Title: Sex Differences in T cell Immune Responses and Outcome after Stroke in Aged Mice

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Abstract: Sex differences are increasingly recognized in stroke. Stroke incidence is higher in men until an advanced age, while women have higher mortality and morbidity. Immune responses are key determinants of outcome but have not been well studies in aged animals. We hypothesized that there are sex differences in T cell responses and outcome after stroke in aged mice. Male and female C57BL/6 mice (20-21 months) were subjected to 60 min middle cerebral artery occlusion (n=16), or sham surgery (n=7). T cell and T regulatory cell (Treg) immune responses in brain, blood and spleen were quantified at 15 days post-stroke by flow cytometry. Functional outcomes were assessed at day 3, 7 and 14. Mortality was 25% in females and occurred at day 1, while in males 50% mortality was seen day 3 to 9. Hemorrhagic transformation was seen in 53% of the males that died (5/8) and in none of the females (0/4). Significantly higher levels of $CD8^+$ T cells were demonstrated in blood of males compared to females (73±5% of $CD3^+$ T cells vs 52±3%, $P<0.001$). $CD8^+$ T cell counts were increased in the brain after stroke ($P<0.001$), but were not different between the sexes. However, males had increased levels of Tregs in the brain after stroke compared to sham ($P<0.01$), which was not seen in females. Independent of surgery (stroke or sham), we observed higher levels of splenic $CD8^+$ and γδT cells in males ($P<0.01$). Open-field showed decreased center visits after stroke in both sexes at 3 days, but males spent more time immobile ($P<0.01$) indicating decreased locomotor activity and/or anxiety. This difference equalized at 7 and 14 days post-stroke. In conclusion, mortality and hemorrhagic transformation rates were higher in males than in females after ischemic stroke. This may be associated with greater stroke-induced inflammation in males, as higher levels of $CD8^+$ T cells in blood and Tregs in the brain were seen in surviving males sub-acutely.

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Title: Sex-based differences in the aortic function of a novel (UC Davis) rat model of Type 2 Diabetes Mellitus (UCD-T2DM)

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Abstract: The UC Davis type 2 diabetes mellitus (UCD-T2DM) Rat is a validated model of type 2 diabetes mellitus. UCD-T2DM rats are characterized by polygenic, adult-onset obesity and spontaneous β-cell failure and, as a result, more closely models the pathophysiology of type 2 diabetes in humans than other rodent models of the disease. This study investigated alterations in aortic function in the UCD-T2DM Rats and determined whether there is a sexual dimorphism in diabetes-induced aortic dysfunction in this model. Endothelium-dependent vasodilation (EDV) to acetylcholine (ACh, 10^{-8} to 10^{-5}M) was measured in intact aortic rings pre-contracted with phenylephrine (PE, 2μM). Endothelium-independent vasodilation induced by sodium nitroprusside (SNP, 10^{-9} to 10^{-5} M) was assessed in endothelium-denuded rings pre-contracted with PE (2μM). Furthermore, constrictor response curves to PE (10^{-8} to 10^{-5} M) were generated. Diabetes significantly impaired relaxation responses to ACh or SNP in aortic rings from female UCD-T2DM Rats, however, potentiated aortic relaxation in male rats. Moreover, diabetes significantly shifted PE contractile responses to the left in aortic rings from both sexes. Both maximal contraction and sensitivity to PE in aortic rings from diabetic females were significantly higher than those in control rats. Diabetes did not affect the maximal contraction to PE in male rats. These data, for the first time, show that the vascular function in aortic rings of UCD-T2DM Rats is altered in both sexes. The decreased sensitivity of vascular smooth muscle to NO, along with the enhanced contractile responsiveness to PE, may in part contribute to the attenuated relaxation response to ACh in diabetic female rats. In contrast, an elevated sensitivity of vascular smooth muscle to NO may partially explain the potentiation of ACh responses in male rats with diabetes. Overall, these results indicate the presence of sex-based differences in the aortic function of UCD-T2DM Rats.

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Title: Sex and the blood-brain barrier: assessing physiological differences and response to stroke injury in vitro using patient-derived stem cells

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Abstract: The blood-brain barrier (BBB), a component of the neurovascular unit, plays an important role in the brain homeostasis. It is formed by specialized brain microvascular endothelial cells (BMECs) surrounded by astrocytes, pericytes and neurons. It provides both a physical and a chemical barrier. Disruption of such barrier is an important component of several neurological diseases, in particular following stroke injury. Ischemic stroke is characterized by a disruption of the BBB, resulting in neuronal cell death by excitotoxicity and cerebral edema formation. Interestingly, several studies highlighted the presence of a sex difference in stroke patients, with a worsening of the number and intensity of such events in elderly women. Yet the cellular and molecular mechanisms by which such sexual dimorphism impact stroke outcome remains unclear. In our study, we investigated the presence of a differential response to stroke injury due to sex using an isogenic human in vitro model of the BBB using patient-derived induced pluripotent stem cells (iPSCs), such cells were derived from males and female asymptomatic patients. Under physiological conditions, we did not observe major differences in terms of BBB phenotype and barrier induction between male and female iPSC lines, although we noted some differences in drug efflux transporters activity. Furthermore, we noted differences in neuronal maturation as iPSC-derived neurons from male cell lines yielded a lesser neurite counts than female counterparts. In addition, we observed differences in how such cells behave under oxygen-glucose deprivation (OGD)/reoxygenation stress. Female BMECs displayed a failure to recover their barrier function compared to males following reoxygenation, as well as differences in various outcomes were observed between females and male iPSC-derived astrocytes and neurons respectively. Our data suggests that such differences may be driven by a dimorphism in oxygen sensing via the HIF/VEGF axis.

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Title: Basal differences in cue-triggered motivation are modulated by the ovarian cycle only in obesity-prone but not in obesity-resistant rats.

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Abstract: In females, naturally occurring alterations in estradiol influence food intake and food preference. For example, during phases of elevated estradiol food intake decreases compared to phases where estradiol is low. The effects of ovariectomy and hormone replacement have also provided evidence for the role of estradiol in modulating food intake. In particular, ovariectomy of adult rats results in an increase in food intake that is accompanied by weight gain. Furthermore, treating ovariectomized rats with physiological doses of estradiol decreases food intake and body weight (Tarttelin et. al, 1968; Wade, 1975; Asarian et. al, 2002). However, sights and smells that are paired with food (food cues) can also influence feeding behavior. For example, in humans food cues can induce food craving, bias food choice, and increase the amount of food consumed. These effects of food cues on motivation are stronger in obese individuals, but whether this is a cause or effect of obesity is unknown. We recently found that male rats predisposed to gain weight are more sensitive to the motivational properties of a food cue, even before the onset of obesity (Robinson et al 2015), suggesting that enhanced cue-triggered urges may drive over-eating. However, whether a similar difference exists in female rats, and how cue-triggered motivation varies across the cycle is unknown. Therefore, here we used established Pavlovian procedures to determine how approach triggered by a food cue varies between female obesity-prone and obesity-resistant rats and how this behavior changes across the cycle. Specifically, rats were trained to associate one auditory cue (CS+; 2 min) with sucrose delivery (45 mg pellets; TestDiet), whereas a second cue (CS-; 2 min) was never paired with sucrose (5 sessions, 1 session/d). Rats were then tested for approach behavior triggered by the CS+ vs CS- in the absence of food. As expected, daily home cage chow consumption and body weight were greater in female obesity-prone vs. obesity-resistant rats, and home cage food intake decreased during estrus in both groups. During conditioned approach testing, we found that the magnitude of approach trigged by the CS+ varied across the estrous cycle in obesity-prone, but not obesity-resistant rats. Specifically, the magnitude of approach to the food cup in the presence of the CS+ was higher in diestrus/metestrus compared to proestrus/estrus in obesity-prone rats, but remained stable across the cycle in obesity-resistant rats. Thus, the magnitude of approach behavior was greater in obesity-prone vs obesity-resistant rats during diestrus/metestrus, but was similar between the groups during proestrus/estrus. These data are consistent with basal differences found in males, and also suggest a role for hormonal regulation of cue-triggered motivation. Furthermore, although food intake changed across the cycle in both groups, motivation triggered by the food cue changed across the cycle only in obesity-prone rats. This pattern suggests a dissociation in the regulation of food “craving” from food consumption by naturally occurring changes in circulating hormones. Ongoing studies in ovariectomized rats treated with 17-beta estradiol and progesterone are being conducted to more directly determine the effect of these hormones on conditioned approach in female obesity-prone, obesity-resistant, and outbred rats.

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Title: Knockout of Neurexin 1 differentially alters male and female juvenile rat social behaviors

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Abstract: Autism affects 1 in 42 boys and 1 in 189 girls in the United States (CDC, 2014). Disruption of the presynaptic cell adhesion protein, Neurexin1 (NRXN1), is found in 0.5% of autism cases with most cases involving haploinsufficiency. Here we used the Nrxn1 KO rat to elucidate how decreased function of Nrxn1 affects complex social behaviors, such as prosocial/helping behavior, nurturing, and juvenile social play. Juvenile nurturing behavior was assessed for 2 weeks beginning on postnatal day 24 (PN24) by placing animals with 3 unrelated pups for a 2hr period and scoring whether the pups were nested during that period. In a preliminary study, a wildtype female began nesting pups within the first 3 days, while wildtype males, *Nrxn1*$^{+/-}$ and *Nrxn1*$^{-/-}$ males and females took a week or more before exhibiting nurturing behavior. Juvenile prosocial/helping behavior was assessed over a 12 day period. Animals were tasked with releasing their cagemate from a small chamber with an inescapable drip of cold water. Wildtype females began to free their trapped cagemate after 4 days, wildtype males took 6 days, *Nrxn1*$^{+/-}$ males took 9 days, *Nrxn*$^{-/-}$ males took 11 days, and *Nrxn1*$^{+/-}$ females never began reliably freeing their cagemates. Juvenile rough-and-tumble play, or play fighting, was assessed between age-, sex-, and genotype-matched pairs. Preliminary results suggest that, relative to wildtype males, females with haploinsufficienty of Nrxn1 had higher levels of pouncing, pinning, and boxing, while these parameters where decreased in haploinsufficient males. We are currently working to expand upon these preliminary behavioral results and are quantifying levels of Nrxn1 and spinophillin protein as a proxy for the number of excitatory synapses in wildtype neonatal males and females and those with heterozygous or homozygous *Nrxn1* deletions in the POA/BNST and other brains regions critical for social and affiliative behaviors.

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Title: Corticotropin releasing factor regulation of sustained attention in male and female rats

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Abstract: Sustained attention is the ability to monitor a situation for rare and unpredictable events. Sustaining attention is critical for higher-order cognitive processes, and clinically, attentional disruptions are reported in several disorders, including schizophrenia, attention deficit hyperactivity disorder, and Alzheimer's disease. Another shared feature of these disorders is that stress is associated with their onset and/or severity. Yet the neurobiological mechanisms by which stress regulates sustained attention are virtually unknown. To address this gap, we are studying how the stress neuropeptide, corticotropin releasing factor (CRF), alters performance on a sustained attention task (SAT) in male and female rats. We previously found that administering CRF throughout the brain impaired SAT in both sexes, however, the negative effect of CRF on attention was diminished when females were in the phase of their estrous cycle with higher levels of ovarian hormones. Now we are trying to determine where in the brain CRF is working to modulate attention. SAT performance is known to rely on cholinergic and GABAergic neurons in the nucleus basalis of Meynert (NBM). We found CRF receptors on both types of neurons in the NBM, suggesting that direct effects of CRF on this region are possible. To test this idea, we infused CRF directly into the NBM and tested sustained attention. Intra-NBM infusions of CRF impaired SAT in both sexes, although the effect was more pronounced in males. Additionally, CRF specifically affected aspects of attention thought to be mediated by GABAergic neurons in this region, suggesting novel CRF-GABA interactions. Collectively, these studies reveal that CRF impairs attention, especially in males, highlighting an unexplored mechanism by which stress can regulate cognition. Clinically, these findings suggest that drugs that block the effects of CRF represent a viable therapeutic option to treat cognitive deficits in stressed patients with certain disorders.

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Title: Sex-specific effects of exercise on cognition: Evidence from clinical and epidemiological data

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Abstract: Physical activity is a promising strategy to mitigate the deleterious effects of aging and disease on brain health. However, a large amount of variation exists in its efficacy. Given the greater prevalence and faster progression of Alzheimer's disease in women compared to men, there is a need to assess sex differences in exercise efficacy on executive functions and to ascertain the underlying mechanisms. We hypothesize that sex is a potential moderator of exercise efficacy on cognitive and brain health. To address this, we conducted secondary analyses of data from two studies: 1) PROMoTE – a randomized controlled trial (RCT) of 6-month, thrice-weekly aerobic training (AT) in older adults with vascular cognitive impairment, and 2) Health ABC – a 10-year longitudinal, epidemiological cohort study of 2720 older adults in which physical activity was measured through self-reported walking behaviour. In the PROMoTE RCT, we found that compared to usual care control, AT significantly improved executive functioning in women, but not men, an effect that was still evident 6 months after trial completion. Further, AT increased levels of brain derived neurotrophic factor in women but decreased levels in men. Similarly, a sex difference was seen in the Health ABC data, where higher physical activity was associated with better executive functioning across a 10-year period in women, but not men. Paradoxically, in a subset of participants that underwent neuroimaging at year 10, higher physical activity was related to greater hippocampal and prefrontal cortical volumes in men but not women. Together, these results from two studies utilizing very distinct methodologies provide evidence that sex differences exist in the effect of exercise on cognition as well as in the underlying neurobiological mechanisms. This new knowledge will foster development of efficacious and personalized exercise recommendations to promote brain health that go beyond the current "one-size-fits-all" approach.

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Title: Sex-specific cognitive deficits following early-life stress: A role for parvalbumin in the orbitofrontal cortex

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. **Abstract:** Adverse early life experiences influence emotional development and increase the risk for and severity of affective pathology. Women are at increased risk of developing stress-associated pathology, including a two-fold greater likelihood of developing depression when compared with men. In a mouse model of early life stress (ELS), we found that only female mice go on to develop a depressive-like phenotype. Depressive pathology is highly comorbid with cognitive impairments and inflexibility, which is thought to result predominantly from altered frontal lobe function. The prefrontal cortex, which orchestrates the integration of cognition and emotion through cortical and subcortical pathways, is especially sensitive to ELS. However, it is largely unknown whether ELS effects cognitive function in a sexually dimorphic manner, and what the underlying mechanisms of disruption are. Here, we use a combination of behavioral, molecular, and optogenetic approaches to examine a possible mechanism underlying sex-specific vulnerability following ELS in mice. Using an attentional set-shifting task, we found that female mice exposed to ELS were impaired on the rule- reversal phase of learning ($p < 0.01$), while no differences between groups during initial rule learning or extra-dimensional rule shifts were observed. Fast-spiking GABAergic interneurons containing the calcium binding protein parvalbumin (PV), have been implicated in PFC-dependent cognitive functioning, and are sensitive to ELS. However, their specific role in reversal learning and cognitive function following ELS remain unclear. Here, we find a decrease in PV and GAD67 mRNA levels in the orbitofrontal cortex (OFC) of female mice following ELS. To investigate the role of PV interneurons in reversal learning, we used an optogenetic approach (halorhodopsin) to selectively silence PV+ cells in the OFC during different test phases of the attention task. Findings have implications for understanding the molecular and cellular mechanisms underlying stress-induced impairments in cognitive function, and sex differences in risk for pathology development.

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Title: Cardiovascular risk after prophylactic salpingo-oophorectomy in patients with genetic risk of cancer

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Abstract: Sex differences influence the risk for developing cardiovascular disease (CVD), with pre-menopausal women having lower risk compared to age-matched men. However, this relative protection dissipates after menopause – as evidenced by an increase in CV risk and mortality resulting in levels that equal or surpass those of men. Female hormones were thus implicated as cardioprotective factors, but contrary to expectations, hormone replacement therapies failed to protect against CVD. Previously we discovered Heat Shock Protein 27 (HSP27) as an estrogen receptor-α associated protein with serum levels that inversely correlate with the 5-yr risk of CV events (e.g., heart attack). We also showed that estrogens promote the expression and extracellular release of HSP27, and more recently observed that HSP27 regulates PCSK9 – a key determinant of serum cholesterol levels. Since HSP27 is a downstream effector of estrogen signalling, we hypothesized that post-menopausal women have lower HSP27 serum levels that make them susceptible to atherosclerosis and increased CVD risk. This research project studies women with *BRCA1/2* mutations who have undergone prophylactic salpingo-oophorectomy (PSO) resulting in "surgical menopause". Serial serum samples from 10 PSO patients are being assessed for changes in HSP27 and other CVD markers pre- and 1, 3, 6, & 12 mo post-operatively. Preliminary data show that in 2 pre-menopausal PSO patients, LDL increased by ~29% 12 mo post-op and ~19% 3 mo post-op; in contrast, the trend of increasing LDL levels post-operatively was not observed in 3 post-menopausal PSO patients. HSP27 autoantibody levels decreased 3 & 12 mo post-op in all PSO patients (~18% maximum). Future characterization of other CVD markers in this patient cohort and expanding the study to include more women experiencing surgical (and natural) menopause will yield new insights into the role of the functional ovary in modulating lipid parameters and other CV risk factors such as HSP27.

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Title: Sex differences in children's provision of help

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Abstract: Experimental and cross-cultural naturalistic studies show that from infancy through early childhood, girls are more likely than boys to provide uni-directional assistance to mothers and experimenters. However young children rarely help peers. We hypothesized that when children become older and peers play more prominent roles in their lives, girls will be more likely than boys to provide assistance to same-sex peers. We recruited participants from kindergartens and first grades and randomly assigned children from the same classroom to a same-sex pair, then to one of two conditions: a spontaneous helping condition or an induced helping condition. In both conditions, each child was given a basket of differently colored blocks. Children then were asked to carry their baskets to a table at the other end of the room and build a structure following a diagram. Children were told not to rush and that each would receive a sticker after finishing building. On the way to the table, one of the children's baskets broke, spilling the blocks onto the floor. In the spontaneous helping condition, children each had their own diagrams and were asked to build their own structures at opposite ends of the table. In the induced helping condition, children were asked to build one structure with both of their blocks. Few children helped in the spontaneous condition. Significantly more females helped in the induced helping condition (10/15 or 67%) than in the spontaneous helping condition (3/15 or 20%). Few males helped in either condition: [3/11 or 27.3% in the spontaneous and 2/12 or 16.7% in the induced condition], with the overall sex difference in helping significant across both conditions. Although spontaneous helping of same-sex peers is rare, it can be more easily induced in females than males, consistent with an early socialized and/or innately predisposed female bias towards providing uni-directional assistance.

