	
WOODLANDS A SESSION III: SEX DIFFERENCES IN DEVELOPMENTAL WINDOWS BY THE GUT MICROBIOME	WOODLANDS B SESSION IV: SEX DIFFERENCES IN BONES, JOINTS AND BODY COMPOSITION – FROM PUBERTY TO OLDER ADULTHOOD	
12:20 Woodlands Ballroom	Lunch	
12:40– 1:50 Regent's / St. Mark's	LUNCH SYMPOSIUM MENTORING SESSION FOR TRAINEES	
2:00 - 3:50	Sessions V and VII	
WOODLANDS A SESSION V: SEX DIFFERENCES IN STRESS RESPONSES	WOODLANDS B SESSION VI: WHAT WE CAN LEARN FROM SEX DIFFERENCES IN MULTIPLE SCLEROSIS	
4:00 - 6:00 Regent's / St. Mark's	WINE & CHEESE RECEPTION POSTER SESSION II	
6:30 - 10:00 Woodlands Ballroom	BANQUET AND AWARDS CEREMONY Live Music and Dancing	
THURSDAY MAY 26, 2016		
7:00 - 8:00 Outside Woodlands Ballroom	Breakfast	
8:00 - 9:50	SESSIONS VII AND VIII	
WOODLANDS A SESSION VII: SEX, INFLAMMATION AND STROKE	WOODLANDS B SESSION VIII: EARLY CELLULAR, SYNAPTIC, AND CIRCUIT-LEVEL BIOMARKERS OF SEX DIFFERENCES IN MEMORY DECLINE	

9:50 - 10:10	Coffee Break	
10:10 - 12:20	Sessions IX and X	
	WOODLANDS A SESSION IX: SEX DIFFERENCES IN CARDIOVASCULAR DISEASE	WOODLANDS B SESSION X: LATE BREAKING RESEARCH IN SEX DIFFERENCES
12:00 Outside Woodlands Ballroom	Lunch	
12:20 - 1:50 Woodlands A/B	LUNCH DISCUSSION RESEARCH METHODS FOR STUDYING SEX	
1:50 - 2:00	Coffee Break	
2:00 - 3:50	SESSIONS XI and XII	
	WOODLANDS A SESSION XI: SEX DIFFERENCES IN ADDICTION	WOODLANDS B SESSION XII: SEX CHROMOSOMES AND SEX-LINKED GENES IN CANCER
3:50 - 4:00	Coffee Break	
4:00 - 5:00 Woodlands A/B	CAPSTONE ADDRESS Sex and aging James L. Kirkland, M.D., Ph.D. Director, Robert and Arlene Kogod Center on Aging Mayo Clinic	
5:00 - 6:00 Woodlands A/B	GENERAL OSSD MEMBERSHIP AND BUSINESS MEETING	

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ORGANIZATION FOR THE STUDY OF SEX DIFFERENCES

Founded by the Society for Women's Health Research

Tenth Anniversary OSSD Annual Meeting

The Causes and Consequences of Sex Differences

May 23 - 26, University of Pennsylvania, Philadelphia, PA

Program Chair: Kathryn Sandberg, Ph.D.

MONDAY MAY 23, 2016

University of Pennsylvania Biomedical Research Building

5:00 - 7:30

BRB Auditorium

OPENING PLENARY & RECEPTION

Sponsored by Penn PROMOTES Research on Sex and Gender in Health and Penn Building Interdisciplinary Research Careers in Women's Health

WELCOMING REMARKS

Tracy L. Bale, Ph.D.
C. Neil Epperson, M.D.
Local Hosts, 2016 OSSD Meeting
University of Pennsylvania

Eve Higginbotham, M.D.
Vice Dean for Diversity and Inclusion
University of Pennsylvania Perelman School of Medicine

Sex differences in the molecular basis of depression

Eric J. Nestler, M.D., Ph.D.
Icahn School of Medicine
BRB Auditorium

BRB 14th Floor

Reception

TUESDAY MAY 24, 2016

The Inn at Penn

7:30 - 9:00 **Outside Woodlands Ballroom**	BREAKFAST
9:00 – 12:00 **Woodlands Ballroom**	WORKSHOP VARIABILITY IN RESPONSE TO DRUGS AND DEVICES: INFLUENCE OF SEX Workshop Chair: Marjorie Jenkins, M.D., MEHP, FACP Director, Scientific Engagement and Medical Initiatives Office of Women's Health, FDA This workshop has been designed and coordinated though the FDA Center for Drug Research and Evaluation Office of Professional Affairs and Stakeholder Engagement and the FDA Office of Women's Health
9:00 – 9:05	WELCOME AND REMARKS Louise McCullough, M.D., Ph.D. President, OSSD University of Texas at Houston Marjorie Jenkins, M.D., MEHP, FACP Office of Women's Health, FDA
9:05 - 9:20	FDA's role in drug development: The intersection of science and regulations Naomi Lowy, M.D. Associate Director, Regulatory Science Office of Drug Evaluation 1, Center for Drug Evaluation and Research, FDA
9:20 – 9:40	FDA's efforts to understand variability to drug response John Whyte, M.D., MPH Director, Professional Affairs and Stakeholder Engagement Center for Drug Evaluation and Research, FDA
9:40 – 10:05	Demographic subset differences Robert Temple, M.D. Deputy Center Director, Clinical Science, Center for Drug Evaluation and Research, FDA
10:05 - 10:30	Moving the needle in drug development Danielle Day, Ph.D. Senior Medical Science Liaison Medical Affairs, Diabetes

Astra Zeneca

10:30 - 10:45	Coffee Break
10:45 - 12:00 Woodlands Ballroom	Sex Differences and the FDA Review Process: Perspectives from FDA Reviewers
10:45 - 11:05	How does FDA incorporate non-clinical studies into drug review? Shiny Mathew, Ph.D. Center for Drug Evaluation and Research, FDA
11:05 - 11:25	FDA Drug Review Process and Transparency Naomi Lowy, M.D. Associate Director, Regulatory Science Office of Drug Evaluation 1, Center for Drug Evaluation and Research, FDA
11:25 - 11:45	Devices and sex differences: An update Kathryn O'Callaghan, Ph.D. Assistant Director (Acting), Strategic Programs Office of the Center Director Center for Devices and Radiological Health, FDA
11:45 - 12:00	Questions and discussion Moderator: Marjorie Jenkins, M.D., MEHP, FACP Office of Women's Health, FDA
12:00 Outside Woodlands Ballroom	LUNCH
12:20 - 1:50 Woodlands Ballroom	LUNCH SYMPOSIUM THINKING ABOUT GENDER IN PRECLINICAL MODELS: EXAMPLES OF MODELS AND BEST PRACTICES Chair: Gillian Einstein, Ph.D. University of Toronto Co-chair: Annie Duchesne, Ph.D. University of Toronto This symposium has been sponsored by the Institute of Gender and Health of the Canadian Institutes of Health Research

12:20 – 12:25	Why should we think about gender in preclinical models and how? Gillian Einstein, Ph.D. University of Toronto
12:25 - 12:30	Gender and preclinical models of cardiovascular disease: Recognizing the urgency Edward O'Brien, M.D., FRCPC Cardiovascular Institute of Alberta, Calgary
12:30 - 12:35	Gender and social stress: From humans to animal models Annie Duchesne, Ph.D. University of Toronto
12:35 - 12:40	Sex or gender? Considering environment and social familiarity in animal models of pain research Loren Martin, Ph.D. University of Toronto
12:40 - 12:45	Identification of sex effects on genes and the gut microbiome Jayne Danska, Ph.D. University of Toronto
12:45 - 12:50	Social status in naked mole rats: Can we tease apart sex and gender in eusocial mammals? Melissa Holmes, Ph.D. University of Toronto
12:50 - 1:45	Questions, comments, and discussion Moderator: Gillian Einstein, Ph.D. University of Toronto
1:45 - 2:00	COFFEE BREAK
2:00 - 3:40 **Woodlands Ballroom**	PRESIDENTIAL SYMPOSIUM GETTING BASIC SCIENTISTS TO THINK ABOUT SEX Chair: Janine Austin Clayton, M.D. National Institutes of Health (NIH) Co-chair: Cara Tannenbaum, M.D., MSc Canadian Institutes of Health Research (CIHR)

2:00 - 2:10	Introductory Remarks Louise McCullough, M.D., Ph.D. President, OSSD University of Texas at Houston
2:10 - 2:40	Development and implementation of a new NIH policy: Consideration of sex as a biological variable Janine Austin Clayton, M.D. Director, Office of Research on Women's Health, NIH
2:40 - 3:10	Impact of the Canadian policy on sex in biomedical research Cara Tannenbaum, M.D., MSc Director, Institute of Gender and Health, CIHR
3:10 - 3:40	Sex and the implications for peer review Richard Nakamura, Ph.D. Director, Center for Scientific Review, NIH
3:45 – 4:00	COFFEE BREAK
4:00 - 5:00 **Woodlands Ballroom**	KEYNOTE ADDRESS
4:00 - 5:00	**Sex differences in multiple sclerosis: Past steps, future paths** Rhonda R. Voskuhl, M.D. University of California, Los Angeles Introduction: Louise McCullough, M.D., Ph.D. President, OSSD University of Texas at Houston
5:00 - 7:30 **Regents/St. Mark's**	WELCOME RECEPTION and POSTER SESSION I

WEDNESDAY MAY 25, 2016

The Inn at Penn

7:00 - 8:20
Outside Woodlands Ballroom

BREAKFAST

8:20 – 10:10

Sessions I and II

WOODLANDS A

SESSION I

SEX DIFFERENCES IN DEVELOPMENTAL ORIGINS OF METABOLIC DISEASE

Chair: Sara E. Pinney, M.D.
Children's Hospital of Philadelphia
Co-chair: Amita Bansal, Ph.D.
University of Pennsylvania

8:20 - 8:30
Overview
Amita Bansal, Ph.D.
University of Pennsylvania

8:30 - 8:50
Sex-specific effects of parental diabetes on offspring
Steven Chernausek, M.D.
University of Oklahoma

8:50 - 9:10
Boys live dangerously in the womb
Kent Thornburg, Ph.D.
Oregon Health Sciences University

9:10 - 9:30
Sex of offspring impacts DNA methylation and placenta gene expression
Sara E. Pinney, M.D.
Children's Hospital of Philadelphia

WOODLANDS B

SESSION II

SEX DIFFERENCES IN SENSORIMOTOR CONTROL

Chair: Jill B. Becker, Ph.D.
University of Michigan
Co-chair: Emily Lawrence, Ph.D. candidate
University of Southern California

8:20 - 8:30
Overview
Chair: Jill B. Becker, Ph.D.
University of Michigan

8:30 - 8:50
One second or less: Sex differences in the organization of movement in non-reproductive behaviors
Evelyn Field, Ph.D.
Mount Royal University

8:50 - 9:10
Sex differences in neuro-muscular function and fatigue
Sandra K. Hunter, Ph.D.
Marquette University

9:10 - 9:30
Sex differences in sensorimotor control of lower extremity function: influences of athletic ability
Emily Lawrence, Ph.D. candidate

	University of Southern California
9:30 - 9:50 Sex differences in body composition in infants and young children David Fields, Ph.D. University of Oklahoma	9:30 - 9:50 Sex differences in performance of athletic tasks: implications for lower extremity injury Susan Sigward, Ph.D. University of Southern California
9:50 - 10:10 Questions and discussion Speaker Panel	9:50 - 10:10 Questions and discussion Speaker Panel
10:10 - 10:30	COFFEE BREAK
10:30 – 12:20	Sessions III and IV
WOODLANDS A SESSION III SEX DIFFERENCES IN DEVELOPMENTAL WINDOWS BY THE GUT MICROBIOME Chair: Tracy Bale, Ph.D. University of Pennsylvania Co-chair: Eldin Jasarevic, Ph.D. University of Pennsylvania	**WOODLANDS B** SESSION IV SEX DIFFERENCES IN BONES, JOINTS AND BODY COMPOSITION – FROM PUBERTY TO OLDER ADULTHOOD Chair: Marcia Stefanick, Ph.D. Stanford University Co-chair: Deepika Laddu, Ph.D. Stanford University
10:30 - 10:40 Overview Tracy Bale, Ph.D. University of Pennsylvania	10:30 - 10:40 Overview Marcia Stefanick, Ph.D. Stanford University
10:40 - 11:00 Sex differences in microbiome and behavior following prenatal stress: Potential role for the intrauterine microbiome Tamar Gur, M.D.,Ph.D. The Ohio State University	10:40 - 11:00 Building bones: male vs. female mice (black vs. white) and men versus women Karl Jepsen, Ph.D. University of Michigan
11:00 - 11:20 Estradiol and diet alter the gut	11:00 - 11:20 Bone and muscle interactions

microbiome in female mice
Marc Tetel, Ph.D.
Wellesley College

11:20 - 11:40

Control of autoimmune disease by genes, sex and the microbiome
Jayne Danska, M.D.
University of Toronto

11:40 - 12:00

Maternal stress reprogramming of the developing gut microbiome-brain axis
Eldin Jasarevic, Ph.D.
University of Pennsylvania

12:00-12:20 pm

Discussion
Speaker Panel

during puberty: girls vs. boys
Mary Leonard, M.D.
Stanford University

11:20 - 11:40

Sex differences in physical activity and body composition in aging cohorts
Deepika Laddu, Ph.D.
Stanford University

11:40 - 12:00

Are men at greater risk for rheumatoid arthritis?
Joshua F. Baker, M.D., MSCE
University of Pennsylvania

12:00-12:20 pm

Discussion
Speaker Panel

12:20
Woodlands Ballroom

LUNCH

12:40– 1:50
Regent's / St. Mark's

LUNCH SYMPOSIUM

MENTORING SESSION FOR TRAINEES*

Chairs: Gretchen Neigh, Ph.D.
Virginia Commonwealth University
Deborah Bangasser, Ph.D.
Temple University

***sign up at registration desk**

2:00 - 3:50

Sessions V and VII

WOODLANDS A

SESSION V

SEX DIFFERENCES IN STRESS RESPONSES

Chair: Seema Bhatnagar, Ph.D.

WOODLANDS B

SESSION VI

WHAT WE CAN LEARN FROM SEX DIFFERENCES IN MULTIPLE SCLEROSIS

Children's Hospital of Philadelphia University of Pennsylvania School of Medicine Co-chair: Laura Grafe, Ph.D. Children's Hospital of Philadelphia	Chair: Anat Biegon, M.D. Stony Brook University Co-chair: Yael Deri, Ph.D. Tel-Aviv University
2:00 - 2:05 Overview Seema Bhatnagar, Ph.D. Children's Hospital of Philadelphia	2:00 - 2:05 Overview Anat Biegon, M.D. Stony Brook University
2:05 - 2:30 Orexins contribute to sex differences in habituation to stress and cognitive function Laura Grafe, Ph.D. Children's Hospital of Philadelphia	2:05 - 2:30 Sex differences in adult multiple sclerosis Patricia Coyle, M.D. Stony Brook University
2:30 - 2:55 Sex differences in the corticotropin releasing factor system: From molecules to circuits Debra Bangasser, Ph.D. Temple University	2:30 - 2:55 What can we learn from sex differences in pediatric multiple sclerosis? Louis Manganas, M.D., Ph.D. Stony Brook University
2:55 - 3:20 Sexually divergent active and passive conditioned fear responses Rebecca M. Shansky, Ph.D. Northeastern University	2:55 - 3:20 Sex differences in animal models of multiple sclerosis Yael Deri, Ph.D. Tel-Aviv University
3:20 - 3:40 Interplay between sex steroids and appetite-regulating hormones in conditions associated with chronic stress Laura Holsen, Ph.D. Harvard Medical School	3:20 - 3:40 Advances in neuroimaging and their impact on the future of multiple sclerosis diagnosis and treatment in men and women Anat Biegon, Ph.D. Stony Brook University
3:40 - 3:50 Questions and discussion Speaker Panel	3:40 - 3:50 Questions and discussion Speaker Panel

4:00 - 6:00
Regent's / St. Mark's

WINE & CHEESE RECEPTION
POSTER SESSION II

6:30 - 10:00
Woodlands Ballroom

BANQUET AND AWARDS CEREMONY

Live Music and Dancing

THURSDAY MAY 26, 2016

The Inn at Penn

7:00 - 8:00
Outside Woodlands Ballroom

BREAKFAST

8:00 - 9:50

SESSIONS VII AND VIII

WOODLANDS A

SESSION VII

SEX, INFLAMMATION AND STROKE

Chair: Halina Offner, M.D.
Oregon Health & Science University
Co-chair: Abby Dotson, Ph.D.
Oregon Health & Science University

8:00 - 8:10
Overview
Halina Offner, Dr. Med.
Oregon Health & Science University

8:10 - 8:30

Sex differences in stroke inflammatory mechanisms
Patricia Hurn, Ph.D.
University of Texas

WOODLANDS B

SESSION VIII

EARLY CELLULAR, SYNAPTIC, AND CIRCUIT-LEVEL BIOMARKERS OF SEX DIFFERENCES IN MEMORY DECLINE

Chair: Jill M. Goldstein, Ph.D.
Harvard Medical School
Co-chair: Emily G. Jacobs, Ph.D.
Harvard Medical School

8:00 - 8:10
Overview
Emily G. Jacobs, Ph.D.
Harvard Medical School

8:10 - 8:30

Estrogenic regulation of hippocampal function in male and female rodents
Karyn M. Frick, Ph.D.
University of Wisconsin-Milwaukee

8:30 - 8:50

The inflammatory response originating from the spleen to the ischemic brain

Keith Pennypacker, Ph.D.
University of South Florida

8:50 - 9:10

Sex, stroke and the immune response

Abby Dotson, Ph.D.
Oregon Health & Science University

9:10 - 9:30

Sex differences in the neuroprotective effect of microRNA on ischemic stroke

Farida Sohrabji, Ph.D.
Texas A&M University

9:30 - 9:50

Questions and discussion

Speaker Panel

8:30 - 8:50

Synaptic health: Implications for cognitive aging

John H. Morrison, Ph.D.
University of California at Davis

8:50 - 9:10

Sex and reproductive aging shape early changes in memory circuitry in midlife

Emily G. Jacobs, Ph.D.
Harvard Medical School

9:10 - 9:30

Fetal programing of sex differences in aging of the memory circuitry

Jill M. Goldstein, Ph.D.
Harvard Medical School

9:30 - 9:50

Questions and discussion

Speaker Panel

9:50 - 10:10 Coffee Break

10:10 - 12:20 Sessions IX and X

WOODLANDS A

SESSION IX

SEX DIFFERENCES IN CARDIOVASCULAR DISEASE

Chair: John N. Stallone, Ph.D.
Texas A&M University
Co-chair: Amutha Selvamani, Ph.D.
Texas A&M University

10:10 - 10:20

Overview

Amutha Selvamani, Ph.D.
Texas A&M University

WOODLANDS B

SESSION X

LATE BREAKING RESEARCH IN SEX DIFFERENCES

Chair: Crystal West, Ph.D.
Georgetown University
Co-Chair: Amrita Pai, Ph.D. candidate
Georgetown University

10:10 - 10:15

Overview

Crystal West, Ph.D.
Georgetown University

10:20 am - 10:40 am

The sex chromosome complement contributes to ischemic stroke sensitivity and inflammatory responses in aged FCG mice

Javiera Bravo-Alegria, Ph.D.
University of Texas Health Science Center

10:40 am - 11:00 am

Role of T cells in the development of sex differences in cardiovascular disease and hypertension

Jennifer Sullivan, Ph.D.
Georgia Regents University

11:00 am - 11:20 am

Effects of age and sex on stroke and cerebrovascular function in the rat middle cerebral artery

John N. Stallone, Ph.D.
Texas A&M University

10:15 am - 10:40 am

Investigating neuroimmune function throughout pregnancy and the postpartum period

Morgan Sherer
University of Delaware

10:40 am - 10:53 am

Sex differences in the effects of microglia on neonatal neurogenesis and behavioral development

Lars Nelson, Ph.D. candidate
Ohio State University

10:53 am - 11:06 am

Cocaine seeking during initial abstinence is driven by noradrenergic and serotonergic signaling in dorsal hippocampus in a sex-dependent manner

Amy Kohtz, Ph.D.
Rutgers University

11:06 am - 11:19 am

Sex-specific mechanisms of songbird audition depend on membrane estrogen receptor activation

Amanda Krentzel, Ph.D. Candidate
University of Massachusetts, Amherst

11:19 am - 11:32 am

An XX sex chromosome complement increases obesity, lipids and atherosclerosis in hypercholesterolemic mice

Yasir Alsiraj, MS
University of Kentucky

11:20 am - 11:40 am

Genomic sex-specific contribution to endothelial cell phenotype heterogeneity: from message to metabolism
Virginia H. Huxley, Ph.D.
University of Missouri

11:40 - 12:00

Questions and discussion
Speaker Panel

11:32 am - 11:45 am

Aged females exhibit enhanced pro-inflammatory leukocyte phenotypes
Meaghan A. Roy-O'Reilly, M.S.
University of Texas Health Sciences Center, Houston

11:45 - 12:00

Role of estrogen and nitric oxide in the sex difference of fat metabolism and survival during fasting
Mika Jikumaru, M.D., Ph.D.
Oita University

12:00 **Outside Woodlands Ballroom**	Lunch
12:20 - 1:50 **Woodlands A/B**	LUNCH DISCUSSION RESEARCH METHODS FOR STUDYING SEX Chair: Susan Phillips, M.D., MSc Queen's University Co-chair: Robert Juster, Ph.D. Columbia University
12:20 - 12:25	Overview Susan Phillips, M.D., MSc Queen's University
12:25 - 12:50	Sex/gender in basic science research and pre-clinical research Stacey Ritz, Ph.D., McMaster University Robert Juster, Ph.D., Columbia University
12:50 - 1:15 pm	Sex/gender in quantitative and qualitative clinical research Susan Phillips, M.D., MSc, Queen's University Katarina Hamberg, M.D., Ph.D., Umea University
1:15 - 1:30	Integrating sex/gender in systematic reviews Sari Tudiver, Ph.D. Cochrane Collaboration

1:30 - 1:50	Discussion Speaker Panel
1:50 - 2:00	Coffee Break

2:00 - 3:50

SESSIONS XI and XII

WOODLANDS A

SESSION XI

SEX DIFFERENCES IN ADDICTION

Chair: Alicia Allen, Ph.D., MPH
University of Minnesota
Co-chair: Erin Emme, MPH
Yale University

2:00 - 2:10

Overview
Erin Emme, MPH
Yale University

2:10 - 2:30

Progesterone and impulsive behavior in cigarette smokers
Alicia Allen, Ph.D., MPH
University of Minnesota

2:30 - 2:50

Stress, addiction and withdrawal symptomatology
Mustafa al'Absi, Ph.D.
University of Minnesota

2:50 - 3:10

Sex and gender differences in addiction: gender sensitive medication development
Sherry McKee, Ph.D.
Yale University

3:10 - 3:30

Progesterone in postpartum smoking relapse
Sharon Allen, M.D., Ph.D.
University of Minnesota

WOODLANDS B

SESSION XII

SEX CHROMOSOMES AND SEX-LINKED GENES IN CANCER

Chair: Christine Disteche, Ph.D.
University of Washington
Co-chair: Joel Berletch, Ph.D.
University of Washington

2:00 - 2:10

Overview
Christine Disteche, Ph.D.
University of Washington

2:10 - 2:30

TSPY and TSPX: an odd couple in human oncogenesis
Chris Lau, Ph.D.
University of California, San Francisco

2:30 - 2:50 pm

Regulation by the histone demethylase KDM6A gene is female biased in development and cancer
Joel Berletch, Ph.D.
University of Washington

2:50 - 3:10

X chromosome regulation in human pluripotent stem cells: potential parallels to female cancer cells
Anna Sahakyan, Ph.D. Candidate
University of California, Los Angeles

3:10 - 3:30

Abnormal X chromosome inactivation of T cells in lupus leads to increased expression from the inactive X

	Jianle Wang Ph.D. University of Pennsylvania
3:30 - 3:50 Questions and discussion Speaker Panel	3:30 - 3:50 Questions and discussion Speaker Panel
3:50 - 4:00	Coffee Break
4:00 - 5:00 **Woodlands A/B**	CAPSTONE ADDRESS Aging and sex James L. Kirkland, M.D., Ph.D. Director, Robert and Arlene Kogod Center on Aging Mayo Clinic Introduction: Kathryn Sandberg, Ph.D. OSSD 2016 Program Chair Georgetown University
5:00 - 6:00 **Woodlands A/B**	GENERAL OSSD MEMBERSHIP AND BUSINESS MEETING

SPEAKER ABSTRACTS

Monday, May 23rd

Transcriptional and Epigenetic Mechanisms of Depression

Eric J. Nestler, M.D., Ph.D (Nash Family Professor and Chair, Department of Neuroscience; Director, Friedman Brain Institute; Icahn School of Medicine at Mount Sinai, New York, NY USA)

Depression is a common, chronic, and debilitating disease. Although many patients benefit from antidepressant medications or other therapies, only about half of depressed patients show a complete remission, which underscores the need for more effective agents. The mechanisms that precipitate depression, such as stress, are incompletely understood. We are interested in the role played by stable adaptations in the brain's limbic regions, mediated by long-lasting changes in gene expression and chromatin structure, in the pathophysiology and treatment of depression. We use several chronic stress paradigms in mice that recapitulate certain symptoms of human depression, which are reversed by administration of antidepressant medications. Importantly, a subset of mice subjected to chronic stress do not exhibit these deleterious behaviors and appear "resilient." We are exploring the molecular basis of stress-induced behavioral pathology, antidepressant action, and resilience by analyzing genome-wide changes in gene expression and chromatin modifications. Our work focuses on several limbic regions of brain which are implicated in controlling mood under normal and pathological conditions. Parallel work has focused on homologous regions in the brains of depressed humans examined postmortem. Together, this work is providing novel insight into the molecular mechanisms underlying depression and other stress-related disorders. Dramatic findings to date include striking differences in depression-associated molecular abnormalities between males and females in both mouse models and human depression. We are now mining these large datasets to identify novel leads for the development of new antidepressant treatments in a sex-specific manner. This includes developing approaches that promote mechanisms of natural resilience and not just those that oppose the deleterious effects of stress.

Tuesday, May 24th

9:00 am – 12:00 pm:
Variability in Response to Drugs and Devices: Influence of Sex

This workshop has been designed and coordinated though the FDA Center for Drug Evaluation and Research (CDER) Professional Affairs and Stakeholder Engagement and the FDA Office of Women's Health (OWH).

9:00 am - 9:05 am: Welcome and Remarks
Marjorie Jenkins M.D., M.E.H.P., F.A.C.P. (Director of Scientific Engagement and Medical Initiatives, OWH FDA)

9:05 am - 9:20 am: FDA's Role in Drug Development: the Intersection of Science and Regulations
Naomi Lowy, M.D. (Associate Director for Regulatory Science, Office of Drug Evaluation 1, CDER FDA)

Regulatory Science is the science of developing new tools, standards, and approaches to assess the safety, efficacy, quality, and performance of all FDA-regulated products. This discussion will educate and inform about how this science is foundational specifically to the development and approval of drugs.

9:20 am - 9:40 am: FDA's Efforts to Understand Variability to Drug Response
John Whyte, M.D., M.P.H. (Director, Professional Affairs and Stakeholder Engagement, CDER FDA)

We know there is biologic variability to drug response; what we don't know is what those major causes of variability are. Directed by Congress, the U.S. Food and Drug Administration (FDA) has been asked to report on the inclusion and analysis of demographic subgroups in applications for drugs, biologics, and devices. With the Drug Trial Snapshots, the FDA has "unveiled the data" to foster conversations and spur discussion on issues concerning variability in response to drugs, clinical trial design, enhancing demographic diversity, and what is the right number of diverse participants to include where appropriate.

9:40 am - 10:05 am: Demographic Subset Differences
Robert Temple, M.D. (Deputy Center Director for Clinical Science, CDER FDA)

Over the years, examples of critical differences have been identified in demographic subsets of the population. This presentation will highlight several of these examples and emphasize the importance of examining these subsets.

10:05 am - 10:30 am: Moving the Needle in Drug Development
Danielle Day, Ph.D. (Senior Medical Science Liaison, AstraZeneca Medical Affairs, Diabetes)

The healthcare industry has an untapped opportunity in addressing sex differences in drug and device development. This presentation will confront the historic and present barriers to bringing a focus on sex differences to industry's scientific and business priorities, highlight examples of recent progress made, and discuss potential future strategies for moving the needle in drug development.

10:45 am – Noon: FDA Expert Panel
Sex Differences and the FDA Review Process: Perspectives from FDA Reviewers

How does FDA incorporate non-clinical studies into drug review?
Shiny Mathew, Ph.D., LT USPHS (Pharmacology/Toxicology Reviewer, CDER FDA)

Studies conducted in animals are required to ensure the safety of drugs to support clinical trials. The Pharmacology/Toxicology Reviewer at the FDA serves an important role in safety decisions on all phases of drug development all the way to its marketing. In instances where clinical data is lacking on sex-dependent differences, animal data can be described in the drug label to inform clinicians and patients about this risk.

FDA Drug Review Process and Transparency
Naomi Lowy, M.D. (Associate Director for Regulatory Science, Office of Drug Evaluation 1, CDER FDA)

Dr. Lowy's presentation will aim to provide the audience with a CDER clinical reviewer's perspective on the FDA's decision-making process.

Devices and Sex Differences: An Update
Kathryn O'Callaghan (Assistant Director for Strategic Programs (Acting), Office of the Center Director, Center for Devices and Radiological Health, FDA

This presentation will highlight the progress that has been made within FDA's Center for Devices and Radiological Health and sex differences. Ongoing and future initiatives will be discussed including ongoing challenges when considering evaluation of sex differences in medical device safety and efficacy.

12:20 pm - 1:50 pm:
Thinking about Gender in Preclinical Models: Examples of Models and Best Practices

Co-chairs: Gillian Einstein, Ph.D. (University of Toronto) and Annie Duchesne, Ph.D. (University of Toronto)

Why We Should Think About Gender in Preclinical Models and How
Gillian Einstein, Ph.D. (University of Toronto)

Sex and gender are inextricably linked. Sex is a major determinant of the worlds in which both humans and animal live affecting hierarchical position, disposition to inflict or suffer violence, degree of social isolation, nurturing responsibilities, that is, social characteristics or, gender. Chromosomal sex does not fully dictate gender, but it is often associated with it. As a first step towards recognizing the importance of sex for human health, NIH and CIHR now mandate the inclusion of male and female animals in basic science and preclinical research, leading to an increasing number of methods/measures for studying the influence of sex differences on diverse clinical issues (e.g., causes of diabetes, mechanisms of cardiovascular disease, etiology and progress of Alzheimer's disease).

Less attention, however, has been paid to sex's social face, gender. Because the social becomes biological, it is important to begin considering gender when establishing animal models for testing that will ultimately take place in humans. Doing so might more strongly predict how illnesses or therapies will play out in humans. Not all gender aspects are easily modeled in animals (e.g., gender identification, sexual orientation), but some are amenable to preclinical study. Gender's relational aspect, for example—interactions among subjects, relations between subjects and experimenter, experimenter question framing, environment—are aspects of gender that could be modeled or taken into account in preclinical studies, making outcome measures such as the efficacy of a given therapy, more applicable to the human case, thereby increasing the rigour, repeatability, and applicability of preclinical science. In this symposium we will consider aspects of gender that could be modeled in animals, hear examples of human disease that are influenced by gender, and discuss some animal models that might be useful in modeling gender in preclinical models of human disease.

Gender and Pre-Clinical Models of Cardiovascular Disease: Recognizing the Urgency
Edward O'Brien, M.D. (Cardiovascular Institute of Alberta, Calgary)

Research conducted in the O' Brien laboratory lead to the discovery of an estrogen responsive protein, Heat Shock Protein 27 (HSP27), as a biomarker for cardiovascular events that also provides novel anti-inflammatory and cholesterol lowering effects that are important in protecting against "hardening of the arteries" (atherosclerosis). The role of ovarian function in modulating the biological effects of HSP27 is proving to be an exciting avenue of research, with potential therapeutic implications for post-menopausal women. Whilst the original approach of his fundamental and clinical research was from the perspective of sex differences, Dr. O'Brien is now exploring the importance (and interdependence) of gender in cardiovascular research.

Gender and Social Stress: From Humans to Animal Models
Annie Duchesne, Ph.D. (University of Toronto)

Sex differences in stress processes are observed across the lifespan and considered central to men's and women's differential health trajectories. These differences are often linked to differences in gonadal hormones and periods characterized by fluctuations in ovarian hormones (e.g. puberty, pregnancy, menopause) correspond to greater stress vulnerability in women. Different socio-cultural characteristics are also associated with men's and women's experience of stress, however these are rarely studied in interaction with biological sex differences. In this presentation, I will question how some relational aspects of gender, such as the type of stress, could be integrated into preclinical models of stress.

Sex or Gender? Considering Environment and Social Familiarity in Animal Models of Pain Research
Loren Martin, Ph.D. (University of Toronto)

Females are at greater risk for developing chronic pain disorders including fibromyalgia and migraine. In clinical laboratory studies, women exhibit greater sensitivity-to-noxious stimuli compared with men and factors such as the gender of the experimenter, prior pain experience and social variables have all been shown to influence pain reports. Here, I will briefly highlight some of our recent findings showing that the gender of the experimenter and prior pain experience modulate pain behaviors in preclinical studies using mice. In both examples, we observe a gender (or sex) interaction with stress and gonadal hormones. The translational potential of these novel models will also be discussed.

Identification of Sex Effects on Genes and the Gut Microbiome
Jayne Danska, Ph.D. (University of Toronto)

Rodent models can be designed to define biological effects of sex, and the impact of medical practice on normal physiology and to model aspects of human disease. An area of current interest is the gut microbial community (microbiome) that forms a critical interface between the mammalian host and a rapidly changing environment and contributes to host metabolic and immunological function. Modern medical practices including C-section delivery and neo/peri-natal exposures to antibiotics impact the establishment of a rich, diverse and resilient microbiome in early life. The use of rodent models to explore the effects of conditions during pregnancy, delivery and early infancy on building a healthy microbiome in both sexes will be discussed.

Social Status in Naked Mole Rats: Can We Tease Apart Sex and Gender in Eusocial Mammals?
Melissa Holmes, Ph.D. (University of Toronto)

I study naked mole-rats, rodents that live in large colonies with rigid social and reproductive hierarchies. Reduced or absent sex differences but striking status differences in many features of neurobiology, gross anatomy, and behavior suggest the social environment is more important than sex for this species. Here I ask whether studying these animals provides a unique opportunity to dissociate gendered environmental elements from biological sex.

2:00 pm – 3:40 pm:
Presidential Symposium: Getting Basic Scientists to Think About Sex

Development and implementation of a new NIH policy: Consideration of sex as a biological variable
Janine Austin Clayton, M.D. (Director, Office of Research on Women's Health, NIH)

The National Institutes of Health (NIH) funds basic, translational, and clinical research. From laboratory research to clinical care, studying both sexes is a guiding principle for experimental design, hypothesis-generation and -testing, and analysis and reporting to expand knowledge toward turning discovery into health for both women and men. Numerous factors prompted the development of new NIH policy, announced in May 2014, to ensure that sex is considered as a basic biological variable in NIH-funded research. These included scientific progress emerging from NIH-funded laboratories, congressional interest and support, and ongoing NIH efforts to enhance the reproducibility of preclinical research through rigor and transparency. NIH grant applications due on or after January 25, 2016, will be evaluated on how they account for sex as a biological variable (SABV) in their research designs, analyses, and reporting in vertebrate animal and human studies. Strong justification from the scientific literature, preliminary data, or other relevant considerations must be provided for applications proposing to study only one sex. Selecting an appropriate preclinical model that considers the role of sex in the context of a specific research question of interest, especially for studies that model human physiology and pathology, is critical to health-related research. A continual growth in knowledge about the influence of sex in biomedicine is imperative to the NIH mission of turning discovery into health.

Impact of the Canadian policy on sex in biomedical research
Cara Tannenbaum, M.D., MSc. (Institute of Gender and Health, Canadian Institutes of Health Research; Université de Montreal)

In 2009 the Canadian government passed its Health Portfolio *Sex and Gender Based Analysis* Policy. By 2010, the Canadian Institutes of Health Research (CIHR), through its Institute of Gender and Health (IGH) introduced mandatory questions on grant applications asking researchers to report whether sex and/or gender was accounted for in their study. Only 25% of basic scientists responded affirmatively. By 2014, this proportion increased to 50% overall, with a wide range of responses across research disciplines.
In its 2015-2019 operations plan, IGH developed a framework to overcome barriers for health scientists to integrate sex and gender in research proposals. Key principles include increasing awareness and skills among researchers, promoting self-efficacy among peer reviewers to provide constructive feedback on applications, requiring a Sex and Gender Champion on teams applying for strategic research funding opportunities, and engaging the Ethics community in the campaign to set checkpoints for researchers to consider sex and gender at all stages of the research process.

The 45-minute IGH online competency certificate program for sex and gender in biomedical research was launched in September 2015 (http://www.cihr-irsc.gc.ca/e/49347.html). The course has now been completed by hundreds of participants from across Canada, the U.S., Europe, Asia and Africa. After completion of the module, self-efficacy scores for assessing research based on its integration or omission of sex and/or gender increased from a mean of 5.8/10 to 8.2/10 ($p<0.001$). Ninety-percent of participants stated that they intended to change their approach for evaluating sex and gender in research and publications. Based on these results, the new CIHR College of Reviewers will require peer reviewers to undergo abridged but mandatory 10-minute CoR training on how to evaluate sex and gender in biomedical research, starting in the Fall 2016.
This presentation will discuss the successes of the Canadian approach, sharing lessons learned along the way and persistent challenges. An important focus will be theories of behaviour change and the mechanisms that bring about culture shift within the health research community.

Sex and the implications for peer review
Richard Nakamura, Ph.D. (Director, Center for Scientific Review, NIH)

NIH has placed increased emphasis on scientific rigor and transparency for grant applications that will be reviewed starting in late May of 2016. How applications will be reviewed will be described with special

emphasis on sex as a biological variable. Dr. Richard K. Nakamura is the Director of the Center for Scientific Review. He leads the review of grant applications of the National Institutes of Health. He was with the National Institute of Mental Health from 1976 to 2011. In 2001, he received the NIH-Asian/Pacific American Organization Outstanding Achievement Award for Administrative Work. In 2002, Dr. Nakamura was elected by the American Association for the Advancement of Science (AAAS) to the status of AAAS Fellow. Also in 2002, Dr. Nakamura was awarded the Presidential Rank Award for outstanding leadership. In 2004 and 2005 respectively, he received leadership awards from the Federation of Behavioral Psychological and Cognitive Sciences, and from the International Society for Behavioral Neuroscience. In 2009 he was awarded the NIH Director's Award for Outstanding Administration.

4:00 pm – 5:00 pm
Keynote Address

Sex differences in multiple sclerosis: Past steps, future paths

Rhonda R. Voshkul, M.D. (Director, UCLA Multiple Sclerosis Program; Jack H Skirball Chair in MS Research, University of California, Los Angeles)

Sex is a naturally occurring, major disease modifier in multiple sclerosis (MS) and many diseases. Effects of sex on disease represent clinical observations for which mechanisms can be disentangled at the basic laboratory bench, with insights translated to clinical trials aiming to recapitulate naturally occurring disease modification. Thus, sex differences represent a powerful opportunity for "bedside to bench to bedside" research in novel drug discovery for diseases, starting with a clinical observation known to be physiologically significant, namely the sex difference.

When studying sex differences in disease, categories include events unique to one sex, such as pregnancy. They also include sex hormones effects, including estrogen, progesterone and testosterone, as well as sex chromosome effects, including XX versus XY chromosome complement. In MS and its preclinical model, all of the above categories have been explored.