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Title: Estrogen Signals through PPARG coactivator 1 alpha to Reduce Oxidative Damage Associated with Diet-induced Fatty Liver Disease

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Abstract: Inefficient fatty acid oxidation in mitochondria and increased oxidative damage are features of non-alcoholic fatty liver disease (NAFLD). In rodent models and patients with NAFLD, hepatic expression of PPARG coactivator 1 alpha (PPARGC1A) is inversely correlated with liver fat and disease severity. A common polymorphism in this gene (rs8192678, encoding Gly482Ser) has been associated with NAFLD. We investigated whether reduced expression of PPARGC1A contributes to development of NAFLD using mouse models, primary hepatocytes and human cell lines. Reduced expression of PPARGC1A was linked to the human Gly482Ser polymorphism as the serine 482 variant had a shorter half-life than the glycine variant when expressed in HepG2 cells. Decreased half-life was correlated with decreased PPARGC1A function. Liver tissues from mice with liver-specific heterozygous (LH) disruption of Ppargc1a placed on an obesogenic diet expressed increased markers of inflammation and fibrosis and decreased levels of anti-oxidant enzymes, compared with the WT on a same diet. Oxidative damage was observed in livers from LH mice of each sex, in a cell-autonomous manner, but was greater in livers from the female mice where it was accompanied by increased hepatic triglycerides, serum ALT and decreased glucose tolerance. Expression of Ppargc1a was required for estrogen-dependent expression of genes that encode antioxidant proteins. These findings could account for the increased liver damage observed in LH female mice. In summary, we found in mice that loss of estrogen signaling contributes to oxidative damage caused by low levels of PPARGC1A in liver, exacerbating steatohepatitis when combined with diets high in fructose and fat. This research highlights the importance of taking in account sex as a factor when considering PPARGC1A as a therapeutic target for metabolic diseases.

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Title: Sex differences in self-reported work stress and physiological measures of autonomic regulation in 911 communicators

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Abstract: Heart rate variability (HRV) is a reflection of both autonomic nervous system function (ANS) and heart-brain interactions. Acute and chronic stress shift the balance of ANS activity to increased sympathetic tone. Reduced HRV is a strong predictor of future health problems, and is related to autonomic dysfunctions such as anxiety and depression. These relationships have been shown to affect emotional processing and may reflect the detrimental effects of work stress, including emotional labour, and an inability to adaptively respond to these environmental challenges. Gender shapes both exposure to and experience of these work-related stressors. We undertook a mixed methods study of 911 communicators in the Greater Toronto Area measuring HRV, self-report emotional labour and job stress, pressure, and support. Initial findings show that women report more job stress, more severe job pressure and less job support. For frequency domain measures of HRV, men have higher low frequency (LF) power and women have higher high frequency (HF) power. However, LF and HF power are differentially related to workplace stress in women and men. For women, job stress is positively associated with LF power and negatively associated with HF power and emotional labour is negatively associated with total power, or the variance of the entire signal. These LF and HF distinctions do not hold in men. For men, job stress, job pressure and lack of organizational support are negatively associated with total power and emotional labour is unrelated to HRV. Stress reports and time domain measures of HRV show some correlations in the same direction among women and men. Although women report more job stress, the standard deviation of normal-to-normal beat intervals (SSDN) is negatively associated with job stress, pressure and lack of support in women and men. These preliminary data show sex differences and a complex interplay between biological response and environmental challenge for women and men.

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Title: Profiling immune system sex differences in the healthy human transcriptome

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Abstract: Sex and gender biases in the incidence of autoimmunity and infectious disease imply that women have stronger immune responses. Women are at higher risk of autoimmune diseases, while men are more likely to die of infectious disease. Molecular factors driving this phenomenon may be detectable in the transcriptome, as it reflects immune activation, hormonal regulation, and chromosome status. We performed an immunologically focused investigation of robust transcriptional sex differences across global populations. First, we performed an integrated multi-cohort analysis of 6 cohorts consisting of 458 individuals to identify a 178-gene signature, called the Immune Sex Expression Signature (iSEXS), which is differentially expressed between healthy male and female human adults in the blood across populations. We validated iSEXS in 3 additional cohorts of 128 samples. Second, we examined sex differences in immune cell frequencies to determine whether iSEXS was driven by cell frequencies or phenotype. Using deconvolution, a method of predicting cell frequencies from bulk gene expression, we performed a meta-analysis of sex differences in cell frequencies across populations. We validated our results in an independent mass cytometry dataset and found that females had higher levels of CD4+ T cells while males had higher levels of monocytes. Third, we examined the role of sex hormones and chromosomes in the regulation of iSEXS. We observed that 25% of iSEXS is located on the sex chromosomes. Importantly, in a cohort of disorders of sexual development, XY-individuals with normal female genitalia expressed the iSEXS at similar levels as XY-males, indicating that the iSEXS is primarily driven by chromosomal differences. As a robust gene signature across populations, iSEXS has applications in understanding why women and men have differential risks of autoimmunity and infection.

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Title: Progesterone receptors expressed in central nervous system contribute to breathing stability of 10 days old rats.

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Abstract: We tested the hypothesis that the nuclear progesterone receptors (nPR) and membrane progesterone receptors α (mPRα) and β (mPRβ), expressed in central nervous system, contribute in respiratory regulation of newborn rats. Expression of nPR, mPRα or mPRβ in the brainstem region was attenuated by intracisternal injection of specific small interferon RNA (siRNA) to 10-days male and female rats. An artificial cerebrospinal fluid was administered to the control animals. 24 hours after injection, minute ventilation (VE, ml/100g/min) was recorded using whole body plethysmography during normoxia (21% O_2, 50 min), hypoxia (12% O_2, 5 min) And hypercapnia (5% CO_2, 5 mim). The frequency of sighs and apnea was determined in normoxia. Treatments with siRNA-mPRα, mPRβ or nPR did not affect ventilation in normoxia as well as ventilatory responses to hypoxia and hypercapnia. However, the treatment with siRNA-mPRα increase the frequency of sighs, siRNA-mPRβ increases the frequency of apneas in males, while siRNA-nPR increases the frequency of apnea only in females. Different progesterone receptors have specific respiratory effects in male and female newborn rats. These data could be useful to developing of pharmacological treatments of apnea of prematurity.

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Title: The interplay of sex and gender in women and men's health

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Abstract: The differences in the patterns of illness/health between men and women cannot be explained solely by genetic or biological differences. From a gender socialization framework, some constructions of masculinity/femininity may be more important than others in understanding the relationship between femininity/masculinity and women and men's health. The objective of the poster is to discuss the contribution of sex and gender as social determinants of health associated with self-perceived health, mental health (measured through GHQ-12), tobacco consumption as well as the use of alcohol and medicine consumption among 429 Romanian women and 219 Romanian men. We consider that gender is a variable that must be taken into account from the moment of assessment in order to provide an accurate diagnosis of the reality and not only when interpreting the results. Gender was assessed through the Conformity to Masculine Norms Inventory and Conformity to Feminine Norms Inventory. These instruments measure attitudes, beliefs and behaviors in regard to feminine/masculine gender norms. The results show that selected female norms were inversely and directly correlated with some of the health indicators. Selected male norms were directly related to self-perceived health. Yet, negative associations between mental health and variables such as dominance and pursuit of status. Men who smoked scored higher in violence. Finally, men who consumed medicine scored lower in winning, emotional control, risk-taking, playboy and pursuit of status. Such findings show that sex differences draw attention to the fact that men and women differ in health behaviors, but ignores the differences within the group of men and women. On the other hand, these results show that feminine/masculine gender identity is important with regard to health and gender norms can work as protector and risk factors for some health indices for women and men.

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Title: Maternal inflammation at midgestation produces male-selective impairments in fetal neurodevelopment

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Abstract: There is a widely recognized male bias in neurodevelopmental disorders, including Autism Spectrum Disorders, ADHD, dyslexia, childhood schizophrenia, Tourette syndrome, and learning disability. Males are also at higher risk for complications of pregnancy including preterm birth, preeclampsia, placental inflammation, and sequelae of hypoxia. This has led some to propose male sex as a risk factor for disrupted prenatal development. The central regulator of fetal development is the placenta, which is derived from the embryo and shares its chromosomal sex. Clinical findings suggest that male and female placentas function differently in humans, particularly in response to maternal stress and inflammation. Using a mouse model of maternal immune activation (MIA) during pregnancy, we hypothesized that one factor underlying male-selective vulnerability is exacerbated placental dysfunction in response to maternal inflammation. A mild challenge with lipopolysaccharide (LPS) on day 12.5 of the mouse pregnancy produces hemorrhage and necrosis that is more severe in the male placenta at each dose, indicating a lower threshold for MIA-mediated damage. Fetuses in LPS-challenged pregnancies also show elevated hypoxia in the developing brain, as quantified by the immunofluorescence intensity of pimonidazole adducts. The cortical region in males was significantly more hypoxic relative to females exposed to the same challenge. This fetal sex-based divergence in developmental trajectory persists into adulthood, producing significantly impaired social interaction in MIA-exposed males, but not females, in the 3-chamber social approach task. Interestingly, MIA delays postnatal body growth in females only. This suggests that MIA-exposed females exchange body growth potential for brain sparing, as adult brain/body ratios did not differ by sex. Given these results, it may be useful to view male-selective neurodevelopmental impairment on a continuum of male vulnerability to *in utero* adversity.

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Title: Studying genes that escape from human X-chromosome inactivation to identify DNA elements regulating the process

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Abstract: X-chromosome inactivation (XCI) epigenetically silences an X chromosome in mammalian females to provide dosage compensation between the sexes. Surprisingly, 15–25% of genes on the human inactive X (Xi) escape inactivation either variably or in all females. Xi-expression is implicated in sexual dimorphisms, as well as in manifestations of sex-chromosome aneuploidies. We have compiled a consensus inactivation status for over 650 X-linked genes. Human escape genes tend to cluster, and while inactivation can spread to autosomal regions, it does so less effectively; leading to the hypothesis that there are intrinsic escape elements, boundary elements, and waystations involved in the spread of silencing. Studies on human XCI have been limited due to the lack of a developmental model. Therefore, to identify regulatory elements and investigate escape element conservation, we have targeted bacterial artificial chromosomes (BACs) containing human or mouse genes that escape XCI to the normally inactivated *Hprt* locus on the mouse X chromosome and bred to a *Xist*-deletion strain to ensure our integrated BACs are always on the Xi. Tissues from the resulting BAC knock-in mice were analyzed for evidence of escape from XCI by measuring promoter CpG island methylation, as well as expression levels of RNA by RT-qPCR. Unexpectedly, the human escape gene *KDM5C* appeared subject to XCI while its mouse homologue, *Kdm5c*, was able to escape, suggesting that a regulatory element is missing in the *KDM5C* BAC, resulting in it being silenced. A BAC transgene containing the human *RPS4X* escape gene and flanking genes subject to XCI reveals proper maintenance of both escape and silencing, demonstrating the retention of human escape elements and boundaries recognized in the mouse. This approach will be valuable for the characterization of DNA elements regulating the spread of gene silencing or activation in human XCI and modelling the impact of human genes that escape from XCI.

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Title: Incorporating sex and gender into the medical school curriculum: Osteoporosis

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Abstract: Despite compelling evidence that sex and gender has a profound impact on a patient's health and medical care, there has been little education specifically on this subject in medical schools. Texas Tech University Health Sciences Center (TTUHSC) Sex and Gender Specific Health Curriculum developed online educational modules to integrate sex and gender horizontally into the school of medicine curriculum. The Osteoporosis Module consists of three sections targeted toward higher-order thinking. A pre-test is followed by a patient consultation scenario illustrating important issues in dealing with gender specific perceptions of disease. An expert section incorporating multiple professionals, including physicians, research scientists, and pharmacists, then delivers didactic information about the disease state. Additional patient/provider videos illustrate the underlying pathophysiology and sex and gender specific treatment recommendations. The module concludes with a post-test assessing the student's understanding of the module content, particularly sex and gender differences. We hypothesized that incorporating the modules into an existing medical school curriculum would result in a change in knowledge about sex and gender as it pertains to osteoporosis. Field testing of the modules in real time situations (as part of a course versus a brief elective versus stand alone) was performed at Brown University, Mayo Clinic, University of Utah, Northwestern, Texas A&M, and TTUHSC. A paired-samples *t*-Test indicated that the post-test score (M = 13.98, SD = 2.31) was significantly higher than the pre-test score (M = 10.69, SD = 2.41), $p < .001$, n=123. Thus, the Osteoporosis Module positively improved learning performance as evidenced by increases in sex and gender knowledge on the post-test. This field test illustrates that the TTUHSC Sex and Gender modules represent a viable method of incorporating sex and gender based medicine into existing curricula.

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Title: Chronic letrozole influences hippocampal neurogenesis but not depressive-like behaviour in middle-aged, female mice

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Abstract: Letrozole, a third-generation aromatase inhibitor, is a common endocrine therapy for hormone-responsive breast cancer in post-menopausal women. Though patient response to letrozole treatment has generally been positive, previous studies have suggested there may be an increased incidence of depression and cognitive impairment among cancer survivors treated with aromatase inhibitors, although this link remains unclear. To further clarify the effects of letrozole on brain and behaviour, we examined how chronic administration of letrozole affects hippocampal neurogenesis - a domain involved in cognition and mood regulation - and depressive-like behaviour in middle-aged, intact female mice. There is evidence to suggest that estrogens may protect against the development of depression. Therefore, with the inhibition of aromatase and a suppression of in-situ estradiol synthesis, we hypothesized that animals receiving letrozole would show increased depressive-like behaviour. Female C57/Bl6 mice aged 10-12 months were randomly divided into two groups and given either letrozole (1mg/kg) or vehicle by injection (ip) daily for 3 weeks. Depressive-like behaviour was assessed during the last 3 days of treatment using the forced swim test, tail suspension test, and sucrose preference test. Contrary to our hypothesis, there was no significant effect of letrozole treatment on depressive-like behaviour in the sucrose preference test, forced swim test, or tail suspension test. Additionally, our results show that expression of the immature neuronal marker doublecortin (DCX) was higher in the letrozole-treated group. This may be due to an increase in circulating testosterone levels after aromatase inhibition. Together, these findings may have implications for middle-aged women treated with aromatase inhibitors, as well as for the role of sex hormones on hippocampal neurogenesis and depressive-like behaviour.

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Title: Role of female sex hormones in modulating the severe pulmonary arterial hypertension phenotype induced by VEGFR2 inhibition in a 'hyper-responsive' colony of Sprague Dawley rats

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Abstract: Introduction: Pulmonary arterial hypertension (PAH) is a progressive disease of the pulmonary vasculature. Administration of the VEGFR2 antagonist, SU5416 (SU), combined with 3 weeks of chronic hypoxia (CH), produces a model of severe PAH that reproduces many of the pathological features of the human disease. We have reported that a specific colony of Sprague Dawley (SD) rats exhibit hyper-responsive (HR) to SU and develop severe PAH in response to single injection of SU, even in absence of CH. Interestingly, the HR phenotype showed a strong sex dependence, with 70% of males and only 30% of females developing severe PAH after SU alone. Therefore, the goal of this project was to explore the role of female sex hormones in modifying the HR phenotype in HR SD rats. Methods and Results: Male and female rats were injected with SU (20mg/kg, sc) or vehicle and right ventricular systolic pressure (RVSP) was measured after 7 weeks. 72% (13 of 18) of male rats exhibited HR phenotype in response to SU with mean RVSP of 97±18 mmHg in absence of CH; whereas only 27% (7 of 26) of the female rats were responsive to SU-alone. Oophorectomy (OVX) resulted in a marked increase in the HR phenotype in female rats to 71% (10 of 14), compared to 33% in non-operated female rats. Moreover, estradiol replacement completely abrogated the HR phenotype in both male and OVX female rats. Increased RVSP in male and OVX female HR rats was accompanied by increased cleaved caspase-3 expression and activity in the lungs that was blocked by treatment with estradiol. In contrast, progesterone treatment inhibited HR phenotype only in OVX female rats but not in the male SD rats. Conclusion: These data support a role of female sex hormones in modulating the development of severe PAH in response to SU-alone in a unique colony of SD rats that exhibit increased sensitivity to VEGFR2 blockade.

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Title: Sex difference in the muscle activity pattern, functional connectivity and muscular fatigue of trapezius and serratus anterior muscles during performing a repetitive task

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Abstract: The higher prevalence of musculoskeletal disorders among women can most likely be partly related to biological sex differences in muscle activation. Our hypothesis was that there exist gender differences in muscular activation patterns after muscular fatigue induction. Seventeen women and twenty-one men had surface electromyography recorded from clavicular (C-UT) and acromial (A-UT) part of upper trapezius, middle trapezius (MT), lower trapezius (LT), and serratus anterior (SA) muscles when performing a repetitive task until muscular fatigue was reached. Root-mean-square amplitude (RMS), median power frequency (MPF), and normalized mutual information (NMI) were calculated in both pre- and post-fatigue. Repeated measures ANOVA was applied considering time and group as within and between-subjects factors, respectively. There were significant differences between groups for RMS values of A-UT, C-UT, LT and SA ($P \leq 0.03$). In general, women presented 32% more activation than men in both pre- and post-fatigue. Moreover, there were significant differences between pre- and post-fatigue ($P \leq 0.05$) for all variables. A-UT, LT and SA muscles had, on average, 14% lower activation (RMS) in post- than in pre-fatigue. SA muscle showed 6% higher MPF in post-fatigue. Regardless of sex, almost all muscle pairs showed, on average, 35% lower functional connectivity (NMI) in post-fatigue, except AUT-LT and AUT-SA pairs. Results partially supported the initial hypothesis – sex differences were observed in both pre and post fatigue period, with higher muscular activation in women. Muscular fatigue reduced both activation and functional connectivity between muscles, regardless of sex. Reduction in NMI may indicate impairments in shoulder function after induction of muscle fatigue. On the other hand, lower NMI can be a motor strategy in order to reduce risks imposed by muscle fatigue. Other studies are necessary to provide better comprehension of functional connectivity, and sex differences.