Pregnancy is protective with an over 70% reduction in relapses in the third trimester. Estriol, the estrogen unique to pregnancy, is anti-inflammatory and neuroprotective in preclinical MS models. Pilot and Phase 2b clinical trials have together shown beneficial effects of estriol treatment on reducing relapses, reducing active brain lesions, and causing a favorable immune shift in peripheral blood immune responses. Exploratory neuroprotective effects included an estriol mediated decrease in fatigue and an improvement in cognition. A Phase 3 trial of estriol in MS is now warranted.

Regarding sex differences in MS, females are over three times more susceptible to disease than males. Testosterone appears to be protective for MS susceptibility through clinical retrospective studies and through anti-inflammatory effects in the MS preclinical model. Additionally, the XX sex chromosome complement is associated with more robust immune responses than XY using the four core genotype mouse system. Together this suggests that the decrease in susceptibility to MS in males is due to a protective effect of testosterone in males and a deleterious, immunostimulatory effect of XX in females.

The other sex difference in MS is that despite the fact that males are less susceptible to disease, men that get MS have worse disability progression as compared to women. Since testosterone treatment slowed brain atrophy in a pilot clinical trial, and retrospective studies correlated higher endogenous testosterone levels with less disability, and neuroprotective effects of testosterone treatment were shown in two preclinical MS models, worse disability progression in MS men is not due to testosterone. Indeed, a placebo-controlled trial of testosterone treatment in MS men is now warranted to investigate its ability to slow disability progression. Instead, it was found that XY, compared to XX, in the central nervous system led to worse neurodegeneration using the four core genotypes in the MS model. Possible etiologies for XX versus XY effects include the presence or absence of the Y chromosome, X dosage effects in XX, or differential

parental imprinting of X genes. Transcriptome and methylome studies in the four core genotype mice are now warranted to discover new targets to prevent disability progression.

In conclusion, the study of sex differences in MS have led to several insights with potential for novel treatments relevant to not only relapsing MS and potentially other autoimmune diseases, but also progressive MS and other neurodegenerative diseases. However, the critical challenge is that many novel treatments that emerge from the research of sex differences will ultimately suggest treatments that are biologics, thereby posing financial feasibility issues for clinical development by the pharmaceutical industry. This issue will grow as more targets are identified through the new NIH policy to encourage researchers to study sex differences. A critical next step is needed in either NIH funding or FDA policy if less expensive biologic treatments are to be developed through clinical trials for FDA approval for patients with these diseases.

Wednesday, May 25

8:20 – 10:10: Sessions I & II

Session 1: Sex Differences in Developmental Origins of Metabolic Disease (Woodlands A)

Co-Chairs: Sarah E. Pinney, M.D. (Children's Hospital of Philadelphia)
and Amita Bansal, Ph.D. (University of Pennsylvania

Sex-specific effects of parental diabetes on offspring
Steven Chernausek, M.D. (Harold Hamm Diabetes Center, CMRI Metabolic Research Program, Department of Pediatrics, University of Oklahoma Health Sciences Center)

There are many aspects of T2DM that are influenced by sex. For example, T2DM is more common among female adolescents than males and the risk of developing T2DM is greater in the offspring of women with T2DM than it is with men with T2DM. The TODAY study is a multicenter clinical trial involving over 700 youths with type 2 diabetes mellitus (T2DM) which offers a rare opportunity to examine sex differences in younger individuals with T2DM. An early report (Copeland et al *J Clin Endocrinol Metab*, 2011) found that males were more likely than females to have a mother with gestational DM (40 vs. 30%, $p < 0.01$) and nearly twice as likely to be born large for gestational age (25 vs. 13 %, $p < 0.01$). This led to subsequent studies of the effect parental DM on these adolescents. (Chernausek et al *Diabetes Care*, 2016) Youth with T2DM that were exposed to maternal diabetes during pregnancy were diagnosed at younger ages (by 0.6 yr. on average), had greater dysglycemia at baseline (HbA1c increased by 0.3% [3.4 mmol/mol]), and had reduced β-cell function compared to those not exposed (c-peptide index 0.62 vs. 0.95).The effect of maternal diabetes on β cell function was observed in non-Hispanic Blacks and Hispanics but not whites. In contrast the effects of paternal diabetes status were minimal. These results support the hypothesis that maternal factors in particular have important and lasting effects on offspring. Fetal exposure to aberrant metabolism producing epigenetic changes is one potential explanation, but transmission of imprinted genes or an altered microbiome (from the maternal colony) could be involved. More targeted research is needed to understand more precisely what is transmitted from mother to child in this circumstance, whether the impact of maternal diabetes is modified by racial-ethnic factors, and whether the pathway to youth-onset T2DM differs by race-ethnicity.

Boys live dangerously in the womb
Kent Thornburg, Ph.D. (Oregon Health Sciences University)

Regardless of sex, growth in the womb is constrained by the ability of the mother to deliver nutrients to the uterus and by the ability of the placenta to transfer nutrients to the developing fetus. Boys are known to die more often in the womb and in early infancy than are girls. Thus, we hypothesized some years ago that the acquisition of nutrients would differ by fetal sex. This speculation has been addressed in many studies in the past decade. For example, we studied 2003 men and women at age 62 in Helsinki, Finland whose birth records were available. We found that 644 had clinically determined hypertension. In women, hypertension was related to a small placental area and mother's height which reflects her protein metabolism. In men, hypertension was related to the width and ovality of the placenta and mother's socioeconomic status, an indicator of dietary quality. In both sexes, hypertension was inversely related to birthweight. Boys are known to grow more rapidly as fetuses than girls but with a lower placental mass. Thus they are more likely to encounter adverse conditions when nutrient flow is reduced. For both sexes, any stressor that reduces the efficiency of the placenta represents a risk for compromised fetal growth. The data for girls suggest that a tall mother who generates a small mismatched placenta has inadequate nutrient flow to her fetus and for boys the combination of a mother consuming a poor diet and an oval shaped placenta signifies even worse nutrient acquisition. This compares to the risk of sudden cardiac death in adult Finnish men who had thin placentas whereas women with sudden death had placentas that were overly large in relation to birthweight.

In both cases, men who have a compromised placental shape pattern are more vulnerable for hypertension and sudden death from cardiac causes. Both hypertension and sudden death are adverse conditions that are found more often in men than in women.

Sex of offspring impacts DNA methylation and gene expression in placenta from women with diabetes during pregnancy

Sara E. Pinney, M.D. (Children's Hospital of Philadelphia)

Background: Offspring of mothers with diabetes during pregnancy (DDP) are at increased risk for obesity and type 2 diabetes, which may be influenced by offspring sex. The mechanisms responsible for the phenomenon are unknown. We hypothesize that that DDP alters genome-wide DNA methylation resulting in differentially methylated loci of metabolically relevant genes and downstream changes in RNA and protein expression.

Methods: We mapped genome-wide DNA methylation with the Infinium 450K HumanMethylation BeadChip using a nested case-control design from a cohort of Native American and Hispanic women with DDP. Term fetal placentae were collected and matched 1:1 with controls based on maternal age, race/ethnicity, and offspring sex (n=17 pairs). Differential methylation (dm) was calculated as $\Delta\beta$ per pair and averaged across all samples. RNA and protein expression were assayed via RNA-Seq and Western Blot.

Results: For genome-wide DNA methylation, 465 CpGs had significant changes for male offspring, 247 for female offspring, and 277 for offspring of both sexes ($p<0.001$). In placentae from male offspring, CYBA, GSTM1, GSTM5, and RASSF2 showed decreased methylation in DDP samples compared to control and a corresponding increase in RNA and protein expression. In placentae from female offspring, KCNE1 and NXN showed increased methylation in DPP samples compared to control and a corresponding decrease in RNA and protein expression. PIWIL3 showed increased methylation in samples from women with DPP compared to control across both offspring sexes, and a corresponding decrease in RNA and protein expression.

Conclusions: In summary, DDP alters placental DNA methylation at metabolically relevant loci in a sex specific manner, with more probes affected in males. Many of these differentially methylated genes corresponded to changes in both RNA and protein expression. This finding may begin to explain the long-term metabolic effects of DDP on offspring.

Sex differences in body composition in infants and young children

David Fields, Ph.D. (Harold Hamm Diabetes Center, CMRI Metabolic Research Program, Department of Pediatrics, University of Oklahoma Health Sciences Center)

Obesity is a known risk factor for a multitude of diseases with body mass index (BMI) the most commonly used clinical measure in evaluation. Early development of body composition (i.e. fat and muscle) is rarely considered, but is important because excessive fat mass is related to other morbidities later in life, namely obesity, diabetes and cardiovascular disease. However, the quality of body weight (i.e. the composition of the body that is fat and muscle) remains unclear when using BMI as a surrogate of adiposity. Until recently, determining body composition in young children (i.e. < 1 year old) has proven difficult if not impossible. In pediatric medicine, there are few to no data comparing sex differences in body composition in the first months of life despite the fact males are typically longer and weigh more than girls at birth with little to no understanding how these potential differences impact future disease risk or are amenable to intervention. This presentation will briefly discuss: i) the normal progression of body composition both in utero and in early life, ii) methodological methods currently available for evaluating body composition, iii) the natural time course of fat deposition in both genders, and iv) gain greater insight and understanding between gender differences in body composition and how these differences may be amenable to intervention to decrease disease risk later in life.

Session II: Sex Differences in Sensorimotor Control (Woodlands B)

Co-Chairs: Jill B. Becker, Ph.D. (University of Michigan)

and Emily Lawrence, Ph.D. Candidate (University of Southern California

One Second or Less: Sex Differences in the Organization of Movement in Non-Reproductive Behaviours

Evelyn Field, Ph.D. (Department of Psychiatry, Mount Royal University)

Whether there are sex differences in the kinematic organization of non-reproductive behaviours is rarely addressed. In this presentation I will discuss sex differences in how male and female rats organize their movements differently during a food protection task, contact righting, skilled reaching and vertical rearing. These differences do not appear to be determined by sex differences in body morphology but rather by changes in gonadal hormone exposure directly after birth. The implications of these sex differences will also be discussed with regards to CNS injury and whether sex differences in non-reproductive behaviours can also be found in other animal species.

Sex differences in neuromuscular function and fatigue

Sandra K. Hunter, Ph.D. (Exercise Science Program, Department of Physical Therapy, Marquette University)

There are sex-related differences in physiology and anatomy that are responsible for some profound differences in skeletal muscle function and fatigability between men and women. While men are stronger and more powerful, women are usually less fatigable than men for similar intensity isometric (static) and slow dynamic fatiguing contractions. This sex difference in fatigability, however, alters depending on the details of the task because different neuromuscular sites will be stressed at different rates for men versus women when the requirements of the task are altered. Task variables that can alter the sex difference in fatigability include the type, intensity and speed of contraction, the muscle group assessed, and the environmental conditions. Some of the relevant physiological mechanisms for the sex differences may include skeletal muscle physiology, muscle perfusion and voluntary activation. However, there are substantial knowledge gaps about the task dependency of the sex differences in fatigability, the involved mechanisms and the relevance to clinical populations. The knowledge gaps are in part due to the significant deficits in the number of women included in neuromuscular fatigability studies despite a gradual increase in the inclusion of women over the last 20 years. This presentation summarizes the current knowledge on sex differences in fatigability, the potential mechanisms across a range of tasks, and highlights emerging areas of opportunity in clinical populations. Examining the underlying mechanisms of sex-based differences in neuromuscular function and fatigability across different task conditions will shed light on the benefits and limitations that muscle fatigue can exert in both men and women during daily activities, ergonomic tasks, exercise performance, training and rehabilitation.

Sex differences in sensorimotor control of lower extremity function: Influence of athletic ability

Emily L. Lawrence, Ph.D. (Department of Biomedical Engineering, University of Southern California, Los Angeles)

Abstract: The ability of the leg to dynamically regulate instabilities during ground contact is essential for participation in athletics and is quantified by the Lower Extremity Dexterity (LED) test. This retrospective analysis quantitatively assesses the differences in LED test performance between 20 skilled elite athletes (10F, 10M, 26.4±3.5 yrs.) and 20 non-skilled recreational athletes (10F, 10M, 24.8±2.4 yrs.). We reconstructed phase portraits from the data using the Takens' theorem and quantified four spatial features to infer differences in sensorimotor control strategies: trajectory length, interquartile range of the Euclidean distance from the centroid, volume, and the sum of edge lengths. A two factor repeated measures ANOVA revealed significant effects of sex and athletic ability on all spatial features. *Post hoc* analyses further indicated that female non-skilled athletes have significantly greater estimated marginal mean values of all spatial features than male non-skilled athletes, indicating greater stochasticity and variability in the way the task is performed. However, these sex differences are not present in skilled athletes. These results show that skilled athletes have increased sensorimotor control for dynamic regulation of instabilities during ground contact compared to non-skilled athletes; and that non-skilled female athletes have the poorest control of the

four groups. Importantly, this may help explain why a prospective study showed that dynamic neuromuscular training in females reduced the risk of knee ligament injury by a factor of 3.6. Our nonlinear approach shows clear differences in sensorimotor control between sexes, and between elite and recreational athletes. But are these differences in sensorimotor control—and risk of injury—due to genetics, athletic training, or both? Future work will explore this important question by determining if exposure to athletic training regimens enhances sensorimotor control for leg dexterity.

Sex differences in performance of athletic tasks: implications for lower extremity injury
Susan Sigward, Ph.D., PT, ATC (Human Performance Laboratory, Division of Biokinesiology & Physical Therapy, University of Southern California)

The disproportionally greater incidence of non-contact anterior cruciate ligament (ACL) tears in female athletes compared to their male counterparts participating in the same sport has garnered a lot of attention over the past 20 years. Many theories have been proposed regarding potential sex specific factors that contribute to the disparate incidence of an injury that does not result from a direct force applied to the knee. Over the years these theories have included sex difference in circulating hormones, anatomic structure, joint laxity, muscle performance and sensorimotor control. While some of these factors do indeed differ between sexes, the link between characteristics commonly observed in females and ACL injury requires an understanding of how they contribute to ligament failure. In this presentation I will describe sex difference in performance of tasks considered high risk for non-contact ACL injuries and how these performance differences relate to mechanics of ACL injury. The overall goal of this presentation is to explore the potential relationships between sex differences in individual systems (endocrine, musculoskeletal and nervous) and potentially injurious task performance with reference to ACL injuries.

10:30 – 12:20: Sessions III & IV

Session III: Sex Differences in Developmental Windows by the Gut Microbiome

Co-Chairs: Tracy Bale, Ph.D. (University of Pennsylvania)
and Eldin Jašarević, Ph.D. (University of Pennsylvania)

Prenatal stress alters intrauterine environment and contributes to adult female microbiome and behavioral changes
Tamar L. Gur M.D., Ph.D. (The Ohio State University Departments of Psychiatry & Behavioral Health, Neuroscience, and Ob/GYN; OSU Institute for Behavioral Medicine Research)

Recent studies demonstrate that exposure to stress changes the composition of the gastrointestinal and reproductive tract microbiota, which is associated with development of stress-induced changes in behavior. Psychiatric disorders have been associated with *in utero* and early neonatal exposure to maternal stress, though the underlying mechanisms are not fully understood. Commensal microbes from the maternal gastrointestinal and reproductive tracts are the first to colonize the developing fetus and newborn, thus raising the possibility that commensal microbes are involved with the transmission of the maternal experience during pregnancy. In this study we address the contribution of maternal stress and commensal microbes on the development of adult psychopathology. Pregnant C57/BL6 females were restrained between embryonic day (E) 10-E16, for a period of two hours using a well-validated restraint stress paradigm, or they were left undisturbed throughout pregnancy as a control. Placental tissue and amniotic fluid were collected from pregnant females and fetuses at E17.5 in one cohort of mice. Microbial diversity was assessed using the Illumina MiSeq platform, for targeted 16S ribosomal RNA gene sequencing. RT-PCR and Bioplex was used to examine placenta. In a second cohort of mice, behavior was assessed with the elevated plus maze (EPM), the tail suspension test (TST) and the novel object recognition test when the offspring reached adulthood. We demonstrate that prenatal stress leads to alterations in the maternal and female offspring intestinal microbial populations, as well as alterations in the placental bacteria. We show alterations in female, but not male, placental gene expression in response to stress, including IL-6, MAO-A and OGT. Furthermore, we show that these changes are associated with alterations in cognition and anxiety, as well as neural gene expression, in the adult female offspring. This suggests that gestation is a critical window in

contributing to the development of adult psychopathology, and that the microbiome may be a key link between early life environment and later life behavioral changes.

Estradiol and diet alter the gut microbiome in female mice
Marc Tetel, Ph.D. (Neuroscience Program, Wellesley College)

The steroid hormone, estradiol, elicits profound effects on growth, reproduction, and metabolism. For example, estrogens act as an anorectic in humans and rodents. In support, postmenopausal women gain fat weight, increasing the likelihood of heart disease, cancers and type 2 diabetes. Work from our lab and others reveal that estradiol treatment protects against high fat diet (HFD)-induced obesity in female mice. However, the mechanism by which estradiol prevents HFD-induced obesity is not well understood. Studies on the human and rodent gut microbiome reveal that microbial communities present in the intestines are a key player in obesity, and diet profoundly affects this microbial structure and activity. Therefore, we tested the hypothesis that estradiol treatment alters the gut microbiome of ovariectomized mice fed a HFD. Mice were ovariectomized and implanted subcutaneously with silastic capsules containing either 17β-estradiol (E2) or oil (Veh). Mice in each group were maintained on a standard rodent chow for 10 days and then fed a HFD for 25 days. Weight measurements and fecal samples were collected over the 35 days. Bacterial DNA from the fecal samples was extracted, and the microbiome from each fecal sample was analyzed by high-throughput 16S rRNA gene Illumina sequencing and compared longitudinally and across treatment groups. Consistent with our previous findings, Veh mice gained 35% more weight than E2 mice over the 35 days. Differences in the gut microbiota were detected between E2 and Veh treated mice on standard chow. Interestingly, when mice were switched to a HFD, the gut microbiota of Veh mice responded to the change in diet immediately, while the gut microbiota of E2 mice were more resistant to the change. We are continuing to explore the effects of estrogens on the gut microbiome in female mice. The identification of estrogen-induced changes in the gut microbiome is critical to understanding the mechanisms by which estrogens protect against obesity.

The autoimmune disease triad: genes, sex and the gut microbiome
Jayne Danska, Ph.D. (Senior Scientist, Hospital for Sick Children Research Institute and Professor, Faculty of Medicine, University of Toronto)

Autoimmune and inflammatory diseases including type 1 diabetes (T1D), multiple sclerosis and inflammatory bowel disease, result from the combined effects of multiple inherited genetic variants that interact with poorly understood environmental factors. The frequency of T1D has increased by >500% in developed countries over the last 50 years. Many immune mediated diseases display sex bias in incidence, progression and severity for which the underlying mechanisms are poorly understood. Danska's research employs mouse models and human cohort studies to examine the impact of host-microbe interactions on autoimmune responses, to define aspects of these pathways that differ between the sexes and to identify biomarkers of disease progression. The talk will discuss manipulations of the gut microbiome that alter host immunity, sex hormone, metabolism and islet-directed autoimmunity in mouse models, and new approaches to define age and sex-specific immune responses to gut microbes in longitudinal studies of children at high-risk, pre-diabetic children display distinct alterations in immune responses to commensal bacteria. The ultimate objective of the work is to identify host and microbial mechanisms that underlie progression to autoimmune disease and to define protective interventions.

Maternal stress reprogramming of sex differences in the developing gut microbiome-brain axis
Eldin Jašarević, Ph.D. (Department of Biomedical Sciences, Center for Host-Microbial Interactions, University of Pennsylvania)

Prenatal stress is associated with an increased risk for neurodevelopmental disorders. In our established mouse model of early prenatal stress (EPS), long-term programming effects on offspring development have been demonstrated, including reprogramming of the hypothalamic-pituitary-adrenal axis, stress responsivity, cognition, and post-pubertal growth. Mounting evidence points to a likely influence of maternal stress experience on reprogramming of the gut-brain axis. As acquisition of the intestinal microbiota begins at birth

and a stable microbial community develops from a succession of these key organisms, disruption to this program is associated with lasting effects on offspring immunity, metabolism, neurodevelopment, and behavior. Using our mouse model of EPS, we show that maternal stress experience disrupts a normal program of microbial dynamics during pregnancy across multiple body sites and these alterations parallel a sex-specific reprogramming of postnatal gut microbiota development in offspring. Colonizing control offspring with stress-altered maternal vaginal microbiota partially recapitulated microbiota patterns of EPS exposed offspring, indicating that the prenatal environment influences subsequent bacterial community assembly. As the mucosal immune system shapes postnatal host-microbe interactions, we tested the hypothesis that EPS disrupts the transcriptional and immune profiles of the fetal gut. Early prenatal stress exposure reprograms fetal intestinal transcriptional profiles in a sex-specific manner, including disruption of genes encoding innate immunity pathways in EPS male offspring. Further, EPS exposure alters sex-specific frequency of myeloid cells in the fetal gut and brain. Taken together, these studies support a link between maternal stress experience during pregnancy and sex-specific programming of the immune system in shaping subsequent host-microbe interactions.

Session IV: Sex Differences in Bones, Joints and Body Composition – From Puberty to Older Adulthood

Co-Chairs: Marcia Stefanik, Ph.D. (Stanford University) and
Deepika Laddu, Ph.D. (Stanford University)

Building bones: male vs. female mice (black vs white) and men versus women
Karl J. Jepsen, Ph.D. (Department of Orthopaedic Surgery, University of Michigan)

The goal of this presentation is to highlight the current state of knowledge regarding sex-specific differences in skeletal structure and mechanical function and how these differences contribute to musculoskeletal injury and disease risk. Secondary sex characteristics such as body size, bone morphology and bone strength differ greatly between men and women, a phenomenon described as sexual dimorphism. On average, female bones are more narrow and have less mass compared to males, even after adjusting for body size. Sexual dimorphism can be attributed largely to differences in skeletal growth, particularly during puberty. Sex-differences in the timing and rate of the decline in sex-hormones also contribute greatly to structural and strength differences between men and women. Together, the sex-differences in growth and aging contribute to the significantly greater risk of fragility fractures in women compared to men.

Although prior work has largely reported how men and women differ on average, recent work will describe how sexual dimorphism based on the behavior of the population mean is only telling part of the story. There is tremendous overlap in bone structure between the sexes, such that boys with slender (narrow) bones show a growth pattern that similar to girls with average to wide (robust) bones. Thus, an analysis of sex-differences based on inter-individual variation provides new insight into sexual dimorphism.

Finally, mouse models are widely used to study gene function and musculoskeletal disease. Recent studies have shown that although female mice have a smaller body weight than male mice, females have a proportionally stiffer and stronger bone relative to their body size compared to males. Thus, secondary sex characteristics in mice do not appear to fully mimic those of the human skeleton. These outcomes provide an important consideration when using the mouse to study sex-specific differences in musculoskeletal injury and disease.

Bone and Muscle Interactions during Puberty: Girls vs Boys
Mary B. Leonard, M.D., MSCE (Department of Pediatrics and Medicine, Stanford University School of Medicine)

Skeletal growth is characterized by sex and maturation- specific increases in cortical and trabecular architecture and volumetric bone mineral density. The anabolic skeletal response to muscle forces (i.e. the functional muscle-bone unit) is greatest during the peri-pubertal years. Early studies suggested that girls develop greater bone mass relative to muscle mass during puberty, compared with males. However, sex

differences in height and body proportions may confound these associations. We conducted a cross-sectional study of 665 healthy participants (310 male) ages 5–35 yr. Tibia peripheral quantitative computed tomography measures were made of cortical bone mineral content (BMC) and bone mineral density (BM.D.), periosteal (Peri) and endosteal circumferences, section modulus (Zp), and muscle area. Regression models were adjusted for tibia length, age, race, sex, and Tanner stage. Cortical BMC, Peri and Zp were lower in females than males in all Tanner stages (all $p < 0.001$), and the sex differences in Peri and Zp were greater in Tanner stage 5 (interaction, $p < 0.02$). Cortical BM.D. was greater ($p < 0.0001$) and endosteal circumference was lower ($p < 0.01$) in Tanner 3–5 females, compared with males. Muscle area was greater in males ($p < 0.001$), compared with females, and was progressively greater in Tanner stages 4 and 5. Adjustment for muscle area attenuated but did not eliminate sex differences in cortical dimensions. Associations between muscle and bone outcomes did not differ according to sex or race. In conclusions, sex was associated with maturation-specific differences in cortical BM.D. and dimensions that were not fully explained by differences in bone length or muscle. No sex differences in the functional muscle bone unit were identified.

Sex Differences in Physical Activity and Body Composition in Aging Cohorts
Deepika R. Laddu Ph.D. (Department of Medicine, Stanford University)

Excess gains in adiposity, and significant loss of lean mass (referred to as sarcopenia when accompanied by low physical function) may be risk factors for chronic disease in old age. Maintaining physical activity (PA) over time may attenuate the age-related body-fat gain and preservation of lean mass in older men and women (≥65yrs). The degree to which age-related changes in PA relate to body composition changes differ in older men versus women remains unclear. Using data from the Osteoporotic Fractures in Men Study (MrOS) and a subset of the Women's Health Initiative (WHI), we aimed to explore differences in the relationships of self-reported PA and body composition changes assessed by Dual-energy x-ray absorptiometry. Group-based trajectory modeling (MrOS) and mixed effects linear regression techniques (WHI) were used to examine the time-varying changes in PA as related to changes in body composition in older men and women, respectively. In 5964 MrOS men (mean age 73 years), *three distinct activity groups (all declining) were identified: low (42.8 %), moderate (50.0%) and high (7.2%)* over an average 6.9year follow-up*; the rate of decline in* PA patterns *appeared constant among each activity group. Joint trajectory modeling illustrated gradual* decreases in body weight, stable fat mass and steep declines in lean mass patterns among all activity groups. Among the 8,352 WHI women, PA levels significantly decreased over a 6-year follow-up ($p=0.0004$). Higher levels of PA attenuated gains in fat mass in women aged 50–59 years, whereas women aged 70-79 years lost fat mass at all PA levels (all $p=0.0006$); however, no significant effects on change in lean mass by PA were reported ($p=0.194$) over the 6-year follow up. These results demonstrate the need to develop appropriately timed, effective and tailored strategies to promote resistance training for the conservation of lean mass in older men and women during aging, for overall health and function in the latter years of life.

Sex-specific assessment of disease-associated muscle loss
Joshua F. Baker, M.D., MSCE (University of Pennsylvania and Philadelphia VA Medical Center)

The impact of systemic inflammatory disease on muscle loss has generally been presumed to be similar in men and women. However, muscle is regulated differently in men and women. Therefore, the use of sex-specific distributions was hypothesized to identify differential effects of chronic disease among men and women. Furthermore, relationships between adiposity and muscle estimates may differ between men and women. Therefore, methods to assess sex-differences should consider the potential for altered relationships between body composition components. We used National Health and Nutrition Examination Survey (NHANES) data to determine Appendular Lean Mass Index (ALMI) Z-Scores and Fat Mass Index (FMI) Z-Scores among 256 patients with RA. We also assessed relationships between ALMI and FMI Z-Scores within age-, sex-, and race-strata within NHANES and demonstrated altered relationships between ALMI and FMI Z-Scores. In two independent cohorts of patients with rheumatoid arthritis (Cohort 1: N=141; Cohort 2: N=115), we demonstrated lower ALMI Z-Scores among men with RA, suggesting greater muscle loss, relative to normative reference data, among men. The mean ALMI Z-Scores were below 0 for cohorts

[Cohort 1: -0.58 (1.17); Cohort 2: -0.15 (1.02)]. However, on average, the mean ALMI Z-Score was 1.17 lower for men in Cohort 1 and 0.51 lower for men in Cohort 2. Furthermore, adjusting for the normal observed muscle-fat association in NHANES, men also demonstrated greater muscle loss relative to the extent of adiposity [Cohort 1: β= -1.92; Cohort 2: Beta: -0.60. Comprehensive methods to define muscle loss in chronic inflammatory conditions should consider that chronic diseases may have sex-dependent effects. Alterations in sex-specific pathways (such as testosterone) may be relevant.

2:00 – 3:50: Sessions V and VI

Session V: Sex Differences in Stress Responses (Woodlands A)

Co-Chairs: Seema Bhatnagar, Ph.D. (Children's Hospital of Pennsylvania, University of Pennsylvania) and Laura Grafe, Ph.D. (Children's Hospital of Pennsylvania)

Orexins Contribute to Sex Differences in Habituation to Stress and in Cognitive Function
Laura A. Grafe, Ph.D. (Department of Anesthesiology Children's Hospital of Philadelphia)

Stress-related psychiatric disorders occur twice as frequently in women compared with men, however, we do not fully understand the neurobiology underlying these sex differences. The neuropeptides orexins are known to promote the acute stress response and are altered in anxious and depressed patients compared to controls. Our studies reveal that female rats exhibit higher orexin mRNA, neural activation, and concentrations in the cerebrospinal fluid compared to male rats under basal conditions. We proposed that these sex differences in orexin expression could explain heightened sensitivity to repeated stress in females. Namely, our experiments revealed that female rats did not habituate as fully to five days of repeated restraint as males assessed by: 1) sustained plasma levels of corticosterone and adrenocorticotropin hormone (ACTH) 2) persistent activation in the paraventricular nucleus (PVN) of the hypothalamus, and 3) continued struggle behavior displayed by females on day 5. To assess the role of orexins in these sex differences in the stress response, we inhibited orexin neuronal activation in females (via Designer Receptors Exclusively Activated by Designer Drugs) during repeated restraint. Orexin inhibition decreased activation in the PVN and basal corticosterone levels in female rats by day 5. Moreover, as stress related pathologies cause cognitive impairments, we tested cognitive flexibility after repeated restraint stress using an Attentional Set Shifting (AST) paradigm. We found that females displayed cognitive deficits, but inhibition of orexins throughout restraint improved subsequent cognitive function. Lastly, using chromatin immunoprecipitation and siRNA methods, we found that the glucocorticoid receptor contributes to the upregulation of orexin expression in females. As orexins regulate stress responses, cognitive function, autonomic responses, and emotional memory, targeting orexins may impact a range of psychiatric symptoms in a sex-specific manner.

Sex Differences in the Corticotropin Releasing Factor System: From Molecules to Circuits
Debra A. Bangasser, Ph.D. (Temple University)

Stress-related psychiatric disorders, such as major depression and posttraumatic stress disorder (PTSD), occur twice as frequently in women as in men. However, the neurobiological mechanisms that can contribute to this disparity are largely unknown. Hypersecretion of the stress-neuropeptide, corticotropin releasing factor (CRF), is linked to the pathophysiology of depression and PTSD. Therefore, our laboratory has been using rodent models to identify sex differences in responses to CRF that could increase female sensitivity to stress. We first identified sex differences in receptors for CRF that, in females, can increase the electrophysiological responses of neurons in the locus coeruleus, a brain arousal system. Next, we found that certain anxiety-related behaviors were greater in female than male rats following central CRF administration, particularly when female rats were in the proestrus cycle phase. Finally, our studies revealed that central CRF induced different patterns of brain network activation in males and females in different estrous cycle stages, suggesting that ovarian hormones alter the way brain regions work together to respond to CRF. Collectively, these studies reveal sex differences in CRF function from the molecular to the systems level. If similar sex differences are present in humans, they may help explain why women are more likely than men to suffer from psychiatric disorders that have CRF hypersecretion as an etiological factor.

Sexually divergent active and passive conditioned fear responses
Rebecca Shansky, Ph.D. (Northeastern University)

An individual faced with a threatening stimulus may respond in many different ways. The selection of either an active or passive response can predict long-term outcomes in clinical populations, but what leads to the selection of one over the other is unknown. Using a classic rodent model of Pavlovian cued fear conditioning, we found that female rats were four times as likely as males to exhibit an active, escape-like "darting" response to the conditioned stimulus, even though escape was not possible. We have now begun to investigate the neural substrates of darting by examining c-fos activity in the medial prefrontal cortex (mPFC) and periaqueductal gray (PAG) after fear conditioning in darting and freezing subpopulations. Darters exhibited greater activity in the dorsolateral PAG, which is consistent with active response strategies. Freezers exhibited greater rostral mPFC activity compared to the ventral mPFC, and the rostral:ventral ratio was tightly correlated to freezing in both Freezers and Darters. These data suggest that darting may be mediated by a shift in the balance of mPFC activity, resulting in enhanced dlPAG activation.

Interplay between Sex Steroids and Appetite-Regulating Hormones in Conditions Associated with Chronic Stress
Laura M. Holsen, Ph.D. (Brigham and Women's Hospital; Harvard Medical School)

Evidence from population-level studies indicates increased comorbidity between obesity and conditions associated with chronic stress, such as major depressive disorder (M.D.D), especially amongst women. The neurobiological substrates underlying this co-occurrence, and sex differences therein, remain unresolved. Recent evidence supports a shared pathway linking metabolic/appetite-regulating hormones with sex steroids, although data have emerged primarily from pre-clinical studies. Our work explores the interaction between stress hormones, sex steroid hormones, and metabolic biomarkers at 2 levels: the population level (Study 1), with men and women who are part of a birth cohort, and (Study 2) through focused clinical fMRI studies designed to characterize hormone-brain relationships associated with comorbid M.D.D and obesity in women. From Study 1, we will present data from analyses examining the interaction between BMI status (healthy weight; obese), M.D.D diagnosis (healthy control; recurrent M.D.D), and sex on biomarkers associated with appetite and metabolism. From Study 2, we will report on relationships between hormone levels and activity in food motivation circuitry, particularly in regions with the highest density of sex steroid hormone receptors. Within Study 2, we will additionally examine differences in these interactions from the perspective of opposing appetite/weight change symptoms during their most severe past major depressive episode, independent of BMI. These studies begin to characterize the hormone and brain phenotypes shared between chronic stress (as experienced in depression) and obesity. Our findings indicate distinct neurohormonal systems related to chronic stress and weight which may assist in developing sex-dependent strategies to treat appetite- and weight-related symptoms in M.D.D.

Session VI: What We Can Learn From Sex Differences In Multiple Sclerosis

Co-Chairs: Anat Bigeon, M.D. (Stony Brook Unviersity)
and Yael Deri, Ph.D. (Tel-Aviv University)

Sex Differences in Adult Multiple Sclerosis
Patricia K. Coyle, M.D. (Department of Neurology, Stony Brook University Medical Center)

Multiple sclerosis (MS) is the major acquired CNS disorder of young adults, short of trauma. There are very important sex differences in MS, which include gene expression demographics, comorbidities, prognosis, disease features, and perhaps therapeutic response. Understanding these sex based differences may provide important insights into MS etiology and management. This talk will review the various sex differences in this disease, current understanding of their significance, and implications for future studies.

Sex differences in pediatric MS
Louis Manganas, M.D., Ph.D. (Department of Neurology, Stony Brook University School of Medicine)

The clinical profile of Multiple Sclerosis (MS) appears similar among children and adults; however, several features differ between the two groups and within the childhood population. First, sex ratios are different between young children (male = female) and adolescents/adults (female>male) thus implicating a role for sex hormones in disease pathogenesis and/or modification of disease expression. These differences are believed to be hormone mediated with the onset of puberty. Second, early puberty in girls but not boys is associated with increased risk of MS. Finally, childhood obesity is independently associated with an increased risk for pediatric onset MS in girls but not boys. This may suggest a correlation between childhood obesity and female hormones or the X chromosome contributing the rise in female-to-male ratios.

Sex differences in animal models of Multiple Sclerosis (MS)
Yael Deri, Ph.D. (The Joseph Sagol Neuroscience Center, Sheba Medical Center, Tel Hashomer, Israel)

For reasons which have not been elucidated to date, young women are 2-3 times more likely to have MS relative to men. Using MOG-induced experimental autoimmune encephalomyelitis (EAE) as a model, we examined the hypothesis that the ambient gonadal hormone environment influences the degree of immune activation (neuroinflammation) in the brain and thereby modulates disease activity.
Male and female mice were induced with EAE and monitored for neuroinflammation, motor deficits and cognitive function over a 4 months period. Intact and adjuvant-injected mice served as controls. Females underwent daily vaginal smears to determined by the stage of the estrus cycle. Regional neuroinflammation was determined by quantitative autoradiography of TSPO (a reliable marker of glial activation) with [^{3}H]PK11195 in brain sections of animals killed at different stages of the disease.
We found that disease onset was significantly delayed in females first immunized in pro-estrus relative to estrus. Subsequently, more than half (56%) of the EAE females developed irregular cycles consistent with persistent estrus and cycle disruption was associated with decreased disease intensity; such that females with regular cycles displayed more severe clinical signs relative to non-cycling females and males.
Significant, region-dependent elevations in PK11195 binding; which correlated with disease activity, were found in both male and female EAE mice relative to sex-matched controls. However, the increases in males (range of reginal means~50-109%) were significantly larger than the increases in females (range 16%-82%) for the same level of clinical deficits. These results demonstrate that gonadal hormone status is a significant modulator of neuroinflammation, disease course and symptom severity in EAE; such that reproductively competent females are more sensitive to neuroinflammation relative to age matched males and fluctuations in estrogen and progesterone levels modulate disease onset and severity.

Advances in Neuroimaging and their impact on the future of MS diagnosis and treatment in men and women
Anat Biegon, Ph.D. (Departments of Neurology and Radiology, Stony Brook University School of Medicine)

In the last few decades, lesion load detected by conventional MRI has become the mainstay of clinical management in multiple sclerosis. Conventional MRI metrics, however, do not reveal specific underlying mechanisms related to disease onset and progression and have not contributed to our understanding of sex differences in MS, while Positron emission tomography (PET) and advanced MRI techniques are poised to offer new insights in this regard. PET is an exquisitely sensitive and quantitative imaging technique; and the number of PET radiotracers targeting specific molecular species is constantly increasing. Tracers successfully used in small cohorts of MS patients include tracers for neuroinflammation, neurodegeneration, demyelination and re-myelination. PET studies of neuroinflammation using the TSPO tracer [^{11}C]PK11195 as well as 2nd generation TSPO ligands show sensitivity to disease progression and preliminary evidence for treatment response. Other tracers such as [^{11}C]PIB and its congeners, originally developed as amyloid imaging agents, are providing highly sensitive and quantitative metrics of white matter integrity. More recently developed tracers likely to be informative in MS as well as relevant sex differences are those targeting estrogen synthesis, adenosine receptors and glutamate NM.D.A receptors. Quantitative measures of molecular targets with PET could also be used in the future to identify novel treatment targets and in clinical trials of new drugs in men and women.

Thursday, May 26

8:00 – 9:50: Sessions VII & VIII

Session VII: Sex, Inflammation and Stroke (Woodlands A)

Co-Chairs: Halina Offner, M.D. (Oregon Health & Science University)
and Abby Dotson, Ph.D. (Oregon Health & Science University)

Sex differences in stroke inflammatory mechanisms
Patricia Hurn, Ph.D. (University of Texas)

Stroke is known to be a sexually dimorphic disease. Increasingly, differences in stroke pathophysiology, disease course and recovery between men and women have come under study. The purpose of this presentation is to highlight some of the key sex differences in clinical and experimental stroke and cerebrovascular disease, with an emphasis on inflammation arising both in the brain and the periphery. Translation from animal and cell models to patients will be discussed.