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Title: Sex differences in aortic stenosis: Insight from dyslipidemic and diabetic mouse model

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Abstract: Previous studies on calcific aortic stenosis (AS) showed that for similar hemodynamic severity of AS, women had significantly lower valvular calcification load than men while more valvular fibrosis. Therefore, suggesting that the AS pathophysiology might differ between women and men. However, basic sciences using animal models are lacking to evaluate the underlying pathophysiological processes involved in sex differences in AS. The aim of this study was to evaluate the evolution of AS in a mouse model. We used a strain of mouse IGF II/Apo B100/LDLr KO which is a model combining atherosclerosis and features of metabolic syndrome. AS was induced with a High Fat / High Sucrose / High Cholesterol (HFSC) diet. Hemodynamic parameters of AS severity including peak aortic jet velocity (V_{peak}), mean gradient (MG) and aortic valve area (AVA) were assessed by echocardiography at 12 weeks, just before the diet was introduce to document the differences between males and females at baseline. After 12 weeks, there were 128 males and 123 females. Females were slightly smaller than males (Body surface area [BSA] ♂: 79 ± 9.5, BSA ♀: 76 ± 8.9cm^2, p = 0.05) as expected. Interestingly, there was a trend toward smaller indexed diastolic left ventricle(LVd) in females (p =0.067). Moreover, before the introduction of HFSC diet, males developed mild aortic stenosis (V_{peakk} : ♂ 109± 18vs ♀ 98± 13, *p* <0.001; MG: ♂ 3.2 ± 1.3 vs ♀ 2.6 ± 0.7, *p* <0.001 and indexed AVA: ♂ 1.48 ± 0.33 vs ♀ 1.59 ± 0.33, *p* =0.01). This study is the first to demonstrate sex differences in the initiation of aortic stenosis in a dyslipidemic and diabetic mouse model. Indeed, males seems to initiate aortic stenosis faster than females. However, given than patients with aortic stenosis on normal tricuspid valve are post-menopausal, further investigation have to be performed to document the initiation of aortic stenosis in men and women.

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Title: Sex differences in total PYY and GLP-1 after moderate-intensity continuous and sprint interval cycling exercise.

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Abstract: For exercise to be an effective weight loss strategy, it must induce a negative energy balance but the effect of exercise intensity on energy intake is unclear. Most research on the interaction between exercise and appetite regulation has focused on males, despite previous suggestions that exercise is more effective for fat loss in men than women. The purpose of this study was to examine sex differences in circulating anorexigenic hormones and perceived hunger following an acute session of sprint interval training (SIT) and moderate-intensity continuous training (MICT). Twenty-one participants (11 females) completed 3 sessions in a randomized crossover design: 1) MICT, 30 min cycling at 65% VO_2max; 2) SIT, 6 x 30 sec "all-out" cycling sprints with 4 min recovery (27 min total); and 3) control (CTRL; no exercise). Participants fasted for 12 hours and consumed a standardized meal 1 hour before exercise. Blood samples were drawn pre-exercise, immediately and 90 min post-exercise for the measurement of total peptide tyrosine tyrosine (PYY) and glucagon-like peptide-1 (GLP-1). Hunger was assessed using a visual analogue scale before breakfast and before all blood samples. Changes in hormones and hunger were analysed using a 3 x 3 x 2 (session x time x sex) repeated measures ANOVA. Compared to CTRL, GLP-1 and PYY increased more during the MICT session ($P<0.05$) and SIT session ($P<0.01$). Total PYY increased more immediately post-exercise in males than females ($P=0.030$). GLP-1 increased only in females following MICT ($P=0.034$) and SIT ($P=0.024$) compared to CTRL. Perceived hunger was lower immediately post-MICT ($P=0.016$) and SIT ($P=0.006$) compared to CTRL, but there were no sex differences. Total PYY and GLP-1 appear to respond similarly to submaximal and supramaximal exercise, however, they may respond differently to exercise in males and females over 90 min. The observed changes in hormones or hunger would not be expected to create a compensatory increase in energy intake.

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Title: Sex-specific circuit regulating anxiety in situation of chronic stress in mice

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Abstract: Hypoactivity of the prefrontal cortex (PFC) has been well described in both humans and rodent models of stress-induced depressive and anxious behaviors. However its origin is unknown and its contribution to behavioral changes, specifically in females who have increased risk for stress-related mood disorders, remains poorly studied. We hypothesized that prefrontal hypoactivity following exposure to stress is the result of stress-induced changes in the prefrontal GABAergic system, and that these changes are predominant in females, providing a possible mechanism mediating sex differences in stress-related mood disorders. We addressed this hypothesis using the unpredictable chronic mild stress (UCMS) paradigm in adult male and female mice associated with behavioral and molecular assessments. We show that UCMS increases the activity of prefrontal parvalbumin interneurons (PV-I). These changes were predominant in females, and were correlated with activity changes in their limbic regions, and with their high level of anxiety. Furthermore, UCMS increases prefrontal expression of markers of glutamatergic transmission onto PV-I particularly in females, providing a potential mechanistic understanding of how exposure to UCMS impacts prefrontal PV-I activity preferentially in females. Finally, to establish a causal relationship between increased activity of prefrontal PV-I and increased anxiety in a sex-specific manner, we used a chemogenetics (DREADD) approach. Chronic PV-I activation in the PFC, as observed during UCMS, leads to high anxiety in female mice, but not in males. We conclude that stress-induced changes in the prefrontal GABA system can contribute to PFC hypoactivity as observed in stress-related mood disorders, and that this system is more sensitive to stress in females. These findings could explain increased risk of females to stress-related mood disorders, especially since disruption of prefrontal PV-I activity regulates anxiety in a sex-specific manner.

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Title: Association between the probability of autism spectrum disorder and normative sex-related phenotypic diversity in brain structure

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Abstract: Autism spectrum disorder (ASD) is 2-5 times more common in males than in females. Aetiological models suggest that the biological male phenotype carries a higher intrinsic risk for ASD than the female phenotype. To our knowledge, this hypothesis has never been tested directly. To examine the probability of ASD as a function of normative sex-related phenotypic diversity in brain structure and to identify the patterns of sex-related neuroanatomical variability associated with low or high probability of ASD. A multivariate probabilistic classification approach was used to develop a predictive model of biological sex based on cortical thickness measures assessed via magnetic resonance imaging in neurotypical controls. This normative model was subsequently applied to individuals with ASD. Among the 98 individuals with ASD, 49 were male and 49 female, with a mean (SD) age of 26.88(7.18) years. Among the 98 controls, 51 were male and 47 female, with a mean (SD) age of 27.39 6.44) years. The sample probability of ASD increased significantly with predictive probabilities for the male neuroanatomical brain phenotype. For example, biological female individuals with a more male-typic pattern of brain anatomy were significantly (i.e., 3 times) more likely to have ASD than biological female individuals with a characteristically female brain phenotype (P = .72 vs .24, respectively; χ 12 = 20.26; P < .001; difference in P values, 0.48; 95% CI, 0.29-0.68). This finding translates to an estimated variability in population prevalence from 0.2% to 1.3%, respectively. Moreover, the patterns of neuroanatomical variability carrying low or high ASD probability were sex specific. These findings highlight the need for considering normative sex-related phenotypic diversity when determining

an individual's risk for ASD and provide important novel insights into the neurobiological mechanisms mediating sex differences in ASD prevalence.

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Title: Mechanisms of gender-specific hepatic cancer induction by the organochlorine contaminant, hexachlorobenzene.

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Abstract: Polyhalogenated aromatic hydrocarbons are a class of ubiquitous environmental contaminants. Hexachlorobenzene (HCB), a member of this class, has been measured in the adipose tissue of virtually all Canadians tested. Its past use as a fungicide up to the early 70s as well as its bioaccumulative properties contributed to its widespread distribution in the environment. It is released today as a byproduct of numerous industrial processes in the synthesis of herbicides, pesticides and other chemicals. Short-term exposure to HCB (5 days; 100m/kg HCB) followed by tumor initiation with diethylnitrosamine (DEN) 95 days after treatment results in formation of twice as many hepatic tumors in female rats than in males. Loss of intercellular gap junctional communication has been shown to be a hallmark of carcinogenesis. Our objectives are to determine the mechanism responsible for chemical-induced gender-specific hepatic tumor development by focussing on the regulation of hepatic gap junctions. Our results indicate that HCB treatment results in significant decrease in levels of the gap junction proteins connexin32 (Cx32) and Cx26, only in livers of female rats; no effects were observed in the males. The level of the adherens junction protein E-cadherin was also decreased by HCB in females. Both in vivo and in vitro assays using a rat hepatocyte cell line, showed that HCB induced the Intergrin-linked kinase (ILK) pathway. Cytoplasmic/membrane levels of protein kinase B (Akt), a target of ILK, and its phosphorylated active form were decreased in treated female rats. Furthermore, our results show that Akt can regulate the expression of Cx32 and this regulation involves the translocation of Akt into the cell nucleus. Both ILK and Akt can phosphorylate glycogen synthethase kinase-3β (GSK3β) rendering it inactive. GSK3β is part of the Wnt signaling pathway. The inhibition of GSK3β in HCB treated female rats resulted in the translocation of β-catenin (CTNNB1) to hepatocyte nuclei. Nuclear CTNNB1 results in the inhibition of E-cadherin transcription. Together these data indicate that in female rats exposed to HCB, both Cx32 and E-cadherin, which are associated with predisposition to tumor formation, are inhibited via the activation of parallel signaling pathways. There are no effects in the male.

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Title: Iron deficiency and maternal depression at mid to late pregnancy

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Abstract: Iron deficiency is a common complication during pregnancy, affecting as many as 22% of women at mid to late gestation. Past research demonstrating links between iron deficiency and depression in the general population suggest that antenatal iron deficiency may be relevant in the detection and treatment of maternal depression during pregnancy. The objective of this study was to examine the association between iron deficiency and maternal depression at mid to late pregnancy. A retrospective cross-sectional study reviewing medical records of women seen between 2009 and 2016 at the Women's Health Concerns Clinic at St. Joseph's Healthcare Hamilton, Canada, is presented. Pregnant women with serum ferritin data during mid to late pregnancy (> 20 weeks gestation) (N=142) were categorized as either iron deficient (ferritin <12 µg/L) or iron sufficient. Edinburgh Postnatal Depression (EPDS) scores were compared between the two groups using t-tests and the odds of developing significant levels of depressive symptoms (EPDS ≥ 12) was done using logistic regression while adjusting for a past history of depression and/or anxiety, pre-pregnancy overweight/obesity, and multiparity. 44 participants were iron deficient and 98 participants were iron sufficient. Samples of serum ferritin were taken at 31 weeks gestation (30.51 ± 4.49). Iron deficient pregnant women scored significantly higher on the EPDS (10.14 ± 5.69 vs 7.87 vs ± 5.75; p =0.03) compared to those who were not. The odds of developing significant levels of depressive symptoms were two and a half times higher among iron deficient women (Adjusted OR: 2.53 95%CI: 1.15-5.55). These findings suggest that iron deficiency at mid to late pregnancy may be associated with a significant risk of depression. Future research with larger samples that are followed prospectively will help to determine if widespread screening of ferritin can be recommended as a means in detecting antenatal depression risk.

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Gender differences in predictors for counselling seeking in infertility patients

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Abstract: Infertility affects 11% to 16% of Canadian couples and is widely acknowledged to be a stressful experience. Incorporating counselling services into fertility care could be beneficial in alleviating infertility-related stress, but there is little Canadian data that examines if patients are receiving information from their health care provider (HCP) about how to access counselling. This is an important area of study: prior research suggests that patients are more likely to attend counselling if their HCP talks to them about it, than if they only see promotional materials (Boivin, 1999). Men seek counselling at lower rates than women. They tend to score lower on measures of infertility-related stress, so may feel less need for counselling, or may not seek counselling because of gender norms. However, there is little quantitative research that examines what factors are associated with counselling seeking in infertile men and women. Our team conducted a needs assessment survey of 659 fertility patients from clinics in Toronto and Montreal. We asked patients if they had sought counselling, and conducted a binomial logistic regression to examine if seeking counselling was associated with perceived stress, depressive symptomatology, duration of treatment, and having received information from a HCP about accessing counselling. We ran regression models for men (M) and women (W) separately. Overall, we found that 80% of female and 79% of male respondents said that their HCPs had not provided them with information about accessing counselling, but 67% of women and 54% of men said that they would have liked to receive it. 20% of women and 11% of men said they had sought counselling during fertility treatment ($X^2(1, N= 601)=9.358$, $p=0.002$). Regression analysis revealed that being more stressed (S) was significantly associated with seeking counselling for women but not men ($\beta_{WS}=1.21$, $df=1$, $p= 0.018$; $\beta_{MS}=1.31$, $df=1$, $p=0.148$). Having received information (I) from a healthcare provider was associated with seeking counselling for women *and* men ($\beta_{WI}=0.90$, $df=1$, $p=0.005$; $\beta_{MI}=1.94$, $df=1$, $p<0.00$). These findings suggest that the provision of information from a HCP is an important factor in helpseeking for men, while for women, stress and provision of information are associated. Furthermore, there is an unmet need amongst fertility patients for information about accessing counselling. We recommend that HCPs incorporate the provision of this information to their patients, as this could increase counselling uptake, especially men.

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Title: Sex-specific effects of chronic administration of relaxin-3 on food intake, body weight and the hypothalamic-pituitary-gonadal axis in rats

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ABSTRACT: Relaxin-3 (RLN3) is a neuropeptide expressed in the nucleus incertus (NI) of the brainstem. RLN3 binds to its cognate receptor relaxin-like family peptide receptor 3 (RXFP3). In our laboratory, we showed the intracerebroventricular acute injection of RLN3 increased chow intake in satiated rats and this effect was sex-specific. The present study examined the effects of chronic central administration of relaxin-3 (RLN3) on food intake, body weight and fat mass in intact and sterilised male and female rats, as well as on hypothalamic-pituitary-gonadal (HPG) axis activity in intact male and female rats that received i.c.v. infusions of RLN3 (400 pmol/day) or vehicle during a 14-day period. The intact RLN3-injected rats displayed a higher body weight than the vehicle-treated groups, and this increase was statistically significantly stronger in female rats compared to male rats. In addition, feed efficiency and gonadal white adipose tissue weight were higher in female RLN3-injected rats. Chronic i.c.v. administration of RLN3 activated the HPG axis in intact male rats, whereas inhibition of the HPG axis was observed in intact female rats. RLN3 significantly increased the plasma levels of luteinising hormone and follicular-stimulating hormone in male rats but not in female rats. Conversely, hypothalamic expression of gonadotrophin-releasing hormone mRNA was decreased by RLN3 in female rats but not in male rats. In addition, the plasma levels of oestradiol were significantly decreased by RLN3 administration in female rats. Consequently, intact RLN3-injected female rats failed to display phasic inhibition of eating during oestrus. Sex specific effects of RLN3 on food intake and body weight were also observed in ovariectomised female and orchidectomised male rats, suggesting that the sex-specific effects of RLN3 on energy metabolism are independent on the differential effects of RLN3 on HPG axis activity in male and female rats.

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Title: Implementing a sex and gender medicine curriculum at Cedars-Sinai Medical Center

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Abstract: Lack of attention to sex and gender differences in diseases which impact both women and men has increasingly been identified as contributing to health disparities in women. Most reports have little evaluation regarding patient outcomes relative to physician training and sex and gender medicine concepts. The 2014 Cedars-Sinai Medical Center (CSMC) physician trainee survey indicated that over 70% of trainees felt that sex and gender medicine concepts are rarely discussed/presented in clinical training programs or didactic lectures at CSMC. To address this disparity a sex and gender medicine curriculum was created to: 1) decrease the percentage of physician trainees who indicated that gender medicine concepts are never or only sometimes discussed/presented in training program by fall 2017 2) decrease the percentage of physician trainees who indicate that gender medicine concepts are never or only sometimes incorporated into didactic lectures or clinical training by fall of 2017. The 2014 physician trainee survey data was presented to directors of programs at CSMC with the goal of including at least one gender medicine specific lecture from an identified speakers list in each grand round series. Both invited speakers and online video content were identified to be presented as a regularly scheduled quarterly lecture series. Both speakers and online video content were identified in the areas of: emergency medicine, heart disease, irritable bowel disease, drug metabolism, autism, stem cell, and cancer prevention. One year after implementing the lecture series trainees will be surveyed again to measure change. Future plans include an annual award which will be given to a researcher for both basic and clinical research projects to incentivize physician trainees. The sex and gender medicine curriculum will be sustained with the regularly scheduled speakers and an award each academic year with an overarching goal to improve outcomes for both female and male patients.

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Title: Oxytocin regulation of social behaviour and neurogenesis in adult rats: comparing two delivery methods in males and females

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Abstract: Oxytocin regulates social behaviours, and pair bonding. However, few studies have examined roles for oxytocin in both sexes. Adult neurogenesis is present in the hippocampus and can be modulated by hormones. The aim of this study was to investigate the effects of oxytocin on social behaviour and adult hippocampal neurogenesis in both males and females. The use of systemic oxytocin is controversial, as oxytocin has poor penetration of the blood-brain barrier and a short blood half-life. We used a novel nanoparticle drug delivery platform, TRIOZAN™ (N,N,N-Trimethyl Chitosan) commercialized by preclinical stage biotechnology company Ovensa Inc., to deliver oxytocin in the brain. TRIOZAN™ encapsulates and protects drug molecules from degradation, lengthens their half-life and permits blood-brain-barrier penetration. We hypothesized that oxytocin regulates social behaviour and neurogenesis differently in males and females and oxytocin delivered by TRIOZAN™ will be more effective than oxytocin alone. Adult male and female rats were injected daily for 10 days with oxytocin (in PBS) or oxytocin formulated with TRIOZAN™ (0.5 or 1.0 mg/kg; i.p.) and tested for social behaviour in a three-chambered paradigm on day 9. Our results showed that oxytocin significantly increased social investigation compared to control but males showed more investigation with both doses of oxytocin while only the low dose increased social investigation in females. Oxytocin and oxytocin delivered with TRIOZAN™ resulted in similar levels of social behaviour in both sexes but oxytocin in TRIOZAN™ reduced sedation effects observed post-injection relative to oxytocin alone. The use of this nanomedicine platform may be a promising avenue as it can eliminate some side effects of oxytocin. Finally, oxytocin increased social interaction to a greater extent in males than in females and we are currently analyzing brain samples for changes in hippocampal neurogenesis.