The inflammatory response originating from the spleen to the ischemic brain
Keith Pennypacker, Ph.D. (University of South Florida)

Many studies have recently demonstrated that the spleen plays a central role in the immune response to stroke, yet few have been successful in describing the precise splenic mechanisms leading to neurodegeneration. Our laboratory was the first to demonstrate that splenectomy decreases infarct volume. Importantly, we have spent the past decade elucidating the inflammatory signals and cell types involved. We have identified the splenic immune cells (monocytes, NK and T) that migrate to the injured hemisphere following experimental stroke. We have also shown that systemic administration of the pro-inflammatory cytokine IFNγ abolished the protective effects of splenectomy, and administration of IFNγ blocking antibodies reduced injury. Moreover, IFNγ activates and induces expression of IP-10 in microglia. IP-10 attracts IFNg-expressing T cells to the injured hemisphere and drives a Th1 response while inhibiting the Th2 one. The spleen-derived neurodestructive signaling involves IFNγ–associated activation of microglia, which leads to a feed forward signal through IP10 to attract more IFNγ-expressing T cells. This leads to the additional expression of IP-10 in M1 microglia to further exacerbate stroke-induced neurodegeneration. Similar studies using female rats indicate their splenic response to stroke differs from males. Thus, targeting this inflammatory pathway as a treatment for stroke could be in a sex dependent manner.

Sex, stroke and the immune response
Abby Dotson, Ph.D. (Oregon Health & Science University)

Ischemic stroke is a leading cause of death and disability in the United States. It is known that males and females respond differently to stroke. The immune response is a critical factor in determining the progress of neurodegeneration after stroke and is fundamentally different for males and females. Additionally, females respond to stroke therapies differently than males, yet are often left out of the basic research focused on developing those therapies. With a resounding failure to translate stroke therapies from the bench to bedside, it is clearer than ever that inclusion of both sexes in stroke studies is going to be essential for future clinical success.

Sex and age differences in the effectiveness of miRNA as stroke neuroprotectants
Farida Sohrabji, Ph.D. (Women's Health in Neuroscience Program, Department of Neuroscience and Experimental Therapeutics, Texas A&M University College of Medicine)

Short non-coding RNA (microRNA) act as translational repressors and regulate large numbers of genes in diverse cell types. As a result, they are an attractive candidate for neuroprotective therapies in diseases such

as stroke which activates cells in central and peripheral tissues. Our studies have focused on identification of neuroprotective miRNA that are effective in older populations, specifically females, a demographic with high stroke prevalence. Two strategies were pursued: a targeted approach and a discovery approach, with the goal of improving stroke outcomes as assessed by infarct volume and sensory motor behavior. In the case of the targeted approach, we focused on miRNAs that regulate insulin-like growth factor (IGF)-1, a peptide hormone that we have previously reported to reduce infarct volume and improve motor behavior in middle aged females. Several microRNA including Let7f and mir1 have a consensus binding site in the 3' UTR of the IGF-1 gene. Using antagomirs to Letf7, we found that this treatment reduced infarct volume and improved sensory motor performance when administered ICV to adult females 4h post stroke. However, this treatment had no effect on adult males, and paradoxically increased stroke impairment in middle aged females. Our second strategy was based on a discovery approach. In this approach, we compared circulating microRNA profiles from groups of adult and middle aged males and females. Adult females have the best stroke-related outcomes, while age-matched males and middle aged males and females have worse outcomes. Six miRNA were found to be directly correlated with stroke outcomes, such that expression of these miRNA was elevated in the group with the best stroke outcomes. We found that inhibition of one of these miRNA (mir363), impaired stroke outcomes in adult females and that mimetics improved stroke outcomes in middle aged females. Surprisingly, these mimetics were ineffective in males. Collectively, our studies show that miRNA can be modulate stroke-induced infarction and behavioral changes, but that their effect is age and sex specific.

Session VIII: Early Cellular, Synaptic, and Circuit-Level Biomarkers of Sex Differences in Memory Decline

Co-Chairs: Jill M. Goldstein, Ph.D. (Harvard Medical School) and
Emily G. Jacobs, Ph.D., Harvard Medical School

Estrogenic regulation of hippocampal function in male and female rodents
Karen M. Frick, Ph.D. (Department of Psychology, University of Wisconsin-Milwaukee)

The risks of age-related memory loss and dementia are greater in women than in men. Although estrogen loss at menopause likely plays a role, estrogen therapy is beneficial for cognition in only some menopausal women and exposes women to dangerous side effects including cancer and stroke. Reaping the beneficial effects of estrogens on memory without these side effects will require a more precise understanding of the molecular mechanisms through which estrogens regulate memory formation. This talk will discuss work in female and male mice identifying cellular processes and receptors in the dorsal hippocampus necessary for estradiol to enhance memory consolidation and increase hippocampal and prefrontal dendritic spine density. Implications of this work for age-related memory loss will be discussed.

Synaptic Health: Implications for Cognitive Aging
John. H. Morrison, Ph.D. (University of California at Davis)

We have been addressing the synaptic basis of age-related cognitive decline as well as the preservation of synaptic health and cognitive performance. In a nonhuman primate (NHP) model, we have found age-related synaptic alterations in prefrontal cortex (PFC) that correlate with cognitive decline. For example, thin spines on pyramidal cell dendrites in PFC suffer a dramatic loss with age that is highly correlated with cognitive decline, whereas mushroom and stubby spines appear unaffected by age. The same class of highly plastic spines that is vulnerable to aging is protected by cyclical estradiol treatment, suggesting that protection against age-related decline is feasible. In addition, the density of pathologic mitochondria at PFC synapses correlates with cognitive impairment, and estradiol decreases the presence of pathologic mitochondria in synapses. Synaptic aging in the hippocampus follows a different pattern. For example, in the dentate gyrus (DG), there is minimal overall synapse loss. However, synaptic complexity decreases with age, and there appears to be an age-related failure of AMPA receptor insertion in the large, stable synapses that correlates with cognitive decline. Thus, the DG and PFC differ in the key elements of synaptic health that are vulnerable to aging, and preserving healthy synaptic phenotypes will help preserve cognitive function.

Sex and reproductive aging shape early changes in memory circuitry in midlife
Emily G. Jacobs, Ph.D. (Harvard Medical School)

Cognitive neuroscience of aging studies traditionally target participants age 65 and older. However, converging preclinical and human evidence indicates that the decline in ovarian estradiol production during the menopausal transition may play a mechanistic role in neuronal changes that occur earlier in the aging process. In this talk, we step back by over a decade to characterize the changes in memory circuitry that occur in early midlife (age ~45-55), as a function of sex, women's reproductive stage and sex steroid hormone concentrations. Functional MRI and behavioral findings from a series of studies demonstrate that reproductive stage, independent of chronological age, impacts regional and network-level connectivity in memory circuitry, most prominently in the hippocampus and prefrontal cortex. Critically, analyzing data without regard to sex obscured group differences in the circuit-level neural strategies associated with successful memory performance. Taken together, these findings underscore the importance of considering reproductive stage, not simply chronological age, to advance our understanding of memory function in the middle decades of life.

Prenatal Immune Programming of Sex Differences in Memory Aging
Jill M. Goldstein, Ph.D. (Harvard Medical School)

Healthy aging and maintaining intact memory function are critical public health concerns as the population becomes increasingly aged. Hypothalamic pituitary adrenal (HPA) axis modulates inflammatory responses and microglial activation across the lifespan, and neuroinflammation is associated with memory circuitry deficits. These immune-HPA connections have given rise to so-called prenatal stress-immune models of aging. We demonstrated that if these fetal stress-immune risk factors occur during a critical period of the sexual differentiation of the brain (2^{nd}-3^{rd} trimester), they impact highly sexually dimorphic brain regions in sex-dependent ways. These regions include stress and memory circuitry regions [hippocampus (HIPP), dorsolateral prefrontal (DLPFC), anterior cingulate (ACC) and inferior parietal (iPAR) cortices]. 200 adult siblings, discordant for preeclampsia (PE) or fetal growth restriction (FGR), were selected from 17,741 pregnancies in the New England Family Study, a representative sample of those receiving prenatal care in Boston-Providence from 1959-1966. Mothers were followed during pregnancy and sera was stored at NIH, from which we assayed TNF-α, IL-1β, and IL-6 (the primary co-activators of HPA axis) and IL-10 for its anti-inflammatory action. Offspring were re-recruited at 45-55 years of age and completed clinical and cognitive assessments, and structural and functional MRI/DTI using working memory and verbal encoding tasks. Using mixed linear models controlled for intrafamilial correlation within sibships and age, we found PE/FGR exposure was significantly related to TNF-α and TNF-α:IL-10, particularly for female offspring. Lower TNF-α :IL-10 ratio was significantly related to worse memory performance in women than men, which was associated with: smaller ACC volume, hyperactivity in inferior parietal cortex and DLPFC in women vs. men, strongest for postmenopausal women. Further, functional connectivity analyses showed low inferior parietal and DLPFC connectivity significantly associated with low TNFa:IL-10 exposure and low DLPFC-Hippocampal connectivity, particularly in postmenopausal women. We consistently found that prenatal exposure to *low* TNFa:IL10 ratio was associated with adverse consequences for the female brain in contrast to a *high* TNFA:IL10 ratio related to adverse impact on the male brain. *Low* TNFa:IL10 was significantly associated with *high* prenatal cortisol exposure suggesting an impact of prenatal glucorticoid excess particularly on the female fetal brain. In adulthood this impact was reflected at both the cognitive (verbal memory) and neural (memory circuitry structure/function) levels, strongest in postmenopausal women, suggesting an impact of reproductive aging on revealing vulnerabilities resulting from prenatal exposures.

10:10 – 12:20: Sessions IX and X

Session IX: Sex Differences in Cardiovascular Disease

Co-Chairs: John N. Stallone, Ph.D. (Texas A&M University) and
Amutha Selvamani, Ph.D. (Texas A&M University)

The sex chromosome complement contributes to ischemic stroke sensitivity and inflammatory responses in aged FCG mice

Javiera Bravo-Alegria, Ph.D. (Department of Neurology, McGovern Medical School, University of Texas Health Science Center)

Aging is the most important non-modifiable risk factor for ischemic stroke; modeling stroke in aged animals is of clinical relevance and translational value. Stroke is a sexually dimorphic disease. Elderly women not only have higher stroke incidence than age-matched men, but also have poorer recovery, higher morbidity and mortality once a stroke occurs. Much of this has been attributed to the loss of estrogen with menopause. However, stroke incidence does not begin to climb until well after natural menopause, suggesting there are hormone independent effects on ischemic sensitivity. As hormone levels are relatively equivalent between sexes at the age post-menopausal, tissue damage and functional outcomes must be influenced by biologic sex (XX vs. XY) in addition to the hormonal milieu. We hypothesized that sex differences in ischemic stroke in aged brains are shaped by the sex chromosome complement. The Four Core Genotype (FCG) mouse model was utilized to dissociate the effects of sex chromosome compliment from hormone exposure on ischemic sensitivity. XXF, XXM, XYF, XYM and XYwt aged mice (18-20 months) were subjected to 90-minute middle cerebral artery occlusion (MCAO). Stroke outcomes were examined at 3d of stroke. Inflammatory responses were also evaluated by flow cytometry and ELISA analysis. At 3d of stroke, more microglial activation and higher levels of pro-inflammatory cytokines (IL-1β and TNF-α) were seen in XXF vs. XYF and in XXM vs. XYM/XYwt mice respectively. Accordingly, XXF and XXM mice had significantly larger infarct volumes than XYF and XYM/XYwt cohorts respectively. There was no significant difference in hormone levels in aged FCG mice. The sex chromosome complement contributes to ischemic sensitivity in aged animals, which is likely mediated in part by innate immune responses.

Role of T cells in the development of sex differences in cardiovascular disease and hypertension

Jennifer C. Sullivan, Ph.D. (Department of Physiology, Augusta University)

There is a growing basic science literature regarding the role of T cells in the pathogenesis of hypertension and blood pressure control, however, the majority of this literature has been performed exclusively in males despite the fact that both men and women develop hypertension. This is especially problematic since hypertension is well-recognized as having distinct sex differences in the prevalence, absolute BP values, and molecular mechanisms contributing to the pathophysiology of the disease. In addition, T cells have been suggested to link multiple organs in the control of blood pressure, and the available data in the literature suggests that T cells may hold the key to explaining the basis of sex differences in hypertension. Both the sex of the subject and sex of the T cell have been shown to impact blood pressure responses to hypertensive stimuli and our group has established that there are distinct sex differences in both the renal T cell profile and how blood pressure impacts the T cell profile. Specifically, female spontaneously hypertensive rats (SHR) have more anti-inflammatory T regulatory cells (Tregs) compared to males. Furthermore, either preventing age-related increases in blood pressure or lowering blood pressure in SHR decreases renal Tregs only in females abolishing the sex difference. These results have led us to hypothesize that female SHR have a compensatory increase in Tregs to limit increases in BP. More studies are needed to better understanding of the role of T cells in blood pressure control in both sexes.

Effects of Age and Sex on Stroke and Cerebrovascular Function in the Rat Middle Cerebral Artery

John N. Stallone, Ph.D. (Texas A&M University)

There is a prominent sexual dimorphism in the incidences of cardiovascular disease (CVD) and stroke and the risk of developing these diseases increases with advancing age. However, most research models of CVD and stroke and studies of the effects of estrogen (EST) on these diseases utilize young animals, raising the question of relevance. In the present studies, the effects of age and sex on cerebrovascular function and the effects of age and exogenous EST on stroke and cerebrovascular function were studied using a rat model that includes mature/multigravid cyclic (MA) and ovarian aged/reproductive senescent acyclic (RS) female rats. While estrogen replacement reduced brain injury and improved neurological

recovery following ischemic stroke in younger (MA) rats, it enhanced brain injury and reduced neurological recovery in aged, senescent (RS) rats. In isolated, pressurized middle cerebral arteries (MCA), vasopressin (VP)-induced vasoconstriction was reduced with age in intact female rats (RS < MA) but was unchanged in male rats. MCA prostanoid (PG) release (PGI_2 and TxA_2) was also reduced with age in female (RS < MA) but not male rats. In ovariectomized (OvX) or OvX+EST-replaced MA and RS female rats, beneficial, protective effects of EST on MCA are evident (decreased vasoconstriction to VP, increased dilator PG) in younger MA, whereas in older RS, detrimental effects of EST begin to appear (enhanced vasoconstriction to VP, increased constrictor PG). These data reveal that vascular effects of EST are distinctly age-dependent in female cerebrovasculature and with ischemic stroke.

Genomic Sex-specific Contribution to Endothelial Cell Phenotype Heterogenity: from Message to Metabolism

Virginia H. Huxley, Ph.D. (Center for Gender Physiology, Departments of Medical Pharmacology and Physiology, and Medicine (Division of Pulmonary and Critical Care), University of Missouri School of Medicine)

Prior to the diagnosis of several cardiovascular diseases displaying sexual dimorphism with respect to incidence, severity, and mortality there is evidence of endothelial dysfunction. In our *in vivo* and isolated vessel studies of barrier function in health and disease we observed sex differences with respect to not only basal microvascular exchange but also permeability responses to a variety of vascular stimuli prior to and following sexual maturity. To further define the mechanisms responsible for sex differences in endothelial cell (EC) phenotype we have concentrated recently on primary culture of EC from aorta (macro-, conduit vessel, extensive literature), skeletal muscle (microvessel, huge surface area, metabolically active and well studied) and mesentery (microvessel, low metabolic demand, site of *in vivo* studies) of sexually mature rats of both sexes. These low passage cells were grown under identical conditions in the absence of additional or oscillating reproductive hormones; the genomic sex of the cells was confirmed by the presence (male) or absence (female) of the SRY gene. After conducting multiple measures of phenotype we first confirmed the previously observed, but little appreciated, fact that EC phenotype is organ-specific. Second, and previously unknown finding is that organ-specificity is accompanied sex-differences in morphology, growth, metabolism, message, protein expression, and wound healing. The emerging picture, while complex, makes it self evident that susceptibility, natural history, and disease progression would be sex-specific necessitating the development of targeted diagnosis and treatment for males and females, respectively.

Session X: Late Breaking Research in Sex Differences

Co-Chairs: Crystal West, Ph.D. (Georgetown University) and
Amrita Pai, Ph.D. candidate (Georgetown University)

Sexual modulation of neural circuits and behavior in the nematode *C. elegans*

Douglas S. Portman, Ph.D. (University of Rochester)

An individual's biological sex can influence the development and function of its nervous system through both cell-autonomous ("genetic") and non-autonomous (hormonal) activities. Simple invertebrate models like the nematode *C. elegans* offer opportunities to understand the biological principles by which these activities sculpt shared regulatory pathways to bring about sex differences in brain and behavior. Though the *C. elegans* nervous system is vastly less complex than that of a mammal, it employs of the same developmental and physiological regulatory pathways. Some sex differences in the *C. elegans* nervous system are anatomical: particular neurons are present only in one sex or the other. Ongoing work in our laboratory and others is investigating the mechanisms by which genetic sex brings about these developmental differences. Other sex differences in the *C. elegans* nervous system are functional. For example, even though the behavioral responses to food and sex pheromones are controlled by shared circuits (sexually "isomorphic" circuits), these behaviors can exhibit marked sex differences. We have found that these sex differences are specified by the genetic sex of the nervous system itself, and that the physiological properties of individual neurons can by "tuned" by sex to modulate behavioral outcomes. Chemosensory neurons are a particular

focus of this regulation: genetic sex modulates gene expression in these cells to modulate their sensory properties, allowing the salience of particular stimuli to be determined by sex differences in perception. Understanding how genetic sex modulates gene expression is an important current challenge. Moreover, even though gene expression differences are determined cell-autonomously by genetic sex, they do not become apparent until adulthood, indicating that yet-unknown mechanisms "gate" this sexual regulation according to developmental stage. We expect that understanding the molecular-genetic mechanisms through which genetic sex and developmental cues intersect to modulate gene expression should shed light onto similar phenomena occurring in more complex systems.

Sex differences in the effects of microglia on neonatal neurogenesis and behavioral development
Lars H Nelson B.S. (Neuroscience Graduate Program, The Ohio State University)

Many neuropsychiatric disorders with developmental onset involve altered immune signaling and show a significant sex bias with prevalence toward males. The male brain has more activated microglia, the innate immune cell in the CNS, during development than the female brain (Lenz et al., 2013, J Neurosci; Schwarz et al., 2012 J Neurochem), but the role of these sex differences in normal brain development is largely unknown. Microglia regulate the number of proliferating cells in the developing cortex by releasing diffusible factors and phagocytizing healthy progenitors (Cunningham et al., 2013, J Neurosci; Shigemoto-Mogami et al., 2014, J Neurosci). During development there is a basal sex difference in hippocampal neurogenesis in rats, with males having more neurogenesis than females (Zhang et al., 2008, Eur J Neurosci), thus we hypothesized that microglia may contribute to sex differences in both normal and abnormal cell genesis during ontogeny. We tested whether microglia regulate the sex-specific rate of proliferation in the neonatal hippocampus by temporarily ablating microglia using central infusion of liposomal clodronate. Clodronate treatment decreased proliferation in the hippocampus of males, but not females, as determined by the number of BrdU+ cells. We also analyzed microglial morphology in the hippocampus at postnatal day 2-3. We found that females had more microglia with phagocytic cups than males and neonatal estradiol treatment masculinized the number phagocytic cups in females. We also found that estradiol increased microglial proliferation in the hippocampus. Ongoing experiments are testing the target of microglial phagocytosis in the developing hippocampus as well as the long-term behavioral consequences of early life microglial ablation. Together, these studies give important insight into the role of neuroimmune cells in normal brain development and thus how perturbations that induce immune activation may disrupt development of brain and behavior.

Cocaine seeking during initial abstinence is driven by noradrenergic and serotonergic signaling in dorsal hippocampus in a sex-dependent manner
Amy S. Kohtz Ph.D. (Brain Health Institute, Rutgers University)

There are substantial sex differences in cocaine addiction, demonstrated by both clinical and pre-clinical studies. Notably, females are more sensitive than males to stress-induced drug-seeking and relapse. Locus coeruleus norepinephrine (LC-NE) and dorsal raphe serotonin (DR 5-HT) systems are involved in stress responses and may drive signaling involved in stress-induced drug-seeking behavior. The dorsal hippocampus (DH) is a stress-sensitive focal brain region in the stress response pathway, and receives strong inputs from LC-NE and DR 5-HT neurons. We hypothesized that NE and 5-HT neurotransmission in DH is involved in drug-seeking behavior during the initiation of abstinence (extinction day 1, ED1), a stressful event involving abstinence from drug. ED1 increased Fos expression in DH, LC, and DR, and these changes in Fos correlated positively to drug-seeking behavior on ED1. We observed decreased drug-seeking behavior on ED1 following combined systemic β-adrenergic (β-AR) and 5-HT receptor antagonism in both male and female rats. We then investigated the effects of specifically blocking 5-HT and β-AR neurotransmission in DH on drug-seeking during ED1 by infusing a cocktail of betaxolol plus ICI-118,551 (β1 and β2 antagonists), WAY100635 plus GR127935 (5-HT1A/1B receptor antagonists), or S-propranolol. In males, 5-HT antagonism was most effective in reducing drug-seeking on ED1, whereas β-AR antagonism was ineffective. In contrast, S-propranolol was most effective in females in reducing drug-seeking on ED1, and 5-HT and β-AR antagonists were each partially effective. We further examined pharmacogenetic effects of inhibiting signaling from the DR to the DH via DREADDs, and found similar effects for inhibition of DR-DH

signaling to decrease ED1 drug-seeking, as in our pharmacological experiments. Our results show that drug-seeking during initial abstinence involves 5-HT and β-AR signaling in female DH, but only 5-HT signaling in male DH.

Sex-specific mechanisms of songbird audition depend on membrane estrogen receptor activation

Amanda A. Krentzel B.S. (Neuroscience and Behavior Program, Center for Neuroendocrine Studies, Department of Psychological and Brain Sciences, University of Massachusetts Amherst)

Auditory processing is essential in both sexes; however, due to the sensitivity that the auditory system has to hormonal influences, auditory mechanisms may differ between the sexes. Estrogen receptors and intracellular signaling pathways are influenced by early sexual development. In the zebra finch brain, the caudomedial nidopallium (NCM) is a secondary auditory region that is involved in song discrimination. This region synthesizes its own estrogens and local estradiol application increases auditory-evoked firing activity rapidly, suggesting mediation from a membrane receptor. It is unknown which estrogen receptor facilitates changes in audition and whether this mechanism is the same between the sexes. We hypothesized that the membrane estrogen receptor GPER1 is both necessary and sufficient in mediating auditory responsiveness in NCM. Using *in vivo* extracellular electrophysiology, males and females were exposed to songs while either an agonist (G1) or antagonist (G36) for GPER1 was infused into NCM. We grouped single units by action potential length into narrow cells and broad cells (putative inhibitory and excitatory neurons, respectively). Our findings show that narrow cells respond more to auditory stimulation than broad cells in males; however, there is a similar response between the cell types in females. During G36 infusion, narrow cells in males and broad cells in females each exhibit diminished auditory-evoked activity such that the cell types resemble the baseline condition of the other sex. Therefore, GPER1 appears to play a role in maintaining a sex difference in higher order sensory processing. Experiments exploring the sufficiency of GPER1 activation using G1 are currently ongoing. In summary, GPER1 is a necessary membrane receptor in coding of complex, auditory physiology, and it contributes to a previously undescribed sex difference of auditory processing in NCM. Rapid estrogenic actions are likely contributing to the maintenance of sexually dimorphic circuits in adulthood.

An XX sex chromosome complement increases obesity, lipids, and atherosclerosis in hypercholesterolemic mice

Yasir Alsiraj, M.S. (Department of Pharmacology and Nutritional Sciences, University of Kentucky)

Background: Obesity, lipids, and atherosclerosis are sexually dimorphic; the underlying mechanisms that contribute to these dimorphisms are not well understood. Most studies have focused on sex hormones as primary mediators of sexual dimorphism of these diseases; however, sex chromosome complement (XX and XY) is another factor differing between males and females that could contribute to sexual dimorphism. We hypothesized that sex chromosome complement influences the response to Western diet-induced obesity, lipids, and atherosclerosis. *Methods and Results:* Transgenic male mice with deletion of Sry from the Y-chromosome expressing Sry on autosomes (8-12 weeks of age) were bred to female *Ldlr*$^{-/-}$ mice to generate female mice with an XX or an XY sex chromosome complement. Female mice (XX and XY) were fed a Western diet for 3 months to induce obesity and atherosclerosis. Body weight was measured weekly, atherosclerotic lesions were measured by enface analysis, and lipids were measured at study endpoint. XX females exhibited significant increases in atherosclerotic lesion surface areas in the aortic arch (XX, 26% ± 2.15; XY, 18% ± 3.3; $P<0.05$) and elevated serum concentrations of cholesterol (XX, 2501 ± 192; XY, 890 ± 141 mg/dl; $P<0.05$), VLDL (XX, 1351 ± 165; XY, 374 ± 67 mg/dl; $P<0.05$), LDL (XX, 1312 ± 58; XY, 525 ± 81 mg/dl; $P<0.05$), and HDL (XX, 147 ± 21; XY, 109 ± 6 mg/dl; $P<0.05$) compared to XY females. Moreover, XX females exhibited significant increases in body weight when challenged with the Western diet compared to XY females (XX, 41.2 ± 2.4; XY, 31.7 ± 2.5 g; $P<0.05$). *Conclusion:* These results demonstrate that when challenged with a Western diet, female XX *Ldlr*$^{-/-}$ mice have increased body weight, serum cholesterol concentrations and atherosclerosis compared to XY females. Future studies will identify gene targets influenced by sex chromosome complement and the role of sex hormones in regulating the XX phenotype.

Aged females exhibit enhanced pro-inflammatory leukocyte phenotypes

Meaghan A. Roy-O'Reilly M.S. (Department of Neurology, University of Texas Health Sciences Center at Houston)

Young women exhibit more robust immune responses than young men, yet it is unknown whether this phenomenon persists in elderly subjects. As the aging population experiences a heavy burden of inflammation-driven diseases such as stroke and atherosclerosis, we examined the effects of sex and age on pro-inflammatory leukocyte phenotypes in mice and humans. Flow cytometry was conducted on peripheral leukocytes isolated from young (3-month) and aged (18-month) C57BL/6 mice, with follow-up studies on cryopreserved samples from human patients. We found that granulocyte percentages were significant increased by age and female sex (p=.0048, p=.0012). Granulocyte and monocyte ROS production, which plays a pathological role in many inflammatory diseases, was also found to be significantly higher in female animals (p=.02). While the proportion of CD8+ T cells increased significantly with age in animals of both sexes (p=<.0001), the proportion of primed, highly cytotoxic *effector memory* $CD8^+$ T cells was significantly higher in aged females (p=.0313). In addition, CD4 T cells expressing CXCR3, which are capable of a TH1-type response, were significantly higher in females regardless of age (p=.0001). Subsequent analysis in samples from elderly human patients revealed similar results, with females exhibiting significantly higher numbers of CD4 TH1-type effector cells. In conclusion, age has significant effects on immune cell populations, including an increase in granulocytes and an increased CD8/CD4 cell ratio. There is a significant interaction of female sex with enhanced levels of pro-inflammatory populations, including ROS-producing myeloid cells, effector memory cytotoxic CD8 T cells and CD8-supporting TH1 CD4 T-cells. Understanding the mechanisms and consequences of the interactive role of age, sex and inflammation may provide insight into novel therapeutic targets for both sexes.

Role of estrogen and nitric oxide in the sex difference of fat metabolism and survival during fasting

Mika Jikumaru M.D., Ph.D. (Department of Biochemistry & Molecular Pathology, Osaka City University Medical School, Osaka, Japan; Department of Neurology, Faculty of Medicine, Oita University, Oita, Japan)

Although various methods for caloric restriction to prolong life time have been proposed, they often impair physiological metabolism and causes various diseases such as anorexia nervosa particularly in female human subjects. To understand the pathologic events induced by caloric restriction, we analyzed the changes in lipid metabolism in male and female mice during fasting. To elucidate the cross-talk of lipid metabolism and inflammation, we compared its effect on wild (WT) and iNOS knock-out (iNOS-/-) male and female mice. Fasting decreased the body weight with concomitant decrease in subcutaneous adipose tissue more rapidly in male than in female. Plasma glucose decreased rapidly within 1 day with concomitant increase in non-esterified fatty acid (NEFA). Although plasma glucose decreased more markedly in iNOS-/- than in WT, all other events occurred significantly slowly in the former. Under fasting conditions, female mice survived longer than male animals. Plasma levels of ketone bodies transiently increased with WT male while those in female maintained fairly high. Although the increase in plasma NEFA was smaller in iNOS-/- than in WT, the increase of ketone bodies was significantly higher with male and female iNOS-/- (♂<<♀) than those with WT groups. Since ketone bodies are important fuels for vital organs, such as brain and heart, their high levels might participate in the survival of animals. In fact, the lifetime of animals showed positive correlation with plasma levels of ketone bodies. All the events observed with female were suppressed by ovariectomy via an estrogen-inhibitable mechanism. These observations suggest that estrogen increases the survival of animals by optimizing fat metabolism to maintain high levels of plasma ketone bodies, major fuels for vital organs, such as the brain and heart, during fasting and that iNOS-derived NO enhances futile metabolisms require for inflammatory reactions that waste fuels in adipocytes.

12:20 – 1:50:

Lunch Discussion: Research Methods for Studying Sex

Co-Chairs: Susan Philips, M.D., M.Sc. (Queen's University) and Robert Juster, Ph.D. (Columbia University)

This symposium will look at rationales and methodologies for including sex/gender in observational studies, cellular and animal experiments, basic science investigations, randomized controlled trials, systematic reviews, and qualitative research. Across all designs we will address ways to incorporate sex/gender in framing the research question, interpreting findings and making evidence-based recommendations. Panelists will draw upon their own scholarly work in these areas and the research of others, and will provide and critique current tools and guidelines from key funders. There will be 5 minutes of questions at the end of each talk and then a general discussion after all talks.

Sex/gender in basic science research and pre-clinical research
Stacey Ritz, Ph.D. (McMaster University) **and Robert Juster, Ph.D.** (Columbia University)

Sex/gender in quantitative and qualitative clinical research
Susan Phillips, M.D., M.Sc. (Queen's University) **and Katarina Hamberg, M.D., Ph.D.** (Umea University)

Integrating sex/gender in systematic reviews
Sari Tudiver, Ph.D. (Cochrane Collaboration)

2:00 – 3:30: Sessions XI & XII

Session XI: Sex Differences in Addiction (Woodlands A)

Co-Chairs: Alicia Allen, Ph.D., MPH (University of Minnesota) and
Erin Emme, MPH (Yale University)

Progesterone and impulsive behavior in cigarette smokers
Alicia Allen, Ph.D., MPH (University of Minnesota)

For unknown reasons, women cigarette smokers are more likely than men to relapse from a quit attempt. While impulsivity and progesterone have both been implicated as predictors of quit outcomes, little is known about the relationship between these two variables and how they may vary by sex. This secondary-data analysis project utilizes data collected at the screening visit from an ongoing larger smoking cessation trial. Participants were men and women (in the follicular phase) who were between the ages of 18 and 40, smoked ≥10 cigarettes/day, were motivated to quit smoking, and were in stable physical/mental health. Use of exogenous hormones and/or psychotropic medications was exclusionary. Progesterone was measured via a plasma sample. Impulsivity was assessed with two tasks (GoStop, Balloon Analog Risk Task [BART]) and one measure of self-report (Barratt Impulsiveness Scale [BIS]). Linear regression models used sex, progesterone and a sex by progesterone interaction to predict impulsivity scores, using an alpha of 0.10. Participants (n=60; 33% female) were, on average, 31.6±0.8 years old and smoked 14.6±0.7 cigarettes/day. Compared to men, women scored significantly lower on "risk taking" per the BART (21.6±15.0 vs. 35.8±15.7, respectively; p=0.002). In both men and women, a higher progesterone level was associated with lower response inhibition on the GoStop task (β=-29.1±15.7, p=0.07). Last, on the BIS Motor Impulsiveness subscale, the association between progesterone and the "tendency to act on the spur of the moment" was stronger in women than in men (β=-10.4±6.0, p=0.09). No other statistically significant trends were observed. These data suggest that within this small cross-sectional sample of smokers, there is a sex difference in risk-taking behavior, and that progesterone may be associated with some aspects of impulsivity. Additional research is needed to explore the sex difference in how these variables may influence cessation outcomes.

Sex difference in stress biology, tobacco addiction, and predictors of relapse
Mustafa al'Absi, Ph.D. (University of Minnesota School of Medicine)

Accumulating evidence demonstrates the role of stress in smoking relapse, and recent findings show sex differences in hypothalamic-pituitary-adrenocortical (HPA), endogenous opioid function, and stress response. Female smokers report more distress after exposure to acute stressful situations than do male smokers. Women also have more difficulties maintaining smoking abstinence than men. This presentation will cover

recent studies focusing on sex differences in biological, psychophysiological, and mood measures during withdrawal and in response to stress in the context of smoking cessation. Results show that women report greater desire to smoke than men after period of abstinence and in response to acute stress. Adrenocorticotropic (ACTH), cortisol, and blood pressure responses are more pronounced in men than in women, and female smokers evidence a blunted cortisol response as compared to female non-smokers. While stress hormones tend to predict smoking relapse in all smokers, the direction of prediction seems to be sex dependent. Lower hormonal responses to stress predict relapse in men, whereas greater response predict relapse in women. Intensity of withdrawal symptoms tend to be a consistent predictor of smoking relapse in women, but not in men. We propose that perturbed stress response exacerbates withdrawal symptoms and may contribute to the rapid relapse observed in the majority of smokers. This prediction is however moderated by sex. The results reinforce the need for sex-specific investigation of mechanistic and interventional strategies to combat the role of stress in smoking relapse.

Gender-sensitive medication development for smoking cessation
Sherry A. McKee, Ph.D. (Department of Psychiatry, Yale School of Medicine)

Tobacco use is the leading cause of preventable morbidity and mortality in the United States. Women, compared to men, have poorer rates of smoking cessation and exacerbated health risks. However, few attempts have been made to develop gender-sensitive smoking cessation treatments. This talk will present an overview of our work identifying sex differences in medication response for smoking cessation. Investigations span epidemiological studies, naturalistic Phase IV investigations, network meta-analysis, human laboratory, and clinical trial designs. Our work identifies that; women have poorer rates of smoking cessation compared to men; women have greater rates of relapse compared to men; there are clear sex differences in the efficacy of FDA-approved smoking cessation medications, and that targeting stress-reactivity may be particularly effective to help women quit smoking. Factoring this knowledge into practice and clinical care guidelines for smoking cessation will be discussed.

Sex hormones and postpartum smoking relapse: A pilot study
Sharon Allen M.D., Ph.D. (Department of Family Medicine and Community Health, University of Minnesota)

Pregnancy is a strong motivator to quit smoking however, postpartum relapse rates are high. Given that the literature supports a potential role of sex hormones in smoking relapse and the abrupt drop in sex hormones at delivery; the potential for role of sex hormones in postpartum relapse is prudent to investigate. This 12-week double blind, placebo-controlled randomized pilot study investigated the potential role of exogenous progesterone to prevent postpartum relapse. We hypothesized there would be no significant differences between participants randomized to progesterone (PRO) versus placebo (PBO) in terms of feasibility and acceptability, as well as adverse events. We also expected to see higher rates of smoking relapse at 4 and 12 weeks postpartum in the PBO group compared to the PRO group. Forty-six abstinent postpartum women with an average age of 26.5 ±5.2 years, and who had previously smoked a mean of 10.1 ±4.5 cigarettes per day were randomized to either PRO or PBO on day 4 postpartum. They took the study medication for 4 weeks and were followed through 12 weeks for smoking relapse, adverse events and acceptability of study procedures. Our overall retention at 12 weeks was 87%. Pill adherence was 68% and clinic visit attendance was 80% with no difference by randomization. No adverse events were related to the study drug. At week 4, 75% of the PRO group and 68.2% of the PBO group were abstinent. At week 12, 54.2% of the PRO group and 40.9% of the PBO group were abstinent. While this study was not powered to evaluate statically significant relapse rates, we did observe a higher prevalence of abstinence at week 4 in the PRO group and high feasibility and acceptability of delivering progesterone to postpartum women. Thus, the results of the pilot study warrant further investigation of progesterone as a potential postpartum relapse prevention strategy.

Session XII: Chromosomes and Sex-Linked Genes in Cancer (Woodlands B)

Co-Chairs: Christine Disteche, Ph.D. (University of Washington) and
Joel Bertlech, Ph.D. (University of Washington)

TSPY and TSPX: an old couple in human oncogenesis

Yun-Fai Chris Lau, Ph.D. (Laboratory of Cell and Developmental Genetics, Department of Medicine, VA San Francisco Health Care System, and Institute for Human Genetics and Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco)

Sexual dimorphisms are prevalent in human cancers. Sex hormones and their receptors could contribute to sex differences in incidence, initiation, progression, and clinical outcomes not only in sex-specific cancers, e.g. breast and prostate cancer, but also in sexually dimorphic cancers, such as liver cancer and melanoma. At present, the roles of the genes on the sex chromosomes, i.e. X and Y chromosome, in sex-specific and sexually dimorphic cancers have not been fully investigated. Expression of a Y-located proto-oncogene will have a positive effect(s) on oncogenesis in males while inactivation of a X-located tumor suppressor will predispose males to oncogenesis, since males have only one X chromosome. Indeed, the testis-specific protein Y-encoded (TSPY) gene on the Y chromosome and its homologue on the X chromosome, TSPX, represent such a pair of homologues on the sex chromosomes that are at the two extremes of the human oncogenic spectrum as results of evolutionary divergence. TSPY is a small repetitive gene mapped to the critical region for the gonadoblastoma locus on the Y chromosome (GBY). It is abundantly expressed in gonadoblastoma, seminoma and carcinoma-in-situ, the precursor for all testicular germ cell tumors. TSPY accelerates cell proliferation, shortens the G_2/M transition and corresponding checkpoints, thereby increasing genome instability. It promotes tumorigenicity in athymic mice and induces gonadoblastoma-like structures in transgenic mice. Hence, TSPY is a male-specific proto-oncogene for the GBY locus on the human Y chromosome. TSPX is a single-copy homologue of TSPY on the X chromosome. TSPY and TSPX originate from the same ancestral gene with similar exon-intron organization at their conserved SET/NAP domain, but diverge significantly at the flanking sequences, especially at their C-termini, in which TSPX harbors a large acidic domain that is absent in TSPY. Various studies showed that TSPX is a tumor suppressor in human cancers, such as prostate, lung and liver cancer, and possesses contrasting properties to those of TSPY. Both proteins interact with the cyclin B/CDK1 complex, but TSPY stimulates and TSPX represses the CDK1 kinase activities, thereby accelerating and arresting cells at G_2/M stage of the cell cycle respectively. TSPX promotes proteosomal degradation of the HBV viral oncoprotein, HBx, while TSPY does not. Significantly, both homologues bind the androgen receptor, AR, and its constitutively active variants lacking the ligand-binding domain, but TSPY stimulates and TSPX represses AR and AR variant transactivation of their target genes in ligand-dependent and independently manner respectively. Molecular characterization shows that the acidic tail in TSPX plays key roles in its inhibition of cell proliferation and repressor functions in AR transactivation. Hence, TSPY and TSPX mediate their oncogenic and tumor-suppressing functions in at least two mechanisms, i.e. promoting and repressing cell proliferation and male sex hormone gene regulatory programs respectively, thereby contributing to the overall human oncogenic processes in sexually dimorphic manners.

Regulation by the histone demethylase KDM6A gene is sex biased in development and cancer

Joel Berletch, Ph.D. (University of Washington)

Epigenetic modifications play important roles in gene regulation during development and in cancer. Some of these epigenetic modifications are controlled by specific genes expressed in a sex biased manner. For example, *KDM6A* is an X-linked gene that encodes a histone H3K27 demethylase and is more highly expressed in females than males because it escapes X inactivation. Investigation into KDM6A's role in cancer etiology has revealed mutations associated with many types of cancers including colon, breast, and renal cancers as well as leukemia. Gender-specific aberrant H3K27 methylation caused by KDM6A inactivating mutations contributes to T-cell leukemogenesis.