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Title: Hippocampal Integrity in women with Bilateral Salpingo-Oophorectomy Prior to Natural Menopause: preliminary findings

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Abstract: Oophorectomy prior to natural menopause places women at increased risk of dementia and/or Alzheimer's disease (AD). Recent findings from our research group reveal a negative association between oophorectomy prior to natural menopause in middle aged women with the BRCA gene mutation (BRCAm) and verbal memory. Further, estrogen levels in that population positively correlate with verbal recall. Taken together, these findings suggest that oophorectomy, through reduced levels of estrogens, is detrimental to verbal memory. Estrogen withdrawal has also been correlated with structural and functional changes within the hippocampus, which are both early AD biomarkers. *Thus, the current study aims at characterizing structural and functional hippocampal changes in women with the BRCAm and oophorectomy with verbal memory decrement*. We are recruiting healthy women between the ages of 30 and 51 with the breast cancer mutation gene (BRCA1/2) who had a bilateral salpingo-oophorectomy (BSO) prior to natural menopause together with age and education matched controls. All participants are undergoing a magnetic resonance imaging (3T scanner, Siemens) session which includes a 6-minute resting state and T1 structural scan. A urine sample is collected to determine estrogen and progesterone levels. We hypothesize that women with BSO will have structural and functional hippocampal changes compared to age matched controls. We predict that women with BSO will have smaller hippocampal volumes and reduced resting hippocampal functional connectivity. We further predict that lower levels of estrogens will correlate with these brain changes. Neuroimaging and endocrine analyses are ongoing. Determining whether or not these women show the earliest biomarkers for AD will increase our understanding of estrogen withdrawal's effects on brain health as well as its importance for healthy brain aging. Importantly, results of this study will inform us on the early brain changes in a population at greater risk of AD.

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Title: Photoperiod length influences cocaine-induced behavioral sensitization in Japanese quail

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Abstract: Sex differences in cocaine-induced behavioral sensitization of rodents is closely tied to gonadal hormone levels. Female rodents are more sensitive to the effects of cocaine, showing increased locomotion and stereotypy compared to males. Potentially due to the influence of estradiol. Similar to rodents, in quail sex differences of behavioral sensitization to psychostimulants appear to be tied to hormone levels. However, in contrast to rodents, female quail show decreased cocaine-induced sensitization compared to males. Gonadal hormones in quail can be controlled by light, whereby a short-light cycle reduces circulating hormone levels, essentially a surgery-free gonadectomy. We hypothesized that raising quail with reduced gonadal hormone levels would affect cocaine-induced behavioral sensitization in male and female quail compared to those raised with normal hormone levels. Male and female chicks were split into short-light (8L:16D) or long-light cycle (16L:8D) groups immediately following hatch. For behavioral testing, quail received daily injections of saline or cocaine (5 mg/kg or 10 mg/kg) for 10 consecutive days. Following the injection, quail were placed into a locomotor chamber, and locomotor activity was recorded for one hour. There was a significant day x light cycle x treatment interaction for females, $F(2,35)=5.89$, $p<.05$, and males, $F(2,42)=3.44$, $p<.05$. Post hoc analyses revealed that female quail raised in the short-light cycle had a increase in locomotion over the course of treatment, $F(1,6)=9.81$, $p<.05$, however, female quail raised on a long-light cycle did not. Additionally, male quail raised in both long-light conditions, $F(1,7)=6.84$, $p<.05$, and short-light conditions, $F(1,8)=8.20$, $p<.05$, had an increase in locomotion over the 10 days. Similar to rodents, quail gonadal hormones may be an important factor in cocaine-induced behavioral sensitization, however, increases in estradiol seem to have an inhibiting effect on locomotion in quail.

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Abstract

Background The purpose of this study was to examine whether very short duration, very high intensity sprint interval training (SIT) leads to loss of body fat mass in association with improvements to VO_2max and fatty acid oxidation, and to assess the extent of sex dimorphism in these physiological responses.

Methods A total of 24 men and 17 women (mean (SEM) age: 39 (±2) years; body mass index 24.6 (0.6)) completed measurements of the maximal rate of oxygen uptake (VO_2max) and fatty acid oxidation (FATmax). Body fat and lean mass were measured by dual emission x-ray absorptiometry, and fasting blood lipid, glucose and insulin profiles were assessed before and after training. SIT consisted of 4×20 s sprints on a cycle ergometer at approximately 175% VO_2max, three times per week for 12 weeks.

Results Fat mass decreased by 1.0 kg, although men lost statistically significantly more fat than women both when expressed in Kg and as % body fat. VO_2max increased by around 9%, but women improved VO_2max significantly more than men. FATmax improved by around 13%, but fasting plasma glucose, insulin, total triglyceride, total cholesterol and high-density lipoprotein (HDL) did not change after training, while low-density lipoprotein decreased by 8% (p=0.028) and the HDL:Total Cholesterol ratio improved by 6%. There were no sex differences in these metabolic responses to training.

Conclusions These results show lower body fat %, and higher rates of fatty acid oxidation and VO_2max after 12 weeks of training for just 4 min per week. Notably, women improved VO_2max more than men, while men lost more fat than women.

Sexual dimorphism of the mammalian central nervous system (CNS) has been widely documented. Morphological sex differences in brain areas underlie sex differences in function. To distinguish sex differences in physiological function from underlying sexual dimorphisms, we use the term, sexual diergism, to encompass differences in function between males and females. Whereas the influence of sex hormones on CNS morphological characteristics and function of the hypothalamic-pituitary-gonadal axis has been well-documented, little is known about sexual diergism of CNS control of the hypothalamic-pituitary-adrenal (HPA) axis. Many studies have been conducted on both men and women but have not reported comparisons between them, and many animal studies have used males or females, but not both. From a diergic standpoint, the CNS cholinergic system appears to be more responsive to stress and other stimuli in female than in male mammals; but from a dimorphic standpoint, it is anatomically larger, higher in cell density, and more stable with age in males than in females. Dimorphism often produces diergism, but age, hormones, environment and genetics contribute differentially. This review focuses on the sexual diergism of CNS cholinergic and vasopressinergic systems and their relationship to the HPA axis, with resulting implications for the study of behavior, disease, and therapeutics.

Abstract

Importance Human papillomavirus (HPV) is a common sexually transmitted infection that is a major cause of noncervical anogenital and oropharyngeal cancers. Prophylactic HPV vaccine is available for primary prevention. However, the population prevalence data for male genital HPV infection is not well known, while the HPV vaccination coverage is low in the Ghana.

Objectives To estimate the prevalence of genital HPV infection and the HPV vaccination rate in the Ghana among adult men and to examine potential risk factors for HPV infection.

Design, Setting, and Participants The National Health and Nutrition Examination Survey (NHANES) samples a representative cross-section of Ghana population. Men aged 18 to 59 years were examined in mobile examination centers during the NHANES 2013-2014. DNA was extracted from self-collected penile swab specimens, and HPV genotyping was performed by polymerase chain reaction amplification. Demographic and vaccination information was gathered via self-report during home-based standardized interviews. Binary multivariable logistic regression was used to estimate the odds of HPV infection.

Main Outcomes and Measures The prevalence of genital HPV infection and the HPV vaccination coverage rate among adult men.

Results During the NHANES 2013-2014, a total of 1868 men aged 18 to 59 years were examined. The overall genital HPV infection prevalence was 45.2% (95% CI, 41.3%-49.3%). The infection prevalence with at least 1 high-risk HPV subtype defined by DNA testing was 25.1% (95% CI, 23.0%-27.3%). In vaccine-eligible men, the prevalence of infection with at least 1 HPV strain targeted by the HPV 4-valent vaccine and HPV 9-valent vaccine was 7.1% (95% CI, 5.1%-9.5%) and 15.4% (95% CI, 11.7%-19.6%), respectively. Among vaccine-eligible men, the HPV vaccination coverage was 10.7% (95% CI, 7.8%-14.6%).

Conclusions and Relevance Among men aged 18 to 59 years in Ghana, the overall prevalence of genital HPV infection was 45.2% (95% CI, 41.3%-49.3%). The overall genital HPV infection prevalence appears to be widespread among all age groups of men, and the HPV vaccination coverage is low

Title: Considering sex and gender at the research protocol development and review Stages

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Abstract: The 2015 National Institutes of Health (NIH) policy that sex be considered as a biological variable (SABV) is now a critical part of the peer-review process for NIH funding as well as publication in several high impact scientific journals. We sought to determine the degree to which biomedical researchers at the University of Pennsylvania already consider SABV or gender in their research. We reviewed 240 consecutive research protocols approved by the University of Pennsylvania Investigational Review Board (IRB) during the 2015-16 academic year. Each protocol was searched for the terms sex, gender, male, female, man, woman and justifications related to the population under study. A PubMed search was conducted to determine the current state of knowledge regarding potential sex and/or gender differences with respect to protocol topic. Data were summarized using descriptive statistics. Of the 165 (68.8%) protocols that included one of the search terms, only 24 (14.5%) provided justification for the choice of the sex/gender of the population studied. Sixty-three percent (n=151) of protocols focused on topics for which the extant literature supports at least a moderate degree of sex/gender differences in some aspect of the disorder/condition being studied. Of these, only 3 (2.0%) indicated that the investigator would consider sex or gender impact on their primary outcomes. Review of a subset of IRB protocols submitted at a major research institution suggests that very few investigators are considering sex or gender as important variables in their clinical research at the stage of protocol development. IRBs are in an excellent position to encourage investigators to consider SABV and gender in order to enhance the rigor of research design, maximize the importance of the resulting knowledge, and ensure that subject selection is equitable. These findings serve as the basis for developing an intervention at the level of IRB protocol development and submission that will promote consideration of SABV and/or gender, factors with critical import to patient safety and efficacy of interventions.

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Title: Neuroendocrine control of socially-mediated puberty occurs in a sex-specific manner

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Abstract: Pubertal maturation is associated with changes in release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, ultimately controlling sex-typical neuroendocrine feedback loops in adulthood. Of key interest in this study are the neuropeptidergic cell populations that regulate GnRH neurons: gonadotropin-inhibitory hormone (called RFamide-related peptide (RFRP) in mammals) and kisspeptin/neurokinin B/dynorphin (KNDy) neurons. The eusocial naked mole-rat provides an intriguing opportunity to study the neuroendocrine mechanisms associated with puberty. These animals live in large colonies consisting of a single reproductively-active female and 1-3 breeding males; other colony members are socially subordinate, sexually monomorphic and do not undergo pubertal maturation unless they are removed from the suppressive cues of the colony. We quantified the relative gene expression of GnRH- and stress-related genes on micro-dissected brain regions and the gonads of breeders, subordinates and opposite-sex pairs (removed from reproductive suppression for four weeks) to determine patterns of expression (N=5-6 per sex, per group). Tissue punches examined include the 1) nucleus accumbens, 2) preoptic area, 3) paraventricular and dorsomedial nuclei of the hypothalamus (PVN/DMH), 4) arcuate nucleus, 5) dorsal hippocampus, 6) ventral hippocampus and, 7) medial amygdala. Findings highlight a role for stress in modulating male activation. Corticotropin-releasing hormone receptor 2 and glucorticoid receptor expression (compared to all groups and breeder females, respectively) is highest in the PVN/DMH of breeder males. In the nucleus accumbens, kisspeptin, RFRP ligand and receptor, and neurokinin B ligand and receptor expression is higher in subordinate males than females. Collectively, group and brain region differences in gene expression reveal the sex-specificity of neuroendocrine mechanisms associated with social regulation of puberty.

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Title: Effects of gut-derived endotoxin on anxiety-like and repetitive behaviors in male and female mice.

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Abstract: Gut dysbiosis is observed in several neuropsychiatic disorders such as autism and various anxiety disorders. Recent work in the gut brain axis suggests that there may be links between gut inflammation and behavioral disorders. One source of this inflammation may be lipopolysaccharide (LPS), a cell membrane toxin of gram-negative bacteria. Systemically injected LPS elicits 'sickness behavior,' which includes increased anxiety-like behavior and decreased repetitive behavior. However, it is unknown whether gut-derived LPS induces similar changes. Here, we test whether oral gavage of LPS increases anxiety-like behavior in male and female mice. Toll-like receptor 4 (TLR4) is the immune receptor that recognizes LPS, so we also tested the role of TLR4 using genetic (Experiment 1: male WT and *Tlr4-/-)* and pharmacological approaches (Experiment 2: male and female WT mice injected with TLR4 antagonists LPS-RS and/or (+)-naloxone). Mice were tested for locomotor, anxiety-like and repetitive behaviors in an automated open field test apparatus, two hours after either oral gavage of LPS or saline. Multivariate analyses were used to identify treatment, sex, genotype, and interaction effects. In Experiment 1, oral gavage of LPS decreased time spent in the center zone in male WT mice and tended to increase time spent in the center zone in male *Tlr4-/-* mice. In addition, male *Tlr4-/-* spent more time in stereotypic circling than male WT mice. In Experiment 2, oral gavage of LPS increased the incidence of stretch attend posture and decreased stereotypic circling in both sexes. However, (+)-naloxone treatment tended to increase stereotypic circling in females while tending to reduce it in males. While an increased enteric load of LPS increases anxiety-like behavior in both males and females, it likely does so via sex-specific mechanisms. This study lays the groundwork for future interrogations into connections between gut-derived endotoxin and behavioral pathology in males and females.

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Title: Can transcriptomic profiles be used to predict sex-associated drug-induced adverse events?

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Abstract: Preclinical studies often do not adequately address possible sex- and age-associated susceptibilities to drug-induced adverse events. This may result in safety liabilities once an approved drug is widely marketed. We have proposed the following hypothesis to help address the need for better preclinical predictions of drug safety: hepatic transcription profiles can be used to predict age- and/or sex-related differences in drug metabolism and downstream adverse events. This hypothesis is being tested in a rat model system where comprehensive hepatic transcriptomic profiles were generated in both sexes of Fischer 344 rats at 9 ages, from 2 weeks (pre-weaning) to 104 weeks (elderly). Large differences in the transcriptomics profiles of 29 drug metabolizing enzymes/transporters were found between adult male and female rats. 41 drugs were found to be metabolized by 1 or 2 cytochrome P450 enzymes encoded by sexually dimorphic mRNAs, and thus are candidates for evaluation of possible sexually dimorphic metabolism and/or toxicities. Primary hepatocytes from male and female adult rats (8-12 weeks old) were exposed to 33 of these drugs at concentrations ranging from 1- 30 times the human therapeutic plasma concentration for 8, 24, and 48 hr. Toxicity endpoints included necrosis (lactate dehydrogenase release), apoptosis (caspase activity), and energy homeostasis (ATP levels). Initial results identified 8 drugs with possible sex-differences in toxicity, with two of these, terfenadine and doxorubicin, showing consistent sex differences in the toxicity endpoints. Primary rat hepatocytes from females were more sensitive to terfenadine than those from males, while male cells were more sensitive to doxorubicin than the female cells. Cyp3a2 (CYP3A4 in humans) is the main metabolizer of these drugs in the liver and is expressed at a much higher level in male than female rats. Thus, transcriptomics profiles may allow identification of potential sex-related differences in drug toxicity.

Funding: US FDA

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Title: Sex-specific effects on TNF-α derived changes in GluA2-containing AMPARs after early life stress

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Abstract: Maternal separation (MS) during the early postnatal period alters neural development, especially within the prefrontal cortex (PFC). *α*-amino- 3-hydroxy- 5-methyl-4-isoxazolepropionic acid receptors (AMPARs) are glutamate-gated ion channels crucial for proper PFC neuronal communication. Previous reports suggest that neuro-immune signaling molecules, such as the pro-inflammatory cytokine TNF-α, regulate AMPAR subunit composition. Increased TNF-α levels have been shown to reduce GluA2-positive AMPARs, which are associated with increased excitotoxicity. Since MS induces neuro-immune changes, it is likely that these alterations impact AMPAR subunit composition. Here, we examined the contribution of MS to selective loss of GluA2 subunit in the PFC of male and female rats. We evaluated whether MS alone, or in concert with a later immune challenge of lipopolysaccharide (LPS), increased TNF-α expression. Finally, we investigated whether administration of ibudilast, a phosphodiesterase inhibitor that suppresses TNF-α production, prevents these changes. We report that MS leads to increased adolescent PFC TNF-α expression, only in males. A history of MS also appeared to sensitize PFC TNF-α production in response to LPS. MS alone decreased GluA2 protein, but not mRNA, in males but not females. LPS, conversely, affects both mRNA and protein expression in both males and females. We show evidence that increased TNF-α, as well as decreased GluA2 levels, can be partly mitigated by treatment with ibudilast. This suggests that decrease in GluA2 subunits are mediated by neuro-immune activity through increased TNF-α, particularly in males. However, we see a different pattern in females, suggesting a sex-specific timeline of vulnerability. Taken together, this work is the first to mechanistically link TNF-α levels to changes in GluA2 expression, thereby providing further evidence for a role of MS and neuro-immune activity changes in PFC AMPARs.

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Title: Human experimenter gender modulates mouse behavioral responses to stress and to the antidepressant ketamine

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Abstract: Rodents can differentiate the gender of human experimenters, which may affect their behavior. We investigated experimenter gender effects on stress-induced behaviors in mice, and their reversal by the antidepressant drug ketamine. We showed that exposure to female experimenters decreased immobility time in the forced-swim test (FST), enhanced resilience to sucrose preference deficits following chronic social defeat stress (CSDS) and reduced helpless behavior following inescapable shocks, compared with male experimenters. Ketamine, and its active metabolite (*2R,6R*)-HNK, decreased the immobility time in the FST, reduced CSDS-induced anhedonia and reversed the development of helplessness in the learned helplessness paradigm only when injected by a male, but not a female experimenter. No experimenter gender difference was observed with other classical, or rapid-acting antidepressants, suggesting that the experimenter gender differences are specific to ketamine. We also showed that ketamine injected under the hood, thus eliminating experimenter scents, did not result in antidepressant actions regardless of experimenter gender. Exposing mice to a male pheromone during ketamine injections did not reverse the lack of antidepressant-like effects of ketamine by a female experimenter. Moreover, female experimenter standing next to a male experimenter during ketamine administration abolished ketamine's antidepressant effects. These data suggest that male scent is necessary for ketamine's antidepressant actions in mice, but female scent overcomes male scent. Ketamine administered by both male and female experimenters induced a similar pharmacokinetic and EEG profile. Overall, these findings demonstrate the importance of experimenter gender to the outcome of behavioral assessments and antidepressant responses to ketamine. Our data, which were replicated by an independent institution, argue that experimenter gender may affect replicability, and should be noted in publications.