Not only does KDM6A function as a tumor suppressor gene, it is also important for normal development. Here, we show that two members of the mouse Rhox homeobox gene cluster, *Rhox6* and *9*, are regulated by de-methylation of histone H3 at lysine 27 by KDM6A in a sex-specific manner. In female mouse ES cells, KDM6A is specifically recruited to *Rhox6* and *9* for gene activation, a process inhibited by *Kdm6a* knockdown in a dose-dependent manner. In contrast, KDM6A occupancy at *Rhox6* and *9* is low in male ES cells and knockdown has no effect on expression. Additionally, in the mouse ovary where *Rhox6* and *9* remain highly

expressed, KDM6A occupancy strongly correlates with expression. Thus, our data implicates *Kdm6a* in the regulation of genes important in reproduction, suggesting that KDM6A plays a role in the etiology of developmental and reproduction-related effects of sex chromosome anomalies. Taken together, it is clear that epigenetic regulators expressed in a gender-biased manner can regulate genes involved in proper development and help maintain normal cell growth and proliferation.

X chromosome regulation in human pluripotent stem cells: potential parallels to female cancer cells
Anna Sahakyan, Ph.D. Candidate (University of California, Los Angeles)

Human pluripotent stem cells (hPSCs) hold great promise in regenerative medicine as they can differentiate to all three lineages. Safety of such therapies is always a concern as cells must be genetically and epigenetically stable to avoid further complications, such as formation of tumors, in the patient. There is a problem specifically with female hPSCs that needs to be solved before clinical use of these cells can be considered. Conventional female hPSCs are in a post X chromosome inactivation (XCI) state, where one of the two X chromosomes is epigenetically inactivated by the expression of the long non-coding RNA *XIST.* However, over time in culture, the inactive X chromosome of female hPSCs loses *XIST* expression and partially reactivates the inactive X chromosome, termed erosion, which does not go away upon differentiation of these cells. Interestingly, certain female breast and ovarian cancers lack an inactive X chromosome either because of loss of the inactive X and gain of an extra active X chromosome, or reactivation of the inactive X chromosome[1,2,3.] Furthermore, experimentally induced deletion of *Xist* in hematopoietic cells in mice results in poor postnatal survival and inevitable development of myelodysplasia with 100% penetrance, due to partial reactivation of the inactive X chromosome[4]. Thus, it appears that both in human and mouse systems, *XIST* expression loss and erosion of the inactive X chromosome are strongly correlated with cell transformation and cancer formation**.**
We have discovered that recently identified naïve culture conditions of hPSCs overcome the X chromosome erosion problem of hPSCs, possibly rendering them safer for future use. This is achieved by pushing the conventional hPSCs, which are in primed developmental state, into post-XCI naïve pluripotency that allows differentiation accompanied with proper X chromosome inactivation.

Abnormal X chromosome inactivation of T cells in female lupus patient and lupus-prone mice leads to increased expression from the inactive X
Jianle Wang, Ph.D. (Department of Biomedical Sciences, University of Pennsylvania School of Veterinary Medicine)

Female mammals use X Chromosome Inactivation to generate a transcriptionally silent inactive X chromosome (Xi) enriched with heterochromatic modifications and Xist RNA, which equalizes gene expression between the sexes. Females have a greater immunological advantage than men, yet they are more prone to autoimmune diseases. This sex bias may be due to the extra X chromosome in females compared to males, which contains many immunity related genes. We have examined the maintenance of XCI in lymphocytes from females in humans and mice and recently discovered that mature naive T and B cells have dispersed patterns of Xist and lack the typical heterochromatic modifications of the Xi. *In vitro* activation of the lymphocytes triggered the return of the Xist RNA transcripts and some chromatin marks. Here we examined the epigenetic characteristics of the Xi in T cells from patients with systemic lupus erythematosus and female-biased lupus mouse model NZB/W F1. We found that lupus patient and lupus-prone mice naïve T cells lack XIST/Xist RNA localization on the Xi. Upon stimulation, XIST/Xist RNA transcripts return to the inactive X in some, but not all, T cells. SLE patient T cells exhibited higher levels of XIST RNA mislocalization compared to healthy controls. We have also investigated the amount of heterochromatin modifications that co-localize with XIST RNA on the Xi. Using single-molecule RNA FISH, we observe biallelic expression of the autoimmunity-related X-linked genes CD40LG and CXCR3 in T cells from lupus patients and NZB/W F1 animals. We propose that the Xi in female lymphocytes is predisposed to become partially reactivated and to overexpress immunity-related genes, providing the mechanic evidence for the enhanced immunity of females and their increased susceptibility for autoimmune diseases, like lupus.

4:00 – 5:00: Capstone Address

Sex and Aging
James L. Kirkland, M.D., Ph.D. (Kogod Center on Aging, Mayo Clinic)

Aging predisposes to most of the chronic diseases that account for much of the morbidity, mortality, and health costs in developed and developing societies, including dementias, cancers, atherosclerosis, diabetes, blindness, and arthritis, among many others. The age of onset and characteristics of these age-related chronic conditions, as well as healthspan and lifespan, differ remarkably between women and men. Sex differences in the timing of age-related phenotypes and lifespan are prominent across the vertebrates, with females outliving males in most mammalian species. Sex-dependent differences across species and genotypes will be reviewed in the context of physiological and molecular parameters related to fundamental aging mechanisms. At the cellular and tissue levels, four interconnected processes are operative in the genesis of aging phenotypes as well as at sites of chronic disease pathology: low-grade inflammation, cellular senescence, macromolecular dysfunction, and stem and progenitor cell dysfunction. What is known about sex-dependent differences in these processes will be reviewed, as will sex-dependent variation in effects of health- or lifespan-extending single gene mutations. Interventions are being developed that target fundamental aging mechanisms. Most of the drugs discovered to date that extend health- or lifespan in lower mammals act in a sex-dependent fashion. Among these drugs are rapamycin and related compounds, ASA, acarbose, 17α-estradiol, senolytic agents, and drugs that interfere with the secretory phenotype of senescent cells (SASP inhibitors). It is important to determine the mechanisms that underlie sex differences in responses to these interventions, as well as in healthspan, lifespan, and the single gene mutations that extend them. Such an understanding is necessary to devise sex- and menopausal state-specific interventions. Furthermore, such knowledge could be exploited to develop new mechanism-based interventions to extend healthspan and delay, prevent, or alleviate chronic diseases and age-related disabilities as a group, instead of one at a time.

POSTER ABSTRACTS

1. **Estrus cycle stage modulation of conditioned contextual fear may involve differential neuronal activation within subnuclei of the bed nucleus of the stria terminalis of female rats**

Author List: Gillian M. Acca[1], Barbara Tsao[2], Stephen Maren[1,2], & Naomi Nagaya[1,2]

Author Affiliations: Institute for Neuroscience[1], Texas A&M University, College Station, TX, USA; Department of Psychology[2], Texas A&M University, College Station, TX, US

Abstract: It is well established that gonadal hormones and their metabolites modulate learned fear in both humans and rodents. We recently demonstrated in naturally cycling female rats that inhibiting the synthesis or action of allopregnanolone (ALLO), a progesterone metabolite and GABAA receptor modulator, through infusions targeting the bed nucleus of the stria terminalis (BNST), increased contextual fear as evidenced by freezing behavior, but had no effect on fear to the auditory conditioned stimulus (CS). The BNST is a site of hormonal modulation in the brain and ALLO levels fluctuate in a similar manner to progesterone during the rat estrus cycle. The potential effects of ALLO on the acquisition and expression of learned fear and its relation to neuronal activation in the BNST can therefore be examined. To this end, we are using the naturalistic model of gonadally intact, cycling female rats in high (late proestrus, P) or low (late diestrus, D2) progesterone states. Animals were trained with 5 CS (2 kHz, 10 s, 80 dB)-footshock (2 s, 1 mA) pairings and subsequently tested for contextual fear for 10 min in the conditioning chamber. Training and testing was timed such that rats were in one of the following combinations of estrus cycle stages: P-P, D2-D2, P-D2, or D2-P. Subjects trained in high progesterone states and tested in low progesterone states appear to have impaired contextual fear recall. Immunoreactivity for the immediate early gene protein, c-fos, was quantified in specific subnuclei of the BNST after context testing to assess neuronal activation following the expression of contextual fear. Preliminary results suggest differential activation of neurons across BNST subnuclei during P and D2 stages. These findings indicate that differences in conditioned contextual fear during high and low progesterone estrus cycle stages may be due to differential neuronal activity within subnuclei of the BNST.

2. **Why sex hormones matter for neural phenotyping**

Author List: Claudia Barth, Christopher J Steele[1,2], Prof. Karsten Mueller[1], Vivien P Rekkas[3], Katrin Arelin[1,4,5], Andre Pampel[1], Inga Burmann[1], Jürgen Kratzsch[6], Arno Villringer[1,4,5,7,8], Julia Sacher[1,4]

Author Affiliations: [1] Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany; [2] Cerebral Imaging Centre, Douglas Mental Health Institute, Department of Psychiatry, McGill University, Montreal, Canada; [3] CAMH Research Imaging Centre and Campbell Family Mental Health Research Institute at the Centre for Addiction and Mental Health and the Department of Psychiatry, University of Toronto, Toronto, Canada; [4] Clinic of Cognitive Neurology, University of Leipzig, Leipzig, Germany; [5] Leipzig Research Center for Civilization Diseases, University of Leipzig, Germany; [6] Institute for Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig, Leipzig, Germany; [7] Integrated Research and Treatment Center Adiposity Diseases, University of Leipzig, Germany; [8] Berlin School of Mind and Brain, Mind and Brain Institute, Berlin, Germany.

Abstract: Sex hormones fluctuate during the female menstrual cycle. Evidence from animal studies suggests similar subtle fluctuations in hippocampal structure, predominantly linked to estrogen. Hippocampal abnormalities have been observed in several neuropsychiatric pathologies with prominent sexual dimorphism. Yet, the potential impact of subtle sex-hormonal fluctuations on human hippocampal structure in health is unclear. We tested the feasibility of non-invasive longitudinal neuroimaging in conjunction with rigorous menstrual cycle monitoring to evaluate potential changes in hippocampal white matter associated with physiological sex hormonal changes. Given the evidence for neurotrophic effects of estrogen in the hippocampus in rodent and human studies, we hypothesized high endogenous estrogen levels to be associated with higher fractional anisotropy (FA) values, a measure sensitive to changes in white matter structure. 30 diffusion weighted imaging (DWI) scans of a single healthy female subject were

acquired across 4 menstrual cycles with a 3-Tesla Magnetom Verio scanner. Diffusion imaging data were processed with FMRIB's software library (www.FMRIb.ox.ac.uk/fsl). We calculated hippocampal FA and investigated potential correlations of sex hormones and FA using a non-parametric permutation-based approach ($p<0.05$, family-wise error corrected for multiple comparisons). We observed a significant correlation between FA values and estrogen in the hippocampus bilaterally, revealing a peak in FA closely paralleling ovulation. This study introduces a novel approach for simultaneously mapping longitudinal characteristics in endogenous hormone and white matter dynamics. In light of recent attempts to neurally phenotype single humans, our findings are the first to highlight menstrual cycle monitoring in parallel with highly sampling individual neuroimaging data to address fundamental questions about the dynamics of human brain plasticity in the adult brain.

3. HIV and symptoms of depression are independently associated with impaired glucocorticoid signaling

Author List: Mandakh Bekhbat[1], C. Christina Mehta[1], Igho Ofotokun[1], Jennifer Felger1, Gina Wingood[1,3], Kathryn Anastos[4], Tracey E. Wilson[5], Seble Kassaye[6], Joel Milam[7], Brad Aouizerat[8], Kathleen Weber[9], Elizabeth T. Golub[10], Michelle Floris Moore[11], Ralph Diclemente[1], Margaret Fischl[13], Mirjam-Colette Kempf1[4], Pauline Maki[15], Gretchen Neigh[1,2]

Abstract: Chronic inflammation caused by HIV infection may lead to deficient glucocorticoid (GC) signaling, thus predisposing people living with HIV (PLWH) to psychiatric disorders linked to GC resistance, such as depression. The female bias in depression renders women living with HIV particularly vulnerable to developing depression. We hypothesized that comorbid symptoms of depression in PLWH would synergistically associate with deficits in GC signaling without stimulation and when stimulated with a synthetic GC, dexamethasone (Dex).This cross-sectional study used PBMCs obtained from participants in the Women's Interagency HIV Study in one of four groups: 1) HIV-neg, non-depressed (n=32); 2) HIV-neg, depressed (n=32); 3) HIV-pos, non-depressed (n=34); and 4) HIV-pos, depressed (n=35). Participants with depressive symptoms had a CES-D score ≥16 at the time of sample collection. Following stimulation of PBMCs with 10-8 M Dex for 12 hrs, expression of the target genes Fkbp5 and Nr3c1 (GR) was assessed via qPCR. A generalized estimating equation model was used to examine the association between gene expression and HIV status and depressive symptoms, controlling for current pot use. When unstimulated, cells from patients with depressive symptoms showed increased FKBP5 ($p=0.036$) and GR ($p=0.005$), these associations were also present in PLWH ($p=0.041$; $p=0.008$). Decreased unstimulated GR ($p=0.004$), but not FKBP5 ($p=0.164$), was observed in cells from PLWH and symptoms of depression compared to HIV-neg non-depressed participants. In Dex-stimulated cells, depression was associated with decreased FKBP5 ($p=0.006$) and GR expression ($p=0.001$) in HIV-neg individuals. In subjects with elevated depressive symptoms, HIV was associated with elevated FKBP5 and GR expression ($p=0.020$; $p=0.049$). These data suggest that both HIV and depressive symptoms are associated with GR and FKBP5 expression but do not appear to exacerbate the impact of either condition when they co-occur.

4. Paternal stress experience reprograms epigenetic trajectory of offspring neurodevelopment

Author List: Jennifer C Chan[1], Ali B. Rodgers[1], Christopher P. Morgan[1], N. Adrian Leu[1] & Tracy L. Bale[1]

Author Affiliations: [1]University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA, USA

Abstract: Epidemiological studies suggest that epigenetic inheritance of parental lifetime experiences can influence neuropsychiatric disease risk in subsequent generations. Notably, parental exposure to trauma has been linked with stress dysregulation, a common feature of neuropsychiatric disease. Whereas the mechanisms by which maternal stress can impact fetal neurodevelopment have been widely investigated, how the paternal environment impacts future offspring health is not well understood. Rodent models, where males do not participate in offspring rearing, provide an exciting model for examining the unique epigenetic germ cell contribution to offspring development. We have developed a paternal stress model in

which both male *and* female offspring show dramatic changes in the programming of their hypothalamus, including a blunted stress response as adults. Mechanistically, sperm analyses identified a significant increase in 9 microRNA (miRs) following paternal stress exposure. Zygote microinjection of these miRs recapitulated the offspring stress phenotype, providing a functional role for sperm miRs. However, how sperm miRs program lasting changes in offspring is not understood. Using single-cell amplification, we found that sperm-derived miRs can regulate maternal mRNA stores in the zygote, and may direct developmental programming by targeting expression of epigenetic regulators. Indeed, preliminary data from RNA-seq analyses demonstrate a massive, brain-specific reprogramming in the zygote-microinjected embryos that include shifts in expression of many chromatin modifiers. Mass spectrometry of the zygote-microinjected embryonic brains corroborate altered abundances in several histone modifications, suggesting sperm miRs can alter the epigenetic landscape of offspring development. These studies confer the importance of paternal experiences in influencing offspring health, and offer an exciting mechanism by which the male germ cell can reprogram offspring neurodevelopment.

5. Conditioning protocol matters: Elimination of sex differences in context fear generalization

Author List: Mara A. Darian[1], Ashley A. Keiser[1], Natalie C. Tronson[1]

Author Affiliations: [1]Department of Psychology, University of Michigan, Ann Arbor, MI, USA

Abstract: Disorders of fear and anxiety, including post-traumatic stress disorder, are more prevalent in wo men than men. A key feature of such disorders is the generalization of fear from a place in which trauma o ccurred, to safe environments. In this project, we aimed to examine sex differences in generalization of co ntext-associated fear memory. Previous work in our laboratory using “foreground” context fear conditionin g (fCFC) demonstrated that females show more generalization of context fear than males. This suggests t hat males learn a more detailed representation of context than females. Here we further examined this eff ect using a “background” context fear conditioning paradigm in which mice are placed in the conditioning c ontext and a tone cue is presented immediately prior to the shock. Given that tone is a more salient conditi oned stimulus than context, we hypothesized that background CFC would reduce learning details of a cont ext and thus result in more generalization of fear. Surprisingly, we found that both males and females sho wed little generalization between training and similar contexts. Importantly, females showed less generaliz ation of fear in comparison with previous experiments. There is some evidence that background CFC resul ts in greater activation of hippocampus compared with fCFC. By strongly activating hippocampus, this par adigm may facilitate learning detailed context representations, particularly in females. To determine wheth er increased hippocampal activity can account for reduced context generalization, we used immunohistoc hemistry to quantify cFos levels in the hippocampus and amygdala. Together, these findings demonstrate that in background fear conditioning, unlike fCFC, females and males show similar patterns of fear conditi oning, context generalization, and activation of hippocampus and amygdala.

6. Sex differences in neural activation following oxytocin administration in awake rats

Author List: Kelly M. Dumais[1], Praveen Kulkarni[2], Craig F. Ferris[2], Alexa H. Veenema[1]

Author Affiliations: [1]Neurobiology of Social Behavior Laboratory, Department of Psychology, Boston College, Chestnut Hill, MA, USA, [2] Center for Translational NeuroImaging, Northeastern University, Boston, MA, USA

Abstract: The evolutionarily conserved neuropeptide oxytocin (OT) regulates social behavior in sex-specific ways in humans and rodents. Importantly, OT has promising effects on improving social deficits in patients with sex-biased neuropsychiatric disorders. However, little is known about potential sexually dimorphic effects of OT on brain function. We investigated neural activation patterns in response to central or peripheral OT administration in adult male and female rats. We used functional magnetic resonance imaging in awake rats to examine positive blood oxygen level-dependent (BOLD) signal intensity changes in the brain within 20 minutes after intracerebroventricular (ICV) or intraperitoneal (IP) administration of

OT. We found that 26 brain regions showed sex differences in activation following ICV OT, with 20 regions showing higher activation in males, and 6 regions showing higher activation in females. Among these were many regions dense in OT receptor (OTR), including the nucleus accumbens and insular cortex which showed higher activation in males, and the lateral and central amygdala which showed higher activation in females. In contrast, IP OT injections elicited fewer sex differences in brain activation (12 brain regions), with only one region (medial amygdala) showing dense OTR. In fact, the most prominent patterns of activation in response to IP OT (activation of olfactory, cerebellum, and brainstem regions) were similar in both sexes. The distinct activation patterns following ICV or IP OT suggest that IP OT may influence brain activity similarly in males and females via sensory afferents from the autonomic nervous system, rather than direct activation of brain OTR. Together, our results indicate that central but not peripheral OT has profound sex-specific effects on neural activation. This knowledge may be informative when using OT as a therapeutic agent to treat social dysfunction in both men and women.

7. Sex differences in TrkB concentrations in the MPNmag

Author List: Nunana A. Gamedoagbao[1], Joe C. Brague[1], Jennifer M. Swann[1]

Author Affiliations: [1]Department of Biological Sciences, Lehigh University, Bethlehem, PA 18104

Abstract: Male and female sexual behavior is regulated by sex specific nuclei. For example, in the male hamster, ablation fot he magnocellular division of the medial preoptic nucleus (MPNmag) eliminates male sex behavior and has no effect on female sex behavior. Exposure to female pheromones triggers male sex behavior. The MPNmag reponds to pheromones in males with circulating testosterone but not in females even in the presence of testosterone. One reason for the sex difference could be sex differences in hormonal regulation of synaptic density. Testosterone increases synaptic density in the male MPNmag but not in the female. As TrkB and its ligand BDNF regulate synaptic plasticity, we hypothesized that the male hamsters have higher levels of TrkB in the MPNmag than females and that testosterone regulates TrkB in the male but not the female. Adult Syrian hamsters were assigned to 5 groups; gonad intact males and females, gonadectomized males and females treated with mineral oil and gonadectomized males treated with testosterone. Animals from each group were sacrificed, their brains removed and tissue containing the MPOA was extracted and homogenized. The proteins were then separated on a polyacrylamide gel transferred to a membrane and labeled with antibodies to TrkB (Santa Cruz). Analtysis indicates that 1) intact and gonadectomized males have lower levels of TrkB than gonadectomized males treated with vehicle. Intact females have less TrkB than males and gonadectomized females treated with mineral oil. Our results, contrary to our hypotheses, can be partly explained by the finding that BDNF downregulates TrkB. Taken together our results suggest that 1) males have more TrkB (and therefore less BDNF) than females and 2) testosterone regulates BDNF/TrkB in both males and females. In conclusion, these findings suggest that TrkB/BDNF may mediate steroid effects on synaptic density in the preoptic area.

8. Neuroimaging the hypothalamus to explain associations between prenatal maternal stress and sexually dimorphic psychopathological symptoms: Project Ice Storm

Author List: Sherri Lee Jones[1,2], Jens Pruessner[1,2,3], David P. Laplante[1], Suzanne King[1,2]

Author Affiliations: [1]Douglas Mental Health University Institute, [2]Department of Psychiatry, McGill University, Montreal, Qc, Canada, [3]McGill Center for Studies in Aging, Department of Psychology, McGill University, Montreal, Qc, Canada.

Abstract: Sex differences in physical traits, neural structures, and behaviors occur at least in part as a result of prenatal androgen exposure, and prenatal maternal stress (PNMS) has been shown to abolish or even reverse certain sex differences. This suggests that PNMS may alter prenatal androgen exposure, including sexually differentiated brain structures that may be implicated in psychopathologies. The human hypothalamus is sexually differentiated (larger in males) largely as a result of prenatal androgen exposure,

and is increasingly recognized as an important brain region for understanding sex differences in psychological health and disease. In a unique prospective, longitudinal study of pregnant mothers that were exposed to Quebec's 1998 Ice Storm, a sudden onset stressor, we have found that PNMS masculinizes digit ratios (a biomarker of prenatal androgen exposure) in girls at 5.5 years old, reverses the sex difference in cortical thickness at 11.5 years, and abolishes the typical sex difference in internalizing symptoms in adolescence. Given these findings, we hypothesize that PNMS interferes with normal sexual differentiation of hypothalamic volume, which in turn may be associated with psychopathological outcomes such as internalizing symptoms. Using T1 and T2 weighted images acquired on a 3T MRI scanner, a novel segmentation protocol is being developed to measure the volume of the hypothalamus and some of its nuclei in the ice storm children and controls born in 1997, for the upcoming 19 years old assessments. Next, we will determine whether the volumetric sex difference is disrupted by PNMS, and whether its volume mediates the association between PNMS and sexually dimorphic outcomes such as internalizing symptoms in adolescence. This will be the first study to determine the effects of PNMS on hypothalamic volume in humans, and to test whether its volume mediates the association between PNMS and sexually dimorphic psychological outcomes in youth.

9. Sex differences in retrieval of context fear memory

Author List: Ashley A. Keiser[1], Mara A. Darian[1], Natalie C. Tronson[1]

Author Affiliations: [1]Department of Psychology, University of Michigan, Ann Arbor, MI, USA.

Abstract: Previous findings suggest that males show stronger memories for fear associated with a specific place. This has been largely attributed to differential memory consolidation, where males form stronger context representations compared with females. Recent evidence, however, suggests that males and females differ in retrieval of emotion-related information. To examine sex differences in memory retrieval we compared male and female mice in 1) retrieval of fear associated memories in the training context and in a similar context (context generalization); 2) retrieval-induced cFos activity in the hippocampus (context representations) and amygdala (context-shock associations); and 3) how retrieval of a context fear memory alters subsequent learning (blocking). We hypothesized that males would show better retrieval of specific context memory and more hippocampus activation compared with females, as well as impaired acquisition of a new association in the blocking paradigm. After conditioning, all mice formed strong context fear memories. Males showed less generalization of fear than females, suggesting retrieval of a more specific memory for context. Consistent with this finding, males showed stronger cFos activity in dorsal hippocampus after retrieval, whereas females showed stronger amygdalar recruitment. In contrast, there were no sex differences during consolidation. Furthermore, prior context-shock associations reduced learning a new tone-shock association in males, a phenomenon known as blocking. We did not observe this effect in females suggesting sex differences in the interaction between a retrieved context memory and formation of a new memory. Collectively these findings demonstrate for the first time that sex differences in context fear conditioning are mediated by differential retrieval mechanisms. Specifically, we show that males use hippocampal-dependent mechanisms for retrieval of context fear, whereas females rely on an amygdala-based strategy.

10. Cocaine seeking during initial abstinence is driven by noradrenergic and serotonergic signaling in dorsal hippocampus in a sex-dependent manner

Author List: Amy S. Kohtz[1] & Gary Aston-Jones[1]

Author Affiliations: [1] Brain Health Institute, Rutgers University, Piscataway, NJ 08854

Abstract: There are substantial sex differences in cocaine addiction, demonstrated by both clinical and pre-clinical studies. Notably, females are more sensitive than males to stress-induced drug-seeking and relapse. Locus coeruleus norepinephrine (LC-NE) and dorsal raphe serotonin (DR 5-HT) systems are involved in stress responses and may drive signaling involved in stress-induced drug-seeking behavior.

The dorsal hippocampus (DH) is a stress-sensitive focal brain region in the stress response pathway, and receives strong inputs from LC-NE and DR 5-HT neurons. We hypothesized that NE and 5-HT neurotransmission in DH is involved in drug-seeking behavior during the initiation of abstinence (extinction day 1, ED1), a stressful event involving abstinence from drug. ED1 increased Fos expression in DH, LC, and DR, and these changes in Fos correlated positively to drug-seeking behavior on ED1. We observed decreased drug-seeking behavior on ED1 following combined systemic β-adrenergic (β-AR) and 5-HT receptor antagonism in both male and female rats. We then investigated the effects of specifically blocking 5-HT and β-AR neurotransmission in DH on drug-seeking during ED1 by infusing a cocktail of betaxolol plus ICI-118,551 (β1 and β2 antagonists), WAY100635 plus GR127935 (5-HT1A/1B receptor antagonists), or S-propranolol. In males, 5-HT antagonism was most effective in reducing drug-seeking on ED1, whereas β-AR antagonism was ineffective. In contrast, S-propranolol was most effective in females in reducing drug-seeking on ED1, and 5-HT and β-AR antagonists were each partially effective. We further examined pharmacogenetic effects of inhibiting signaling from the DR to the DH via DREADDs, and found similar effects for inhibition of DR-DH signaling to decrease ED1 drug-seeking, as in our pharmacological experiments. Our results show that drug-seeking during initial abstinence involves 5-HT and β-AR signaling in female DH, but only 5-HT signaling in male DH.

11. Sex-specific mechanisms of songbird audition depend on membrane estrogen receptor activation

Author List: Amanda A. Krentzel[1,2,3] & Luke Remage-Healey[1,2,3]

Author Affiliations: [1]Neuroscience and Behavior Program, [2]Center for Neuroendocrine Studies, [3]Department of Psychological and Brain Sciences, University of Massachusetts Amherst, Amherst, MA, USA.

Abstract: Auditory processing is essential in both sexes; however, due to the sensitivity that the auditory system has to hormonal influences, auditory mechanisms may differ between the sexes. Estrogen receptors and intracellular signaling pathways are influenced by early sexual development. In the zebra finch brain, the caudomedial nidopallium (NCM) is a secondary auditory region that is involved in song discrimination. This region synthesizes its own estrogens and local estradiol application increases auditory-evoked firing activity rapidly, suggesting mediation from a membrane receptor. It is unknown which estrogen receptor facilitates changes in audition and whether this mechanism is the same between the sexes. We hypothesized that the membrane estrogen receptor GPER1 is both necessary and sufficient in mediating auditory responsiveness in NCM. Using *in vivo* extracellular electrophysiology, males and females were exposed to songs while either an agonist (G1) or antagonist (G36) for GPER1 was infused into NCM. We grouped single units by action potential length into narrow cells and broad cells (putative inhibitory and excitatory neurons, respectively). Our findings show that narrow cells respond more to auditory stimulation than broad cells in males; however, there is a similar response between the cell types in females. During G36 infusion, narrow cells in males and broad cells in females each exhibit diminished auditory-evoked activity such that the cell types resemble the baseline condition of the other sex. Therefore, GPER1 appears to play a role in maintaining a sex difference in higher order sensory processing. Experiments exploring the sufficiency of GPER1 activation using G1 are currently ongoing. In summary, GPER1 is a necessary membrane receptor in coding of complex, auditory physiology, and it contributes to a previously undescribed sex difference of auditory processing in NCM. Rapid estrogenic actions are likely contributing to the maintenance of sexually dimorphic circuits in adulthood.

12. Sex differences in the expression of mineralocorticoid and glucocorticoid receptor mRNA in response to acute mate pair separation in zebra finches (*Taeniopygia guttata*)

Auhor List: Farrah N. Madison[1], Andrew J. Kesner[2], Gregory F. Ball[1]

Author Affiliations: [1]Department of Psychology, University of Maryland College Park, M.D. 20742; [2]Johns Hopkins University, Baltimore, M.D. 21218

Abstract: The highly social zebra finch forms and sustains long-term monogamous pair bonds in the wild and in the laboratory, making them an appropriate species to investigate the physiological and neurochemical consequences of mate pair separation. Studies in zebra finches as well as in mammals have shown that mate separation stimulates stress responses, eliciting changes in behavior and triggering the release of glucocorticoid (GC) hormones. The underlying cellular and molecular events that are modulated in association with mate pair separation have not been elucidated. In this study, we investigated possible changes in mineralocorticoid receptor (MR), glucocorticoid receptor (GR), and corticotropin releasing hormone (CRH) mRNA expression in response to acute mate pair separation in male and female zebra finches. Males and females were maintained on long days (14L:10D) and established mate pairs were confirmed using pair bond specific behavioral criteria. Birds were assigned to one of the following treatment groups: (1) male and female birds that were separated from their respective mate, (2) males and females were allowed to stay with their mates but were separated from the stimulus female that had been introduced to their cage, or (3) the subjects were handled but not separated from their mate (control). After 36 hours in the new housing condition, brains were collected, frozen, and later micropunches from the paraventricular nucleus (PVN) and hippocampus (HP) were analyzed via qPCR for MR, GR, and CRH mRNA. Blood was collected prior to, and at the end of the study. No significant differences in plasma CORT levels, as well as in MR, GR, or CRH mRNA expression in PVN were observed in response to any treatment for both males and females. In addition, females showed no differences in MR or GR mRNA expression in the HP. However, males showed a marked down regulation of both MR and GR expression in the HP as a result of mate separation treatments, but not other treatments or control treatments. This study provides evidence for the presence of a sex difference in the neural mechanisms related to the regulation of the hypothalamic-pituitary-adrenal axis during the stressful social scenario of mate separation in the pair bond-forming zebra finch.

13. Using GECIs to measure sex differences in calcium dynamics in hippocampal primary cultures

Author List: Amanda H. Mahnke[1,2], Shelby D. Weyand[1], Ursula H. Winzer-Serhan[1,2]

Author Affiliations: [1]Neuroscience and Experimental Therapeutics, TAMHSC, Bryan, TX; [2]Women's Health in Neuroscience Program, TAMHSC, Bryan, TX

Abstract: Calcium plays a pivotal role within cells as a second messenger, propagating signals from both external stimuli and intracellular events. Genetically-encoded calcium indicators (GECIs) are powerful tools for the visualization of calcium dynamics. Here, we use the highly sensitive GECI (GCaMP6s) to quantify spontaneous and evoked intracellular calcium signals in primary hippocampal cultures with the goal to study sex differences in both neuronal and glial calcium activity. Sex-segregated cultures were generated from postnatal day 1 mouse pups, transduced after 2-3 days with an AAV5 viral vector containing GCaMP6s, and imaged at 14-15 days *in vitro* using confocal microscopy. GCaMP6s expression was found in both neurons and glia, with functional calcium signals detected in the soma and far into cellular processes. Neurons, but not glia, were responsive to KCl (20 mM) stimulation, with a rapid rise in fluorescence intensity in soma and dendrites. Neurons responsive to KCl were analyzed for their response to increasing doses of nicotine (0.01 - 10 µM) in the presence and absence of bicuculline (10 µM). We found female neurons reached peak calcium responses at 0.1 µM nicotine, whereas males continued to show increased calcium responses up to 10 µM nicotine. There were significantly different calcium spike amplitudes between male and female neurons in response to 1.0 µM nicotine without bicuculline co-administration and at 0.1 µM nicotine with bicuculline. Preliminary data suggest that male and female glia might also have different baseline calcium dynamics. Subsequent antibody staining for the GFP domain of GCaMP6s allowed more detailed characterization of cell types present in the cultures and viral vector transduction rates. In general, this methodology provides a novel approach to assay calcium dynamics of both spontaneous and drug-induced activity in different cell types *in vitro*, and allows for new insights into sex differences in neuronal and glial function.

14. Pericytes improve motor recovery after spinal cord injury in both sexes

Author List: Jamie N. Mayo[1] & Shawn E. Bearden[2]

Author Affiliation: [1]University of Melbourne, Parkville, Australia, [2]Biology, Idaho State University, Pocatello, ID, USA

Abstract: New capillary growth can improve recovery after spinal cord injury (SCI). Pericytes, microvascular cells, can be stimulated in vitro to induce new capillary growth. The hypothesis was tested that stimulated pericytes would improve motor recovery in both sexes in a pediatric model of SCI. Pericytes and pericytes stimulated with cobalt chloride were injected into the site of a thoracic hemi-section of the spinal cord in rat pups on postnatal day three (P3). Hindlimb motor recovery was evaluated on P10 in addition to vessel and neurofilament density. Pericyte, but not stimulated pericyte, treatment improved motor recovery in both sexes ($p<0.05$). Pericyte treatment supported a stable vascular bed in both sexes and greater neurofilament density. There were significant interactions between treatment and sex in vessel density after SCI ($p<0.05$). In the control group, females had a higher vessel density seven days after injury than males ($p<0.05$). This pattern persisted following pericyte injection ($p<0.05$). With stimulated pericyte injection, males had a greater vessel density then their sex-matched controls ($p<0.05$) and no longer had lower vessel density then females. Sex was a significant source of variation ($p<0.05$) for endothelial proliferation. Within the control group there was less endothelial proliferation in female tissue than there was in male tissue ($p<0.05$). Injected directly after injury, naïve pericytes as opposed to stimulated pericytes have more potential to improve motor recovery. It appears that the timing of, or capacity for, the initiation of an angiogenic program following CNS injury might differ between males and females. Additionally, it is possible that females may be better able to maintain vessel viability after initial injury. The results from this study highlight the importance of appreciating that even when males and females reach the same gross functional end measurements, they might do so by different structural and molecular mechanisms.

15. The neurosteroid 3α-androstanediol protects SH-SY5Y human neuroblastoma cells against the neurotoxic effects of amyloid β peptide

Author List: Ari L. Mendell[1] & Neil J. MacLusky

Author Affiliations: [1]Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada

Abstract: Low levels of free testosterone have been associated with increased incidence of Alzheimer's disease (AD) in men (Hogervorst *et al*. *Curr Drug Targets CNS Neurol Disord* **4**, 531-40, 2005). The protective effects of sex steroids against neurodegenerative diseases may involve conversion to active metabolites, including neurosteroids that modulate neuronal sensitivity to gamma-aminobutyric acid (GABA). 5α-androstane-3α,17β-diol (3α-diol), a metabolite of testosterone, allosterically potentiates the activity of GABA at the $GABA_A$ receptor. We hypothesized that this might contribute to sex differences in the incidence of AD. Dysregulation and prolonged phosphorylation of extracellular signal-regulated kinase (ERK) is an indication of cellular toxicity, and has been implicated in amyloid-induced nicotinic cholinergic receptor (nAChR)-dependent neuronal deficits in AD. Therefore, we sought to determine whether 3α-diol could protect against neurotoxic ERK phosphorylation induced by hydrogen peroxide (H_2O_2) and amyloid β peptide 1-42 (Aβ42) in SH-SY5Y human female neuroblastoma cells. In addition, we determined whether 3α-diol influences the physiological phosphorylation of ERK by acetylcholine via nAChR, pre-treating with atropine in order to prevent muscarinic receptor involvement. ERK phosphorylation changes were evaluated using western blots. 3α-diol prevented the increase in ERK phosphorylation induced 24 hours after treatment with H_2O_2 or Aβ42 treatment, without having any effect on the short-term activation of ERK by acetylcholine. 3α-diol also blocked caspase 3 cleavage that was induced by treatment with Aβ42 for 48 hours. These results indicate that 3α-diol protects against oxidative stress induced by Aβ42 without disrupting normal neuronal ERK signaling, suggesting that it may play an important role in the prevention of amyloid-induced neurotoxicity.

16. Preadolescent adversity programs a disrupted maternal stress reactivity in humans and mice

Author List: Kathleen E. Morrison[1,2], Dina H. Appleby[2,3], Mary D. Sammel[2,4], Claudia Iannelli[2,3], Jessica L. Snell[2,3], C. Neill Epperson[2,3,5], & Tracy L. Bale[1,2]

Author Affiliations: [1]Department of Biomedical Sciences, School of Veterinary Medicine, [2]Penn Center for the Study of Sex and Gender in Health, [3]Department of Psychiatry, Perelman School of Medicine, [4]Department of Biostatistics and Epidemiology, [5]Obstetrics and Gynecology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Abstract: Adverse childhood experiences (ACEs) are one of the greatest predictors for affective disorder presentation across the lifespan for women. Dynamic periods of hormonal flux like pregnancy exacerbate the risk for affective disturbances and promote hypothalamic-pituitary-adrenal (HPA) axis stress dysregulation, a key feature of affective disorders. However, little is understood as to how stress alters the programming unique to this window of brain maturation and its interaction with the hormonal changes that occur during pregnancy and postpartum. Preadolescent female mice were exposed to chronic stress and then examined for programming changes in their HPA stress axis during pregnancy and postpartum, including assessment of maternal-specific stress responsiveness and transcriptomics of the paraventricular nucleus of the hypothalamus (PVN). Translationally, we recruited pregnant women with low or high ACE experiences and examined their maternal stress responsiveness in a manner similar to that of the mouse model. As predicted, preadolescent stress in mice resulted in a significant change in the HPA stress response specifically during pregnancy. Using transcriptomics analysis of the PVN, we found long-term reprogramming by preadolescent stress of immediate early genes and their transcriptional target genes. Importantly, high ACE women showed a similar change of HPA response to a maternally relevant stressor. This unique mouse model recapitulates clinical outcomes demonstrating that childhood adversity is an important underlying risk factor for stress-related affective disruption during periods of dynamic hormonal changes, such as pregnancy, and suggests that long-term reprogramming of the PVN by preadolescent stress may be responsible for this outcome.

17. Sex differences in the effects of microglia on neonatal neurogenesis and behavioral development

Author List: Lars H Nelson[123], Spencer Warden[4], Anabel Galan[24], Kathryn M Lenz[234]

Author Affiliations: Neuroscience Graduate Program[1], Group in Behavioral Neuroendocrinology[2], Dept. Neuroscience[3], Dept. of Psychology[4], The Ohio State University

Abstract: Many neuropsychiatric disorders with developmental onset involve altered immune signaling and show a significant sex bias with prevalence toward males. The male brain has more activated microglia, the innate immune cell in the CNS, during development than the female brain (Lenz et al., 2013, J Neurosci; Schwarz et al., 2012 J Neurochem), but the role of these sex differences in normal brain development is largely unknown. Microglia regulate the number of proliferating cells in the developing cortex by releasing diffusible factors and phagocytizing healthy progenitors (Cunningham et al., 2013, J Neurosci; Shigemoto-Mogami et al., 2014, J Neurosci). During development there is a basal sex difference in hippocampal neurogenesis in rats, with males having more neurogenesis than females (Zhang et al., 2008, Eur J Neurosci), thus we hypothesized that microglia may contribute to sex differences in both normal and abnormal cell genesis during ontogeny. We tested whether microglia regulate the sex-specific rate of proliferation in the neonatal hippocampus by temporarily ablating microglia using central infusion of liposomal clodronate. Clodronate treatment decreased proliferation in the hippocampus of males, but not females, as determined by the number of BrdU+ cells. We also analyzed microglial morphology in the hippocampus at postnatal day 2-3. We found that females had more microglia with phagocytic cups than males and neonatal estradiol treatment masculinized the number phagocytic cups in females. We also found that estradiol increased microglial proliferation in the hippocampus. Ongoing experiments are testing the target of microglial phagocytosis in the developing hippocampus as well as the long-term behavioral consequences of early life microglial ablation. Together,

these studies give important insight into the role of neuroimmune cells in normal brain development and thus how perturbations that induce immune activation may disrupt development of brain and behavior.

18. The prepubertal ovary contributes to sex differences in juvenile social play behavior of Siberian hamsters

Author List: Matthew J. Paul[1], Clemens K. Probst[2], & Geert J. de Vries[3]

Author Affiliations: [1]Psychology Department, University at Buffalo SUNY, Buffalo, NY, USA; [2]University of Massachusetts Amherst, Amherst, MA, USA; [3]Neuroscience Institute, Georgia State University, Atlanta, GA, USA

Abstract: The ovary is widely considered to be quiescent prior to puberty. However, the prepubertal ovary secretes measureable levels of hormones, and some evidence suggests it programs sexually dimorphic adult behaviors. Ascribing effects of ovarian manipulations to the prepubertal ovary, however, is difficult and often contested when behaviors are measured in adulthood. Siberian hamsters are seasonal breeders and their gonadal function is regulated by photoperiod. Hamsters reared in a long day length (LD) typical of spring/summer undergo rapid pubertal development, whereas those reared in a short day length (SD) typical of fall/winter delay puberty by several months to synchronize breeding with the next spring. In the present study, we used both photoperiod and surgical manipulations to assess the role of the ovaries and testes in the development of a sexually dimorphic juvenile behavior, social play. Male and female hamsters were reared in a LD or SD, and social play behavior was assessed every 10 days from 20 days of age (P20) until adulthood (P60 in LD hamsters, P120 in SD hamsters). LD hamsters displayed the typical sex difference seen in other species (males > females). As expected, SD hamsters delayed pubertal development. This photoperiod-modulation of gonadal development did not affect play of males but increased play of females. Notably, this female-specific effect eliminated the sex difference in play. To confirm the role of the prepubertal ovary in this photoperiod-modulation of female play, LD-reared male and female hamsters were gonadectomized (GONX), sham-operated (SHAM), or left un-operated (UNOP) at P15, and their play behavior was assessed at P30. As with photoperiod, GONX increased play of females but not males, thereby eliminating the sex difference. This finding indicates that the prepubertal ovary contributes to sex differences in behavioral development and challenges the notion that the ovary is functionally quiescent prior to puberty.

19. Opposing effects of male sex chromosome complement and circulating testosterone on pre-pubertal and adult expression of somatostatin in the basolateral amygdala

Author List: Rachel Puralewski[1,2], Georgia Vasilakis[1,2], Marianne L. Seney[1,2]

Author Affiliations: [1]Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA 15213; [2]Translational Neuroscience Program, University of Pittsburgh, Pittsburgh, PA 15213

Abstract: Women are twice as likely to be diagnosed with a mood disorder compared to men, but the molecular mechanisms underlying this sex difference are unclear. Using the Four Core Genotypes (FCG) mouse model, in which genetic and gonadal sex are decoupled, we recently showed an opposing effect of male sex chromosome complement and male-like testosterone levels on anxiety-like behavior (i.e., higher anxiety-like behavior in XY mice and lower anxiety-like behavior in testosterone-treated mice). We hypothesized that molecular features in the basolateral amygdala (a brain region involved in mood regulation) underlie this opposing behavioral effect of male sex chromosome complement and male-like testosterone levels. In adult FCG mice, we examined basolateral amygdala expression of somatostatin (*Sst*), a marker of GABA interneurons that regulate excitatory input onto pyramidal neurons that have been implicated in mood regulation (N = 12-20/group). We also examined basolateral amygdala *Sst* expression in gonadally intact pre-pubertal FCG mice to determine whether any sex-related factors influenced gene expression before the onset of puberty (N = 19-26/group). We found that adult basolateral amygdala expression of *Sst* mirrored the behavioral effect [i.e., lower *Sst* expression in XY compared to

XX mice ($p < 0.05$) and higher expression in mice treated with testosterone ($p < 0.05$)]. We found a similar pattern in gonadally intact pre-pubertal mice, with XY mice having lower *Sst* expression than XX mice ($p = 0.076$) and a correlation between circulating testosterone levels and *Sst* gene expression ($p = 0.032$). Due to the role of SST neurons in mood regulation, we speculate that testosterone opposes the XY gene effect at the level of basolateral amygdala SST cell function.

20. Sex differences in the fluid intake and pressor response to angiotensin II (AngII): influence of body weight

Author List: Jessica Santollo[1], Ann-Marie Torregrossa[1], Derek Daniels[1]

Author Affiliations: [1]Department of Psychology, University at Buffalo, SUNY, Buffalo, NY, USA

Abstract: Sex differences in fluid intake have been observed, but the direction of the effect is inconsistent and may relate to differences in body weight. To address this question, male and estrous female rats were given 0, 1, 10, 50, or 100ng AngII and resultant water intake was measured. Analysis of raw intake revealed that males drank more than females after treatment with 10, 50, and 100ng of AngII ($p<.05$). When normalized to body weight, however, the sex difference was reversed ($p<.05$). We also used weight-matched males and females and found no sex differences in intake (p=ns). Using a mixed model regression, we found that intake significantly varied with sex and weight. We further probed these data for differences in the effect of weight on fluid intake by testing for correlations between weight and fluid intake for each sex, and comparing the regression coefficients after treatment with 10, 50, and 100ng AngII. This analysis found no effect of body weight and no difference between the curves after treatment with 10 and 50ng AngII, but did reveal a significant difference after 100ng AngII. Specifically, at this dose, weight was a predictor of intake in males but not in females. We, therefore, generated an equation to correct for the differences between the sexes and found that corrected intake was still lower in females than in males. AngII also increases blood pressure and the pressor response is greater in males than in females. It is, however, unclear if sex differences in weight are a contributing factor. Ongoing studies are, therefore, measuring changes in blood pressure after AngII in age- and weight-matched male and estrous female rats. Our results suggest that fluid intake in males is greater than in females, regardless of weight differences. Weight, however, does influence drinking under certain conditions, in males but not females. This is an important finding to consider when designing experiments on fluid intake when sex is used as a biological variable.

21. Interactive effects of Adverse Childhood Experiences and Tryptophan Depletion on Executive Function in Healthy Menopausal Women

Author List: Sheila Shanmugan[1,2], James Loughead[1], Dina Appleby[1,2], Wen Cao[1], C. Neill Epperson[1,2,3]

Author Affiliations: [1]Department of Psychiatry, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA; [2]Penn Center for Research on Sex and Gender in Health, University of Pennsylvania, Philadelphia, PA, USA; [3]Obstetrics and Gynecology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA

Abstract: Hypogonadal women frequently report a subjective decline in executive function. Loss of estradiol modulation of serotonin has been proposed as one mechanism contributing to this decline. However, Adverse Childhood Experiences (ACE) are highly prevalent and exert lasting effects on serotonin function. Thirty-six healthy, menopausal women with high ACE vs low ACE have undergone fMRI and tryptophan depletion (TD), a paradigm used to lower central serotonin levels, as part of an ongoing study aimed at characterizing the interaction between ACE and TD on executive system activation during performance of the n-back task. We hypothesized that active TD will unmask differences in activation between high vs low ACE groups not present during sham TD. Results demonstrate main effects of both ACE ($p<0.03$) and TD ($p<0.03$), as well as a trend for the interaction between ACE and TD ($p<0.08$) in the right dorsolateral prefrontal cortex (DLPFC) during the 2-back condition. In this region, the

greatest increase in activation was observed in high ACE group under active TD while the least increase was in low ACE group under sham TD. Main effects of ACE were also observed in the right ($p<0.03$) and left parietal cortex ($p<0.03$), while main effects of TD were observed in the middle frontal/cingulate gyrus ($p<0.02$), and left DLPFC ($p<0.03$). Additionally, a significant interaction between ACE and TD was detected in the left insula ($p<0.03$). In this region, TD increased activation in high ACE but not low ACE individuals. Moreover, TD-induced change in overall task performance, as measured by d', was positively correlated with TD-induced change in BOLD signal in two regions: the left DLPFC ($r=0.35$, $p<0.05$) and VMPFC ($r=0.38$, $p<0.03$). Therefore, we conclude that ACE and TD exert an interactive effect on executive function in hypogonadal women. Further research is needed to determine whether estradiol modulates ACE-by-TD interactions on brain and behavioral measures of executive function.

22. Pre-pubertal emergence of sex differences in oxytocin and vasopressin V1a receptor binding in the rat brain

Author List: Caroline J. Smith[1]**,** Max L. Poehlmann[1], Sara Li[1], Aarane M. Ratnaseelan[1], Remco Bredewold[1], Alexa H. Veenema[1]

Author Affiliations: [1]Neurobiology of Social Behavior Laboratory, Boston College, Chestnut Hill, MA

Abstract: Oxytocin (OT) and vasopressin (AVP) regulate various social behaviors and often do so in sex-specific ways. This may be due to sex differences in OT and AVP systems in the brain. In support, sex differences in OT receptor (OTR) and the AVP V1a receptor (V1aR) binding density have been shown in the adult brain. However, the sex-specific regulation of social behavior already occurs at pre-pubertal ages, suggesting that these sex differences in OTR and V1aR may be present prior to puberty. To test this, we compared OTR and V1aR binding density between males and females in numerous brain regions (33 for OTR and 27 for V1aR) at both juvenile and adult ages. We found sex differences in binding density in 11 brain regions (8 for OTR and 3 for V1aR) and in 5 of these regions, the sex difference was significant in both juveniles and adults. In detail, compared to females, juvenile and adult males showed higher OTR binding density in the posterior bed nucleus of the stria terminalis, posterodorsal medial amygdala, and posteroventral medial amygdala, and lower V1aR binding density in the arcuate nucleus and ventromedial thalamus. Sex differences in the other 6 brain regions were age-specific. In detail, juvenile males showed higher OTR binding density than juvenile females in the paraventricular nucleus, lower OTR binding density in the perirhinal cortex and lateral septum, and lower V1aR binding density in the lateral septum. On the other hand, adult males showed higher OTR binding density than adult females in the ventromedial hypothalamus and lower OTR binding density in the insula. Overall, these findings demonstrate the brain region-specific emergence of sex differences in OTR and V1aR binding density. These sex differences appear to be stable across the pubertal phase in several brain regions, while being juvenile- or adult-specific in other brain regions. The functional significance of these sex differences in OTR and V1aR binding density awaits further study.

23. Neonatal administration of testosterone masculinizes brain characteristics of SF-1 knockout mice

Author List: Tanja Spanic[1], Stuart Tobet[2], Gregor Majdic[1, 3]

Author Affiliations: [1] Center for Animal Genomics, Veterinary faculty, University of Ljubljana, Ljubljana, Slovenia; [2] Department of Biomedical Sciences, Colorado state University, Fort Collins, CO, USA; [3] Institute of Physiology, Medical school, University of Maribor, Maribor, Slovenia

Abstract: During early life, testosterone has an important organizational role in the brain. In rodents, the testosterone metabolite estradiol causes masculinization and defeminization. The present study examined the influence of testosterone administration in neonatal life in mice in which the *steroidogenic factor 1* gene (*sf-1; NR5a1*) was disrupted. SF-1 is a member of the nuclear receptor superfamily with important roles in the development and function of endocrine organs. Mice with disrupted *sf-1* alleles (SF-1 KO) are born without adrenal glands and gonads, and are phenotypically females regardless of genetic sex. Due to

adrenal insufficiency, they normally die shortly after birth but can be rescued by adrenal transplantation. These mice are not exposed to endogenous sex steroids during development and provide a model to study hormonal influences on sex differences in the brain and behavior. In the present study, one group of mice was treated with testosterone before birth, another group after birth, and a third group before and after birth (all relatively low doses). In adulthood, all mice were tested for intermale aggressive behavior. Brains were fixed and processed by immunohistochemistry for calbindin-D28k (Calb) and arginine vasopressin (AVP). Both behavioral and immunohistochemical data showed that postnatal testosterone treatment was sufficient to masculinize brain characteristics of agonadal SF-1 KO mice. Postnatal, but not prenatal treatment, increased aggressive behavior in SF-1 KO mice of both sexes to the levels seen in WT control males. Similarly, postnatal, but not prenatal, treatment was sufficient to produce the appearance of a calbindin immunoreactive cell group in the preoptic area, and increased the number of vasopressin immunopositive fibers in the lateral septum in SF-1 KO mice of both sexes to levels, seen in WT control males. The data is consistent with postnatal exposure to testosterone being sufficient to masculinize brain characteristics in agonadal SF-1 KO mice.

24. Sex differences in the relationship between depressive symptoms and risk of amnestic mild cognitive impairment

Author List: Erin E Sundermann[1], Mindy J Katz[1], Richard B Lipton[1]

Author Affiliations: Einstein Aging Study and the Department of Neurology, Albert Einstein College of Medicine, Bronx, NY

Abstract: The relationship between depressive symptoms and subsequent cognitive impairment in older adults is controversial. Sex differences and the differences in the method of categorizing depressive symptoms may contribute to the inconsistencies. We aimed to examine the effect of severity of baseline depressive symptoms on risk of incident amnestic mild cognitive impairment (aMCI) separately in men and women. Non-demented, community-dwelling older adults (aged≥70 years) from the Einstein Aging Study completed the 15-item Geriatric Depression Scale (GDS) at their baseline visit. Participants were categorized into “no or low symptoms” (GDS score=0-2), “mild symptoms” (GDS score=3-5) and “moderate/severe symptoms” (GDS score>6) groups. Sex-stratified Cox proportional hazards models, adjusted for age, education and antidepressant medication estimated hazard ratios (HR) and 95% confidence intervals (CIs) for incident aMCI as a function of depressive symptoms group. We followed 572 women (mean age=78) and 345 men (mean age=77) for 4.2 years on average (range=1.0-14.6 years). Ninety women and 64 men developed aMCI. Compared to no/low depressive symptoms, mild symptoms were associated with a two times greater risk of developing aMCI (HR=2.22, p=0.006) in men but not in women (HR=1.26; p=0.36). Conversely, moderate/severe depressive symptoms were associated with a two times greater risk of developing aMCI in women (HR=1.99, p=0.03) but not in men possibly due to low statistical power in this subgroup (HR=0.29 p=0.22). Results indicate that mild depressive symptoms in men and moderate/severe symptoms in women may represent a marker for future cognitive impairment. Results indicate a need to examine whether there is a gender bias in reporting depressive symptoms. Men may be more reluctant to disclose symptoms and, thus, reported symptoms may be more extreme in men versus women.

25. Sex differences in microglia number and activation in the developing rat brain

Author List: Alexandra Turano[1], Jennifer H. Lawrence[1], Jaclyn M. Schwarz[1]

Author Affiliations: [1]Department of Psychological and Brain Sciences University of Delaware, Newark, DE

Abstract: Microglia are the resident immune cells of the brain. During development, microglial progenitor cells migrate and infiltrate into the nervous system from the periphery. This period of migration and infiltration is critical to the maturation of the developing brain. Microglia in the developing brain are quite

distinct from microglia in the adult brain as they have a round, amoeboid shape with short, thick processes and they produce elevated levels of cytokines and chemokines imperative for many processes of normal brain development. Activation of microglia in the developing brain, via neonatal infection or immune activation, can often lead to long-term neuronal and cognitive dysfunction. Furthermore, microglial activation is associated with multiple neurodevelopmental disorders including autism, ADHD, schizophrenia, and cerebral palsy – disorders also known or suspected to have immune etiologies. All of these disorders exhibit a strong sex bias in males. We have previously seen that male rats have significantly more microglia in the developing hippocampus, cortex and amygdala than female rats on postnatal day 4 (P4) (Schwarz et al. J. Neurochem., 2012). More so, females do not show the same vulnerability that males show to infection at P4 (Bilbo et al. J. Neuroimmune Pharmacol., 2011). Given these rodent data and well-known human epidemiological data, we hypothesize that male rat pups will be more vulnerable to an immune challenge during the critical period in which microglia are infiltrating the nervous system. To test this hypothesis, we treated male and female rat pups with a mild E.coli infection on P4 and observed the presence of activated microglia in the developing hippocampus. Also, using an in vitro method, we treated isolated sex-specific microglia cultures from the hippocampus of male and female rat pups with a mild lipopolysaccharide challenge on P4 and examined whether immature microglial cells exhibit a sex-specific response to immune activation.

26. Sex differences in facial affect recognition, psychiatric symptoms, and social functioning in the general population and individuals at psychometric-risk for psychosis

Author List: Deborah J. Walder[1,2,3], Anastasia Pluish[1], Marta Statucka[1,2,3,4], Irina Rydca[1], Beril Yaffe[1,2,3]

Author Affiliations: [1]Department of Psychology, Brooklyn College of The City University of New York (CUNY), Brooklyn, NY, USA; [2]The Graduate Center of CUNY, New York, NY, USA; [3]Queens College of CUNY, Queens, NY, USA; [4]Currently at Krembil Neuroscience Centre, University Health Network, 399 Bathurst Street, Toronto, Canada

Abstract: Sex differences in clinical phenomenology, social cognition, and functional impairment are well established in psychosis. The few high-risk studies reporting sexual dimorphisms suggest an analogous pattern. Despite evidence that facial affect recognition (FAR; an important aspect of social cognition) is normally sexually differentiated (females outperform males) and predicts functional outcome, patients with psychotic disorders, their healthy relatives, and individuals at risk for psychosis typically fail to show this female FAR advantage. The current study aimed to extend our recent finding that individuals at psychometric high- (versus low-) risk for psychosis performed worse on a FAR task and social functioning (Statucka & Walder, in preparation), by examining sex effects in these factors. Based on prior literature, we predicted (in the total sample) greater disorganized symptoms among males, and better FAR performance and social functioning among females. Among individuals at psychometric high-risk for psychosis, we expected comparable FAR performance between sexes, as well as more severe negative and disorganized (and less severe depression) symptoms among males. Participants included 850 (584F/266M) young adults who completed measures of schizotypal traits and social functioning, and a computerized FAR task. Consistent with our hypotheses, in the total sample, disorganized and affective symptoms were greater among males, whereas depression symptoms and FAR performance (accuracy) and overall social functioning were better among females. Furthermore, as hypothesized, among individuals at psychometric high-risk, FAR performance was comparable between sexes, and depression was less severe among males. Contrary to expectation, disorganized symptoms were not sexually differentiated. Results suggest consideration of sex effects in FAR, symptoms and social functioning in the general population is warranted. Findings have potentially important implications for understanding sexually dimorphic etiological underpinnings of psychosis risk.

27. Sex differences in inhibitory control among at-risk drinkers

Author List: Jessica Weafer[1]**,** Jessica De Arcangelis[1], Harriet de Wit[1]

Author Affiliations: Department of Psychiatry and Behavioral Neuroscience, University of Chicago,

Chicago, IL, USA

Abstract: Alcohol abuse has been traditionally considered a male-oriented problem and as a consequence research on risk factors specific to women has been minimal. However, the sex gap in substance abuse is closing rapidly, and findings from both animal and human studies suggest that females are actually more vulnerable to drug use than males. As such, it is important to identify sex differences in risk factors for alcohol abuse in order to develop sex-specific prevention and treatment efforts. The current study examined sex differences in one well-established risk factor for alcohol abuse, poor inhibitory control. Men (n=218) and women (n=322) were classified according to alcohol risk status based on scores on the Alcohol Use Disorders Identification Test (8+ = at-risk; <8 = non-risk). Inhibitory control was assessed using a go/no-go task, in which participants were instructed to respond as quickly as possible to 'go' targets and to inhibit responses to 'no-go' targets. Most (85%) of the trials were go trials, establishing the 'go' response as prepotent, and making it more difficult to inhibit when the no-go targets occasionally appeared. The number of inhibitory failures (i.e., failure to inhibit a response to a no-go target) provided the dependent measure of interest. Sex differences were observed in the high-risk group only (sex × risk status interaction, $F(1, 536) = 5.1$, $p = 0.024$). In the at-risk group, women committed significantly more inhibitory failures than men, $t(118) = 3.2$, $p=0.002$; whereas men and women did not differ in the non-risk group ($p = 0.17$). Additionally, among women only, there was a trend for more inhibitory failures in the at-risk compared to non-risk drinkers ($p = 0.08$). These findings suggest that heavy drinking women have poorer inhibitory control than heavy drinking men. Further research is needed to determine whether impaired inhibition in these women is a cause or consequence of heavy drinking.

28. Sex differences in corticotropin releasing factor-evoked behavior and activated networks

Author List: **Kimberly Wiersielis**, Brittany Wicks, Hannah Simko, Sarah R. Cohen, Sabina Khantsis, Nausheen Baksh, David E. Waxler, Debra A. Bangasser

Author Affiliations: Neuroendocrinology and Behavior Laboratory, Department of Psychology and Neuroscience Program, Temple University, Philadelphia, PA 19122, USA

Abstract: Hypersecretion of corticotropin releasing factor (CRF) is linked to the pathophysiology of post-traumatic stress disorder and major depression, disorders that are more common in women than men. Preclinical studies have identified sex differences in CRF receptors that can increase neuronal sensitivity to CRF in female compared to male rodents. These cellular sex differences suggest that CRF may regulate brain circuits and behavior differently in males and females. To test this idea, we first evaluated if there were sex differences in anxiety-related behaviors induced by the central infusion of CRF. High doses of CRF increased grooming more in female than male rats, and the magnitude of this effect in females was greater in the proestrus phase of their estrous cycle (higher ovarian hormones) compared to the diestrus phase (lower ovarian hormones), suggesting ovarian hormones potentiate the anxiogenic effect of CRF. Brain regions associated with CRF-evoked grooming were identified by correlating a marker of neuronal activation, cFOS, with time spent grooming. Because CRF regulates a number of regions that work together to coordinate different aspects of responding to stress, we then examined more broadly whether CRF-activated functional connectivity networks differed between males and cycling females. Interestingly, hormonal status altered correlations for CRF-induced neuronal activation between a variety of brain regions, but the most striking differences were found when comparing proestrus females to males. These results suggest that ovarian hormones alter the way brain regions work together in response to CRF, which could drive different strategies for coping with stress in males versus females. These sex differences in stress responses could also help explain female vulnerability to psychiatric disorders characterized by CRF hypersecretion.

29. TrkB phosphorylation is neuroprotective only in estrogen receptor α wild-type female hippocampal neurons after in-vitro ischemia

Author List: Dila Zafer[1], Vishal Chanana[1], Douglas B. Kintner[1], Jayadevi H. Chandrashekhar[1], Eshwar

Udho[1], Ayse Canturk[1], Peter Ferrazzano[1,2], Robert A. Shapiro[3], Jon E. Levine[3], Pelin Cengiz [1,2]

Author Affiliations: [1]Waisman Center, University of Wisconsin, Madison, WI, USA; [2]Department of Pediatrics, University of Wisconsin, Madison, WI, USA; [3] Department of Neuroscience, University of Wisconsin, Madison, WI, USA

Abstract: Male neonate brains are more susceptible to the effects of hypoxia-ischemia (HI) related 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 neonate mice through phosphorylation of the TrkB post-HI (in-vivo). Differential hippocampal TrkB phosphorylation is associated with increased hippocampal ERα expression in ERα+/+ female mice and gets ablated in ERα$^{-/-}$ female mice. These results suggest a role of ERα in conferring responsivess to TrkB phosphorylation in female mice only. We hypothesized that differential ERα expression followed by TrkB phosphorylation and neuroprotection takes place in hippocampal neurons after in-vitro ischemia. Sexed hippocampal primary neuronal cultures were prepared from P1 C57BL/6J ERα$^{+/+}$ and ERα$^{-/-}$ mice in estrogen free medium and exposed to either normoxia or OGD for 4 h at DIV 7 followed by VC or 7,8-DHF. After 24 h REOX, cells were stained for cell survival and p-TrkB. For multiple comparisons ANOVA was used. 7,8-DHF enhanced TrkB phosphorylation in a dose responsive manner and promoted cell survival only in ERα$^{+/+}$ female hippocampal neurons following OGD-REOX ($p < 0.05$). HI and 7,8-DHF mediated increases in TrkB phosphorylation was ablated in ERα$^{-/-}$ male and female hippocampal neurons. Sexually differentiated TrkB phosphorylation in response to in-vitro ischemia enhanced with TrkB agonist therapy in the female hippocampal neurons is dependent on ERα. Future research will attempt to identify the time-course of ERα, bcl-2 and aromatase expressions following in-vitro ischemia in hippocampal neurons. By understanding the sexually differential role of ERα-TrkB interaction in neuronal survival, we hope to provide novel insights into the etiology and targeted therapies post- HI.

30. Characterization of factors that predispose to the metabolic syndrome in middle-aged male and female mice

Authors: Hilda Ahnstedt[1], Louise D McCullough[1]

Author Affiliations: University of Texas Health Science Center at Houston, Department of Neurology, Houston, Texas

Abstract: The metabolic syndrome is a clinical condition defined as the presence of multiple risk factors for diabetes, heart disease and stroke. Women are more likely to present with the metabolic syndrome. Women also suffer from worse stroke outcomes and more stroke death than men, especially after menopause. Young female mice experience less histological injury after stroke compared to males, while in middle-aged cohorts this scenario was reversed, and greater brain damage and stroke-related inflammation was seen in females. The underlying mechanisms for the sex differences in stroke outcome in middle-aged mice are not known but might originate from baseline differences in the metabolic syndrome. The current study characterized multiple factors involved in the development of the metabolic syndrome in middle-aged male and female mice including obesity, lipids and glucose. Body weights as well as abdominal white adipose tissue, liver and spleen weights were compared in 14-15 month old C57BL/6 mice. Glucose, triglycerides and high density lipoprotein (HDL) and low density lipoprotein (LDL) concentrations were determined in plasma after 6 h fasting. We further aimed to study whether sex differences exist in white adipose tissue derived immune cell populations as this change with obesity and may set the stage for an inflammatory milieu prior to stroke. Middle-aged males had higher body weights than females, as well as higher liver and abdominal white adipose tissue weights, while no difference in spleen weights were observed. Plasma glucose concentrations were elevated in both sexes. Lipid profiling using colorimetric assays demonstrated no differences in triglycerides or LDL levels while lower concentrations of HDL, the “good cholesterol”, were measured in females. Flow cytometry on adipocyte-

derived immune cells indicate that middle-aged males and females may have different subsets of T lymphocytes. Ongoing studies are examining these changes in aged male and female mice.

31. The sex chromosome complement contributes to ischemic stroke sensitivity and inflammatory responses in aged FCG mice

Author List: Javiera Bravo-Alegria[1], Mehwish Mirza[2], Kathryn Bentivegna[2], Eleanor B. Steffens[2], Yan Xu[1], Louise D. McCullough[1], Fudong Liu[1]

Author Affiliations: [1]Department of Neurology, McGovern Medical School, University of Texas Health Science Center, Houston, Texas; [2]Department of Neuroscience, University of Connecticut Health Center, Farmington, Connecticut

Abstract: Aging is the most important non-modifiable risk factor for ischemic stroke; modeling stroke in aged animals is of clinical relevance and translational value. Stroke is a sexually dimorphic disease. Elderly women not only have higher stroke incidence than age-matched men, but also have poorer recovery, higher morbidity and mortality once a stroke occurs. Much of this has been attributed to the loss of estrogen with menopause. However, stroke incidence does not begin to climb until well after natural menopause, suggesting there are hormone independent effects on ischemic sensitivity. As hormone levels are relatively equivalent between sexes at the age post-menopausal, tissue damage and functional outcomes must be influenced by biologic sex (XX vs. XY) in addition to the hormonal milieu. We hypothesized that sex differences in ischemic stroke in aged brains are shaped by the sex chromosome complement. The Four Core Genotype (FCG) mouse model was utilized to dissociate the effects of sex chromosome compliment from hormone exposure on ischemic sensitivity. XXF, XXM, XYF, XYM and XYwt aged mice (18-20 months) were subjected to 90-minute middle cerebral artery occlusion (MCAO). Stroke outcomes were examined at 3d of stroke. Inflammatory responses were also evaluated by flow cytometry and ELISA analysis. At 3d of stroke, more microglial activation and higher levels of pro-inflammatory cytokines (IL-1β and TNF-α) were seen in XXF vs. XYF and in XXM vs. XYM/XYwt mice respectively. Accordingly, XXF and XXM mice had significantly larger infarct volumes than XYF and XYM/XYwt cohorts respectively. There was no significant difference in hormone levels in aged FCG mice. The sex chromosome complement contributes to ischemic sensitivity in aged animals, which is likely mediated in part by innate immune responses.

32. The association of Loss of the Y chromosome in blood with cardiovascular death in men

Author list: Saskia Haitjema[1], Daniel Kofink[2], Jessica van Setten[1], Sander W. van der Laan[1], Saskia C.A. de Jager[1], Gerard Pasterkamp[1,3], Folkert W. Asselbergs[4,5,6], Hester M. den Ruijter[1]

Author affiliations: [1] Laboratory of Experimental Cardiology, University Medical Center Utrecht, Utrecht, The Netherlands, [2] Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands, [3] Laboratory of Clinical chemistry and Haematology, University Medical Center Utrecht, Utrecht, The Netherlands, [4] Department of Cardiology, University Medical Center Utrecht, Utrecht, The Netherlands, [5] Durrer Center for Cardiogenetic Research, ICIN-Netherlands Heart Institute, Utrecht, The Netherlands, [6] Institute of Cardiovascular Science, University College London, London, United Kingdom

Abstract: The Y chromosome has long been considered genomic wasteland, containing only few genes implicated in sex determination. However, recent studies found Y-chromosomal dosage-sensitive whole-genome regulators, an immunoregulatory role for the Y chromosome, and a relation between loss of Y (LOY) and a higher risk of cancer and mortality. The effect of LOY on mortality could not be explained fully by the higher risk of cancer alone. Given the involvement of immune cells in atherosclerosis, we hypothesized that (a subset of) the remaining effect of LOY on mortality may be explained by cardiovascular disease. Therefore, we conducted a pilot study and show a negative effect of LOY on cardiovascular survival in a severely diseased patient group (368 men) undergoing carotid endarterectomy. To test the hypothesis that LOY is associated with cardiovascular death in men, we included 71,773 men from the UK Biobank for whom raw intensity genotyping data were available so LOY

could be determined. We will correlate LOY to cardiovascular death (ICD 10: I10-I79) using death registry linkage data as supplied for this cohort. This way, we aim to answer the question whether LOY is negatively associated with cardiovascular death in men.

33. Sex-specific differences in DNA methylation of 488 atherosclerotic plaques from patients undergoing carotid endarterectomy

Author list: Saskia Haitjema[1*], Marten A. Siemelink[1*], Sander W. van der Laan[1], René Luijk[2], Koen F. Dekkers[2], Michal Mokry[3], Gert Jan de Borst[4], Hamid el Azzouzi[5], Bas Heijmans[2], Gerard Pasterkamp[1,6] & Hester M. den Ruijter[1]

Author affiliations: [1] Laboratory of Experimental Cardiology, University Medical Center Utrecht, Utrecht, The Netherlands, [2] Department of Molecular Epidemiology, Leiden University Medical Center, Leiden, The Netherlands, [3] Laboratory of Pediatric Gastroenterology, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands, [4] Department of Vascular Surgery, University Medical Center Utrecht, Utrecht, The Netherlands, [5] Department of Cardiology, University Medical Center Utrecht, Utrecht, The Netherlands, [6] Laboratory of Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht, The Netherlands (* authors contributed equally)

Abstract: Sex-differences in the etiology of cardiovascular disease (CVD) and atherosclerosis have been well defined, yet the extent to which a sex-specific epigenetic signature exists within the atherosclerotic vessel is unknown. We present an epigenome-wide association study (EWAS) into sex-differentially methylated regions in carotid atherosclerotic plaques of men and women undergoing carotid endarterectomy. In addition, we investigated whether the resulting loci could be involved in differences in CVD pathophysiology. We included carotid plaque specimens of 488 patients (148 women, 340 men). DNA was isolated, bisulfite converted and used to interrogate DNA methylation (DNAm) by means of the Illumina Infinium HM450 Beadchip Array. Results were validated in 92 whole blood samples (31 women, 61 men). Analysis was confined to autosomal chromosomes. DNAm was associated with sex using linear modelling corrected for age. Gene-promotor methylation was determined based on local CpGs. Additionally, differentially methylated genes were associated to measures of CVD severity. We identified 311 differentially methylated CpGs between women and men in atherosclerotic plaques. We found the majority of CpGs to overlap with previously reported sex-differentially methylated CpGs in other tissues. Analysis of promoter methylation identified 4,568/14,456 gene-promoter regions (31.6%) that were differentially methylated between the sexes. We found no indication for confounding through risk factors or atherosclerotic plaque characteristics and no association with measures of atherosclerotic disease severity. This epigenome-wide association study shows that DNAm profiles are highly sex-specific, even in the severely diseased atherosclerotic vessel wall. Given the sex-specificity of DNAm profiles, our results suggest that research into DNAm of atherosclerotic tissue and its relation with CVD should take sex into account, preferably in a sex-stratified study design.

34. Sex differences in inflammatory response to acute stress among HIV positive individuals

Author List: Liisa Hantsoo[1], Neill Epperson[1]

Author Affiliations: [1]The University of Pennsylvania Perelman School of Medicine, Department of Psychiatry

Abstract: Chronic immune activation and heightened inflammatory processes may contribute to increased risk for cardiovascular disease, metabolic syndrome, and/or affective disorders among HIV+ individuals. To examine sex differences in how HIV status may impact stress response, we assessed HIV+ and HIV- individuals using a laboratory stressor. We hypothesized that HIV+ women would be more physiologically reactive in terms of startle, endocrine and immune response to the stressor. Participants aged 40-55 years were recruited from the community, and were screened for current psychiatric disorders, drug abuse, steroid, and beta-blocker use. Participants completed threat of shock testing, in which mild shocks were

applied during a 40 minute test session. Acoustic startle response, serum cortisol, and serum cytokines (interleukin (IL)-6, IL-8, IL-10, IL-1β, C-reactive protein (CRP), tumor necrosis factor (TNF)-α, interferon (IFN)- γ) were assessed in response to the stressor. Participants (n=15) included 8 HIV- controls and 7 HIV+, similar in age, race, and education. HIV+ participants trended toward a larger cortisol response than HIV- (*p*=0.09), and a sex x HIV status interaction showed that HIV+ men had greater maximum cortisol response than other groups (p=0.05). Among HIV+ participants, cortisol 30 minutes post-stressor correlated negatively with magnitude of eyeblink (electromyography; EMG) response to acoustic startle (p's <0.05). There was no statistically significant difference in cytokine response to stressor between HIV+ and HIV- individuals. However, men showed larger response in TNF-α (p=0.05), IL-10 and IL-1β (trend: p=0.08) post-stressor compared to women. Contrary to our hypotheses, HIV+ men had greater maximum cortisol response, and men in general had greater cytokine response, compared to women. Further work should be done to assess the impact of stress on inflammatory response in HIV+ individuals, particularly men.

35. Role of estrogen and nitric oxide in the sex difference of fat metabolism and survival during fasting

Author List: Mika Jikumaru[1,2], Hirohisa Miyashita[1], Kouji Azuma[1], Eisuke F. Sato[1],,Keiichi Hiramoto[1], Emiko Kasahara[1], Masayasu Inoue[1], Etsuro Matsubara[2]

Author Affilations: [1] Department of Biochemistry & Molecular Pathology, Osaka City University Medical School, Osaka, Japan, [2] Department of Neurology, Faculty of Medicine, Oita University, Oita, Japan

Abstract: Although various methods for caloric restriction to prolong life time have been proposed, they often impair physiological metabolism and causes various diseases such as anorexia nervosa particularly in female human subjects. To understand the pathologic events induced by caloric restriction, we analyzed the changes in lipid metabolism in male and female mice during fasting. To elucidate the cross-talk of lipid metabolism and inflammation, we compared its effect on wild (WT) and iNOS knock-out (iNOS-/-) male and female mice. Fasting decreased the body weight with concomitant decrease in subcutaneous adipose tissue more rapidly in male than in female. Plasma glucose decreased rapidly within 1 day with concomitant increase in non-esterified fatty acid (NEFA). Although plasma glucose decreased more markedly in iNOS-/- than in WT, all other events occurred significantly slowly in the former. Under fasting conditions, female mice survived longer than male animals. Plasma levels of ketone bodies transiently increased with WT male while those in female maintained fairly high. Although the increase in plasma NEFA was smaller in iNOS-/- than in WT, the increase of ketone bodies was significantly higher with male and female iNOS-/- (♂<<♀) than those with WT groups. Since ketone bodies are important fuels for vital organs, such as brain and heart, their high levels might participate in the survival of animals. In fact, the lifetime of animals showed positive correlation with plasma levels of ketone bodies. All the events observed with female were suppressed by ovariectomy via an estrogen-inhibitable mechanism. These observations suggest that estrogen increases the survival of animals by optimizing fat metabolism to maintain high levels of plasma ketone bodies, major fuels for vital organs, such as the brain and heart, during fasting and that iNOS-derived NO enhances futile metabolisms require for inflammatory reactions that waste fuels in adipocytes.

36. Sex-specific expression of beta-adrenergic receptors in rat aorta

Author List: Irakli Kopaliani[1], Suzan Al-Gburi[1], Birgit Zatschler[1], Kristina Lorenz[2], Silvio Weber[3], Andreas Deussen[1]

Author Affiliations: [1]Department of Physiology, Medical Faculty Carl Gustav Carus, Dresden University of Technology, Germany; [2]Department of Pharmacology and Toxicology, Medical Faculty Carl Gustav Carus, Dresden University of Technology, Germany; [3]Department of Pharmacology and Toxicology, Würzburg Medical Faculty, Julius-Maximilians University of Würzburg, Germany

Abstract: Estrogen can modify adrenergic responsiveness in vasculature during hypertension. Even though it has been demonstrated that aortas from male rats constrict stronger in response to norepinephrine than females, mechanisms behind this effect are incompletely understood. We hypothesized that aorta of female rats express more dilatory beta-adrenergic receptors (β) than males by that counteracting constrictive effects of alpha-adrenergic receptors. Spontaneously hypertensive rats (SHR) were used as a model of hypertension. Sex-specific expression of β1, β2 and β3 adrenergic receptors in aorta was investigated both functionally (Mulvany Myograph) and using molecular techniques (Western blot, RT-qPCR and tracer assay). Norepinephrine and isoproterenol were used to induce constrictive and dilatory responses, respectively, with or without selective β blockers. We show that selective blockade of β1 (CGP20712) and β3 (SR59230A) receptors markedly increased aortic constriction by norepinephrine in female, but not in male SHRs. Selective β2 (ICI118,551) blockade, however, markedly increased aortic constriction by norepinephrine equally both in female and in male SHRs. Removal of endothelium also increased aortic constriction by norepinephrine in female, but not in male SHRs. Consistently, the β1 and β2 agonist isoproterenol, as well as selective β3 agonist (BRL37344) induced stronger aortic relaxation in female than in male SHRs. The selective β2 agonist (salmeterol) induced equal aortic relaxation both in female and in male SHRs. Unlike β2, protein and mRNA of aortic β1 and β3 receptors were markedly higher in female than in male SHRs. Finally, tracer assay revealed higher binding to aortic β1 and β3 receptors in females than in males, but no difference in β2. Our results demonstrate that aortas of female SHRs have more dilatory β1 and β3 receptors than males, which may account for a weaker aortic constriction by norepinephrine in females. At least in part these receptors act via the endothelium.