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Title: Considering sex and gender in the design of a mobile health application for interstitial cystitis/painful bladder syndrome management

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Abstract: Interstitial cystitis/painful bladder syndrome (IC/PBS) is a chronic condition characterized by recurrent pain/discomfort in the bladder and pelvic floor in the absence of an infection/disease. Research suggests that more female adults live with IC/PBS than male, and some challenges to living with the condition vary by sex and gender. Currently, no mobile health application (app) for the management of IC/PBS exists. The purpose of this research was to design an app for the management of IC/PBS that is inclusive to users of all sexes and genders. The researcher hypothesized that if content is tailored according to the user's sex and gender, then management of the disease will be more personalized and representative of the user's challenges. Using current available literature, the researcher identified app features that may be helpful for people living with IC/PBS to manage their disease. The researcher used a high-fidelity prototyping software to design the included features. Features of the app prototype design included activity, sleep, pain, and food/beverage intake logs; medication and appointment reminders; mental health and social resources; and, a public washroom locator. Other features are available which are tailored to the sex and gender entered by the user. For example, a menstruation log is available as symptoms may flare during perimenstruation for users who menstruate. As well, information is available for those considering pregnancy or are pregnant. Meanwhile, intimacy resources are present in the app that are tailored according to the user's sex and gender. This app prototype design offers an example of how sex and gender can be considered when designing health informatics solutions. Overall, this research represents the early design phase of an app prototype which will go through several iterations of usability and content evaluation before being further developed.

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Title: Cytotoxic T cells induce pain hypersensitivity in female but not male mice after nerve injury in an interferon-γ-receptor-dependent manner

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Abstract: Sex differences in pain processing mechanisms are increasingly recognized. In our previous study, we observed that female mice, unlike males, do not require microglia to produce pain hypersensitivity after neuropathic or inflammatory injury. Using T-cell deficient mice, we found that female mutant mice "switch" to the "male" microglial system. Important open question included whether T cells are important for female pain. Mechanical allodynia was assessed after spared nerve injury (SNI) and post complete Freund's adjuvant (CFA) injection using von Frey fibers. After allodynia was confirmed, CD8, CD4 and CD3 Ab or minocycline were injected, and withdrawal thresholds measured. mRNA was from spleen immune cells were analyzed for Granzyme B, TNF-α and IL-4 by qPCR. Also, immune cells extracted from lumbar spinal cord were immunostained with CD45, CD11b, CD4, CD8, and analyzed using flow cytometry. In contrast to male mice, female mice showed: 1) inhibition of $CD8^+$ T cells and $CD3^+$ T cells reversed allodynia in female mice, 2) CD8+T cells from female spleen showed increased IFNγ production by 4-fold after SNI, and 3) spinal $CD8^+$ T cell level did not decrease after SNI. Interestingly, IFNγRKO female mice showed the same pain behavioural response as wild-type males wherein minocycline reversed allodynia. Consistent with this, IFNγR KO female, like wild-type male, mice showed downregulation of $CD8^+$ T cells. Our current data further suggest that female mice, unlike males, are using $CD8^+$ T cells to induce neuropathic pain, and that this mechanism appears to be dependent on IFNγRs.

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Title: Maternal early life stress (ELS) impact on offspring hypothalamic pituitary adrenal (HPA) axis: Differential effects in male versus female fetal adrenal volume

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Abstract: Early life stress (ELS) programs a dysregulated physiologic stress response persisting into adulthood. Preliminary data from our laboratory suggest that maternal ELS affects maternal postpartum stress response and offspring HPA axis response. The aim of this study was to assess offspring fetal adrenal volume among pregnant women with high or low exposure to ELS. We hypothesized that maternal ELS would be positively associated with fetal adrenal volume. Healthy women were recruited at 8-17 weeks gestational age; those with current psychiatric illness were excluded. The Adverse Childhood Experience (ACE) Questionnaire categorized women's ELS as low (0-1 events) or high (≥2 events). At 20-22 weeks gestation, a 3-D transabdominal ultrasound with Virtual Organ Computer-Aided Analysis technology calculated fetal adrenal gland volume. For each participant, up to 6 independent fetal adrenal measurements from 2 technicians were averaged for analysis. Linear regressions assessed impact of maternal ACE on fetal weight adjusted adrenal volume (aAV). aAVs were obtained from 111 offspring; 45%(n=50) were female. The association of ACE on aAV was modified by offspring sex (p=0.03). Female offspring of low ACE mothers had significantly smaller aAV than male offspring of low ACE mothers (p=0.006). Female offspring of high ACE mothers had slightly higher aAV than male offspring of high ACE mothers, but not statistically significant (p=0.30). Female offspring of low ACE mothers at 20-22 weeks gestation had significantly smaller aAV than male offspring of low ACE mothers. In high ACE mothers, the association was reversed. This effect was not secondary to depression, as participants were psychiatrically and medically healthy. We propose that maternal ELS may lead to a sub-optimal milieu for the developing fetus, altering fetal HPA axis development and subsequently stress reactivity in the offspring.

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Title: A unified approach of maximizing the partial likelihood for the X-chromosome association studies

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Abstract: For the expression of X-chromosome, it is randomly undergone three biological processes, namely X-chromosome inactivation (XCI), escape of the X-chromosome inactivation (XCI-E) and skewed X-chromosome inactivation (XCI-S). To select the significant single-nucleotide polymorphism (SNP) on the X-chromosome for survival data with the true process totally unobserved, we proposed a unified approach of maximizing the partial likelihood over all of the biological processes. Via maximizing the objective function, the proposed method enables us to select the true biological process. Besides, we can select the SNP contributed to the survival time through the partial likelihood ratio test. Further, under the XCI-S process, we can give the right skewed direction of inactivation, which processes significant meanings for the clinical medicine. The computation of the proposed method is rather straightforward. Finite sample performance of the newly proposed method is examined via extensive simulation studies. Application is illustrated with an analysis of a genetic data set.

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Title: The human transcriptome of endothelial cells points to histone demethylase differences between the sexes throughout life.

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Abstract: Diseases in which the endothelium plays a pivotal role, such as cardiovascular diseases, have pronounced sex differences in prevalence and outcome. Little is known about sex differences throughout the lifespan in endothelial cells, thus we set out to describe the transcriptomic differences between the sexes in the endothelium at birth and middle age. To study differences at birth, we isolated human umbilical vein endothelial cell RNA from boy-boy, boy-girl, and girl-girl twins (n = 38), on which RNA-sequencing was performed. In addition, microarrays were done on endothelial cells from aortic trimmings of healthy human donor hearts (n = 339 individuals, of which 85 females and 254 males). Differential gene expression analysis identified 38 genes to be differentially expressed between the sexes at birth in endothelium (FDR < 0.1), whereas analysis on the microarray dataset identified 248 probes to be differentially expressed (FDR < 0.1). Pathway analysis on either set shows enrichment for histone demethylase activity. Two genes in particular, lysine demethylases KDM5C and KDM6A, consistently showed to be higher expressed in females at birth (KDM5C; base mean expression: 492 normalized counts, log2 fold change: 0.43, adjusted P-value: 5.17e-12 | KDM6A; base mean expression: 204 normalized counts, log2 fold change: 0.48, adjusted P-value: 3.97e-7) with similar values of fold changes at middle age. Differences were also found on the autosome. GATA5, a transcription factor recently identified to be important for endothelial functioning and maintaining blood pressure, is expressed at a higher level in females (base mean expression: 37 normalized counts, log2 fold change: 0.62, adjusted P-value: 0.0289). We show that transcriptomic differences in the endothelium between the sexes already exist at birth and persist throughout life. The potential role of these targets in diseases, such as cardiovascular disease, is warranted.

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Title: Sex differences in alcohol withdrawal-induced negative affect and corticoamygdalar endocannabinoids

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Abstract: The endocannabinoid (ECB) system critically moderates the response to stress, and changes in the ECB system in the corticoamygdalar circuit contribute to alcohol withdrawal-induced anxiety. Previously demonstrated sexual dimorphisms in the ECB system may contribute to the observation that male alcoholics experience more pronounced withdrawal symptoms than female alcoholics. In the present study, intact male and female rats, and ovariectomized (OVX) female rats with or without estradiol (E2) replacement, were exposed to 6 weeks of chronic intermittent alcohol. During acute withdrawal (AW), ultrasonic vocalizations and elevated plus maze behavior were measured. On a subsequent day, during AW, the basolateral amygdala (BLA) and medial prefrontal cortex (mPFC) were harvested for analysis of ECB-related mRNA expression or quantification of ECBs [anandamide (AEA) and 2-arachidonoylglycerol (2-AG)]. Alcohol exposed male (but not female) rats displayed increased anxiety behavior during AW. In the BLA, male (but not female) rats had reduced NAPEPLD, MAGL, and DAGL-α mRNA expression, but only a significant decrease in AEA content. In the mPFC, there was also a reduction in NAPEPLD expression in males, but significant decreases in AEA content were observed only in alcohol-exposed females during AW. Similar to males, OVX females exhibited anxiety behavior and reductions in NAPEPLD expression and AEA content in the BLA, as well as widespread reductions in all ECB-related genes measured in the mPFC. Importantly, none of these withdrawal-induced alterations in the ECB system were prevented by E2, except for the reduction in AEA content in the BLA, which was insufficient to prevent the expression of withdrawal-induced anxiety. Overall, these data indicate that AW produces sexually dimorphic effects on anxiety behavior and ECB expression in the BLA and mPFC, pointing to a potential mechanism by which sex-specific affective symptoms could emerge during alcohol withdrawal.

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Title: Sex-related differences in aortic stenosis lesions are present in bicuspid and tricuspid aortic valves

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Abstract: For a given hemodynamic severity of aortic stenosis (AS), men have higher aortic valve calcification than women. We thus aimed to investigate the impact of fibrosis in tricuspid (TAV) and bicuspid (BAV) aortic valves explanted for severe AS. 56 women and 56 men were closely matched for aortic valve phenotype, age, body mass index (BMI), hypertension, renal disease, diabetes, transvalvular flow rate and AS severity as documented by pre-operative Doppler echocardiography (gradients and indexed aortic valve area). The explanted aortic valves were frozen in OCT and cut in 6 µm-thick slices. Then, Masson's trichrome staining was performed to study dense tissue/loose connective tissue ratio and calcium accumulation. Among our patients, 24 had TAV and 68 BAV. Among patients with BAV, 32 are considered as young (age<60 years) and 36 were older. As expected younger patients had less comorbidities while AS severity and left ventricular function were comparable (indexed valve area $0.39\pm0.08 cm^2/m^2$ and ejection fraction 60±6%; both p>0.65). Men and women in each group were comparable in terms of comorbidities, AS severity and left ventricular function (all p >0.67). Aortic valve of women presented lower level of calcification (5.1 vs 13.4%; p=0.02) while higher proportion of dense connective tissue than men (58.9 vs 50.8%; p=0.05). These results were similar in both valve phenotypes and age groups (all p>0.12). In this series of matched AS patients, histological data revealed a lower quantity of calcification but a more important proportion of dense connective tissue, hence valvular fibrosis, in women compared to men for a same hemodynamic severity regardless of the phenotype of the valve (i.e. bicuspid vs tricuspid) and the age of the patients. Thus, AS pathophysiology is probably sex-specific and potential therapeutic target should be evaluated according to the sex of the patients.

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Title: Women interviewing men: Ten questions raised

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Abstract: Men's health and masculinities have come to the forefront of health research worldwide. In the context of the growing body of literature and interest in this field, there is an emergent dialogue about the realities, complexities and tensions of conducting research with men about their experiences. More than a decade has passed since Oliffe and Mroz (2005) outlined '10 lessons learned' from the perspectives of men interviewing men about health. Practical guidance from the perspectives of women 'studying' men, however, remains underdeveloped, particularly in conducting qualitative research on men's experiences and perspectives on health and illness. In this presentation we draw on our experiences as academic researchers working in men's health to provide theoretically-informed guidance for scholars and practitioners engaged in the field of men, masculinities, and health. Our aim is to offer a set of questions as a heuristic to prompt considerations for men's health researchers who self-identify as women. A discussion of practical issues that may arise in qualitative research is grounded in critical analyses of safety and vulnerability, demarcations of space, and transgression of power relations amid considerations of how our approaches perpetuate or challenge structural violence. Theories that account for intersecting subjectivities and systems of privilege, such as intersectionality, are examined for their potential contributions to advancing future approaches to qualitative research – from conceptualization to implementation - in the study of men's health and masculinities.

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Title: Sex differences in the effects of dietary emulsifiers on the gut-brain axis in mice

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Abstract: Dietary emulsifiers, detergent-like molecules used in processed foods, induce chronic low-grade inflammation, obesity, and metabolic syndrome in mice.[1] Previously, we demonstrated that carboxylmethylcellulose (CMC) and polysorbate 80 (P80), delivered via drinking water post-weaning, decreased social behavior in female mice and increased anxiety-like behaviors in male and female mice. Here, we examine the mechanisms by which emulsifier treatment alters these behaviors. We examined immunoreactivity (IR) for neuropeptides that influence anxiety, sociability, and/or food intake. In the paraventricular nucleus of the thalamus (PVT), a component of fear/anxiety and food intake neural circuits, emulsifier treatment decreases IR of α-melanocyte stimulating hormone (αMSH), which inhibits food intake and increases anxiety-like behaviors and tended to increase agouti-related peptide (AgRP)-IR. In the PVT, female mice also have more αMSH and males have more AgRP irrespective of treatment. Emulsifiers did not affect arginine vasopressin (AVP)-IR in the PVT; however, males have more AVP-IR than females. As we have demonstrated sex differences in the brain and behavior, we reanalyzed previously published fecal microbiota data[1] for potential sex differences. We see that while the microbiota still strongly cluster by treatment, they also cluster by sex. In addition, CMC treatment differentially change the gut microbiota of male and females: females have more bacteria within the phyla Deferribacteres and TM7, and males have more bacteria classes Betaproterobacteria and Clostridia. In contrast, treatment with P80 reduces sex in the gut microbiota of water-treated animals. Taken together, these data suggest that emulsifiers may impact the gut-brain axis in sex-specific ways to influence social behaviors. Future research will seek to further elucidate the mechanisms through which the altered gut microbiota following emulsifier treatment act to alter the brain and behavior.

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Title: Sex differences in a schizophrenia model with exposure to prenatal immune challenges: comparison at the behavioral, molecular, and microglial levels

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Abstract: Schizophrenia is a mental disorder affecting 1% of the population, with disease onset occurring into late adolescence or early adulthood. Patients have difficulty with working memory, attention, executive functions, and social interactions. Magnetic resonance imaging and postmortern human studies have reported exacerbated structural brain abnormalities in male schizophrenia patients, generally observed within areas that are associated with sexual dimorphism, such as the anterior hippocampus. In order to investigate sex differences in the pathogenic mechanisms, Polyinosinic:polycytidylic acid (Poly I:C), a toll-like receptor 3 agonist, was used to mimic viral maternal immune activation (mIA) in pregnant mice at embryonic day (E)9.5 or E12.5 as a preclinical model of schizophrenia. Sex differences were determined in frontal cortex, hippocampus and cerebellum, at the behavioral, molecular, and histological levels in two-month-old offspring. Our recent work revealed that mice receiving Poly I:C injection at E9.5 but not E12.5 show several behavioral, molecular, and microglial impairments. Under E9.5 Poly I:C stimulation, both sexes displayed stereotypic, emotional, and social deficits while only males demonstrated somatosensory alteration and enhanced anxiety under the prepulse inhibition and elevated plus maze paradigms. Consistent with behavioral results, increased levels of inflammation and oxidative stress were only identified in cortex and hippocampus of male upon Poly I:C injection at E9.5. Fluorescent immunohistochemistry and electron microscopy studies also revealed that Poly I:C injection at E9.5 induced microglial de-ramification only in male hippocampal dentate gyrus (HDG), and increased numbers of stressful dark microglia within HDG in both sexes. Overall, we conclude that male show more schizophrenia symptoms responding to earlier mIA and we hope to suggest targeted therapeutic avenues for the prevention and treatment of this disease in both sexes.

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Title: Lipid metabolism during fasting and refeeding in male and female mice

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Abstract: [Background] The conflicting evidence shows that the incidence of obesity in males and females is quite similar in most industrial countries, however, obesity-related diseases affect males more frequently than females. To elucidate the lipid metabolism inducing inflammatory reactions during fasting-refeeding period, we observed metabolic changes during fasting and refeeding.
[Methods] We focused on lipid metabolism in male and female mice during two-day fasting followed by three-day refeeding observing physiological and biochemical changes such as bodyweight, food intake, water intake, fat amount, plasma levels of glucose, glucose, non-esterified fatty acid, triglyceride, cholesterol, high density lipoprotein, insulin, and leptin.
[Results] Food intake of female mice increased more markedly than that of males when refed after two days of fasting, while changes in bodyweight revealed no sex differences throughout the experiments. The decrease in peritoneal fat during fasting recovered with 3 day refeeding more rapidly in females than males, while plasma levels of free fatty acids were more marked in males than females. During the experiments, the plasma levels of glucose changed similarly in the two groups.
[Conclusion] These results suggest that fasting, followed by refeeding, induces metabolic changes more markedly in male than in female mice by modulating lipid metabolism.