37. Sex-specific risk for cardiovascular disease: Intravascular cellular activation and carotid intima-medial thickness in middle aged women with and without history of preeclampsia

Authors: Virginia M. Miller[1,2], Vesna D. Garovic[3], Michelle M. Mielke[4,5], Brian D. Lahr[6], Kent R. Bailey[6], Muthuvel Jayachandran[2]

Author affiliations: [1]Departments of Surgery, [2]Physiology and Biomedical Engineering, [3]General Internal Medicine, Division of Nephrology and Hypertension, [4]Health Science Research, [5]Neurology, [4]Department Health Science Research, Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN

Abstract: Intravascular factors contributing to accelerated progression of cardiovascular disease in women with a history of preeclampsia (PE) remain to be clarified. This study tested the hypothesis that inflammation-stimulated intravascular cellular activation and procoagulant blood microvesicles (MV) would characterize carotid intima-medial thickness (CIMT) in women with a history of PE. Caucasian women (40 with a history of PE and 40 without a history of gestational hypertension (GHTN) matched for parity and age at index birth confirmed by review of medical records) were recruited from women of Olmsted County, MN who delivered between 1976 and 1982. Blood chemistries, cells, intravascular cellular interactions and populations of MV were measured in venous blood. CIMT was measured by B-mode ultrasound. CIMT was significantly ($P<0.01$) greater in women with a history of PE compared to without a history of GTHN. Body mass index, waist circumference, insulin resistance, levels of high-sensitive C-reactive protein and use of antihypertensive, statin and non-statin, aspirin and anti-inflammatory medications were greater in women with PE compared to without a history of GHTN. Numbers of granulocytes ($P=0.04$), tissue factor positive ($P=0.03$) and stem cell antigen positive MV ($P=0.004$) were nominally greater in women with a history of PE. Seven principal components (PC), reflecting percentage of activated platelets and interactions among platelets, granulocytes, monocytes, lymphocytes and endothelium, differed nominally between groups ($P<0.047$). Two of the PC's nominally correlated with CIMT but only in women without GHTN. Women with a history of PE may have inflammatory-stimulated cellular activation even 30 years after the incident event. Differences between groups may reflect different pathways of disease processes and effects of anti-inflammatory and anti-coagulant medications on progression of CIMT at this point in time after the incident event.

38. Hypertension in female Dahl rats involves cytotoxic $CD8^+$ interleukin (IL)-$17a^+$ T-cells

Author List: Amrita V Pai[1], Crystal A West[2], Aline Souza[2], Hong Ji[2], Xie Wu[2], Kathryn Sandberg[2]

Author Affiliations:[1]Department of Biochemistry & Molecular and Cellular Biology, 3900 Reservoir Road, NW, Washington DC, 20057. [2]Department of Medicine, 4000 Reservoir Road, NW, Washington DC, 20057

Abstract: Background and Objective: Oophorectomy is associated with increased body weight (BW) gain and a higher incidence of hypertension (HT) compared with age-matched women. Obesity and HT coincide with increased inflammatory biomarkers. We investigated the effect of ovariectomy on T-cell subpopulations in normotensive Dahl (DR) and hypertensive Dahl (DS) rats to distinguish immune responses due to HT from those due to BW gain. **Methods:** Six week old DS and DR rats were ovariectomized (Ovx) or had sham surgery (Sham). Mean arterial pressure (MAP) was measured in anesthetized rats after 4 and 10 months (mo) by indwelling vascular catheters. T-cell populations were quantitated by flow cytometry at 10 mo. **Results:** Ovariectomy increased the MAP in DS but not DR rats at 4 mo [MAP (mmHg): DS-Ovx, 143±1.0 vs DS-Sham, 129±2.9; p=0.0002 & DR-Ovx, 123±4.4 vs DR-Sham, 126±1.3; ns n=10-12/group]. At 10 mo, the MAP remained higher in the ovariectomized DS compared to DR rats [MAP (mmHg): DS-Ovx, 190±12 vs DR-Ovx, 114±1.2; n=5-8/group; p=0.002]. At 10 mo, ovariectomy increased BW to a similar extent in DS and DR rats [BW gain (g): DS, 179±13 vs DR, 202±14; ns]. The frequency of $CD8^+$ IL-$17a^+$ T-cells increased in the ovariectomized DS but not DR rats [Frequency (%) at 10 mo: DS-Ovx, 9.6±2 vs DR-Ovx, 1.3±0.4; n=5-8/group; p<0.0001]. **Conclusion:** Numerous studies in males suggest the T helper $CD4^+$ IL-$17a^+$ T-cell population contributes to HT. In contrast, our study in female DS rats suggest the cytotoxic $CD8^+$ IL-$17a^+$ T-cell population plays a role in the HT-specific inflammatory response.

39. Aged females exhibit enhanced pro-inflammatory leukocyte phenotypes

Author List: Meaghan A. Roy-O'Reilly[1], Louise D. McCullough[1]

Author Affiliations: [1]Dept. of Neurology, University of Texas Health Sciences Center at Houston, Houston, Texas

Abstract: Young women exhibit more robust immune responses than young men, yet it is unknown whether this phenomenon persists in elderly subjects. As the aging population experiences a heavy burden of inflammation-driven diseases such as stroke and atherosclerosis, we examined the effects of sex and age on pro-inflammatory leukocyte phenotypes in mice and humans. Flow cytometry was conducted on peripheral leukocytes isolated from young (3-month) and aged (18-month) C57BL/6 mice, with follow-up studies on cryopreserved samples from human patients. We found that granulocyte percentages were significant increased by age and female sex (p=.0048, p=.0012). Granulocyte and monocyte ROS production, which plays a pathological role in many inflammatory diseases, was also found to be significantly higher in female animals (p=.02). While the proportion of CD8+ T cells increased significantly with age in animals of both sexes (p=<.0001), the proportion of primed, highly cytotoxic *effector memory* $CD8^+$ T cells was significantly higher in aged females (p=.0313). In addition, CD4 T cells expressing CXCR3, which are capable of a TH1-type response, were significantly higher in females regardless of age (p=.0001). Subsequent analysis in samples from elderly human patients revealed similar results, with females exhibiting significantly higher numbers of CD4 TH1-type effector cells. In conclusion, age has significant effects on immune cell populations, including an increase in granulocytes and an increased CD8/CD4 cell ratio. There is a significant interaction of female sex with enhanced levels of pro-inflammatory populations, including ROS-producing myeloid cells, effector memory cytotoxic CD8 T cells and CD8-supporting TH1 CD4 T-cells. Understanding the mechanisms and consequences of the interactive role of age, sex and inflammation may provide insight into novel therapeutic targets for both sexes.

40. Female opiate exposure during adolescence sex-specifically induces metabolic dysfunction in F1 progeny when maintained on a diet high in fat or sugar

Author List: Anika Toorie[1], Fair Vassoler[1], Annie Kuberek[1], Cristina Wyse[1], Kevin Huynh[2], Giovanni Widmer[2], & Elizabeth Byrnes[1]

Author Affiliations: 1- Department of Biomedical Sciences, Tufts University, Cummings School of Veterinary Medicine, North Grafton, MA, USA; 2- Department of Infectious Disease and Global Health, Tufts University, Cummings School of Veterinary Medicine, North Grafton, MA, USA

Abstract: There has been a dramatic increase in the misuse/abuse of opioids amongst adolescents. Opioids regulate reward- and motivation- related behaviors and chronic opioid use results in dysfunctions in the brain's reward system. Data from animal models suggest that opioid exposure in females can impact their future progeny, even when that exposure occurs prior to conception. A number of neurobehavioral and endocrine effects have been observed in the offspring of morphine exposed females (Mor-F1) when compared to the offspring of saline exposed females (Sal-F1). Amongst these effects is increased proopiomelanocortin (POMC) gene expression within the hypothalamus arcuate nucleus (ARC) of Mor-F1 males. POMC is an inactive precursor polypeptide that is differentially processed into several bioactive neuropeptides which play key roles in homeostatic and adaptive mechanisms (Fig. 1). The POMC-derived peptides, β- endorphin (β-EP) and α- melanocyte stimulating hormone (α-MSH) regulates reward/motivation and metabolic pathways, thus influencing both reward liability and energy balance. In addition, both opioid exposure and diet composition have known effects on gut microbial community, and microbiota composition influences ones susceptibility to a number of metabolic disorders. Thus, F1 gut microbiota was sequenced to assess for changes in bacterial species organization as a function of maternal history of adolescent opiate exposure both prior to and after 6 weeks on a control, 45% high fat diet (HFD), or 45% high sucrose diet (HSD). Preliminary data indicate that when maintained on a standard diet F1 animals show no alterations in body weight nor do they display overt metabolic dysfunction. However, when maintained on a HFD or HSD, Mor-F1 animals sex-dependently display impaired fasting glucose levels and they gain more weight than Sal-F1 controls. Collectively, results demonstrate that offspring born to females adolescently exposed to morphine display sex-specific metabolic disturbances. In particular, Mor-F1 males exhibit baseline changes to POMC protein levels within the mediobasal hypothalamus (MBH); while female Mor-F1's display an altered microbial profile relative to Sal-F1 females, an effect that was not observed in male F1's. Currently, on-going studies are: (1) examining the role of the POMC-derived peptides in mediating the Mor-F1 metabolic phenotype, and (2) identifying the specific microbial shifts that occur in F1 offspring with a maternal history of adolescent opiate exposure.

41. Regulation by the histone demethylase KDM6A gene is sex biased in development and cancer

Author List: **Joel B. Berletch[1]**, Xinxian Deng[1], Di Kim Nguyen1, Christine M. Disteche[1,2]

Author Affiliations: [1]Department of Pathology, School of Medicine, University of Washington, Seattle, Washington, United States of America, [2]Department of Medicine, School of Medicine, University of Washington, Seattle, Washington, United States of America

Abstract: Epigenetic modifications play important roles in gene regulation during development and in cancer. Some of these epigenetic modifications are controlled by specific genes expressed in a sex biased manner. For example, *KDM6A* is an X-linked gene that encodes a histone H3K27 demethylase and is more highly expressed in females than males because it escapes X inactivation. Investigation into KDM6A's role in cancer etiology has revealed mutations associated with many types of cancers including colon, breast, and renal cancers as well as leukemia. Gender-specific aberrant H3K27 methylation caused by KDM6A inactivating mutations contributes to T-cell leukemogenesis. Not only does KDM6A function as a tumor suppressor gene, it is also important for normal development. Here, we show that two members of the mouse Rhox homeobox gene cluster, *Rhox6* and *9*, are regulated by de-methylation of histone H3 at lysine 27 by KDM6A in a sex-specific manner. In female mouse ES cells, KDM6A is specifically recruited

to *Rhox6* and *9* for gene activation, a process inhibited by *Kdm6a* knockdown in a dose-dependent manner. In contrast, KDM6A occupancy at *Rhox6* and *9* is low in male ES cells and knockdown has no effect on expression. Additionally, in the mouse ovary where *Rhox6* and *9* remain highly expressed, KDM6A occupancy strongly correlates with expression. Thus, our data implicates *Kdm6a* in the regulation of genes important in reproduction, suggesting that KDM6A plays a role in the etiology of developmental and reproduction-related effects of sex chromosome anomalies. Taken together, it is clear that epigenetic regulators expressed in a gender-biased manner can regulate genes involved in proper development and help maintain normal cell growth and proliferation.

42. Genetic Variation in Chromosome Y Regulates Susceptibility to Influenza A Virus Infection and Associated Sexual Dimorphism

Author List: Laure K. Case[1], Oliver Dienz[2], Abbas Raza[1], Dimitry N. Krementsov[1], Jonathan Boysen[2], and Cory Teuscher[1]

Author Affiliations: [1]Department of Medicine and [2]Surgery, Center for Immunobiology and Infectious Diseases, University of Vermont, Burlington, VT USA

Abstract: Males of many species, ranging from humans to insects, are more susceptible than females to parasitic, fungal, bacterial, and viral infections. One mechanism which has been proposed to account for this difference is the 'immunocompetence handicap model' which posits that the higher infectious disease burden in males is due to testosterone (T), which drives the development of secondary male sex characteristics at the expense of suppressing immunity. However, the causal link between elevated T levels and immunosuppression is only observed within certain taxa and for certain measures of immunocompetence, suggesting that cell-intrinsic (chromosome X and Y) sex-specific factors may also contribute to the sexual dimorphism in infectious disease burden. In humans, this is supported by the fact that in epidemiological studies on influenza A virus (IAV) infection where age and sex are included as covariates, the data indicate that incidence and severity change as a function of age, with more males being affected thru adolescence, and more females affected across all post-adolescence age ranges. Using a murine model of IAV infection and panel of ChrY consomic strains on the C57BL/6J background, we present data showing that natural genetic variation in chromosome Y (ChrY) influences IAV pathogenesis in males and the resulting sexual dimorphism in IAV pathogenesis, independent of serum T levels. Additionally, we present data showing that susceptibility to IAV segregates independent of copy number variation (CNV) in multicopy ChrY gene families that influence susceptibility to other immunopathologic phenotypes, including survival following infection with Coxsackie virus B3. These results demonstrate a critical role for ChrY in regulating the sexual dimorphism in susceptibility to infectious disease.

43. Prediction of sex-associated susceptibilities to drug-induced adverse events in F344 rats based on transcriptomic profiles

Author List: James C. Fuscoe[1], Kejian Wang[1], Qiang Shi[1], and Vikrant Vijay[1]

Author Affiliations: [1]Division of Systems Biology, National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR

Abstract: Preclinical safety assessments are a crucial step in assuring the development of safe and effective medical products. Despite large investments of resources in this process, drugs can enter the market with safety liabilities that result in patient injury and even death. A crucial step in developing a safe and effective drug is assessing drug metabolism. During non-clinical drug development, a drug candidate is often evaluated in adult animals of a single sex (males). Age- and/or sex-differences in the enzymes that metabolize the drug may result in unrecognized age- and/or sex-related differences in the disposition, safety, and efficacy of the drug. Drug metabolizing enzymes plus transporters (DME/T) play a major role in a drug's detoxification, excretion, and/or activation, and thus differences in the DME/T expression

profiles may play a key role in drug safety. A rat model was used to identify differences in the basal hepatic transcriptional profiles of 298 DME/T genes in adult males and females. Genes were considered to be differentially expressed between females (F) and males (M) if the t-test p-value <0.05 and fold ratio (F/M or M/F) >2. The expression of 29 genes was differentially expressed between the sexes with 17 being more highly expressed in males and 12 being more highly expressed in females. The genes coding for Cyp3a9, Sult2a1, Adh6 and Cyp2c12 were expressed at 5 to 378-fold higher levels in females than males. The genes coding for Sult1e1, Cyp2c11, Cyp2c13 and Cyp3a2 were expressed at >1000-fold higher levels in males than females. The 29 enzymes encoded by these differentially expressed genes metabolize more than 600 drugs, with 40 drugs metabolized by 1 or 2 sexually dimorphic DME/Ts. Based on these findings, the disposition of these drugs may be different in the two sexes. Confirmation of these results by in vivo studies may allow the prediction of sex-related susceptibilities to adverse drug events from easily obtained transcriptomics data.

44. Epigenome-wide methylation profile in normal human kidney: sex, age, and ethnic differences

Author List: Beverly D. Lyn-Cook[1], Stancy Joseph[1], Nysia George [2], Bridgette Green-Knox[3], Beverly Word[1], Tamara Nicolson[4], George Hammons[1], Shiew-Mei Huang[5]

Author Affiliations: [1]Division of Biochemical Toxicology, [2]Division of Bioinformatics and Biostatistics, [3]Division of Systems Biology, National Center for Toxicological Research, FDA, Jefferson, AR 72079-9502, [4]Drug Discovery and Development, London, UK, and [5]Center for Drug Evaluation, White Oak, Maryland, 20993.

Abstract: Background: A wealth of clinical data has shown that women and men respond differently to various drugs. Sex differences can be due to a number of factors that influence pharmacokinetics, pharmacodynamics, adverse reactions, drug efficacy, and safety. Differences have been mainly attributed to genetic polymorphisms in drug metabolizing genes; however, emerging evidence is also showing that regulation of gene expression could be due to epigenetic mechanisms. Three major organs, the liver, kidney, and small intestine, play important roles in drug disposition, clearance, drug-drug interactions, absorption, and toxicity. This study focused on the kidney, an organ in which disease and toxicity can affects more than 20 million adults and cause significant mortality. **Results:** To understand the role of epigenetics for sex differences in the kidney, baseline data on epigenetic status are required. Here we used measurements from more than 450,000 CpG islands (CGI). Over 24,000 CGI were identified with sex-based differences in non-diseased, age-matched kidney tissues, of which 582 significantly methylated regions were identified. Hierarchically clustered technical replicates were highly correlated (r> 0.99). Furthermore, statistical analyses showed significant interaction between sex and ethnic group and between sex and age for specific genes. **Conclusions:** This study provides insights into the epigenetic regulation of genes that may play important roles in kidney function, transport, and clearance, which can ultimately provide guidance in improving drug efficacy and safety and reducing adverse reactions.

45. Uncovering buried sex differences in gene expression using cell-type specific RNA sequencing of actively translated mRNAs

Author List: Bridget M. Nugent[1], Tracy L. Bale[1]

Author Affiliations: [1]Department of Biomedical Sciences, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, 19104, USA

Abstract: Large-scale genomic analyses of sex differences in gene expression can provide valuable insight into sex differences in cellular signaling, function, and ultimately disease risk. Due to technical limitations, most analyses of sex differences in transcriptomic profiles have been performed on tissue with heterogeneous cell populations, often masking important cell type specific sex differences in gene expression. The placenta is one such heterogeneous tissue, in which surprisingly few sex differences in

gene expression have been reported despite its fetal origin and sex-specific chromosome complement. The placenta is a critical arbitrator between the mother and fetus, providing necessary substrates for fetal growth and is widely regarded as an important contributor to healthy neurodevelopment. Identifying sex differences in gene expression within fetally-derived placental trophoblast cells could enhance our understanding of why prenatal insults preferentially impact male offspring neurodevelopment. Using RiboTag technology and trophoblast-specific Cre-expressing transgenic mice, we isolated mRNAs within ribosomes specifically in fetally-derived placental trophoblast cells. RNA-Seq analysis revealed widespread sex differences in gene networks associated with cellular metabolism, suggesting that sex differences in placental metabolic function might contribute to male-biased vulnerabilities to prenatal insults. Previously, we identified O-linked-N-acetylglucosamine transferase (OGT) as a sex-specific placental biomarker of gestational stress. OGT, an X-linked metabolic and epigenetic regulator, escapes X inactivation in female trophoblasts – providing the female placenta with two copies of OGT and the male placenta with one copy. Genetically reducing OGT copy number in female trophoblasts dramatically reduces trophoblast-specific sex differences in gene expression, suggesting that OGT plays a critical role in determining sex differences in placental function. As OGT reduction in the female placenta also mimics the male-specific effect of prenatal stress on neurodevelopment, we believe that OGT orchestrates sex differences in gene networks that safeguard the female fetus from the neurodevelopmental consequences of prenatal stress exposure.

46. Differences in gene expression and regulation across the male and female genome

Author List: Rachael Werner[1], Bryant Schultz[1], Jozef Madzo[1], Jaroslav Jelinek[1], Nora Engel[1]

Author Affiliations:[1]Fels Institute for Cancer Research and Molecular Biology, Lewis Katz School of Medicine at Temple University

Abstract: Mortality rates in males and females differ as early as newborn stages. Male embryos have a higher growth rate at preimplantation stages, before overt sexual differentiation and exposure to sex hormones. X inactivation, a drastic epigenetic event exclusive to females, likely affects the embryonic transcriptome and epigenome in a sex-specific manner. Thus, male and female genomes are epigenetically poised for their divergent pathways early on. To determine whether there is differential expression at preimplantation stages, we derived male and female mouse embryonic stem (ES) cell lines and performed RNA-sequencing. When XY and XX cell lines were compared, over 400 genes were differentially expressed ($\alpha < 0.01$). A substantial number of these are transcription factors and epigenetic enzymes that are predicted to be dosage sensitive, indicating that there are regulatory differences between male and female embryos that depend solely on their chromosomal composition. Differences within key pathways, based on gene ontology and pathway analysis were validated by qPCR and luciferase. To determine whether these expression differences translated into epigenomic differences, we conducted focused chromatin immunoprecipitation analyses and observed significant sex-dependent variation in chromatin accessibility in specific genes. We are exploring whether these sexual dimorphisms impact lineage determination by directed differentiation of the ES cells and whether they predict distinctions in response to environmental signals. The results from these objectives will have implications in understanding the developmental origins of disease, will impact disease treatment and stratification and, importantly, will have significance in the field of regenerative medicine.

47. Sexual differentiation of juvenile social play behavior and development of the amygdala is modulated by CB1 and CB2 receptors in the rat

Author List: Kathryn J Argue[1], Margaret M McCarthy[1]

Author Affiliations: [1]Department of Pharmacology and Program in Neuroscience, University of Maryland Baltimore School of Medicine, Baltimore, M.D., USA.

Abstract: Rough-and-tumble behavior is a common form of juvenile play in many species. A universal feature is higher frequency and longer duration of play by males relative to females. We previously reported that administration of a combined CB1 and CB2 agonist, WIN, on postnatal days 0-3 masculinizes play behavior in females (PNAS 107; 2010). This change in play correlates with a sex difference in the number of BrdU+ cells in the developing amygdala, an important brain region for the sexual differentiation of juvenile play behavior. Females have more BrdU+ cells compared to males and treatment with WIN decreases the number to that of males. We now seek to discern the relative role of CB1 versus CB2 receptor activation in both play behavior and cell genesis in the developing amygdala. Treatment with highly selective agonists for either CB1 (ACEA) or CB2 (GP1a) significantly decreased the number of BrdU+ cells in the medial amygdala of females to levels observed in males (ANOVA $F(3, 80) = 16.76$, $p < 0.001$), but had no impact on subsequent play behavior. Surprisingly, co-administration of ACEA and GP1a masculinized juvenile female play behavior (Kruskal-Wallis$(3,289) = 14.07$, $p < 0.001$) and neonatal co-antagonism of CB1 (AM281) and CB2 (AM630) feminized male play behavior (Kruskal-Wallis$(4,192) = 16.29$, $p = 0.001$). Despite reports that CB2 is not detected in the brain, our immunohistochemical analysis demonstrated CB2 co-localization on neurons, astrocytes, and microglia in the developing and adult amygdala. Furthermore, quantitative analysis of CB2 immunohistochemical data suggests both age- and sex-dependent changes in expression. A similar analysis of CB1 immunohistochemistry is currently underway. Additionally, we are using flow cytometry to analyze sex differences in proliferation of different cells types and reconstructing neuronal morphology in the neonatal amygdala to determine how these are altered with specific manipulation of CB1 or CB2.

48. Sex differences in morphine-induced analgesia on sensory and affective components of acute and inflammatory pain

Author List: Alexander Armendariz[1]; Lisa Harton[1]; Arbi Nazarian[2]

Author Affiliations: [1]Master of Science in Pharmaceutical Sciences graduate program, College of Pharmacy, Western University of Health Sciences, Pomona, CA, USA; [2]Department of Pharmaceutical Sciences, College of Pharmacy, Western University of Health Sciences, Pomona, CA, USA

Abstract: The experience of pain is characterized by the presence of an aversive sensory stimulus combined with negative affect, which is often mediated clinically through administration of analgesics such as morphine or other prescribed opioids. The pain-relieving effects of opioids however, have been shown to vary across sexes. The present study investigates sex differences in the effects of morphine on sensory and affective components of pain following administration of Complete Freund's Adjuvant (CFA), a model of chronic inflammatory pain, and formalin, a model for acute, spontaneous pain. An intraplantar injection of CFA or formalin was administered into the left hind paw of male and female Sprague-Dawley rats. For CFA-treated animals, Hargreaves tests for thermal nociception and conditioned place preference (CPP) data were obtained following subcutaneous administration of varying morphine doses (0, 1, 4, 8 mg/kg). Hargreaves Test results revealed sex differences in paw withdrawal latencies (PWL) in a dose dependent manner, with females being less sensitive to morphine than males. CPP results revealed sex differences in the preference for the morphine-paired chamber after one-day post-CFA, but not seven days post-CFA. Intraplantar formalin results also revealed sex differences in morphine analgesia but only at the 4.0mg/kg dose. Conditioned place aversion (CPA) studies in formalin treated animals revealed no sex differences in morphine-induced decrease in aversion due to formalin. These results reveal sexually dimorphic properties of morphine analgesia on the affective and sensory components of pain. Considering these findings, further investigation into the underlying mechanisms of morphine analgesia in the affective and sensory component of acute and inflammatory pain is needed.

49. Sex differences in spontaneous morphine withdrawal-induced behaviors and CREB activation in the tail of the ventral tegmental area

Author List: Samara A. Morris Bobzean[1], Linda I. Perrotti[1]

Author Affiliations: [1]Department of Psychology, College of Science, University of Texas at Arlington, Arlington, Texas, USA

Abstract: Opiate withdrawal syndrome is a feature common to chronic opiate use that often serves as a powerful motivator of continued drug use. GABA-ergic neurons in the tail of the ventral tegmental area (tVTA) are implicated in mediating responses to opiates, including withdrawal. The tVTA regulates the effects of opiates on VTA dopamine neurons and a number of earlier studies have shown that changes in levels of CREB within the VTA affect drug-motivated behaviors. To date, the mechanisms underlying morphine withdrawal have been studied almost exclusively using men and male animals. The aims of the current study were to investigate sex effects on the expression and duration of spontaneous somatic morphine withdrawal behavior; and identify the relationship(s) between spontaneous somatic morphine withdrawal behavior and CREB activity in tVTA GABA-ergic neurons. Morphine-dependence was induced in adult, male and female Long Evans rats using twice-daily injections of escalating doses of morphine for 10 days. Spontaneous somatic morphine withdrawal behavior was recorded every 12 hours for 72 hours after the last morphine treatment. Animals were sacrificed via exsanguination after the last behavioral observation (72 hours). Both male and female morphine-dependent rats developed somatic symptoms of withdrawal, however, males expressed more severe symptoms earlier in withdrawal (in the first 36 hours) compared to females. While, females demonstrated lower overall symptom severity, these symptoms persisted for a longer period of time; as a result, withdrawal symptoms in females were more severe than males' at the 72-hour time point. CREB activation in tVTA GABA-ergic cells was significantly higher in morphine-withdrawn females compared to controls 72 hours after the end of treatment. These results demonstrate sex differences in the timing of the expression of somatic withdrawal. Moreover, our data suggest sex differences in the timing of withdrawal-induced activation of tVTA CREB.

50. Sex and age differences in forebrain distribution of vasopressin and oxytocin fibers in the rat

Author List: Brett T. DiBenedictis[1], Elizabeth R. Nussbaum[1], Harry K. Cheung[1], and Alexa H. Veenema[1]

Author Affiliations: [1]Neurobiology of Social Behavior Laboratory, Department of Psychology, Boston College, Chestnut Hill, MA, USA

Abstract: The neuropeptides vasopressin (AVP) and oxytocin (OT) have been implicated in the regulation of numerous social behaviors. AVP and OT signaling occurs within a circuit of interconnected brain regions known collectively as the 'social behavior neural network' (SBNN). Importantly, AVP and OT signaling within the SBNN differentially regulate various social behaviors depending on the sex and/or age of the animal. We hypothesized that variation in the display of these behaviors could be due to sex and age differences in AVP and OT fiber innervation within the SBNN. To test this, we conducted immunohistochemistry to visualize AVP- and OT-immunoreactive fibers in juvenile and adult male and female rats and subsequently quantified AVP and OT fiber density throughout the SBNN. We found robust sex (higher in juvenile and adult males) and age (higher in adult males and females) differences in AVP fiber density in the lateral septum, anterior bed nucleus of the stria terminalis, medial amygdala and preoptic area of the hypothalamus, while no sex or age differences were found in the anterior hypothalamus or ventromedial hypothalamus. In contrast, there were fewer sex and age differences in OT fiber density, and the direction of these differences was brain region-specific. In detail, OT fiber density was higher in adult females compared to adult males in the medial lateral septum and anterior hypothalamus, lower in adult males compared to juvenile males in the rostral lateral septum, and higher in adult males compared to juvenile males in the anteroventral medial amygdala. These findings suggest that AVP signaling in discrete SBNN nuclei may be particularly critical for behaviors expressed by adult males, while OT signaling may slightly favor adult female-typical behaviors, but in a brain region-specific manner. More work is needed to determine the causal involvement of sex and age differences in AVP and OT fiber density in the sex- and age-specific regulation of social behavior.

51. Systemic inhibition of oxytocin receptors blocks anxiolytic effects of social stress in female California mice

Author List: Natalia Duque-Wilckens[1], Michael Steinman[2], Rebecca Hao[3], Brian C. Trainor[1,3]

Authors Affiliations: [1]Animal Behavior Graduate Group, University of California, Davis, CA 95616; [2]Molecular, Cellular, and Integrative Physiology Graduate group, University of California, Davis, CA 95616; [3]Department of Psychology, University of California, Davis, CA 95616.

Abstract: Oxytocin (OT) is often considered as pro-social and anxiolytic, but recent evidence suggests that the effects of OT are context-specific. It has been proposed that OT increases the salience of social cues, which can explain why OT can either enhance or inhibit social behaviors. We previously discovered in the California mouse (*Peromyscus californicus)* that three episodes of social defeat stress reduce social interaction (SI) behavior in females but not males. We also found that in females but not males, stress induces hyperactivity of OT neurons in the bed nucleus of the stria terminalis (BNST), and intranasal infusions of OT reduce SI in females that are naïve to defeat (which mirrors the effects of stress on this behavior). Here we used a pharmacological approach to determine whether increased activation of OT receptor (OTR) or V1a receptor (V1aR) in stressed females contributes to stress-induced decreases in SI. First we examined the nucleus accumbens shell (NAcsh). Here, stress increases V1aR binding in females, but infusion of V1aR antagonist in NAcsh had no effects on SI in stressed females. Next we focused on OTR. Control and stressed females were randomly assigned to receive an IP injection of saline or OTR antagonist (L-368,899, Sigma) 30 min before behavior testing. Control females showed high levels of SI, and in an odor preference test (OP) preferred an odor of an unfamiliar individual vs. the familiar odor of a cage mate. Stress not only reduced SI, but also reversed the OP such that stressed females showed a preference for a familiar odor over an unfamiliar odor. Interestingly, OTR antagonist treatment rapidly blocked the effects of stress on both SI and OP. This suggests that stress-induced increases in OTR activation may drive females away from unfamiliar and towards familiar social contexts (and possibly safer). These results suggest that the use of OTR antagonists could have unanticipated benefits as an anxiolytic agent in social contexts.

52. Sex differences in cocaine self-administration and reinstatement after adolescent stress exposure

Author List: Anne Q. Fosnocht[1], Lisa A. Briand[1]

Author Affiliations: [1]Department of Psychology and Neuroscience Program, Temple University, Philadelphia, PA 19122

Abstract: Adolescence is a formative period, during which stressful experiences can influence behavior in adulthood, most notably by increasing the likelihood of current and future substance abuse. Chronic adverse life events, low socioeconomic status and social isolation during adolescence greatly increase vulnerability to addiction. Further, females are more sensitive to chronic stress than males, exhibiting higher rates of stress-related disease. Drugs that target the sympathetic nervous system seem to more effectively decrease craving in female addicts, suggesting there are sex difference in the interaction between stress and addiction. However, the neural mechanisms underlying this interaction remain unclear. The current study utilizes adolescent social isolation as model of early life stress to determine its effect on cocaine self-adminstration, extinction, and reinstatement behavior in mice. Although adolescent stress exposure did not alter the acquisition of food self administration in either sex, we found that isolation stress led to an increase in both cocaine instake and responding. This increase in responding was driven primarily by the males that received social isolation rather than the females. However, when we examined their responding on a progressive ratio schedule, both male and female isolated animals exhibited an increased motivation for cocaine compared to group-housed controls. In contrast to what was seen during the cocaine self-administration phase, we found that socially isolated female mice exhibited an increase in cocaine seeking during a cue-induced reinstatement session whereas males did not differ from controls. Future studies will examine whether these sex differences are driven by differences in gonadal hormone levels as well as which brain regions might be critically involved in these behavioral effects.

53. Menstrual cycle phase effects on neural responses and cigarette craving in naturally cycling, cigarette-dependent women

Author List: Reagan R. Wetherill[1], Nathan Hager[1], Kanchana Jagannathan[1], Melanie Maron[1], Emily Guthier[1], **Teresa R. Franklin**[1]

Author Affiliations: [1]University of Pennsylvania, Department of Psychiatry, Philadelphia, PA 19104, USA

Abstract: Cigarette smoking is the leading cause of preventable morbidity and mortality. The prevalence of smoking is greater among men than women, yet women are less successful at quitting. Preclinical and clinical research suggests that ovarian hormones (i.e., estradiol, E and progesterone, P), which fluctuate over the course of the menstrual cycle (MC), may contribute to these sex differences. Specifically, research suggests that P may protect against nicotine seeking/smoking behavior; whereas E may enhance vulnerability by increasing the reward experienced from nicotine and the people, places and things associated with smoking (i.e., smoking cues). Here, we present neuroimaging data supporting the extant literature. While women are in the follicular phase of their MC (high E) and are exposed to smoking cues, they exhibited greater neural activity in regions of the brain associated with reward. Further, cigarette craving was enhanced by smoking cue exposure and correlated with activity in the insula, a brain region known to moderate craving. These activations were not observed in women who are in the luteal phase (high P). We further report that executive control regions of the brain were less functionally connected to reward-related brain regions during the follicular phase, and the greater the disconnect within these circuits, the greater the attentional bias to smoking cues. Collectively, these findings may indicate that while in the follicular phase, women have less cognitive control over rewarding activities (reduced ability to 'Just Say No'). Understanding how MC phase and its associated hormonal fluctuations affect neural processes, cognition, and behavior is a critical step in the development of treatment strategies for women. For example, given that not smoking during the first 3 days after quitting is the strongest predictor of smoking cessation success, timing Quit Dates to occur based on the natural hormonal milieu could substantially reduce relapse rates.

54. Sex differences in the effect of aromatase inhibition on cognition and behavior of gonadectomized common marmosets (*Callithrix jacchus*)

Author List: Nicole J. Gervais[1], Kathryn Pilar Workman[1], Matthew. LaClair[1], & Agnès Lacreuse[1]

Author Affiliations: [1]Psychological and Brain Sciences, University of Massachusetts Amherst, Amherst, MA, 01003

Abstract: Sex steroids, in particular estradiol (E2) and testosterone (T), modulate several aspects of cognition and behavior in nonhuman primates and are thought to contribute to cognitive sex differences. Recent rodent findings suggest that estrogens synthesized in the brain, via conversion of T to E2 by the enzyme aromatase, may also have an important role in modulating cognition. The purpose of the present study was to examine the effects of E2 synthesis inhibition on working memory, as assessed by the Delayed Response and Delayed-Matching-to-Position (DMP) tasks in gonadectomized (GDX) common marmosets. Sixteen marmosets (*n* = 7 females) were tested for one week at baseline and then administered the aromatase inhibitor Letrozole (20 µg, p.o.; *n* = 3 females, *n* = 5 males) or vehicle (*n* = 3 females, *n* = 5 males) daily for four weeks. In addition to cognitive performance, affiliative and anxious behaviors (duration and frequency during 10-min video recorded session) were assessed daily. While there were no sex differences in DMP task performance, there was a tendency for females to outperform males on the DR task. Letrozole reduced accuracy on the DMP task in both sexes but had not effect on DR. In addition, Letrozole increased agitated locomotion in males, but not females. These results suggest that Letrozole impairs hippocampus-dependent working memory, and may increase anxiety in male, but not female GDX marmosets. These data lend support to the idea that brain-derived E2 influences some

aspects of cognition and behavior in primates, and that this effect may be sex-dependent. Further studies are needed to expand on these findings.

55. Sex differences in the effects of dietary emulsifiers on physiology and behavior in mice

Author List: Mary K. Holder[1], Benoit Chassaing[2,3], Nicole V. Peters[1], Jack J. Whylings[1], Andrew T. Gewirtz[2,3],Geert J. de Vries[1]

Author Affiliations: [1]Neuroscience Institute, Georgia State University, Atlanta, GA, USA; [2]Center for Inflammation, Immunity, and Infection, Georgia State University, Atlanta, GA, USA; [3]Institute for Biomedical Sciences, Georgia State University, Atlanta, GA, USA

Abstract: Dietary emulsifiers, detergent-like molecules commonly used in processed foods such as peanut butter and ice cream, alter the composition of the intestinal microbiota and induce chronic low-grade inflammation, obesity, and metabolic syndrome in mice.[1] As alterations in the gut microbiota can lead to changes in anxiety-like and altered social behaviors, we hypothesized that emulsifier consumption would affect these behaviors as well. Upon weaning, male and female mice were placed into a new cage with water or a 1% solution of either carboxylmethylcellulose or polysorbate80 in the drinking water. Body weights were recorded weekly and behavior was assessed using a battery of standard tests (one test per week) including open field (OF), elevated plus maze (EPM), light/dark box (L/D), and three-chambered sociability. In addition, the length of colon and weights of the colon, spleen, liver, and adipose tissue were recorded at the end of the experiment. Emulsifier treatment altered anxiety-like behavior in male mice, as indicated by decreased time in the center of the OF and increased distance travelled in the EPM. Emulsifier consumption did not affect anxiety-related behaviors in females; however, it reduced the preference for a novel conspecific mouse in the three-chambered sociability test in female, but not male, mice. Emulsifier treatment caused a 60-80% increase in adiposity and chronic intestinal inflammation, characterized by shortened colons, in both male and female mice compared to water-treated controls. Emulsifiers also increased total body weight of males, but not females. Taken together, these results indicate that emulsifier treatment leads to more severe physiological and behavioral alterations in male mice, compared to females. Future research will seek to elucidate the mechanisms through which emulsifiers act to produce these sexually dimorphic effects.

56. Prenatal maternal stress accelerates menarche onset through increased in body mass index at 5½: Project Ice Storm

Author List: Sherri Lee Jones[1,2], Annie Duchesne[3], Aihua Liu[1], David P. Laplante[1], Suzanne King[1,2]

Author Affiliations: [1]Douglas Mental Health University Institute. [2]Department of Psychiatry, McGill University, Montreal, Qc, Canada. [3]Department of Psychology, University of Toronto, Toronto, ON, Canada.

Abstract: Age at menarche is a biomarker of reproductive, physiological and psychological health, and its early onset is associated with adverse outcomes such as increased risk for vascular disease, depressive and anxious symptomatology, and risky sexual behaviors. Factors that accelerate menarche onset include childhood adiposity and childhood stress. Stress experienced during pregnancy has been shown to have long-lasting effects on the offspring's psychological, neural, and metabolic function, including increased adiposity in childhood. Yet, it is unknown whether prenatal maternal stress also influences daughter's age at menarche. We hypothesized that an association does exist between prenatal maternal stress and age at menarche, either directly and/or indirectly through its effects on childhood body mass index. A group of girls (n=31) whose mother's were pregnant with them during the 1998 ice storm in Quebec, Canada was followed from 6 months to 15½ years old, in a prospective longitudinal study. Within 6 months of the storm, the mother's level of objective hardship and subjective distress was collected. Daughters' BMI was measured at 5½, and age at menses onset was collected through self-report at 13½ and 15½. Mediation analyses were used to test direct and indirect effects, controlling for maternal smoking and ponderal index

at birth. We found that higher levels of objective prenatal maternal stress indirectly accelerated the onset of menses through an increase in BMI at 5½ years of age. No direct effects were found. We conclude that objective prenatal maternal stress can accelerate menarche onset through its effects on metabolic measures (specifically BMI) in childhood. The findings increase our understanding of pathways through which prenatal maternal stress can lead to adverse health outcomes. Future research should examine whether prenatal maternal stress influences other measures of reproductive function in humans.

57. The microRNA miR-124 is higher in the neonatal female hippocampus and impacts parameters mediating neuronal excitation

Author List: Katherine E. Kight[1], Margaret M. McCarthy[2,3]

Author Affiliations: [1]Program in Molecular Medicine, and Departments of [2]Pharmacology and [3]Psychiatry, University of Maryland School of Medicine, Baltimore, Maryland

Abstract: GABA is the dominant inhibitory neurotransmitter in the adult brain, but during development it depolarizes immature neurons, resulting in excitation and regulating multiple developmental processes including cell proliferation, migration, and neuronal maturation. The shift in GABA activity from depolarizing to hyperpolarizing occurs at different ages in males and females, and is determined by the relative abundance of chloride channels on the developing neuron. In particular, high expression of the bumetanide-sensitive Na-K-2Cl cotransporter, NKCC1, confers a depolarizing action of GABA on immature neurons, while decreased NKCC1 expression is a hallmark of mature neurons that are hyperpolarized by GABA. In the neonatal hippocampus of rats, the developmental shift in GABA activity occurs earlier in females, during the first week of life, and in males during week two (Galanopoulou, 2008; Nuñez and McCarthy, 2007). Here we report that females had lower levels of NKCC1 protein compared to males in the hippocampus during the first week, but no sex difference in NKCC1 transcripts, implying posttranslational regulation. We hypothesized that NKCC1 expression is downregulated by miR-124, a microRNA that promotes neuronal maturation and has putative binding sites in the NKCC1 transcript. miR-124 is elevated in female hippocampus during week one, compared to males, and antagonism of miR124 *in vivo* and *in vitro* upregulated NKCC1 expression. The higher levels of miR124 in females and the inhibitory effect of miR124 on NKCC1 expression suggests a mechanism for the earlier developmental shift to hyperpolarizing GABA seen in the hippocampus of females. In this way, miR124 may be a key regulator of sex differences in the developing hippocampus through downstream effects on neuronal physiology.

58. CNS autoimmunity is regulated by the interaction of estrogen receptors and p38 MAP kinase in a sex- and cell type-specific fashion

Author List: Dimitry N. Krementsov[1], Cory Teuscher[1]

Author Affiliations: [1]Department of Medicine, Immunobiology Division, University of Vermont, Burlington, VT USA

Abstract: Multiple sclerosis (MS) is a debilitating central nervous system (CNS) neuroinflammatory disease of autoimmune etiology. MS prevalence in females has continued to increase, and women with MS currently outnumber men 3:1. Sex hormones have been implicated in this sexual dimorphism, and evidence from experimental autoimmune encephalomyelitis (EAE), the principal animal model of MS, suggests that administration of *exogenous* estrogens may suppress MS. However, little is known about how *endogenous* estrogens interact with cellular signaling pathways to regulate sexual dimorphisms in autoimmunity, and such knowledge can provide the rationale for the design of sex-specific therapeutics. In this regard, we have previously shown that the p38 MAP kinase (MAPK) signaling pathway, a key cellular sensor of inflammation and environmental stress, promotes EAE in female, but not male mice. Myeloid cell-specific genetic deletion of p38apha, the main isoform of p38 MAPK, ameliorated EAE in females, but not males. This sexual dimorphism depended on the presence of adult sex hormones, as it was

completely reversed by gonadectomy. Thus, we hypothesized that signaling by estrogen, a key female sex hormone, via the nuclear estrogen receptors (ERs) alpha and beta, was co-regulating the role of p38 in EAE. To test this hypothesis, we used either global or myeloid cell-specific deletion of the two ERs. We found that the disruption of either ER did not affect the pathogenic role of p38 in females, i.e. deletion of p38 was still protective in the absence either ER. Unexpectedly, global or myeloid cell-specific deletion of ERalpha in males rendered the ablation of p38 protective in EAE. Strikingly, global deletion of ERbeta had the opposite effect, whereby ablation of p38 resulted in disease exacerbation, and this was not recapitulated by myeloid cell-specific deletion of ERbeta. Taken together, our results reveal opposing roles of the two nuclear ERs and their complex cell- and sex-specific interactions with the p38 pathway in EAE. Moreover, our results suggest that the p38 MAPK not only represents a potential sex-specific therapeutic target, but that there is potential for targeting this pathway in a combinatorial manner with sex hormone receptors, which are already an established target in ongoing clinical trials.

59. The effect of nicotine pretreatment on cocaine reward: Is there a sex difference?

Author List: Prableen Singh[1], Paul Marquez[1], Abdul Hamid[1], **Kabirullah Lutfy[1]**

Author Affiliations: [1]Department of Pharmaceutical Sciences, College of Pharmacy, Western University of Health Sciences, Pomona, California, USA

Abstract: Preclinical and clinical data have shown that nicotine intake during the adolescent period is associated with increased use and abuse of licit and illicit drugs. In the present study, using the conditioned place preference (CPP), as an animal model of reward, we examined whether nicotine pretreatment during adulthood would alter the rewarding action of cocaine and whether there is a sex-related difference in this response. Adult male and female C57BL/6 mice were treated with saline or nicotine (0.25 or 1 mg/kg) twice daily for seven consecutive days. Mice were then tested for baseline place preference, received conditioning with saline/cocaine (15 mg/kg) or cocaine/saline twice daily for three days, 30 min each, and then were tested for CPP. Mice then received extinction training followed by a test for the extinction of CPP and, the next day, for reinstatement of CPP following a priming dose of cocaine (7.5 mg/kg). Our results revealed that cocaine induced a robust CPP in both male and female mice pretreated with saline, but there was no sex difference in this response. In contrast, there was a sex-related difference in cocaine CPP in mice pretreated with nicotine, i.e., cocaine induced CPP in female but not male mice pretreated with nicotine. Male and female mice pretreated with saline on days 1-7 also exhibited extinction and reinstatement with no significant difference in the magnitude of these responses between male and female control mice (i.e., mice pretreated with saline on days 1-7). On the other hand, mice pretreated with nicotine exhibited altered reinstatement of CPP following the same priming dose of cocaine. The magnitude of the reinstated CPP response was reduced in male but not female mice. Together, these results suggest that pretreatment with nicotine during adulthood reduced cocaine-induced CPP in male but not female mice. Likewise, the reinstatement of CPP response was reduced by pretreatment with nicotine in male but not female mice.

60. The motivational effect of nicotine and the effect of prior nicotine treatment on cocaine reward: Is there a sex difference?

Author List: Paul Marquez[1], Prableen Singh[1], Abdul Hamid[1], **Kabirullah Lutfy[1]**

Author Affiliations: Department of Pharmaceutical Sciences, College of Pharmacy, Western University of Health Sciences, Pomona, California, USA

Abstract: There is accumulating clinical evidence suggesting age and sex differences with regards to the rewarding action of nicotine. In this study, using the place conditioning paradigm, we assessed if there is any sex-related difference in the motivational effects of nicotine. We also examined if prior conditioning with nicotine would alter the subsequent cocaine-induced conditioned place preference (CPP), and whether there is a sex-related difference in these responses. To this end, adult male and female C57BL/6

mice were tested for baseline place preference on day 1. Mice were then received conditioning with saline/saline or saline/nicotine (0.25 mg/kg) or nicotine/saline once in the morning and once in the afternoon, 15 min each, for three consecutive days. Mice were then tested for post-conditioning place preference (CPP)/aversion (CPA) the following day. Mice were then left untreated for the next two days, and then received another set of conditioning, as described above. Mice were then tested for nicotine-induced CPP/CPA. The following week, mice were treated with saline/cocaine (15 mg/kg) and confined to the vehicle-paired or drug-paired chamber for 30 min, respectively. In the afternoon, mice were injected with the alternate treatment and conditioned to the opposite chamber. Mice were tested for CPP the next day, and then received conditioning on this day as well as on the next day, and were tested for CPP 24 h later. Our results showed that female mice exhibited a significant CPA in response to nicotine conditioning, but this response appeared to be attenuated in male mice. Cocaine induced a significant CPP response in both male and female mice, but the magnitude of this response was not altered in mice with prior nicotine exposure or by sex of the mice. Together, the present results demonstrate that there may be some sex-related differences in the motivational effects of nicotine but not in the ability of nicotine to alter cocaine CPP.

61. The role of sex and beta-endorphin in the rewarding action of cocaine

Author List: Prableen Singh[1], Abdul Hamid[1], Paul Marquez[1], **Kabirullah Lutfy[1]**

Author Affiliations: [1]Department of Pharmaceutical Sciences, College of Pharmacy, Western University of Health Sciences, Pomona, California, USA

Abstract: We have previously shown that beta-endorphin plays a functional role in the rewarding action of acute cocaine in male mice. In this study, we assessed the role of beta-endorphin in cocaine-induced conditioned place preference (CPP), its extinction and reinstatement in mice of both sexes. To this end, mice lacking beta-endorphin and their wild-type controls were tested for a baseline place preference on day 1. On day 2, mice were treated with saline/cocaine (15 mg/kg) and confined to the vehicle-paired or drug-paired chamber for 30 min, respectively. In the afternoon, mice were injected with the alternate treatment and conditioned to the opposite chamber. Mice were tested for CPP on day 3, then received conditioning on this day as well as on day 4, and were tested on days 5 and 8. Mice then received extinction training (saline in both chambers) on day 9 and tested for CPP on day 10. The following day, mice were tested for reinstatement of CPP following a cocaine priming dose (7.5 mg/kg). Both male and female wild-type mice exhibited a CPP response following one cocaine conditioning, and this response was blunted in mice lacking beta-endorphin regardless of the sex of the animals. Interestingly, male but not female mice lacking beta-endorphin showed a blunted CPP response compared to their controls even following repeated conditioning with cocaine. When mice exposed to extinction training, both male and female wild-type mice exhibited no significant CPP response but expressed a robust CPP following a priming cocaine dose, showing that the CPP response was reinstated in wild-type mice. In contrast, mice lacking beta-endorphin failed to demonstrate a significant CPP response following the same priming dose of cocaine. The present results illustrate that endogenous beta-endorphin plays a critical role in the acquisition and reinstatement of cocaine CPP response, and there may be an interaction between beta-endorphin and sex during the induction of cocaine CPP.

62. Sex differences in fear extinction: The role of estradiol within the fear network and across fear phenotypes

Author List: Lisa Y. Maeng[1,2], Kara K. Cover[1], Mohamad Taha[1,2], Aaron J. Landau[1], Mohammed R. Milad[1,2], Kelimer Lebron-Milad[1,2]

Author Affiliations: [1]Behavioral Neuroscience Program, Department of Psychiatry, Massachusetts General Hospital, Charlestown, MA, USA; [2]Department of Psychiatry, Harvard Medical School, Boston, MA, USA

Abstract: It is well established that women are twice as likely as men to develop stress and anxiety disorders. Fear conditioning and extinction paradigms are commonly used to study the processes underlying the development and treatment of these disorders. Interestingly, sex differences in fear extinction appear to emerge depending on female hormonal status. Females that undergo extinction training during the low-estradiol metestrus phase of the estrous cycle exhibit poor extinction recall compared to males and females that are extinguished during the high-estradiol proestrus phase. It has also been shown that estradiol (E2) administration in metestrus rats significantly improves extinction memory. We hypothesized that E2 influences critical brain regions within the fear network to elicit effects on extinction retention. In this study, we examined the effect of E2 on immediate early gene c-fos expression within the fear extinction circuitry. One day after fear conditioning, female rats received subcutaneous injections of estradiol (15ug/kg) or vehicle during metestrus prior to extinction training. Half of the rats were sacrificed after extinction, and half were sacrificed after recall 24h after extinction. E2 significantly increased centrolateral amygdala (CeL) c-fos activity compared to vehicle treatment during extinction. Interestingly, centromedial amygdala (CeM) activity was significantly reduced by E2 during recall. We also discovered that the modulatory influence of E2 on extinction memory consolidation may depend on fear phenotype. E2 enhanced extinction retention in females expressing the highest fear during fear conditioning, but not in those that expressed moderate and low fear. Together, these data suggest that E2 (1) modifies critical nodes in fear extinction circuitry, (2) engages the CeL during training, reducing CeM activity during recall, and (3) exerts its greatest influence in females that are predisposed to high fear responses.

63. Childhood trauma as a predictor of obsessive-compulsive severity in women with anxiety or obsessive-compulsive disorder in the perinatal period

Author List: Gabriella F. Mattina[1,2], Lauren Mak[3], Karen Rowa[4], Luciano Minuzzi[1,2,5], Calan Savoy[1,2], Meir Steiner[1,2,5]

Author Affiliations: [1]McMaster Integrative Neuroscience and Discovery & Study (MiNDS), McMaster University, Hamilton, Ontario, Canada, [2]Women's Health Concerns Clinic, St. Joseph's Healthcare, Hamilton, Ontario, Canada, [3]Centre for Neuroscience Study, Queen's University, Kingston, Ontario, Canada, [4]Anxiety Treatment and Research Centre, St. Joseph's Healthcare, Hamilton, Ontario Canada, [5]Department of Psychiatry and Behavioural Neurosciences, McMaster University, Hamilton, Ontario, Canada

Abstract: A multitude of changes occur within a female's body during the perinatal period, creating a critically vulnerable time period where women are at risk for developing anxiety symptoms. Obsessive-compulsive disorder (OCD) is a mental disorder more prevalent in postpartum women compared to the general population. We explored whether obsessive-compulsive (OC) symptoms change from pregnancy to postpartum in women diagnosed with an anxiety disorder and/or OCD, with possible comorbid depressive disorder, and whether OC severity was predicted by childhood trauma. 26 women diagnosed by the CIDI-Venus were seen during 2nd-3rd trimester of pregnancy and 3-9 months postpartum. Perinatal Obsessive-Compulsive Scale (POCS) and Yale-Brown Obsessive Compulsive Scale (Y-BOCS) scores were collected at each time point. Childhood trauma information was collected using the Childhood Trauma Questionnaire (CTQ). Data was analyzed using paired samples t-test. Linear regression models examined whether CTQ subtypes predicted OC symptoms, with age and comorbidity as covariates. Comparing postpartum to pregnancy, there was no significant change detected for POCS or Y-BOCS scores. Emotional abuse (EA) scores, defined by the CTQ, predicted POCS symptom severity in pregnancy, $p=0.006$, $R^2=0.34$, 95%CI [0.4, 1.42], and EA scores predicted POCS scores postpartum when parity was included, $p=0.006$, $R^2=0.38$, 95%CI [0.45, 1.56]. Y-BOCS scores in pregnancy were predicted by EA scores, $p=0.003$, $R^2=0.38$, 95%CI [7.7, 29.9], but no CTQ subtype significantly predicted Y-BOCS scores postpartum. When examining a subset of women with confirmed OCD (n=13), Y-BOCS scores significantly decreased postpartum ($p=0.03$), but the POCS did not. This may reflect a difference in the underlying nature of OCD in the perinatal period, as POCS addresses symptoms involving the well-

being of the fetus/newborn. Results suggest that emotional abuse may be a predictor of perinatal OCD in a clinical sample of women.

64. Excitatory synaptic input differs by sex in the nucleus accumbens core but not shell

Author List: Jinyan Cao[1,2], Jaime A. Willett[1,2,3], Caitlin A. Hauser[1], Tyler A. Will[1], David M. Dorris[1], **John Meitzen**[1,2,4,5]

Author Affiliations: 1. Department of Biological Sciences, North Carolina State University, Raleigh, NC; 2. W.M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC; 3. Graduate Program in Physiology, North Carolina State University, Raleigh, NC; 4. Center for Human Health and the Environment, North Carolina State University, Raleigh, NC; 5. Comparative Medicine Institute, North Carolina State University, Raleigh, NC

Abstract: Sex differences exist in how the brain mediates motivated behavior and reward, both in normal and pathological contexts. Investigations into the underlying neural mechanisms yield accumulating evidence of sexually different dendritic spine morphology and neuromodulator and steroid sex hormone action in the striatal brain regions, including the nucleus accumbens core and shell. How these sex differences influence the electrophysiological properties of neurons in the nucleus accumbens to ultimately modulate this region's function is an area of active research. One current hypothesis is that the excitatory synaptic input onto medium spiny neurons (MSNs), the primary output neurons of the nucleus accumbens, differs by sex. Here we test this hypothesis by performing whole-cell recordings of MSNs in acute brain slices from pre-pubertal male and female nucleus accumbens core and shell. We assess intrinsic neuronal electrophysiological properties through the application of current stimuli in current-clamp and excitatory synaptic input through recording of miniature excitatory post-synaptic currents (mEPSCs) in voltage-clamp. mEPSC frequency is higher in female than in male MSNs in the core but not shell. No sex difference was found in mEPSC amplitude or time of decay. MSN intrinsic excitability and action potential properties are stable across sex in both the core and shell. This data implicates excitatory synaptic input as a potential mechanism underlying sex differences in nucleus accumbens-mediated behaviors, and that sex differences in excitatory synaptic input is generated before puberty.

65. Gender dysphoria and patient-initiated gender change in persons with ovotesticular syndrome

Author List: Heino F. L. Meyer-Bahlburg[1,2]

Author Affiliations: [1]Division of Gender, Sexuality, and Health, New York State Psychiatric Institute, [2]Department of Psychiatry, College of Physicians & Surgeons of Columbia University, New York, NY, USA

Abstract: Among psychological sex differences, gender identity has a particularly large effect size. It usually develops in line with the gender assigned at birth on the basis of the apparent genital sex. A minority of individuals develops gender dysphoria and may transition to the 'other' gender. The prevalence of this phenomenon is increased in somatic intersexuality, but varies with syndrome, syndrome severity, and initial gender assignment. In intersex newborns, gender assignment aims at minimizing the risk of later gender dysphoria and gender change. The evidence base to guide such decisions is lacking for many rare syndromes, including for ovotesticular syndrome (OTS; formerly labeled "true hermaphroditism"). This poster reviews the literature on gender outcome in OTS. Using the search terms OTS and true hermaphroditism, PubMed was searched for pertinent papers from the last 10 years (since the Intersex Consensus Conference of 2005) in several languages, and the reference lists were scanned as well. Among 491 individuals with OTS listed (with gender stated for only 140), only 6 cases of gender reassignment based on psychological evaluation were found (all with 46,XX karyotype): 2 from male to female, and 4 from female to male; 2 earlier reports had shown gender dysphoria in two additional female-assigned cases, one in a 46,XY child that resolved with psychotherapy, and the other unresolved in a 46,XY child. These data likely represent underestimates, as OTS is more prevalent in countries with poor health-care resources, where many children are lost to follow-up. The details provided do not allow clear

statements regarding specific features (such as degree of genital masculinization) that may serve as cut-points for male versus female assignment. We conclude that more detailed standardized assessments of masculinization status at birth in conjunction with systematic assessment of long-term gender outcome are needed to improve the clinical decision making.

66. Limited nesting stress (LNS) alters maternal behavior and *in vivo* intestinal permeability (IP) in a sex-dependent manner and is associated with loss of fiber-digesting and butyrate-producing bacteria in the intestinal microbiome at weaning in Wistar rat

Author List: Nabila Moussaoui[a#], Jonathan Jacobs[a#], **Muriel Larauche[a],** Mandy Biraud[a], J. Molet[b,c], Mulugeta Million[a], Emeran Mayer[a], Yvette Taché[a]

Authors Affiliations: [a] Oppenheimer Family Center for Neurobiology of Stress, CURE: Digestive Diseases Research Center, Digestive Diseases Division, Department of Medicine and Brain Research Institute, University of California, and VA Greater Los Angeles Healthcare System, Los Angeles, California 90073, USA. [d] Department of Anatomy/Neurobiology, University of California-Irvine, Irvine, CA 92697-4475, USA. [#] Contributed equally

Abstract: Early life adverse events predispose to stress related intestinal disorders such as irritable bowel syndrome (IBS). The aim of our study was to investigate alterations of maternal behaviors induced by LNS from postnatal day (PND) 2 to 9 in rats and the impact on stress hormone, glycemia and *in vivo* IP on pups and fecal microbiota. Dam Wistar rats were housed under control (direct contact bedding n=9) or LNS conditions (wire bottom floor + ½ paper towel, n=9). Maternal behavior was assessed once daily between 9:00-10:00 am. At PND10 and PND21, blood was collected and IP was assessed 4-h after fluorescein isothiocyanate–dextran 4kDa (FD4) oral gavage. Feces of PND21 rats underwent DNA extraction and amplification of the V4 region of the 16S rRNA gene. Paired end 2x150bp sequencing was performed using an Illumina MiSeq. Alpha and beta diversity analysis was performed at 97% operational taxonomic units (OTUs) and multivariate analysis with DESeq2. Dams with LNS spent similar time licking and grooming pups and eating/drinking while spending more time building a nest (118%), self-grooming (69%), and bringing pups to the nest (167%) compared to CTL. Frequency of pups outside of nest was 3.7 times higher in LNS than CTL. At PND10, LNS male and female pups had reduced body weight (4-5%), adrenal weights/100g BW (17-18%), plasma corticosterone levels (64-62%) and blood glucose (11%-12%) vs. same sex CTL. IP was increased only in male LNS pups by 2.7-fold. At weaning, the LNS group maintained the body weight reduction and hypercorticosteronemia (males 67 %; females147% vs. CTL) and had increased IP only in females (1.7-fold vs CTL, $P<0.01$). CTL showed no sex difference either PND10 or 21. At weaning, the LNS group showed increased fecal microbial diversity using three metrics: Chao1 ($p=0.001$), phylogenetic diversity ($p=0.002$), and Shannon index ($p=0.001$). Unweighted UniFrac analysis indicates that LNS rats had a distinct fecal microbiome composition compared to controls ($p=0.0008$) with decreased abundance of 24 genera ($q<0.05$): fiber-degrading microbes: *Oscillospira* (47 OTUs), *Ruminococcus* (17), and *Lachnospira* (3); butyrate-producing microbes: *Roseburia* (27), *Coprococcus* (10), and *Eubacterium dolichum*; and mucus-resident bacteria *Akkermansia mucinophila* and *Mucispirillum schaedleri*. There was increased abundance of 20 genera, of which 8 were Gram positive cocc. Additional enriched genera included *Clostridium* (4), *Corynebacterium* (4), *Desulfovibrio* (1), *Granulicatella* (2), *Rothia* (1), and *Proteus* (1). No genera and only 2 OTUs were significantly sex associated. In conclusion, LNS delayed the HPA maturation of PND10 followed by elevated corticosteronemia at weaning. The alterations of fecal microbiota with expansion of several genera of Gram positive cocci along with sex dependent elevation of intestinal permeability induced by LNS at PND10 and weaning may contribute to the sex-related susceptibility to IBS.

67. An investigation into the contribution of sex on the developing immune system and long-term learning deficits

Author List: Brittany F. Osborne[1], Jaclyn M. Schwarz[1]

Author Affiliation: [1]Department of Psychological and Brain Sciences, University of Delaware, Newark, DE, USA

Abstract: Epidemiological data show a higher prevalence and severity of developmental disorders in males compared to females. Data suggest the etiology of these disorders involves activation of immune factors in early development, however the contribution of sex on the developing immune systems of the brain and periphery and how these factors influence the onset of learning disorders are not well understood. Microglia, the immune cells of the brain, survey the environment and respond to infection by changes in morphology and release of proinflammatory cytokines. The immune cells of the periphery, white blood cells, respond to infection by increasing cell production and releasing proinflammatory cytokines. Neonatal males have more microglia in the hippocampus, amygdala and cortex than females, suggesting this may be one mechanism underlying the sex difference rendering males more vulnerable to early immune activation. We examined long-term consequences of neonatal immune activation either alone or following a second immune challenge during the onset of hippocampal-dependent learning. We hypothesized that neonatally infected males would show exaggerated immune responses compared to females either alone or following a second immune challenge. Using the Context Pre-exposure Facilitation Effect paradigm, we examined whether animals would exhibit developmental delays in learning following neonatal immune activation either alone or following a second immune challenge. Males did not show exaggerated immune responses compared to females, however, both sexes showed increased immune responses following neonatal infection in the presence of a second immune challenge. Males and females that were neonatally infected showed learning delays only in the presence of a second immune challenge. Our data suggest that early immune activation may program immune cells to exhibit increased responses following secondary immune activation and that this activation may contribute to learning delays.

68. Stronger consequences of TLR5 knockout on physiology and behavior in males

Author list: Nicole V. Peters[1], Benoit Chassaing[2,3], Mary K. Holder[1], Jack J. Whylings[1], Andrew T. Gewirtz[2,3] , Geert J. de Vries[1]

Author Affiliations: [1]Neuroscience Institute, Georgia State University, Atlanta, GA, USA; [2]Center for Inflammation, Immunity, and Infection, Georgia State University, Atlanta, GA, USA; [3]Institute for Biomedical Sciences, Georgia State University, Atlanta, GA, USA

Abstract: Toll-like receptor 5 (TLR5) is a pattern recognition receptor that detects bacterial flagellin and contributes to intestinal homeostasis. TLR5 knockout (T5KO) animals have an altered intestinal microbiota composition characterized by an increased pro-inflammatory potential that leads to intestinal inflammation, resulting in symptoms of metabolic syndrome. As changes in the gut microbiota composition are associated with changes in social and anxiety-like behaviors, we hypothesized that T5KO animals would display an altered behavioral phenotype, due to differences in microbiota. Adult (12-14 weeks) male and female C57Bl/6 WT and T5KO mice were behaviorally assessed in a battery of tests, including the open field, elevated plus maze, light/dark test, marble burying and three-chambered sociability. In addition, parameters of intestinal inflammation and metabolic syndrome were determined. T5KO mice present altered anxiety-like behavior (indicated by decreased time in the dark side of the light/dark box), increased grooming and altered social behavior (indicated by increased time investigating both novel and familiar mice). T5KO mice consistently showed a decrease in distance traveled in each apparatus; however we typically found that female T5KO traveled more than male T5KO mice. Sex and genotype interacted in the marble burying test, with the knockout increasing time digging only in males. T5KO animals have an increased body weight, adiposity, and colon weight, with each alteration being more severe in males than in females. We conclude that knockout of TLR5 causes a more severe behavioral and physiological phenotype in males than in females. Analysis of the intestinal microbiota composition may ultimately lead to the identification of the mechanism of protection in females from the effects of TLR5 deficiency.

69. Sex-differences in grey matter volume in cocaine use disorder: voxel-based morphometry study

Author List: Rebecca Preston-Campbell[1], Gabriela Gan[1], Anna Zilverstand, Scott, J. Moeller[1,2], Muhammad A. Parvaz[1], Nelly Alia-Klein[1,2], Rita Z. Goldstein[1,2]

Author Affiliations: [1]Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [2]Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Abstract: Individuals with cocaine use disorders (iCUD) show grey matter volume (GMV) reductions in regions implicated in reward/punishment, goal-directed behavior, and cognitive control [e.g., prefrontal cortex and subcortical limbic regions]. Sexually dimorphic neuro-morphological alterations contribute to addiction severity; however, most research has focused on men. Here we explored whether sex modulates the grey matter volume reductions commonly observed in iCUD. Twenty-two iCUD (13M/9F) and 25 matched healthy controls (12M/13F) underwent MRI (3T Skyra), providing T1-weighted anatomical images acquired with a 3D MPRAGE sequence. Baseline craving was assessed. Independent and interactive effects of diagnosis and sex on GMV were examined using a whole-brain 2 (diagnosis: iCUD, control) x 2 (sex: M, F) ANOVA in SPM8, with follow-up comparisons computed. Clusters with >20 contiguous voxels, with a Puncorr<0.005 search threshold, were considered significant. Results showed the reliable bilateral OFC and inferior frontal gyrus GMV reductions in iCUD were modulated by sex: iCUD women had lower GMV in the bilateral superior and midfrontal gyri, and left OFC relative to control women; iCUD men had lower GMV in the R rectus gyrus relative to control men. Compared with iCUD men, iCUD women had greater GMV in the bilateral dorsal anterior cingulate cortex and amygdala, with the latter (left side) correlating with more baseline craving (r=0.66 p=0.04).Our results suggest that OFC morphological differences between iCUD and controls may be driven by females. There was also a novel sex × diagnosis effect in the amygdala, which may mark addiction-mediated changes in the brain's stress/alarm system that may contribute to, or potentiate, aversive emotions directly linked to drug craving in women iCUD. Future longitudinal studies can test whether these GMV sex-differences reflect a predisposition to drug use, and/or morphologic changes secondary to chronic drug use are accentuated in women.

70. Sex differences in associations of arginine vasopressin and oxytocin with resting state brain function

Author List: Leah H. Rubin[1], Li Yao[2], Sarah K. Keedy[3], James L. Reilly[4], Jeffrey R. Bishop[5], C. Sue Carter[6], Hossein Pournajafi-Nazarloo[6], Lauren L. Drogos[7], Carol A. Tamminga[8], Godfrey D. Pearlson[9], Matcheri S. Keshavan[10], Brett A. Clementz[11], Wei Liao[12], Gongjun Ji[12], Su Lui[2], John A. Sweeney[8,2]

Author Affiliations: [1]Department of Psychiatry, University of Illinois at Chicago, Chicago, IL; [2]Huaxi MR Research Center (HMRRC), Department of Radiology, West China Hospital of Sichuan University, Chengdu, People's Republic of China; [3]Psychiatry, University of Chicago, Chicago, IL; [4]Department of Psychiatry and Behavioral Sciences, Northwestern University, Chicago, IL; [5]Pharmacy and Psychiatry, University of Minnesota, Minneapolis, MN; [6]Kinsey Institute, Bloomington, IN; [7]University of Calgary, Calgary, Alberta, Canada; [8]Psychiatry, University of Texas Southwestern Medical Center, Dallas, TX; [9]Departments of Psychiatry and Neurobiology, Yale University and Olin Neuropsychiatric Research Center, Hartford, CT; [10]Department of Psychiatry, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; [11]Department of Psychology, University of Georgia, Athens, G; [12]Center for Cognition and Brain Disorders and the Affiliated Hospital, Hangzhou Normal University, Hangzhou, People's Republic of China

Abstract: Oxytocin (OT) and arginine vasopressin (AVP) exert robust and often sexually dimorphic influences on brain social processing systems. We examined sex differences in the association of OT and AVP with functional brain networks using resting-state fMRI. We predicted that hormones would exert sexually dimorphic effects in brain networks important for social behavior including prefrontal, temporal, and insular cortices and the limbic system. OT and AVP serum concentrations were assayed in sixty healthy individuals (24 men; 36 women) aged 16- 60. Brain functional networks were examined using graph theory-based approaches to assess sex differences in the interrelationships between hormones (OT

or AVP) and functional brain connectivity. Graph theory methods characterize brain networks as connected nodes and properties of the connections are the key metrics. In men, higher AVP was associated with less nodal degree (connectedness) and less nodal efficiency (information propagation capacity) in left inferior frontal gyrus (IFG; pars orbitalis) and left superior temporal gyrus and less nodal efficiency in left IFG (pars triangularis) (p's<0.05). In women, higher AVP was associated with less nodal betweenness (information flow across the network) in left inferior parietal gyrus and higher OT was associated with less nodal degree in left IFG (pars orbitalis) (p's<0.05). Findings suggest a differential role of these hormones in complex brain networks important in emotion regulation and cognition for men and women. In men, higher AVP, which is in part androgen- dependent, may help to program the "male" brain for emotional reactivity and decreased emotional regulation. Conversely, in women, higher OT may help to program the "female" brain for decreased emotional reactivity given its ability to dampen the effects of AVP in the brain.

71. Sub-clinical anemia during the antenatal period: A silent risk factor for mood disruption in pregnancy?

Author List: Calan Savoy, Gabriella Mattina, Lauren Wright, William Simpson, Meir Steiner

Author Affiliations: Women's Health Concerns Clinic, St. Joseph's Healthcare, Hamilton, ON; Department of Psychiatry and Behavioural Neurosciences, McMaster University; McMaster Integrated Neuroscience Discovery and Study Program, McMaster University, Hamilton, ON

Abstract: Approximately 23% of Canadian women are diagnosed with anemia during pregnancy, while prevalence of Major Depressive Disorder and anxiety disorders are also dramatically elevated in the antepartum period. In this preliminary investigation, we evaluate the relationship between depressive and anxiety symptoms and measures of anemia included in the complete blood count in a physically healthy population of women. Identification and aggressive treatment of anemia may prove to be a useful first step in addressing antenatal mood and anxiety symptoms. 38 women were recruited during their third trimester of pregnancy from the Women's Health Concerns Clinic, St. Joseph's healthcare Hamilton. They completed the CIDI-Venus semi-structured diagnostic interview, the Edinburgh Postnatal Depression Scale (EPDS), the Perceived Stress Score (PSS), the State-Trait Anxiety Inventory (STAI), and received a complete blood count (CBC). Linear regression models were used to assess variance in CBC measures accounted for by EPDS scores. After adjusting for age and weeks gestation, there was a negative relationship between EPDS scores and hemoglobin (β=-.34, 95%CI= -.33- -.01), hematocrit (β=-.45, 95%CI= -31.13- -2.39), mean corpuscular volume (β=-.45, 95%CI= -.75- -.14,), mean corpuscular hemoglobin (β= -.48. 95%CI=-1.90- -.41)and mean corpuscular hemoglobin concentration (β= -.42, 95%CI=-.57- -.09). An association between PSS and Hemoglobin ((β=-.38, 95%CI= -.69- -.05), as well as between STAI and Hemoglobin (β=-.45, 95%CI= -.42- -.07) and Hematocrit (β=-.33, 95%CI= -.004- -.0001) was also observed. Self-report antenatal depression and anxiety scores are associated with CBC measures indicative of clinical and sub-clinical anemia, perhaps partially explaining increased incidence of mood disruption during pregnancy.

72. Sex differences in hypocretin modulation of dopamine signaling in the nucleus accumbens

Author List: Jessica K. Shaw[1], Rodrigo A. España[1]

Author Affiliations: Department of Neurobiology & Anatomy, Drexel University College of Medicine, Philadelphia, PA, USA

Abstract: Women progress to cocaine dependence more rapidly and are more prone to stress-induced relapse than men. Preclinical evidence is consistent with this, indicating that females acquire self-administration more readily, display higher motivation for cocaine and are more likely to reinstate cocaine use than their male counterparts. The mechanisms underlying these sex-differences have yet to be fully elucidated but likely involve the mesolimbic dopamine (DA) system—a pathway that is modulated by

hypocretin (HCRT) projections to DA neurons in the ventral tegmental area (VTA). Although increased HCRT expression in the female hypothalamus has been described, sex differences in HCRT regulation of dopamine transmission have not been sufficiently investigated. Here we used *in vivo* voltammetry to detect HCRT-mediated DA release and uptake in anesthetized females. We implanted female WT or HCRT knockout (KO) mice and female rats with a carbon fiber microelectrode, where DA release was electrically evoked via a stimulating electrode implanted in the VTA. Following the determination of baseline levels of DA release, mice were treated with 10mg/kg cocaine i.p. and DA neurotransmission was monitored. Rats were treated with either intra-VTA SB-334867 (a HCRT receptor 1 antagonist) or vehicle and changes in DA signaling were recorded for 30 minutes prior to i.v. infusion of 1.5mg/kg cocaine. Whereas HCRT KO males demonstrate reduced evoked DA relative to WT males under baseline conditions and in response to cocaine, KO females instead showed higher concentrations of evoked DA than WT females under all conditions. In concordance with this and contrary to the well-characterized effects of HCRTr1 antagonists in males, female rats treated with intra-VTA SB-334867 displayed increased DA release compared to rats treated with vehicle. Taken together, these observations suggest a differential role for HCRT regulation of DA reward pathways based on sex.

73. Early predator odor exposure exerts sexually dimorphic effects on juvenile social play behavior

Author List: Sara L. Stockman[2,3], Margaret M. McCarthy[1]

Author Affiliations: [1]Deptartment of Pharmacology, [2]Program in Neuroscience and [3]Medical Scientist Training Program, University of Maryland School of Medicine, Baltimore, M.D.

Abstract: Juvenile social play behavior is one of the earliest sexually differentiated behaviors to emerge through development. In rats, male animals engage in more rough-and-tumble play compared to females. Participation in social play during the juvenile period is critical for appropriate development. Exposure to early life adversity is a major driver of later health and behaviors. Research suggests that adverse early life event exposure can have differing effects on males and females, however, how such exposures alter social play behavior in the juvenile period is poorly understood. To address this question, male and female neonatal rats were exposed to predator odor (PO) for 5 min on PN1-PN3. When tested on an additional fourth day, in the presence of PO, the rat pups were found to reduce both ultrasonic vocalization (USV) call frequency and amplitude of emitted calls. Additionally, video recordings over the three day exposure indicated that PO exposed pups display elevated freezing behavior. This suggests neonatal rat pups modulate their behavior in response to fearful stimuli. Following exposure, animals were normally raised to PN26 and social play and anxiety-like behaviors were tested. Social play was evaluated for the frequency of pouncing, pinning, chasing and boxing behaviors, as well as, the total time engaged in play and number of play events. Under the play paradigm utilized, early predator odor produced sexually dimorphic alterations of play, whereby males exposed to PO exhibited a decrease in all parameters measured and females exposed to PO increased play behavior across all measures. PO exposure did not alter anxiety-like behavior assessed in the open field test, the elevated plus maze or the light/dark box. Knowing that social behaviors are disrupted in several neuropsychiatric disorders, this work provides some insight into how sex may interact with adverse early life event exposure to contribute to the etiology of these disease manifestations.

74. Role of Microglia in Sexual Differentiation of the Amygdala

Author List: Jonathan W. VanRyzin[1], Stacey J. Yu[2], Kathryn J. Argue[2], Margaret M. McCarthy[1,2]

Author Affiliations: Program in Neuroscience[1] and Department of Pharmacology[2], University of Maryland, Baltimore, School of Medicine, Baltimore, M.D., USA

Abstract: Microglia are the dominant resident immune cells of the brain and function in multiple ways outside their traditional capacity of responding to insult. During development microglia regulate tissue homeostasis, neuronal precursor populations, and synaptic circuitry. We implicated microglia as an

integral component of sexual differentiation of the preoptic area and control of male copulatory behavior, suggesting these immune cells can also organize sex-specific brain structure and function (Lenz *et al. J Neurosci* 33(7), 2013). The amygdala is also a sexually dimorphic brain region that regulates social behaviors known to differ in males and females. We reported a sex difference in the number of newly born cells in the developing rat amygdala mediated by endocannabinoids, with females having higher numbers of newly born cells than males and a lower endocannabinoid tone. The differences in newly born cells correlated to behavioral changes, as treating newborn females with the CB1/2 agonist WIN55,212-2 masculinized juvenile social play behavior and reduced cell genesis in the amygdala (Krebs-Kraft *et al. PNAS* 107(47), 2010). Further investigation suggests microglia may be central to establishing the observed sex difference in newly born cells, as males have more Iba1+ microglia exhibiting phagocytic morphology compared to females. We are now deciphering the relative contributions of microglia phagocytosis and trophic signaling in the regulation of newly born cells. Preliminary data using methods to selectively deplete microglia suggest that animals whose microglia were depleted during early postnatal life display marked deficits in the early expression of social behaviors. Moreover, these animals exhibit a pervasive reduction in anxiety-like behaviors and innate fear. Ultimately, these studies will provide valuable insight into new facets of immune regulation of brain sexual differentiation and the development amygdala-dependent circuitry.