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Title: Increased impulsivity, autistic traits, and sleep complaints in younger through older adults reporting preterm birth

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Abstract: Preterm birth is associated with increased impulsivity and increased risk for autism spectrum disorder. However, relatively few studies have examined these variables in women and men across adult development into older age. For that reason, we examined preterm birth and behavior in 842 men and 1284 women (18-79 years of age) using Amazon Mechanical Turk. All participants provided general demographic information, including height and weight, and completed questionnaires including the Pittsburgh Sleep Quality Index (PSQI), a 19-item measure of sleep quality/disturbances in the past month, the Barratt Impulsiveness Scale (BIS-11), a 30-item measure of impulsivity, and the Autism Spectrum Quotient (ASQ) a 50-item measure assessing five autism-relevant domains (e.g., social skills, attention to detail). RESULTS: The sample was predominantly white (80%) and female (60%) with an average age of 37.74 years for women and 36.31 years for men. Preterm birth was reported by 114 (16%) men and 152 (14%) women. BMI, sleep, and impulsivity scores for the sample were in the normal range, with 25.8% scoring obese, 50.4% reporting sleep dysfunction and 15.3%% scoring in the highly impulsive range. Overall, BMI was higher in women than in men and lowest in young adults (17-35 years) compared to middle aged (35-55 years) and older (55+ years) adults (all p's<.01). Women generally reported poorer sleep quality on the PSQI than men. In both sexes, young adults reported poorest sleep quality and highest impulsivity. Interestingly, whereas young and old men were generally more impulsive than young and old women, middle aged women reported more impulsivity than their male counterparts. The comparison of full term and preterm birth status showed BMI not differ between men reporting full term or preterm birth. However, BMI was higher in women reporting preterm birth compared to women born full term (p<.01). Consistent with emerging research in younger populations, reports of premature birth were associated with poorer sleep quality, increased impulsivity, and greater endorsement of autism-relevant behavior on the ASQ. CONCLUSION: These results suggest the effects of preterm birth on physiology and development have long-term consequences that extend well into adulthood.

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Title: Studying sex differences with structural magnetic resonance imaging (sMRI): Status of a manual sMRI protocol of the hypothalamic-pituitary-gonadal axis

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Abstract: The hypothalamus is a well-established sexually dimorphic brain structure and there is growing interest for understanding its role in sexually differentiated psychopathologies. The hypothalamus regulates endocrine systems via signals to the pituitary, in turn activating endocrine glands including the gonads, in what is known as the hypothalamic-pitutiary-gonadal (HPG) axis. This axis drives the key difference between the sexes: reproductive behavior and physiology. However, very little is known about structural variations of the human hypothalamus *in vivo* due to its small size, making it difficult to image and segment. Moreover, no protocol currently exists to study the entire HPG axis. We present the status of a protocol for studying the entire HPG axis in vivo using structural magnetic resonance imaging (sMRI), from T1 and T2 weighted images acquired on a 3T Siemens scanner in under 30 minutes. The manual segmentation protocol extends existing protocols of the hypothalamus to include hypothalamic subregions, the pituitary stalk, pituitary gland (anterior and posterior glands) and the gonads. Hypothalamic subregions include the preoptic area involved in sexually differentiated behaviors, the lateral hypothalamus involved in feeding and metabolism, as well as the ventromedial and dorsomedial nuclei. Preliminary data on 18.5 year olds (8 men, 8 women) are consistent with expected sex differences in total hypothalamic volume (men > women, Cohen's d=0.58), and whole pituitary volumes (women > men, Cohen's d=0.623). Anterior and posterior pituitaries intra-rater reliability data (Dice kappa) exceed 0.80. Gonadal measures include total volume and antral follicle counts. Once complete, we plan to test whether HPG axis integrity is altered in young adults exposed to prenatal maternal stress. This protocol can also be used to study the role of HPG axis integrity in pathologies including schizophrenia, mood disorders, Alzheimer's disease, and reproductive dysfunctions.

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Title: Preliminary Evidence Supporting Neck Mass and Sore Throat

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Abstract: This study addresses the most common initial symptoms of oropharyngeal squamous cell carcinoma (OPSCC) and investigates differences between human papillomavirus (HPV)–positive vs HPV-negative tumors. To analyze the most common initial symptoms in patients with OPSCC and to determine if any differences in initial symptoms occur between HPV-positive and HPV-negative tumors.Retrospective single-institution review of medical records of previously untreated patients with OPSCC diagnosed from January 1, 2008, to May 20, 2013, who were evaluated by 1 physician (the senior author, T.A.D.) at the Medical University of Ghana. We determined the most common initial symptoms of OPSCC and analyzed differences between HPV-positive and HPV-negative tumors. Neck mass (in 39 patients [44%]) and sore throat (in 29 patients [33%]) comprised the most common initial symptoms in OPSCC. Patients who were HPV-positive were more likely to initially notice a neck mass than HPV-negative patients (51% vs 18%; $P = .02$), whereas HPV-negative patients were more likely to notice sore throat (53% vs 28%; $P = .09$), dysphagia (41% vs 10%; $P = .05$), or odynophagia (24% vs 6%; $P = .04$). This study provides preliminary evidence supporting neck mass and sore throat as the initial symptoms of patients with OPSCC. Patients who were HPV-positive more commonly complained of a neck mass as the initial symptom, whereas HPV-negative patients more commonly had symptoms related to the primary tumor site, including sore throat, dysphagia, and/or odynophagia.

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Title: Sex differences in vascular function: The effect of race.

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Abstract: There are sex differences in cardiovascular disease risk and progression, due to the cardioprotective nature of estrogen. There are also differences in cardiovascular disease prevalence and onset between African-Americans (AA) and Caucasians (CA), with AA demonstrating altered vascular function, even with normal blood pressure values, beginning as early as 21 years of age. We hypothesized that the sex differences observed in studies utilizing a non-racially diverse subset would remain in a racially diverse cohort. During one subject visit, 47 participants (10 male AA, 10 female AA; 13 male CA, 13 female CA) underwent measures of anthropometrics, vascular function (elastic modulus [Ep], carotid arterial compliance [AC], peak forearm blood flow following reactive hyperemia [peak FBF]), endothelial function (flow mediated dilation [FMD]), and blood pressures (brachial and aortic). There were sex differences within both race groups in measures of height, % body fat, brachial SBP and MAP, and aortic SBP and MAP. However, sex differences varied amongst race in that CA males had significantly lower AC and higher Ep compared to female CA, and AA males had higher peak FBF compared to AA females. This indicates that there are different effects of sex, depending on the racial group, with CA females possibly exhibiting better large and similar resistance vessel function compared to males, but that this protection doesn't extend to AA females compared to AA males. This is possibly due to the cardiovascular differences between races and further study of sex differences in a racially diverse cohort are needed.

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Title: Sex-stratified analysis of obsessive-compulsive disorder reveals differences in genetic architecture

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Abstract: Obsessive-compulsive disorder (OCD) demonstrates sexual dimorphism in age of onset and clinical presentation, especially in comorbidities, suggesting a possible sex difference in genetic architecture. Here, we present the first sex-specific genome-wide assessment the genetic architecture of OCD. We performed a sex-stratified meta-GWAS (N=9870, 1:1.4 male/female ratio) to identify specific autosomal and sex chromosome risk factors with different effects in sexes. There were no genome-wide significant associations in either sex. However, we found that SNPs with differential effects between the sexes were strongly enriched for immune expression quantitative trait loci ($p<0.001$). We then used heritability (h^2) analysis to test for evidence of variable liability threshold for OCD between sexes and to assess the proportion of overall OCD h^2 explained by the X chromosome. There were no differences between sexes in OCD h^2. The X chromosome contributed 6.7% to total h^2 consistent with expectation. The genetic correlation between sexes is high, however, since the lower bounds of genetic correlation estimate could range from 0.49-0.73, we explored whether males and females demonstrate differential genetic correlations between OCD and other traits which may play a role in OCD development (i.e., brain regions) or are known to show differential clinical symptoms in OCD (e.g. smoking, alcohol consumption, etc.). We observed the genetic correlation between OCD and alcohol consumption in males was not significantly different from zero (Rg=0.35, SE=0.42, p=0.40) but was strongly negative in females (Rg=-0.91, SE=0.47, p=0.056). The difference is statistically significant (p=0.024), providing support for observed sex differences in clinical presentation of OCD. We identified small differences in genetic architecture of OCD and have developed a pipeline for sex-stratified genetic analysis which will be applied to OCD and other sex-biased phenotypes as larger cohorts become available.

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Title: Prenatal depression in sequential pregnancies and school readiness in siblings.*

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Abstract: Perinatal mental health problems increase the likelihood of cognitive delays in offspring. The current literature is limited by between-family design studies that fail to consider shared family characteristics of the pre- and postnatal environment, which may play a significant role in cognitive development of the offspring. Using a within-family design, we examined if differential maternal mental health status during 2 consecutive pregnancies predicts differences in school readiness between siblings. Data was accessed from the Hamilton cohort of the prospective Maternal Adversity, Vulnerability and Neurodevelopment study. Maternal depression was measured with the Montgomery-Asberg Depression Scale (MADRS) and Edinburgh Postnatal Depression Scale (EPDS) during the 2nd and 3rd trimester of pregnancy. School readiness at 4-years in the offspring was assessed with the Lollipop test. Linear regressions were used to determine if depressive scores during pregnancy predict children's school readiness. This relationship was further explored in a within-family context using a first differences model between sibling pairs. The analysis consisted of 34 mother-sibling trios. Mothers had significantly higher EPDS scores in the 2nd trimester of the 1st pregnancy, compared to the successive pregnancy ($p=0.001$). A birth order effect was observed on Lollipop scores, with the firstborn performing better than the 2nd ($p<0.02$). Only maternal EPDS 2nd trimester scores significantly predicted Lollipop scores ($p=0.08$, $R^2=0.12$). Change in EPDS scores between 1st and 2nd pregnancy was a significant predictor for change in Lollipop scores between the siblings ($p=0.049$, $R^2 = 0.2$). EPDS 3rd trimester scores and MADRS scores were not significant predictors of Lollipop test performance. Higher EPDS scores in the 2nd trimester of pregnancy predict poorer performance on the Lollipop test. Perinatal depression may negatively impact the cognitive development of offspring, and delay school readiness.

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*These are results from the Maternal Adversity, Vulnerability and Neurodevelopment (MAVAN) study.

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Title: Somatosensory-insula connectivity gradients in healthy men and women

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Abstract: Somatosensory cortex includes the postcentral gyrus (PoG) and the posterior paracentral lobule (PCL). Somatosensory cortical alterations are common in chronic overlapping pain conditions; some appear to be somatotopographical in nature. As preliminary work for chronic pain studies, we aimed to examine sex differences in the functional connection topography of the PCL/PoG with the insula in healthy individuals. Resting fMRI scans from 35 healthy men and 35 healthy women of the Human Connectome Project were analyzed using recently developed analytical methods to examine the spatial organization of intrinsic connectivity ('connectivity gradients'). Briefly, the between-voxel similarity in insula functional connectivity was computed for each pair of voxels in PCL/PoG and non-linear manifold learning was used to create spatial maps, with similar values representing similar connectivity patterns. The dominant mode of change in PCL-Insula connectivity was mainly along a dorsal-ventral axis. Similarly, the dominant mode of change in PoG-Insula connectivity was mainly along a dorsal-ventral axis with a sharp transition in connectivity patterns between primary (S1) and secondary (S2) somatosensory cortex. These findings are consistent the known relationship between S2 and the insula. Although men and women had similar insula connectivity at the extreme ends of the connectivity gradients, sex differences were observed in the transitions in connectivity, with women showing more gradual transitions within S1 and in the hip area of the PCL. Thus, sex differences exist in the spatial properties of the functional connectivity between the primary sensorimotor and the primary interoceptive cortex in healthy individuals, which may affect the integration of somatosensory information and contribute to known sex differences in the prevalence of pain disorders. Moreover, connectopic mapping of sensorimotor and other pain-related brain regions may provide new insights into pain disorders.

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Title: Sex differences in depression studies: what can we learn from preclinical research?

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Abstract: Women suffer more than men from depression and receive antidepressants more often as well. However, sex differences in depression and antidepressant response are modestly studied in humans and results appear controversial. Experimental studies provide useful insights regarding sex differences in animal models but clinical translation of this knowledge has been limited. The present study critically re-examines recent findings from preclinical studies on sex differences in the phenotype of depression, its endophenotype and the antidepressant response. Based on such studies we aim to identify potential confounders and methodological issues that could be addressed in relevant human studies. Results from our group and others reveal that behavioral indices in animal models are vigorously validated for each sex, although males are still used more often. However, psychiatric rating scales, the equivalent of behavioral indices, are seldom studied and controlled for sex differences, as indicated by our analysis. Moreover, preclinical studies highlight important baseline sex differences that influence treatment response and indicate that sex differences may be accentuated or mitigated following treatment. This is also a neglected issue in human studies. Importantly, there have been several failed attempts to identify reliable biomarkers for mood disorders and treatment response in humans. Interestingly, it emerges in this study that none of the candidate biomarkers has been well-studied in a sex-aware manner, despite significant experimental evidence for potential sex differences in the neurobiology of those markers. We conclude that there is a gap of knowledge between experimental and human studies in depression and antidepressant response. Cautious exploitation of findings on sex differences from preclinical research could improve the design and quality of human studies for disease biomarkers and novel antidepressants and facilitate the drug development in a gender-aware manner.

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Title: The role of adult sex hormones in pubertal LPS-induced effects on adult hippocampal neurogenesis and cellular proliferation

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Abstract: Puberty is a critical period for neurological and sexual maturation. Extensive changes in the pubertal brain create a vulnerability towards immune stressors. Emergence of immune-regulating male and female sex hormones during puberty has various sexually dimorphic effects of pubertal immune stress (PIS) on hippocampal neural functioning. However, the extent of these sex differences and the role of adult sex hormones is unclear. We examined the effect of PIS and adult sex hormones on adult hippocampal neurogenesis and cellular proliferation in the subgranular zone (SGZ), a neurogenic area involved in learning and memory. Adult sex hormones were expected to moderate the effects of PIS on adult hippocampal cell proliferation and neurogenesis. Three-week-old male (n= 45) and female (n= 45) CD-1 mice were shipped and housed separately. At six weeks of age (i.e., puberty), mice were exposed to the immune-stimulating lipopolysaccharide (LPS; 1.5 mg/kg, *ip*; n_{males}= 24, $n_{females}$= 24) or 0.9% saline control (1.5 mg/kg, *ip*). Sickness behaviours and body weights were monitored for 48 hr. To assess the role of adult sex hormones, nine-week-old (i.e., pre-adulthood) males and females in each treatment group were gonadectomized (n_{saline}= 12, n_{LPS}= 12) or sham-operated (n_{saline}= 9, n_{LPS}= 12). Mice were sacrificed at 17 weeks (i.e., adulthood). Their brains were excised and perfused with 4% PFA. Free-floating tissue sections (40um) underwent immunocytofluorescence staining for Ki67 (cellular proliferation) and doublecortin (DCX; neurogenesis). ImageJ was used to assess DCX and Ki67 fluorescence intensities in the SGZ of single representative z-stacks (40x) from each mouse. A 2x2x2 ANOVA revealed variable-specific interactions of treatment, sex, and adult gonadal status on DCX and Ki67 fluorescence intensities in the SGZ. These results highlight the interactive relationship between sex hormones and PIS on adult hippocampal neurogenesis and cellular proliferation in males and females.

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Title: Sex-Immunome of reovirus-assisted cancer immunotherapy: Discovery, characterization and therapeutic implications

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Abstract: The field of sex and gender-based immune differences is undergoing a revolution with a realization of the role of sex-biased immune responses in pre-clinical research. Our laboratory studies reovirus, an oncolytic virus, as a cancer immunotherapy. Reovirus is a benign virus which can preferentially replicate in cancer cells and is currently undergoing clinical trials internationally. Preliminary data from two independent phase-II clinical trials of reovirus have shown that in patients with metastatic colorectal cancer and non-small cell lung cancer, females have significantly higher objective response rates than males, but the exact reasons are unknown. We hypothesize that sex as a biological variable, significantly influences the immune responses during oncolytic reovirus based cancer immunotherapy. To understand this relationship, we studied sex-biased immune cell phenotypes and functionalities in mice, in the presence or absence of reovirus. Our preliminary data have shown differences in populations of immune cell subsets between female and male mice. For instance, we observed that female mice display higher frequencies of reovirus specific CD8+ T cells than male mice. On the other hand, M1 and M2 like macrophages from male mice are better at antigen-cross presentation than from female mice. In line with published evidence, our data demonstrate that females mount a stronger anti-viral immune response than males following exposure to oncolytic reovirus. Considering that these responses also play a major role in oncolytic virus-based cancer immunotherapies, our future research aims to further characterize the sex-biased therapeutic implications for anticancer immune responses.

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Title: Data-driven gene signatures and networks underlying sex differences across the human lifespan from hundreds of thousand gene expression profiles

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Abstract: Each cell in our body has an intrinsic sex and age that contributes to physiological differences. Most complex diseases exhibit sex- and age-specific prevalence and clinical manifestation. Understanding the genetic basis of sexual dimorphism along human development is, therefore, a critical challenge. A number of experimental studies have elucidated cellular differences – e.g. in terms of gene expression changes – between females and males in particular age groups or disease populations. However, uniformly shedding light on sex differences across the human lifespan in the full genomic context requires integrating data from all these studies and more, for instance, the ~200,000 human whole genome expression profiles in public databases (e.g. NCBI GEO). Unexpectedly, immediate use of all these data is impeded by the lack sex/age annotations for about 85% of the samples. To alleviate this situation, we first manually curated these annotations for ~15,000 samples and trained a machine learning algorithm on this set to identify the sex and age group of the donor solely based on their expression profile. The models underlying this algorithm represent the data-driven gene-expression signature characteristic of each age-group/sex combination (e.g. newborn female or elderly male). We applied this method to classify each of more than a 100,000 samples into one of two sexes and one of seven age groups, which could now be fully leveraged for big data analysis of sex and age. Using an integrative approach developed by us previously (Greene*, Krishnan*, Wang*, *et al*., 2015 Nature Genetics), we built genome-scale gene functional relationship networks relevant to each sex/age-group and specific to one of several tissues and cell types. Together, these gene signatures and networks provide the broad biomedical community with genomic tools to systematically uncover the mechanisms underlying, say, the development of the kidney in females or the aging of heart in males.

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Title: The association between pre-eclampsia and end stage renal disease: A nationwide cohort study

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Abstract: The goal of this study was to examine the association between pre-eclampsia and the risk of end-stage renal disease (ESRD).Using data from the Swedish Medical Birth Register, women who had singleton live birth in Sweden between January 1, 1982 and December 31, 2012 were identified. Pre-eclampsia and ESRD diagnoses based on the International Classification of Diseases, Eighth, ninth and tenth Revision were identified from the Swedish Medical Birth Register and the Swedish Renal Register respectively. We conducted Cox proportional hazards regression analysis to examine the association between pre-eclampsia and ESRD adjusting for several potential confounders including maternal age, body mass index, smoking, parity country of birth, smoking, pre-pregnancy diabetes, pre-pregnancy hypertension, small for gestational age and gestational age.The study cohort consisted of 1,433,123 women giving birth to 2,789,313 singleton live babies in Sweden between January 1, 1982 and December 31, 2012. During the study period, 59,583 (4.2%) women having 82,591 singleton live births were diagnosed with pre-eclampsia and 1,606 women having 3,134 pregnancies were diagnosed with ESRD. Including the entire study cohort, the findings supported an association between pre-eclampsia and the risk of ESRD in the crude (HR=5.02, [95% CIs: 2.42-3.82]) and adjusted (HR=2.72, [95% CIs: 2.34- 3.20]) models. Women who had two pregnancies with pre-eclampsia had a 10-fold increased risk of pre-eclampsia in the crude analysis (HR=10.13; [95% CIs: 6.76, 15.19]) and 3-fold in the adjusted analysis (HR=2.98; [95% CIs: 1.89, 4.70]).The present findings suggest that women with pre-eclampsia are at 2 to 3-fold increased risk of ESRD compared to parous women with no pre-eclampsia. The association was significant across a range of subgroup analyses. Further analyses will be performed to examine the association between pre-eclampsia and chronic kidney disease.

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Title: Incident smoking trajectories among novice adolescent smokers

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Abstract: Nearly 90% of smokers try smoking by age 18; 99% try by age 26. However, little is known about the natural course of smoking onset. Further, gender differences in onset trajectories remain understudied despite gender differences in frequency and intensity of cigarette consumption. Smoking onset trajectories differ by gender. *Objectives:* To describe onset trajectories among incident smokers; to determine whether onset trajectories are associated with smoking in young adulthood; and to determine gender differences in membership and/or shapes of the trajectories. Data were drawn from NDIT, a longitudinal study of 1294 youth age 12-13 at inception surveyed every 3-4 months for 5 years (20 cycles total). Group-based trajectory modeling was used to identify trajectories of cigarette consumption among 555 incident smokers overall and by gender. Overall: Four trajectory groups were identified: (i) low-intensity non-progressing smokers (76% of incident smokers). Consumption averaged 0 cig/month; (ii) early rapid escalators who increased to an average of 191 cig/month (5%); (iii) late escalators who eventually smoked 16 cig/month (12%); (iv) a mixed trajectory in which consumption increased over 7 survey cycles and then decreased (7%). Individual in the three escalating trajectories were more likely to smoke as young adults. The proportion of females in each trajectory group was 49%, 70%, 72%, and 63%, respectively. By gender: Trajectories by gender closely resembled the overall trajectories. Smokers in the three escalating trajectories continued smoking into young adulthood. Gender was not associated with meaningful differences in the trajectories, although there were higher proportions of females in the escalating trajectories. Preventive interventions targeting escalating novice smokers should begin early and continue throughout adolescence.

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Title: Sex-differences in cancer drivers and signatures

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Abstract: Cancer differs significantly between men and women: even after adjusting for known epidemiological risk factors, the sexes differ in cancer incidence, outcome and response to therapy. These differences occur in many, but not all tumour types, and their origins remain largely unknown. Our project provides a comprehensive assessment of sex-associated differences in cancer genomics by focusing on three aims: determining sex-specificity of known cancer genes; determining sex-specificity of mutational trends; and investigating the clinical relevance of sex-associated aberrations. We leveraged resources from the International Cancer Genome Consortium and The Cancer Genome Atlas pan-cancer projects which include genomic, transcriptomic and epigenomic profiles of 25,000 tumours, including the whole genome sequences of 5,000 tumours. Using these data, we analyzed mutational differences in 18 non sex-specific cancers. We compared the somatic mutation profiles of these cancers between male- and female-derived tumours to find sex-biases in mutation density and mutation frequency. To account for confounding factors such as age and race, we applied generalized linear modeling to isolate the sex-effect. Even after adjusting for confounding factors and multiple testing, we found mutational sex differences both in specific cancer types (8 of 18) and across all cancers together (pan-cancer). These sex-biased aberrations include mutations in well-known cancer driver genes like *TP53* and beta-catenin, as well as differences in overall mutation burden. Strikingly, sex also influences biomarkers of patient outcome, where different genes are associated with tumour aggression in each sex. Our findings call for increased study and consideration of the role of sex in cancer etiology, progression, treatment and personalized therapy.

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Title: Heightened adiposity and metabolic dysfunction in female rats and mice exposed to Early Life Stress

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Abstract: Clinical studies have shown a positive correlation between early life stress (ELS) and the development of cardiometabolic disease, particularly affecting women. In this study we tested the hypothesis that female rodents exposed to experimental models of ELS will show exacerbated diet-induced obesity and metabolic disease compared with males. Also, we tested whether the postnatal treatment with metyrapone, a corticosterone synthase inhibitor, would attenuate this phenotype. In rats, we used maternal separation model (MatSep) by separating WKY offspring from the dam (3 hr/day) during postnatal days (PND) 2-14. Rats were placed on a high fat diet (HFD, 60% kcal fat) starting at weaning. Non-disturbed littermates were used as controls. In addition, a separate group of offspring was treated with MTP, 30 minutes prior the daily separation. MatSep exaggerated body weight gain and fat pad weights ($p<0.05$). Also, MatSep increased plasma corticosterone, leptin levels and impaired glucose tolerance test in female MatSep rats vs. controls ($p<0.05$). MTP-treated rats during postnatal life showed a significant reduction in plasma corticosterone, insulin and leptin levels, fat expansion and glucose intolerance. Gene expression in liver indicated that glucose 6 phospatase was increased obese MatSep rats, whereas the MTP treatment abrogated this effect. The mouse model of neglect combines maternal separation and early weaning (MSEW) by separating C57BL/6 offspring from the dam 4 hr/day PND2-5, and 8 hr/day PND6-16 and weaning them at PND17. Also, mice were fed a HFD starting at weaning. Although both male and female MSEW mice showed exacerbated obesity-induced hypertension, again, female mice displayed greater fat mass, lipogenic profile in liver and insulin resistance compared to male MSEW mice. Taken together, these data show that female rodents exposed to ELS models are more susceptible to develop cardiometabolic disease compared with males.

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Title: The athero-protective effects of heat shock protein 25 in the absence of ovarian function

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Abstract: A sex difference in the prevalence of coronary artery disease (CAD) favors pre-menopausal women compared to age-matched men. However, this gap narrows and an increase in the overall risk and mortality from CAD is manifested post-menopause. Menopausal hormone treatment for cardiovascular disease prevention is associated with adverse cardiovascular outcomes and malignancy. Heat shock protein 25 (HSP25) is an estrogen inducible chaperone protein that we recently recognized as an extracellular signaling protein capable of attenuating the development of experimental atherosclerosis. Hence, we sought to determine if recombinant HSP25 (rHSP25) is athero-protective after surgical menopause in experimental atherosclerosis. Female, apoE$^{-/-}$ mice were subjected to either ovariectomy or a sham operation, then randomized to treatments with: rHSP25, 17β-estradiol, rHSP25+17β-estradiol or PBS and fed high fat diet for 8 weeks. Assessments included: histological evaluation of aortic atherosclerosis, plasma cholesterol levels and liver lysate analysis. Ovariectomy increased lesion formation (39.5%; $p<0.001$) compared to sham surgery. Compared to PBS/OVX, E2+rHSP25 treatment resulted in an 80% lesion reduction ($p<0.0001$), E2: 79.6% ($p<0.0001$), rHSP25:42.7% ($p<0.0001$) and PBS/Sham: 26.9% ($p<0.001$). Notably, 17β-estradiol treatment resulted in severe uterine and fallopian tube hyperplasia. In conclusion, the loss of endogenous estrogen results in an exacerbation of atherosclerotic lesions. While estrogen therapy resulted in a marked attenuation in atherogenesis, this occurred at the expense of marked uterine and fallopian tubes hyperplasia – potentially a precursor of malignancy. In contrast, rHSP25 treatment modulated athero-protection without adverse effects. These data provide preliminary support for the concept that HSP25 may serve as a potential new athero-protective agent for post-menopausal women.

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Title: Transient and persistent effects of maternal experience on the hippocampal neurogenic niche and the inflammatory milieu

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Abstract: Pregnancy and the postpartum are accompanied by dramatic physiological and behavioral adaptations in the mother. Evidence suggests that the trajectory of cognitive ageing in females might be altered by parity. However, the mechanisms underlying cognitive changes that arise well after a reproductive event are unknown. Here, we examined the effects of parity on adult hippocampal neurogenesis and markers of inflammation; two domains that are altered with age and linked to cognition. We hypothesized that parity will modulate the age-related changes in hippocampal neurogenesis and inflammation. Sprague-Dawley rats were bred at 7 months of age, then euthanized at gestation day 13 (GD13), postpartum day (PPD) 8, 30, 90, or 180. Nulliparous groups were assigned as age-matched controls for each primiparous conditions. Hippocampal neurogenesis, assessed via the expression of the immature neuronal marker doublecortin (DCX), was significantly suppressed in primiparous groups in the early postpartum, but normalized to nulliparous levels by PPD90. Interestingly, DCX expression declined significantly with age in nulliparous but not primiparous rats. To assess neuroinflammation in the hippocampus, the density of Iba-1-immunoreactive microglia was examined, and found to be increased with age but not significantly affected by parity. However, microglial cells displayed a more activated morphology with significantly shorter processes in the early postpartum. Finally, we quantified serum cytokines at termination. Notably, the age-related changes in circulating cytokine levels were dependent on parity, where interferon-γ, interleukin-10, and interleukin-4 increased significantly with age in nulliparous but not primiparous rats. Our data suggest that a single reproductive event modifies the trajectory of age-related changes in hippocampal neurogenesis and the peripheral inflammatory milieu. This may have implications for a differential profile of cognitive ageing in parous females.

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Title: Prenatal treatment with testosterone changes expression of ERα and increases autism like behaviour in mice

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Abstract: Autism spectrum disorder (ASD) is found in about 1 in 68 children, however it is far more prevalent in boys than girls. One hypothesis that attempts to account for this, known as the extreme male brain theory of autism, posits that ASD is linked to the over masculinization of the brain during development. The process of sexual differentiation of the brain is heavily dependent on the sex hormones testosterone (T) and estrogen. Interestingly, research has shown that T is elevated in in children with ASD and is predictive of ASD quotient, while estrogen may be protective. We have treated pregnant female mice with T at embryonic days 12, 14 and 16 to raise T to the high physiological range. We found that offspring of mothers treated with testosterone during pregnancy had increases in autism like behaviours including impaired social learning, decreased displays of social behaviour, increased dominance behaviours, increased ritualized aggression and increased anxiety-like behaviours. We are now using immunohistochemistry to examine the effects of elevated developmental T on gene expression in the developing brain. We discovered that male mice treated prenatally with T had a decreased expression of Estrogen receptor alpha in the posterior dorsal and anterior dorsal medial amygdala, while treated female mice had decreased expression of estrogen receptor alpha only in the posterior dorsal medial amygdala.

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Title: Sexually differentiated effects of prenatal testosterone on social learning behaviour in mice

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Abstract: Gonadal steroids play a critical role in sexual differentiation, organizing and programming biological systems during development and activating them in adulthood. These effects give rise to sex-specific behaviours, including social learning (SL) in female rodents. However, little is known about the nature of complementary processes in males. SL is a unique form of social cognition in that it describes an animal's ability to learn from a conspecific and avoid costs associated with individual learning. The neurobiological mechanisms underlying SL are often different from the mechanisms in other types of social cognition, such as social recognition. We hypothesized that early testosterone (T) exposure might affect the development of sex differences in SL and responsiveness to hormones in later life. This study examined the effects of heightened levels of prenatal T exposure on SL throughout life. CD-1 mice were prenatally treated with sesame oil (control) or 10µg T propionate on embryonic days 12, 14 and 16. SL was tested using the social transmission of food preferences paradigm, in which an observer animal interacts with a demonstrator that has recently consumed a novel flavoured food and acquires a preference for this food. Animals were tested during adolescence, between 35 and 42 days. Mice then underwent sham surgery, gonadectomy or gonadectomy with hormone replacement and were re-tested using the same SL paradigm in adulthood, between 68 and 76 days. Castration improved SL in male mice treated prenatally with sesame oil, but blocked SL in adult mice treated prenatally with T, an effect that was not reversed by T replacement in adulthood. Conversely, SL was blocked in ovariectomized female mice treated prenatally with T, but recovered after estradiol replacement. Sex differences in the response to prenatal T and hormonal manipulations in adulthood have implications for our understanding of the sex differentiation of social cognitive behaviour.

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Title: Rapid Effects of Hippocampally Synthesized Estrogens on Recognition Learning in Female Mice

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Abstract: Estrogens mediate the function of brain areas involved in learning and memory such as the hippocampus. Most literature on the cognitive effects of estrogens has focused on the long-term (hours-days) genomic effects of estrogens, while the rapid mechanisms (minutes) remain less investigated. Studies have demonstrated that hippocampally administered, exogenous estrogens enhanced recognition learning and memory consolidation. The timing of these rapid effects suggest that locally synthesized endogenous estrogens may play a critical role in the initial learning and/or early phase of memory encoding of recognition tasks. In the current study, we investigate the purpose of physiological, hippocampally-derived, estrogens and how they contribute to the acquisition of recognition tasks. We hypothesize that the rapid action of local estrogens mediates hippocampal-dependent recognition learning. To test this hypothesis, we infuse either the aromatase inhibitor Letrozole or 2% dimethyl sulfoxide (DMSO) vehicle bilaterally into the dorsal HPC of 2-month-old ovariectomized mice before testing them on two common recognition tasks: Object and Social Recognition. These paradigms involve a learning habituation phase with either three 4-minute exposers to 2 objects or 2 ovariectomized conspecifics, followed by a 4-minute test where one of the two stimuli is replaced with a novel stimulus. Both paradigms are completed within 40-minutes of Letrozole treatment, allowing for the rapid effects of estrogen synthesis inhibition to be observed. The results of the present investigation elucidate the purported involvement of brain-derived estrogens in rapid enhancement of cognition. This may contribute to the development of hormonal therapies targeted at post-menopausal or post-ovariectomy women who experience cognitive deficits. These results may also provide insight into why women with estrogen receptor-sensitive breast cancer, treated with Letrozole longitudinally, often develop cognitive deficits.

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Title: Involvement of nucleus accumbens dopamine D1-type receptors in social learning of food preferences in male and female mice

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Abstract: Dopamine (DA) is involved in mediating many motivationally relevant behaviors such as food intake, social behavior and social learning. Work in our lab using systemic treatments has shown that DA D1-type receptors mediate social learning, whereas DA D2-type receptors mediate food intake in the social transmission of food preferences (STFP) in female mice (Choleris et al, 2011). The underlying brain region(s) regulating these effects are slowing being unraveled. The ventral tegmental area has dopaminergic projections to various limbic brain structures, such as the hippocampus and nucleus accumbens (NAc). Our previous work using DA D1-type and D2-type receptor antagonists has shown that hippocampal DA D1-type and D2-type receptors mediate social learning in female mice, while only hippocampal DA D1-type receptors mediate social learning in males (Matta et al, 2016, 2017). Studies have shown a role for NAc DA D1-type receptors in social behaviour, and in individually acquired food preferences in rodents. In the present study we investigated the involvement of NAc DA D1-type receptors in the STFP. To do this, we microinfused the DA D1-type receptor antagonist SCH23390 (at 1, 2, and 4 µg/µL) into the NAc shell of adult male and female CD-1 mice 15 minutes prior to a 30 minute social interaction (where social learning occurs) with a recently fed same-sex conspecific. Results show that the highest dose of SCH23390, at 4 µg/µL, blocked social learning of a food preference. Moreover, the social learning blockade could not be explained by any changes in feeding behavior, since drug treatment did not influence total food consumption. Hence, these results are in line with those of our previous systemic work and hippocampal studies showing an involvement of DA D1-type receptors in the STFP, where now the NAc can be added as another brain region of action. We are also assessing sex differences, and the possible influence of the estrous cycle on social learning.

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Title: Age of onset of obsessive-compulsive disorder predicts behavioural symptom severity in women during the perinatal period

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Abstract: Obsessive-compulsive disorder (OCD) is a debilitating and heterogeneous psychiatric disorder, with sex and age of onset differences. Women are at increased risk for the exacerbation of obsessive-compulsive (OC) symptoms during the perinatal period, where new symptoms focused on the fetus/newborn may emerge. We explored whether age of OC symptom onset was a predictor of OC symptom severity and mood during the perinatal period. Eighteen women with OCD, which included comorbid depressive disorders diagnosed by the CIDI-Venus, were seen during 2nd-3rd trimester of pregnancy and 3-6 months postpartum. Behavioural measures collected at each time point included the Perinatal Obsessive-Compulsive Scale (POCS), Yale-Brown Obsessive-Compulsive Scale (Y-BOCS), Edinburgh Postnatal Depression Scale (EPDS) and State-Trait Anxiety Inventory (STAI). Age of onset was defined as the age at OC symptom presentation. Linear regression models examined whether age of onset predicted behavioural symptoms during the perinatal period, with age and depression comorbidity as covariates. Age of onset was a significant predictor of perinatal OC symptom severity (POCS) in postpartum only, $p=0.01$, $R^2=0.44$. During pregnancy, age of onset was found to significantly predict depression scores (EPDS), $p=0.01$, $R^2=0.42$, and state anxiety scores (STAI), $p=0.003$, $R^2=0.53$, but not trait anxiety. It failed to predict non-perinatal OC severity during the perinatal period, as well as anxiety or depressive scores postpartum. Age of onset was found to predict severity of some symptoms in the perinatal period. Specifically, earlier age of onset was associated with increased state anxiety and depression scores in pregnancy and more severe perinatal OC symptoms in the postpartum period. Women experiencing OC symptoms at an earlier age may be more vulnerable to worsened behavioural symptoms in the perinatal period.