75. Adolescent social stress results in sex-specific transcriptional reprogramming throughout the reward circuitry in adult mice

Author List: Deena M. Walker[1], Immanuel Purushothaman[1], Hannah M. Cates[1], Rose C. Bagot[1], Cate J. Peña[1], Marie A Doyle, Efrain Ribeiro[1], Pamela J. Kennedy[2], Eric J. Nestler[1]

Author Affiliations: [1]Fishberg Department of Neuroscience and Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, New York 10029, USA; [2] Department of Psychology, The University of California Los Angeles, Los Angeles CA, 90095, USA.

Abstract: *Background:* Adolescent social isolation (SI) alters neuronal morphology, physiology, and gene expression throughout the reward circuitry and increases preference for drugs of abuse in male rodents. However, few studies have investigated if there are sex differences in response to adolescent SI as well as the molecular mechanisms underlying such long-term changes in reward-associated behaviors. Our preliminary data suggests that SI results in the reversal or reduction of sexually dimorphic reward- and anxiety-related behaviors. Therefore, we tested the hypothesis that SI results in persistent transcriptional changes that underlie sex differences in reward. *Methods:* Mice were socially isolated or group housed (GH) from postnatal day (P) 22 - P42, then GH until adulthood (~P90). Micropunches from 4 reward-associated brain regions (meAMY, VTA, NAc, and PFC) were collected and transcriptome-wide changes investigated by RNA-sequencing after acute or chronic cocaine or saline (7.5mg/kg) (n = 5 – 8/group). *Results:* Similar to the differences observed in behavior, SI reversed baseline sex differences in gene expression in all 4 brain regions. Hierarchical clustering revealed that GH males cluster with SI females and vice versa for those genes displaying a sex X stress interaction (meAMY (869 genes), VTA (260 genes), PFC (495 genes) and NAc (1444)). Additionally, sexually dimorphic genes were enriched in those genes reversed by SI in all 4 brain regions ($p < 0.001$), an effect not observed in genes altered by SI alone. Finally, significant overlap of sexually dimorphic genes across all 4 brain regions was observed. These data suggest that adolescence is a critical period for development of sex differences in the reward circuitry and that adolescent stress causes sustained reversal of sexually dimorphic gene expression. Future analysis will focus on sex differences in response to cocaine to identify targets underlying long-term alterations in cocaine-associated behaviors.

76. Sex differences in the antidepressant effect of scopolamine in rats

Author List: Brittany Wicks, Marni Shore, Sabina Khantsis, Attilio Ceretti, Sarah Cohen, Alexa Fritz, Yushi Kawasumi, Debra A. Bangasser

Author Affiliations: Neuroendocrinology and Behavior Laboratory, Department of Psychology and Neuroscience Program, Temple University, Philadelphia, PA 19122, USA

Abstract: Scopolamine, a non-selective muscarinic receptor antagonist, has recently been found to have rapid antidepressant effects, even in a subset of patients who are resistant to other treatments. Compellingly, women have a greater antidepressant response to scopolamine than men, an effect that suggests heightened female sensitivity to the drug. Most sex differences in drug efficacy are thought to be attributable to circulating ovarian hormones. To test whether scopolamine efficacy is regulated by ovarian hormones, we utilized rat models of antidepressant efficacy. First we found that in both the Forced Swim and the Novelty Suppressed Feeding (NSF) tests of antidepressant efficacy, scopolamine had a greater antidepressant effect in female than in male rats. Next using the NSF task we determined that the magnitude of scopolamine's efficacy changed across the estrous cycle. To further examine a role for circulating ovarian hormones we tested the effects of scopolamine in ovariectomized female rats utilizing NSF. Female sensitivity to scopolamine was reduced in ovariectomized compared to gonadally intact female rats. Collectively, these results indicate that circulating ovarian hormones contribute to the sex differences in scopolamine efficacy in rodents. Scopolamine has a narrow therapeutic index and can cause severe side effects (e.g. psychosis). These preclinical findings suggest that clinical studies into the efficacy and safety of scopolamine should consider hormonal status of women in case different dosing recommendations are needed for women during their reproductive years.

77. Sex-specific effects of pubertal, but not post-pubertal, stress on pre-pulse inhibition and depressive-like behavior

Author List: Jari Willing[1]**,** Laura R. Cortes[1], Carly M. Drzewiecki[2], Janice M. Juraska[1,2]

Author Affiliations: [1]Psychology Department, University of Illinois at Urbana-Champaign, Champaign, IL, USA; [2]Program in Neuroscience, University of Illinois at Urbana-Champaign

Abstract: Adolescence, the period between adolescence and adulthood, is characterized by many important neuroanatomical changes that coincide with altered behavior to meet changes in environmental demands. We have shown previously that within adolescence, the period of pubertal onset is associated with neuronal and synaptic pruning in the prefrontal cortex (PFC), and a rapid change in performance on a PFC-dependent task. This collectively suggests a heightened susceptibility to external stressors, specifically during the pubertal period. In the present study, we use a combined isolation plus restraint stress paradigm in male and female rats during the period of pubertal onset (Females: P32-38, Males: P41-47) and compared them with post-pubertally stressed rats (Females: P40-46, Males: P49-55) and unstressed controls. When subjects reached young adulthood, they were tested in the Elevated Plus Maze, Forced Swim Test, Pre-Pulse Inhibition (PPI) task and Novel Object Recognition task. While there were no effects of stress in either sex on object recognition performance, females stressed during pubertal onset displayed an increased latency to enter an open arm in the elevated plus maze and displayed increased immobility time during the forced swim test. Additionally, males stressed during the pubertal period showed a deficit in PPI compared to controls and animals stressed after puberty. These results show sex-specific effects of stress during, but not after, pubertal onset, suggesting sex differences in neural responses to stress at puberty.

78. Molecular mechanisms underlying sex differences in the brain oxytocin system

Author list: Nicholas B Worley[1], Kelly M. Dumais[1], Jingting C Yuan[1], Laura E Newman[1], Andrea G. Alonso[1], Alexa H Veenema[1]

Author affiliations: [1]Neurobiology of Social Behavior Laboratory, Boston College, Chestnut Hill, MA, USA

Abstract: The neuropeptide oxytocin (OT) often regulates social behavior in sex-specific ways. This may be due to sex differences in the brain OT system. In support, our lab has recently demonstrated that adult

male rats have higher OT receptor (OTR) binding density than females in various forebrain regions, including the posterior bed nucleus of the stria terminalis (BNSTp). Understanding the origin of this sex difference may advance our understanding of the development and regulation of sex-specific social behavior. Sex differences in the brain are organized by testosterone (T), often via actions of its metabolite estradiol during critical (perinatal and pubertal) periods in development. To define the critical period for the sex difference in OTR binding density in the BNSTp, we determined the age of onset of this sex difference. We show that the sex difference in OTR binding density is present before and after puberty in the BNSTp, and that this sex difference corresponds with a sex difference in OTR mRNA expression. This suggests that the sex difference is organized during the perinatal critical period. To test this, we are currently determining whether neonatal T treatment masculinizes OTR binding density in the BNSTp of females and if so, whether this is due to T-dependent androgen receptor or estrogen receptor activation. We predict that neonatal T treatment will increase OTR binding density in the pBNST of females to the level seen in males and that this effect is mediated by the estrogen receptor, but not androgen receptor. Outcomes will help elucidate the biological mechanisms underlying the sex difference in OTR expression.

79. Chronic stress exposure during adolescence results in cross- and trans-generational changes in response to nicotine administration in mice

Author List: Nicole L. Yohn[1], Julie A. Blendy[1]

Author Affiliations: [1]Department of Pharmacology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Abstract: Transgenerational effects of short term parental stress exposure on anxiety, depression, and physiological stress response has been documented. However, there has been no research on the effect of long-term parental stress exposure on response to drugs of abuse including nicotine, despite the well-known impact of stress on addiction. Further, the importance of stress exposure during a critical period for gamete development has not been evaluated. Therefore, we used a chronic stress exposure paradigm in adolescents to determine the effects of nicotine in several generations of offspring. Male and female C57BL/6 mice underwent chronic unpredictable stress for 2 weeks starting at 4 weeks of age. Following CUS, both male and female mice were mated with naïve partners to produce F1 offspring. In addition, mice of both sexes from the F1 generation were mated with naïve partners to produce F2 offspring. All generations were administered a series of tests for anxiety-like behavior, stress response, and response to nicotine administration between 10-14 weeks of age. Offspring whose parents were exposed to adolescent stress show altered locomotor response to chronic nicotine in a sex and lineage dependent manner (F1 generation). F1 male offspring of paternal or maternal adolescent stress exposure produce male offspring (F2 generation) with increased locomotor response to nicotine administration. RNA-Seq on F1 male offspring of paternal or maternal stress was performed to determine changes in gene expression in the amygdala. Differentially expressed genes implicated in stress, reward, and immune signaling within the amygdala of F1 male offspring whose fathers experienced stress were identified. However, maternal stress did not impact gene expression.

80. Cc2d1a loss of function disrupts cognitive and social behaviors and CREB signaling in a sexually dimorphic manner

Author list: Marta Zamarbide[1], Adam W. Oaks[1], Heather L. Pond[1], M. Chiara Manzini[1]

Author Affiliations: [1] Department of Pharmacology and Physiology and Integrative Systems Biology, The George Washington University School of Medicine and Health Sciences, Washington, DC, USA

Abstract: Neurodevelopmental disorders such as Intellectual Disability (ID) and Autism Spectrum Disorder (ASD) are more commonly diagnosed in males, with ratios for 2:1 male:female for ID to 4:1 for ASD. Multiple theories have been put forth to explain such sex bias, from a female-protective effect to increased male susceptibility. However, the lack of an appropriate model has hindered our ability to test

such hypotheses. While developing a novel mouse model of ID and ASD by generating animals deficient for the ASD/ID gene Cc2d1a we found striking sex bias. Namely, male Cc2d1a knockout (KO) mice show cognitive and social deficits in the Morris Water Maze, the Novel Object Recognition Test, the Open Field Test, the Three Chamber Test and the Social Approach Test, while female KO mice only display mild cognitive phenotypes. Cc2d1a is a protein scaffold regulating intracellular signaling of multiple pathways involved in learning and memory, including CREB signaling. We asked whether sexually dimorphic signaling could be responsible for behavioral differences in Cc2d1a KO males. We investigated cAMP/PKA/CREB pathway activation by Western blot in cortex and hippocampus adult tissue using phospho-specific antibodies whereas cAMP levels were examined by ELISA. Finally, we tested the dynamics of CREB activation in primary hippocampal and cortical neuron cultures from wild type and KO animals. The sex-specific behavioral differences in male KO mice correlate with impairments in CREB activation. The activation of the PKA pathway is dysregulated in the hippocampus of adult male KO mice as well as in male primary hippocampal neuron cultures. We hypothesize that this sexually dimorphic regulation of CREB signaling in the Cc2d1a loss of function mouse model may contribute to ASD/ID-like features.

81. An XX sex chromosome complement increases obesity, lipids, and atherosclerosis in hypercholesterolemic mice

Author List: Yasir Alsiraj[1], Sean Thatcher[1], Heba M. Ali[1], Joseph Layne[1, 2], Ryan Temel[1, 2], Alan Daugherty[2,3], Lisa Cassis[1]

Author Affiliations: Department of Pharmacology and Nutritional Sciences[1], Saha Cardiovascular Research Center[2], Department of Physiology[3], University of Kentucky, Lexington, KY.

Abstract: *Background:* Obesity, lipids, and atherosclerosis are sexually dimorphic; the underlying mechanisms that contribute to these dimorphisms are not well understood. Most studies have focused on sex hormones as primary mediators of sexual dimorphism of these diseases; however, sex chromosome complement (XX and XY) is another factor differing between males and females that could contribute to sexual dimorphism. We hypothesized that sex chromosome complement influences the response to Western diet-induced obesity, lipids, and atherosclerosis. *Methods and Results:* Transgenic male mice with deletion of Sry from the Y-chromosome expressing Sry on autosomes (8-12 weeks of age) were bred to female *Ldlr*$^{-/-}$ mice to generate female mice with an XX or an XY sex chromosome complement. Female mice (XX and XY) were fed a Western diet for 3 months to induce obesity and atherosclerosis. Body weight was measured weekly, atherosclerotic lesions were measured by enface analysis, and lipids were measured at study endpoint. XX females exhibited significant increases in atherosclerotic lesion surface areas in the aortic arch (XX, 26% ± 2.15; XY, 18% ± 3.3; $P<0.05$) and elevated serum concentrations of cholesterol (XX, 2501 ± 192; XY, 890 ± 141 mg/dl; $P<0.05$), VLDL (XX, 1351 ± 165; XY, 374 ± 67 mg/dl; $P<0.05$), LDL (XX, 1312 ± 58; XY, 525 ± 81 mg/dl; $P<0.05$), and HDL (XX, 147 ± 21; XY, 109 ± 6 mg/dl; $P<0.05$) compared to XY females. Moreover, XX females exhibited significant increases in body weight when challenged with the Western diet compared to XY females (XX, 41.2 ± 2.4; XY, 31.7 ± 2.5 g; $P<0.05$). *Conclusion:* These results demonstrate that when challenged with a Western diet, female XX *Ldlr*$^{-/-}$ mice have increased body weight, serum cholesterol concentrations and atherosclerosis compared to XY females. Future studies will identify gene targets influenced by sex chromosome complement and the role of sex hormones in regulating the XX phenotype.

82. Bisphenol A (BPA) and Bisphenol S (BPS) increase Coxsackievirus B3 myocarditis in female and male BALB/c mice by activating mast cells, increasing immune cell infiltrate and activating the inflammasome

Author List: Katelyn A. Bruno[1,2,3], Jessica Mathews[2], Alex Yang[1], Henry Greyner[1], Frank Molina[1], Adriana Bucek[1], DeLisa Fairweather[1,2,3]

Author Affiliations: 1. Environmental Health Sciences, The Johns Hopkins Bloomberg School of Public Health, Baltimore, M.D., United States. 2. Department of Immunology, Mayo Clinic, Jacksonville, FL, United States. 3. Department of Cardiology, Mayo Clinic, Jacksonville, FL, United States.

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 could increase myocarditis through deleterious actions of the ERβ rather than beneficial effectsA 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 in females. In females we found BPA significantly decreased ERα and VDR, 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. In male mice, we found that at the high dose of BPA increased myocarditis and increased all immune cell markers and the inflammasome. We also investigated the compound Bisphenol S (BPS) which has replaced BPA in a number of products and found that BPS also increases myocarditis. Interestingly this effect was only seen in Balb/c mice and not BL/6 suggesting that race could be a factor in evaluating ED role in disease. We have found that ED exposure is having a significant effect on myocarditis and could potentially influence a number of human diseases.

83. Outcomes of Antitachycardia Pacing Therapy (ATP) in patients with ICDs: Is there any gender-based difference in success?

Author List: Dalia Giedrimiene[1,2]**,** Danette Guertin[1], Jeffrey Kluger[1]

Author Affiliations: [1] Division of Cardiology, Section of Arrhythmia Services, Hartford Hospital, Hartford, CT, USA; [2] University of Saint Joseph School of Health and Natural Sciences, West Hartford, CT, USA

Abstract: The implantable-cardioverter device (ICD) therapy has been shown to improve survival of patients with ischemic or non-ischemic heart disease and is used for primary or secondary prevention of sudden cardiac death (SCD). The incorporation of ATP into ICDs has provided a better tolerated alternative to shocks and has been shown to be highly effective for termination of ventricular arrhythmias (VAs). We conducted a retrospective case-control analysis of patients implanted with an ICD for VAs at a single institution between 2000 and 2011, and who had at least 12 months of post-ICD implantation follow-up. Cases included all patients experiencing ICD-delivered therapy, including ATP-therapy and ICD shocks. A total of 936 patients experienced at least one ICD delivered episode of therapy during follow-up 1.6 – 18.5 years (av. = 7.95); of them 789 (84.3%) - men and only 147 (15.7%) – women have received ICD therapy; age: 19.5 – 95.5 y (mean - 71.9±13.9 y.). In 594 (63.5%) cases ATP was delivered for ventricular tachycardia (VT) event. It was successful in 498 (83.8%) of cases and in 96 (16.2%) cases due to ATP failure the delivery of ICD shock was required. In men – success of ATP therapy was achieved in 425 out of 508 (83.7%) cases, in women – in 73 out of 86 (84.9%). Patients 75 years older and older had the same success rates of ATP therapy as younger, and there was no gender-based difference in cases of ATP therapy success or failure. The study results showed that ATP therapy successfully terminates ventricular tachycardia-based events in patients with ICDs. Success of ATP therapy is high in both gender groups, however significantly less women received ICD implantation, and consequently less ICD-delivered ATP therapies have been observed.

84. An ignored group: men with peripheral fat distribution

Author list: Kalypso Karastergiou[1], Dylan Thomas[2], Susan K. Fried[1]

Author affiliations: [1]Boston University School of Medicine, Boston, MA, USA; 2Boston Medical Center, Boston, MA, USA

Abstract: Peripheral fat distribution (preferential deposition in the gluteofemoral area or pear shape) is protective of cardiometabolic diseases and characteristic of premenopausal women. The prevalence of pear shape in men, and whether their cardiometabolic health benefits from it, are unknown. We analyzed data from subjects >18 years old and free of type 2 diabetes who participated in the 2003-04 and 2005-06 cycles of the National Health and Nutrition Examination Survey (n=10,124). Fat distribution was assessed by dual energy X-ray absorptiometry. Pear-shape was defined as android:gynoid fat mass ratio < 0.33 which corresponds to the bottom quartile of its distribution in women.By this definition, 17.15% of Hispanic, 33.07% of non-hispanic white, and 29.63% of non-hispanic black women were classified as pear-shaped, as did 5.84% of Hispanic, 8.80% of non-hispanic white, and 20.39% of non-hispanic black men. Pear-shaped men had lower fasting glucose and insulin, HbA1c, blood pressure (BP), CRP, 2h-glucose after oral glucose tolerance test, triglycerides and total- and LDL cholesterol, and higher HDL-cholesterol compared to non-pear men and women. Compared to pear-shaped women, these men had lower CRP, insulin, HDL- and total cholesterol, but higher fasting glucose and diastolic BP (all p<0.0001, full factorial two way ANOVA). In multiple linear regression models, pear shape remained an independent predictor of total, LDL-, HDL-cholesterol, triglycerides and diastolic BP after controlling for age, gender, BMI and ethnicity. Significant interactions between body shape and sex were noted for total cholesterol and BP.In conclusion, up to 5-20% of men of different ethnicities show peripheral fat distribution, comparable to the 25% most pear-shaped women and they appear metabolically healthy. Further studies of their physiology and adipose tissue characteristics will add insight into whether and how sex interacts with fat distribution to influence disease risk.

85. Changes in Blood Pressure and Angina in the Women's Ischemia Syndrome Evaluation Coronary Vascular Dysfunction

Author List: Melody Michaelson[1]**,** Janet Wei[2]**,** Quanlin Li[2]**,** Puja Mehta[3]**,** Chrisandra Shufelt[2]**,** Daniel S. Berman[2]**,** Louise E. J. Thomson[2]**,** Carl J. Pepine[4]**,** C. Noel Bairey Merz[2]

Author Affiliations: [1]Tufts University School of Medicine, Boston, MA; [2]Barbra Streisand Women's Heart Center, Cedars Sinai Medical Center, Los Angeles, CA; [3]Emory Women's Heart Center, Emory Clinical Cardiovascular Research Institute, Atlanta, GA; [4]Division of Cardiology, Department of Medicine, University of Florida, Gainesville, FL

Abstract: Among women with signs and symptoms of ischemia and no obstructive coronary artery disease (CAD), prior work demonstrated that angina improves over time. We hypothesized that angina improvement may be related to change in blood pressure (BP). Baseline and 1 year follow-up Seattle Angina Questionnaires (SAQ) were available in 53 WISE subjects with retrospective chart review of clinical BP and medication data. Paired Student t test was used to test the mean 1 year differences, while Fisher's exact test was used to test association between SAQ and medication changes. Mean age was 54.5±10.7 yrs, BMI 29.0±7.5, 36% had a history of hypertension, 42% had a history of smoking, 15% had dyslipidemia, 9% had diabetes, and 70% were postmenopausal. Angina reduction was seen across all 5 scales of the SAQ. However there was no difference in systolic or diastolic BP, and number of BP medications did not differ (Table 1). Change in SAQ was not associated with medication changes. Among symptomatic women with normal BP at baseline, there appears to be no relationship between BP and reduced angina at follow up. This initial data suggests that angina improvements may not be related to BP in women with signs and symptoms of ischemia and no obstructive CAD. Further investigation is needed to determine mechanisms of angina in this population.

86. Does sex moderate the relation between mood and diabetes-related distress in adults with impaired sleep?

Author List: **Jonna L. Morris**[1], Susan M. Sereika[1, 2], Eileen R. Chasens[1]

Author Affiliation: [1]University of Pittsburgh, School of Nursing, [2]University of Pittsburgh, School of Public Health

Abstract: People with diabetes frequently struggle with their self-care. It is unclear if impaired mood or sleep disorders (i.e. obstructive sleep apnea [OSA], insomnia) further worsens distress. Research suggests women are more vulnerable to poor mood and insomnia, whereas men are more vulnerable to OSA. It is unknown if sex moderates these effects on diabetes-related distress. The purpose of this study was to examine sex as a moderator of distress in diabetes self-care as predicted by mood, OSA severity, and insomnia in people with type 2 diabetes. This study employed a cross-sectional design using baseline data from the ongoing Diabetes Sleep Treatment Trial (R01 DK096028). Instruments include the: Problems Areas in Diabetes questionnaire (PAID), Profile of Mood States (POMS), and Insomnia Severity Index (ISI). Demographic information included age, sex, marital status, and race. Clinical evaluations included height and weight to calculate BMI (kg/m^2), A1C levels, and a home ApneaLink sleep study to determine OSA severity (AHI). Analysis included descriptive statistics, one-way ANOVA, and hierarchical linear regression to test moderation. The sample (N=122) was 55.6% male sex, 46% marriage/partnered, 64% non-white, middle aged (Mean ± SD = 55.4±10 years), and obese (Mean BMI±SD = 34.8±6.7) with suboptimal metabolic control (mean A1C±SD= 8.1±2.0%). There were no significant differences by sex on the PAID, POMS, ISI or AHI (p≥.05). Diabetes-related distress was significantly associated with poor mood (F= 38.391, p = .000) and insomnia (F= 20.902, p=.000), but not OSA severity
(F 1.017, p = .468).

Sex did not moderate the relations between diabetes-related distress with mood (ΔR^2=.003, p = 0.457) or with insomnia (ΔR^2=.009, p = .236). While mood and insomnia are associated with diabetes self-management distress, sex does not moderate these relations. More research is needed examining these relations in a larger sample.

87. Unusual maintenance of X-chromosome Inactivation predisposes female lymphocytes for increased expression from the inactive X

Author List: Jianle Wang[1], Camille Syrett[1], Marianne C. Kramer[2], Arindam Basu[3], Michael Atchison[1], and **Montserrat C. Anguera**[1]

Author Affiliations: [1] Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia PA 19104, USA; [2] Department of Biochemistry and Biophysics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104; [3] Pennsylvania State University, Brandywine Campus, Media, PA 19163

Abstract: Females have a greater immunological advantage than men, yet they are more prone to autoimmune disorders. The basis for this sex bias lies in the X-chromosome, which contains many immunity-related genes. Female mammals use X-Chromosome Inactivation (XCI) to generate a transcriptionally silent inactive X chromosome (Xi) enriched with heterochromatic modifications and XIST/Xist RNA, which equalizes gene expression between the sexes. We examined the maintenance of XCI in lymphocytes from females in mice and humans. Strikingly, we found that mature naïve T and B cells have dispersed patterns of XIST/Xist RNA and they lack the typical heterochromatic modifications of the Xi. *In vitro* activation of lymphocytes triggered the return of XIST/Xist RNA transcripts and some chromatin marks (H3K27me3, ubiquitin-H2A) to the Xi. Single-cell RNA FISH analysis of healthy female lymphocytes revealed that the X-linked immunity genes *CD40LG, TRL7,* and *CXCR3* are biallelically-expressed in some cells. Using knock-out and knock-down approaches, we found that Xist RNA-binding proteins, YY1 and hnRNPU, are critical for recruitment of XIST/Xist RNA back to the Xi in female lymphocytes in humans and mice. Furthermore, we examined T and B cells from patients with systemic lupus erythematosus, an autoimmune disorder with a strong female bias, and observed mis-localized XIST RNA transcripts and evidence of biallelic expression of immunity-related genes from both X-chromosomes. We propose that the Xi in female lymphocytes is predisposed to become partially

reactivated and to overexpress immunity-related genes, providing the first mechanistic evidence for the enhanced immunity of females and their increased susceptibility for autoimmunity.

88. Abnormal X chromosome inactivation of T cells in female lupus patient and lupus-prone mice leads to increased expression from the inactive X

Author list: Jianle Wang[1], Camille M. Syrett[1], Montserrat C. Anguera[1]

Author Affiliations: [1]Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104

Abstract: Female mammals use X Chromosome Inactivation to generate a transcriptionally silent inactive X chromosome (Xi) enriched with heterochromatic modifications and Xist RNA, which equalizes gene expression between the sexes. Females have a greater immunological advantage than men, yet they are more prone to autoimmune diseases. This sex bias may be due to the extra X chromosome in females compared to males, which contains many immunity related genes. We have examined the maintenance of XCI in lymphocytes from females in humans and mice and recently discovered that mature naive T and B cells have dispersed patterns of Xist and lack the typical heterochromatic modifications of the Xi. *In vitro* activation of the lymphocytes triggered the return of the Xist RNA transcripts and some chromatin marks. Here we examined the epigenetic characteristics of the Xi in T cells from patients with systemic lupus erythematosus and female-biased lupus mouse model NZB/W F1. We found that lupus patient and lupus-prone mice naïve T cells lack XIST/Xist RNA localization on the Xi. Upon stimulation, XIST/Xist RNA transcripts return to the inactive X in some, but not all, T cells. SLE patient T cells exhibited higher levels of XIST RNA mislocalization compared to healthy controls. We have also investigated the amount of heterochromatin modifications that co-localize with XIST RNA on the Xi. Using single-molecule RNA FISH, we observe biallelic expression of the autoimmunity-related X-linked genes CD40LG and CXCR3 in T cells from lupus patients and NZB/W F1 animals. We propose that the Xi in female lymphocytes is predisposed to become partially reactivated and to overexpress immunity-related genes, providing the mechanic evidence for the enhanced immunity of females and their increased susceptibility for autoimmune diseases, like lupus.

89. The SP-A2 male alveolar macrophage miRNome in response to O_3 is associated with inflammatory pathways, regulation of reactive oxygen species, and apoptosis

Author List: George T. Noutsios[1], Xuesheng Zhang[1], Todd M. Umstead[1], Faryal Durrani[1], David S. Phelps[1] and Joanna Floros[1,2]

Author Affiliations: [1]Center for Host Defense, Inflammation, and Lung Disease (CHILD) Research, Department of Pediatrics and [2]Department of Obstetrics and Gynecology. Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033, USA.

Abstract: Pulmonary surfactant protein A (SP-A) is an innate host defense molecule produced by alveolar type II cells. Human SP-A1 and SP-A2 differentially affect the function and proteome of alveolar macrophages (AM). We hypothesized that SP-A genes differentially regulate the AM miRNome and this may explain the differences in AM function and proteome. SP-A knock out (KO) and humanized transgenic mice (hTG) expressing SP-A1 and SP-A2 (3/group, n=36) were exposed to 2 ppm O_3 or filter air (FA) for 3 h, sacrificed 4 h later and AM isolated. Total RNA was extracted, miRNAs purified, and cDNA generated and used for qRT-PCR. Levels of 372 most abundantly expressed miRNAs were determined. Ingenuity pathway analysis was employed to identify networks and genes regulated by miRNAs >2x in hTG compared to KO AM miRNome in response to O_3 or FA. Five genes were identified (IL6, STAT3, BCL2, NF-kB, FOXO1) that were directly associated with SP-A2-regulated miRNAs in OxS. These SP-A2-regulated miRNAs include: 1) miR-191-5p that was downregulated and binds IL6; this may increase IL6 levels (supported by our preliminary findings), leading to the activation of STAT3. 2) miR -21-5p and 181a-5p (downregulated) and -1195 (upregulated). These may affect STAT3 activation leading to

its translocation to the nucleus, and transcription of pro-inflammatory genes. 3) miR -21-5p, -16-5p and -195a-5p (downregulated) and miR-153-3p and -15b-5p (upregulated) bind the anti-apoptotic gene BCL2 which is upregulated by STAT3. Thus, these miRNAs may play a role in apoptosis. 4) miR -9-5p (downregulated) and -183-5p (upregulated) target NF-kB mRNA. 5) miR -9-5p, -21-5p, -16-5p, -183-5p, and -153-3p were either up- or down- regulated and influence FOXO1, which is involved in the homeostasis of ROS. These observations were only found in the male SP-A2 AM miRNome in response to OxS and not in females, indicating sex-specific differences, consistent with our previous AM proteomic findings.

90. Biological sex is a major determinant of *Toxoplama gondii* pathogenesis

Author List: Dionne P. Robinson[1], Beth Gregg[1], Patricia Sikorski[1], Michael E. Grigg[1]

Author Affiliations: [1]The National Institute of Allergy and Infectious Disease, Laboratory of Parasitic Diseases, Molecular Parasitology Section, Bethesda, M.D. 20892

Abstract: Across species, females respond immunologically to infectious agents differently than males. In the majority of protozoan parasite infections, females are better protected from severe disease than males. This is not the case during *Toxoplasma gondii* (*T. gondii*) infection where excessive immune responsiveness (e.g., increased IFN-gamma), coupled with decreased immune regulation (e.g., regulatory T cells; Tregs), contributes to immunopathology. Using adult male and female C57BL/6J mice inoculated with *T. gondii*, we observe greater mortality in females than males following peroral, but not intraperitoneal (i.p.) inoculation that is not a result of differential parasite burden, but correlated with distinct microbial, endocrine, and immune responses. To determine whether greater *T. gondii* susceptibility in females correlates with differential microbial dysbiosis in the small intestine, which exacerbates toxoplasmosis, small intestine contents were assessed by RT-PCR, revealing greater gamma-proteobacteria outgrowth in females than males, which may influence inflammation and endocrine function. Quantification of hormone-responsive tissues to assess endocrine function revealed no changes in seminal vesicle mass in males, regardless of infection route; however, females inoculated perorally, but not i.p., experience a significant loss in uterine horn mass, which is indicative of reduced estradiol concentrations. Immunologically, males have greater serum concentrations of regulatory cytokines, IL-5 and IL-13, compared with females, suggesting sex-dependent immune polarization. We also observe a Treg collapse in the small intestine of both sexes; however, males experience greater collapse than females, despite being better protected against *T. gondii*. These data suggest the existence of a protective 'male-typic' response profile that modulates inflammation and microbial dysbiosis, highlighting the intricate connection between the endocrine, immune, and microbial systems.

91. Investigating the impact of immune activation on neuroimmune function during various time-points throughout pregnancy and the postpartum period

Author List: Morgan L. Sherer[1], Jaclyn M. Schwarz[1]

Author Affiliations: [1]University of Delaware, Department of Psychological and Brain Sciences, Newark, DE 19716.

Abstract: It is well known that the immune system changes dramatically during pregnancy in order to prevent the developing fetus from being attacked by the maternal immune system. As an example, many women who suffer from autoimmune disorders actually find that the severity or symptoms of their disease is significantly changed throughout pregnancy due to these changes in immune function. Despite this evidence, no one has examined whether changes in neuroimmune function also occur during pregnancy and the postpartum period. It is well known that changes in immune function are often linked to the onset of certain mental health disorders, including depression. Thus, we hypothesize that changes in immune function that are associated with pregnancy and the postpartum period might increase the risk of postpartum depression following birth as a result of changes in the neuroimmune system. Previous

studies in our lab have found significant changes in inflammatory molecules within both the prefrontal cortex and the hippocampus of postpartum rats. In the current study, we investigated the impact of an immune challenge (LPS) during various time points throughout pregnancy and postpartum period on the expression immune molecules in the brain with future experiments examining how these changes in immune function may influence mood and anxiety during pregnancy and the postpartum period.

92. Investigation of the epigenetic features of the inactive X chromosome in B cells from the female-biased lupus model NZB/W F1 mice

Author List: Camille M. Syrett[1], Jianle Wang[1], Arindam Basu[1, 2], Michael L. Atchison[1], & Montserrat C. Anguera[1]

Author Affiliations: [1]Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104; Pennsylvania State University, Brandywine Campus, Media, PA 19163

Abstract: The X chromosome has many immune-related genes, and females (XX) have an immunological advantage over males (XY). Some autoimmune disorders such as lupus exhibit a strong female-bias, and often some X-linked immunity-related genes are over-expressed in female (but not male) lupus patients and mouse models of lupus. The mechanism underlying the sex-specific differences in lupus is unclear, and hypotheses suggest an important role for the X chromosome. Female mammals use X Chromosome Inactivation (XCI) to equalize X-linked gene expression between sexes, which is initiated by the long noncoding RNA Xist. This generates a transcriptionally silent inactive X chromosome (Xi) that is maintained after each cell division into adulthood. We have recently found that XCI is maintained differently in female lymphocytes, and that the Xi has some euchromatic features. We hypothesized that XCI may contribute to the observed female bias in the classic lupus mouse model NZB/W F1. To test this, we examined the maintenance of XCI in female B cells from healthy wild type and NZB/W F1 mice. Strikingly, we find that mature naïve CD23+ B cells from both wildtype and NZB/W F1 mice have dispersed patterns of Xist RNA, and they lack the typical heterochromatic modifications of the Xi. In vitro activation of lymphocytes triggers the return of Xist transcripts and some chromatin marks to the Xi in wild type mice. However as symptoms of lupus-like disease progress, Xist localization to the Xi is perturbed. Furthermore, typical heterochromatin marks of the Xi are reduced in lupus female mice compared to healthy females. Using knockout techniques, we found that the Xist binding protein YY1 is required to recruit Xist RNA back to the Xi during B cell activation. These findings are the first to link the unusual XCI in female B cells to female-biased autoimmune susceptibility.

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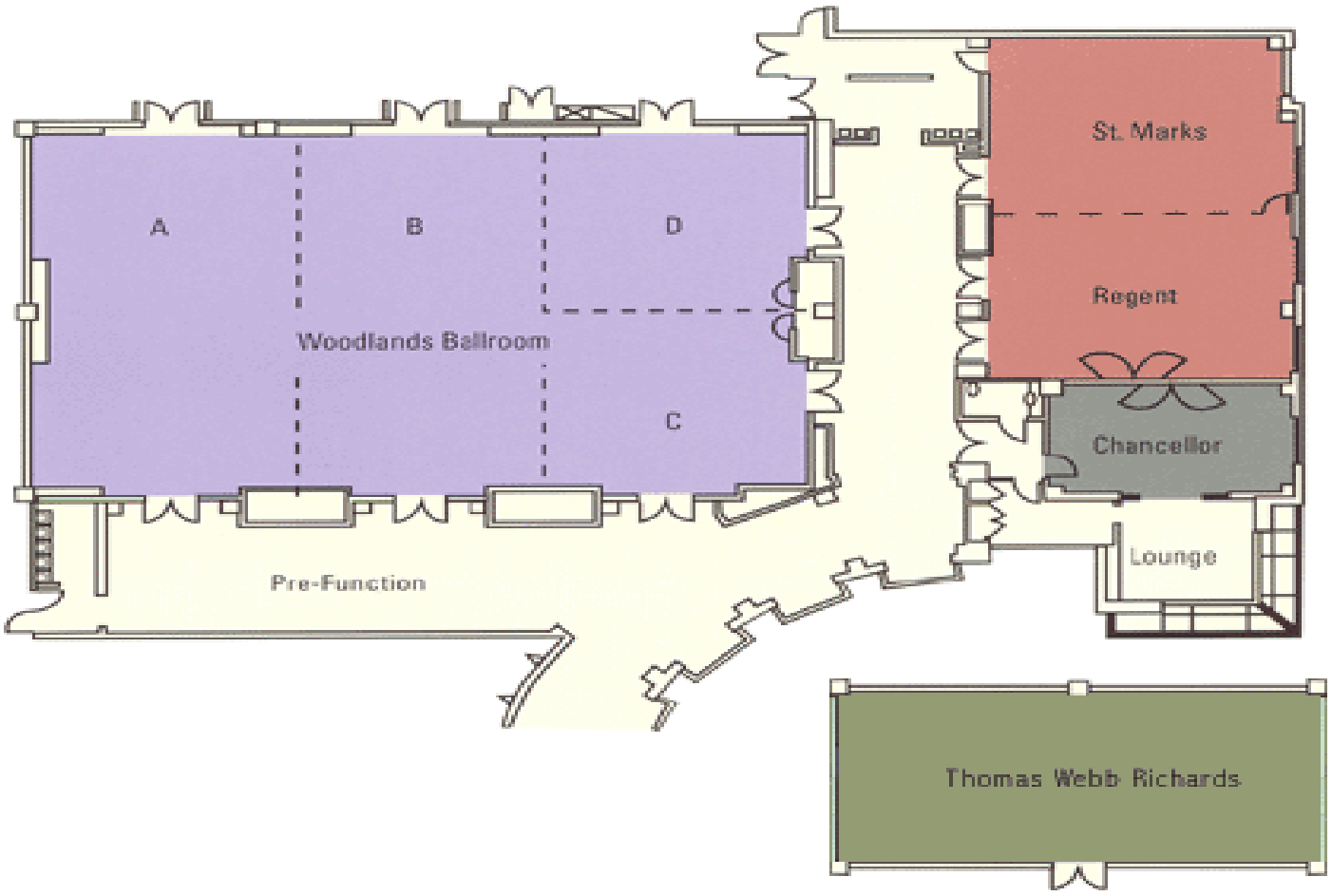

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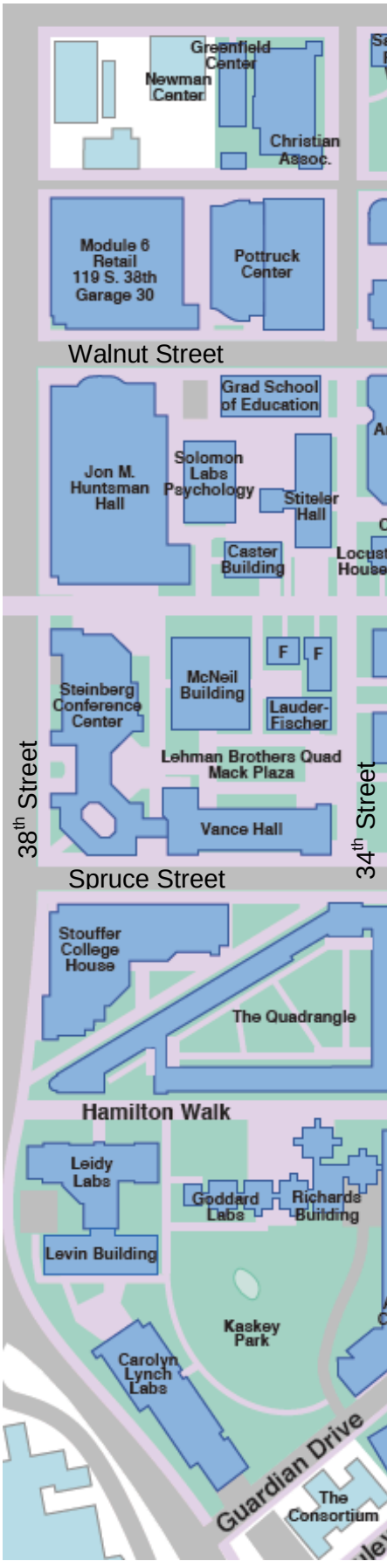
University of Pennsylvania Campus Map



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