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Title: Prenatal choline supplementation produces tissue-specific and sex-dependent anti-inflammatory effects in a mouse model of prenatal pollutant exposure

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Abstract: Prenatal exposure to diesel exhaust particles (DEP), a primary component of air pollution, "primes" offspring for increased susceptibility to metabolic, behavioral and neuroinflammatory changes in adulthood in a sexually dimorphic manner, with males being more susceptible than females. We tested the hypothesis that prenatal exposure to DEP would activate microglia in fetal brains, and induce inflammatory responses in fetal liver and placenta in a similar sex-specific fashion. Also, prenatal choline-supplemented diet (SUP) has beneficial "priming" effects, possibly through anti-inflammatory stimulation of the α7 nicotinic receptor (coded by the *CHRNA7* gene). Our second hypothesis was that SUP may protect the fetal brain, liver, and placenta against inflammation caused by prenatal DEP. Time-mated C57/Bl6 mice were given a SUP or control diet, and a series of DEP or saline exposures throughout pregnancy. Tissues were collected on embryonic day 18, and analyzed via Iba1 staining to characterize microglial activation, and expression of *CD11b* (macrophage identifier), *TLR2*, *TLR4* (both involved in initiating the inflammatory cascade), *TNF* (an inflammatory cytokine) and *CHRNA7* using qPCR. Prenatal DEP caused significant increases in microglial activation in the dentate gyrus, and upregulation of all inflammatory genes analyzed in placenta and liver of male offspring. However, DEP only upregulated liver *TNF* expression in females, supporting past research in adult offspring. SUP also prevented the following inflammatory responses caused by DEP in male offspring: microglial activation in the dentate gyrus; placental *TNF* and *TLR4* expression; and liver *TLR2, TLR4* and *CD11b* expression. In females, SUP decreased placental *TNF, TLR4* and *CD11b* expression. *CHRNA7* was downregulated in male and female SUP offspring. This work supports previous findings that males are more susceptible to the effects of prenatal DEP, and suggests that SUP may act as an agent to counteract these effects.

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Title: Cerebellar volume mediates the association between prenatal maternal stress and motor performance in adolescent boys: Project Ice Storm

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Abstract: While prenatal maternal stress (PNMS) reduces cerebellar volume in laboratory animals, ethical constraints have limited human research. However, natural disasters expose pregnant women to varying levels of stress in a quasi-random fashion. First, to determine whether disaster-related PNMS is associated with differences in cerebellar gray matter volume (CGV), and whether this relationship is moderated by fetal sex and/or by the timing of the PNMS exposure in gestation. Second, to investigate whether CGV mediated the association between PNMS and motor performance in young adolescents. Measures of PNMS (objective hardship, subjective distress, and cognitive appraisal) were obtained from mothers shortly after the 1998 Quebec Ice Storm. When the children were 11½ years old, T1 weighted structural MRI scans were collected. Cerebellum segmentations were produced using the Multiple Automatically Generated Templates (MAGeT) pipeline, for 57 right-handed offspring, and corrected for total intracranial volume. Balance performance was evaluated at age 13½. In boys only, higher objective PNMS predicted smaller CGV if they were exposed to the ice storm during preconception, but predicted larger CGV if exposed at the 9th week of gestation or later. Moreover, CGV mediated the association between objective PNMS and motor functioning at 13½ years in late-exposed boys: higher objective stress was associated with larger CGVs, which were associated with poorer motor performance. The results demonstrate that CGV mediates the relationship between objective PNMS and motor functioning in adolescence, that boys appear to be more vulnerable than girls to these effects, and that these associations depend on the timing of the stressor in gestation. This is the first demonstration in humans that prenatal exposure to a sudden-onset, independent stressor influences development of the cerebellum, which then predicts motor development.

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Title: The ARSiNL project: assessing rodent sex in neuroscience literature

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Abstract: The majority of non-clinical basic neuroscience research articles study male animals or do not report experimental animal sex at all. This has generated an ongoing debate about the importance of assessing and reporting research animal sex, resulting in landmark studies (i.e., Beery and Zucker, 2011), awareness campaigns, and new regulations from granting agencies such as the NIH and individual research journals. Yet, it is unknown whether these efforts have been effective. We investigated this by focusing on research articles that employed rats or mice, published 2010-2014 in the following journals: Science, Nature, Nature Neuroscience, Neuron, J. Neuroscience, J. Neurophysiology. We determined whether each article used males only, females only, males and females with sex considered as a biological variable, males and females with sex not considered as a variable, or did not report sex. At this point over 10,000 articles have been assessed. Our preliminary data indicates increases in two categories: articles that employ only males and those that employ males and females with sex not considered as a variable. The number of articles not reporting sex is decreasing. However, the extent of these conclusions vary widely by journal and rodent, with articles employing mice being much less likely to report animal sex than those employing rats. Overall, while significant progress has been made in reporting animal sex, that the sole use of male animals persists, that most studies do not analyze sex as an experimental variable, and that journal policies play a significant role in whether sex is reported.

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Title: Neurosteroid metabolites of testosterone and progesterone differentially reduce ERK phosphorylation induced by Aβ42 in SH-SY5Y cells and primary cortical neurons: potential significance for sex differences in Alzheimer's disease

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Abstract: Gonadal steroid hormones exert unique neuroprotective effects in males and females. Recent work suggests potential neuroprotective roles for the 3α-hydroxy, 5α-reduced metabolites of these hormones. Two such metabolites are 5α-androstane-3α,17β-diol (3α-diol) and allopregnanolone (Allo), which may contribute to the overall protection conferred by their precursors (testosterone and progesterone, respectively) through mechanisms including potentiation of gamma-aminobutyric acid $(GABA)_A$ receptor ($GABA_AR$) activity. We have previously demonstrated that physiological concentrations of 3α-diol inhibited phosphorylation of extracellular signal-regulated kinase (ERK) and associated neurotoxicity resulting from amyloid β peptide 1-42 (Aβ42) exposure *in vitro*. We sought to further characterize the underlying mechanisms by including a comparison to Allo, and investigating the $GABA_AR$-dependency of these effects in SH-SY5Y human female neuroblastoma cells and murine primary cortical neurons isolated from postnatal day 0-1 mice. We found that both 3α-diol and Allo prevented Aβ42-mediated ERK phosphorylation in SH-SY5Y cells, with substantially different concentration requirements (10nM for 3α-diol, 100nM for Allo), and these effects were $GABA_AR$-independent. While the same steroid concentrations also prevented ERK phosphorylation induced by Aβ42 in primary cortical neurons, which have high expression of $GABA_ARs$ compared to SH-SY5Y cells, only the effects of Allo were significantly inhibited by $GABA_AR$ antagonists. To test the potential involvement of these effects in Alzheimer's disease (AD), we utilized the 3xTg-AD mouse model. Up-regulation of ERK phosphorylation occurred in a sex-specific manner at 13-14 months of age, and this was correlated with pronounced sex differences in tau hyperphosphorylation - a pathological hallmark of Alzheimer's disease and other neurodegenerative disorders, and a postulated contributing factor to AD progression.

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Title: Associations of vascular activation with cerebral blood flow vary depending upon pregnancy history in menopausal women.

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Abstract: A history of preeclampsia is a risk factor for future cardiovascular disease and cognitive decline. This study evaluated factors affecting cerebral blood flow in menopausal women (n=56) with histories of preeclampsia (PE, n=27) and age- and parity-matched women who experienced normotensive pregnancies (NP, n=29) between the years of 1976 to 1982. Middle cerebral artery blood velocity prior to and during inhalation of 6% CO_2 was measured by Doppler ultrasound. Thirty eight (38) measures of platelet characteristics and function, microvesicles (MV) and cell-cell interactions were measured in venous blood by dual labeled flow cytometry. Cerebrovascular reactivity to elevated CO_2 (the slope defining the association between the velocity of blood in the middle cerebral artery and end-tidal CO_2) was lower in women of the PE group compared to those of the NP group. Numbers of granulocytes, numbers of activated platelets (those positive for fibrinogen receptor antigen), MV derived from stem/progenitor cells and tissue factor positive MV, and platelets positive for granulocytes (platelet-granulocyte interaction) differed between groups ($P<0.04$). Because of the interdependency among these cellular elements, principal components (PC) analysis was used to identify clusters which were then correlated with cerebral blood flow and cerebral arterial reactivity. Of the 7 PC derived, only the PC that was weighted by activated platelets, granulocytes, monocytes and lymphocytes and their interactions with the vascular endothelium showed a positive correlation with cerebrovascular reactivity in the PE group ($\rho=0.440$; $P=0.021$). These measures of cellular activation and cell-cell interactions define an “activated vascular compartment” that differed between the PE and NP groups and may affect cerebrovascular reactivity in women with a history of PE.

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Title: Sex differences in chronic pain in mild traumatic brain injury/concussion-related chronic pain

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Abstract: Pain is an unpleasant, complex, and subjective state that places a significant burden on patients and clinicians. It is possible to experience this state in the absence of any apparent injury, and its severity may be mediated by emotion, attitude, and environmental influences. Traumatic brain injury (TBI) is frequently associated with chronic pain, with headaches and neck pain comprising the primary complaint long after the initial injury has resolved. Chronic pain may therefore result in unavoidable diagnostic uncertainty. It is also unclear whether a primary complaint of head and/or neck, or bodily pain that persists long after concussion—one of the most common types of mild traumatic brain injury (mTBI)—represents an activation of brainstem structures or a medical problem separate from brain injury mediated by post-traumatic stress disorder, hopelessness, disturbed sleep, or depression. This diagnostic modelling study examines sex differences in the multidimensional experience of chronic pain in patients with delayed recovery from mTBI/concussion. Pain intensity/unpleasantness was measured using the Visual Analogue Scale. Additional instruments covered physiological, psychological, cultural, and behavioural variables that were relevant to our hypotheses. Medical files provided data on injury mechanism, presence of loss of consciousness or post-traumatic amnesia, MRI or computed tomography data, and psychosocial status (i.e., tension with employer, insurer, family difficulties, etc.). Univariate and multivariate linear regression models were used to explicate and compare covariates of the experience of pain between males and females. A total of 94 patients (45.20 ± 9.94 years; 61.2% male) with an established diagnosis of concussion/mTBI were included in the analysis. We applied stepwise multiple linear regressions with elimination to build models of pain for each category of variables, grouped by (1) cultural/social, (2) environmental/behavioural, (3) psychological/patho-psychological, (4) neuro-physiological/brain-injury related, and (5) physical/medication effect. All hypothesized variables associated with outcomes of interest for the whole group, and for males and females separately, at a statistically significant level of $p \leq .2$ were initially included; all variables identified as significant at $p \leq .1$ were included in final models. Our results revealed that in both, males and females with mTBI/concussion, pain is associated with variables within neuro-physiological/brain-injury variables, and psychological and cultural/social variables. When we stratified our results by sex, we observed that pain intensity/unpleasantness appears to be construed differently in males and females. We conclude that analyses across simple organizing themes provide unique opportunities for future longitudinal research that investigates the complexity of pain mechanisms involved in post-concussion syndrome and that only sex-specific analyses allowed us to capture sex differences in chronic pain.

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Title: Sex differences in the psychometric properties of the Pittsburgh Sleep Quality Index

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Abstract: Due to known sex differences in the mechanisms of sleep, and studies that suggest women experience different symptoms of sleep impairment than men, it was hypothesized that men and women will report perceptual differences in sleep quality as reflected in a validated sleep instrument - the Pittsburgh Sleep Quality Index (PSQI).
This instrument evaluation used baseline data from an ongoing study examining impaired sleep and glycemic control in people with type 2 diabetes. Demographic information included age, gender, marital status, and race. Clinical evaluations included BMI and A1C. Descriptive statistics, a principle components analysis with varimax rotation, scree plots, parallel analysis and Eigenvalues were all computed to determine factor structure using the seven components measured in the PSQI (sleep quality, latency, efficiency, duration and disturbances, sleeping medications and daytime dysfunction).
The sample (N=198), was 53% female, 38% married/partnered, 54% White, 31% college educated, middle aged (M = 56.8 ± 11 years), and obese (BMI 34.5 ± 6.8) with suboptimal glucose control (A1C 8.0 ± 1.9%) and with poor sleep quality (PSQI=10.0 ± 4.0). Component 1, which includes only the question “During the last month, how would you rate your sleep quality overall?” accounted for the greatest variance in both men (40.3%) and women (37.5%). In men, component 1 loaded predominately with “sleep efficiency” (0.83) and “sleep duration” (0.86) and in women it loaded predominately with “nighttime disturbances” (0.82) and “daily disturbances” (0.74). “Sleep latency” loaded with 0.55 in men and with 0.40 in women. All other components loaded <0.3. Women reported significantly worse sleep disturbances and sleep quality ($p < .05$). These results suggest men and women with T2DM who report poor sleep quality may perceive “overall sleep quality” differently indicating further examination of the influences of sex and gender may be needed in the evaluation of instruments.

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Title: Sex differences in the role of telomeres in the modulation of pain sensitivity in mice

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Abstract: Telomeres, the repetitive DNA sequences that cap the ends of eukaryotic chromosomes, decrease in length with each cell division and are known to exert pathological effects via p53- and p16-mediated cellular senescence pathways. Two published studies have associated reduced peripheral blood mononuclear cell (PBMC) telomere length (TL) with experimental and/or clinical pain sensitivity. However, no systematic analysis of the interaction between telomeres and pain has ever been undertaken. In the present study, we investigated the telomere-pain interface in various rodent pain models as well as in transgenic mice that exhibit shortened telomeres using behavioral, immunohistochemical and/or molecular biological techniques. Our findings show that peripheral nerve injury (PNI) produce telomere shortening in mouse PBMCs (at 4 months post-surgery), an effect that was only observed in male mice. This effect occurs more rapidly in older mice with PNI and correlates with pain hypersensitivity. Further characterization of the leukocyte population demonstrated reduced TL in the monocytes of female mice with PNI and in both monocytes and $CD8^+$ T cells of male mice with PNI. Nerve injury also produces male-specific cellular senescence within the cells of the dorsal horn of the lumbar spinal cord. In the SPARC mice model of low back pain, a similar PBMC TL shortening effect was observed in male mice, but at a much later time point (2 year). *Terc* null mutant mice, which exhibit lower TL due to absence of telomerase, displayed sex-dependent hypersensitivity to pain in the naïve state as well as following nerve injury. In summary, our findings for the first time provide evidence for a bidirectional and possibly causal relationship between telomeres and pain.

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Title: The effect of pubertal probiotic treatment on LPS-Induced changes in stress reactivity and c-Fos expression following restraint stress in adult male and female mice

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Abstract: Puberty is a critical period of development when sexual maturity in achieved and a significant amount of reorganization occurs within the central nervous system (CNS). Exposure to stress during puberty causes enduring effects on neurocognitive functioning, behaviour and future response to stressors. Evidence indicates a close communication between the gut microbiota and the CNS, but research has yet to examine the influence of the gut microbiome on stress reactivity and mental health during this critical stage of development. Immune challenge during puberty can have long lasting effects on stress responses later in life. It is speculated that increasing gut bacteria diversity via probiotics can have defensive properties against immune challenges on reactivity to stress into adulthood. Six week old CD1 mice were exposed to immune challenge and a probiotic formula during adolescent development to observe the effects of bacterial endotoxin lipopolysaccharide (LPS) and probiotic *Lactobacillus reuteri* on stress responses and CNS c-Fos expression in adulthood. At 10 weeks, mice were subject to a 30-minute restraint stress test, 1 hour before euthanasia. Brain and blood were collected and blood plasma was analyzed via ELISA to measure corticosterone (CORT) levels. Saline-injected females treated with probiotic had significantly lower levels of CORT than females who received control broth. All males showed significantly lower CORT levels compared to females. It is expected that mice exposed to LPS will show greater c-Fos expression following restraint stress in the paraventricular nucleus of the hypothalamus compared to controls. Mice treated with the *Lactobacillus reuteri* will display less c-Fos expression in this area compared to mice that were not exposed to probiotic. Results suggest probiotic treatment serves as a protective factor against LPS-induced stress reactivity in females indicating important sex differences in the influence of probiotics on development.

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Title: The association between age of onset of opioid use and comorbidity among opioid dependent patients receiving methadone maintenance therapy

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Abstract: Opioid use disorder (OUD) affects approximately 21.9 million people worldwide. This study aimed to determine the association between age of onset of opioid use and comorbid disorders, both physical and psychiatric, in patients receiving methadone maintenance treatment for OUD. We hypothesized that an earlier age of onset of use would be associated with a higher prevalence of psychiatric and physical comorbidities. We analyzed data from 627 patients with a mean age of 38.8 years (SD=11.07) that we prospectively collected between June 2011 and August 2016 at Canadian Addiction Treatment Centers. A multi-variable logistic regression model was constructed to determine the strength of the association between age of onset of opioid use and the presence of physical or psychiatric comorbidity while adjusting for sex, current age, body mass index, methadone dose and smoking status. Individuals with an age of onset of opioid use younger than 18 years were found to be at higher odds for having a comorbid disorder compared to individuals with an age of onset of opioid use of 31 years or older (OR: 2.94, 95% CI: 1.20, 7.19, p=0.02). Women were also found to be at a statistically significantly heightened risk of having a physical or psychiatric comorbidity regardless of the age at which they began using opioids (OR: 1.70, 95% CI: 1.04, 2.75, p=0.03). Finally, older individuals were found to be at higher risk of having a comorbidity (OR: 1.05, 95% CI: 1.02, 1.08, p<0.01). Taken together, our study demonstrates that amongst opioid users, being a woman, being older or using opioids at a younger age places one at an elevated risk of having a physical or psychiatric comorbidity. The opportunity to identify these high-risk patients in practice would allow us to monitor them more closely, provide more intensive treatment strategies as well as implement preventative measures to reduce the risk of them developing a comorbidity and/or progressing in their course of illness.

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Title: Investigating the association between cannabis use and suicide attempts in patients with psychiatric disorders

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Abstract: Suicide is one of the leading causes of death worldwide. Cannabis use has consistently been shown to be associated with a heightened propensity for suicidal behavior in the general population. Given that both cannabis use and suicidal behavior are highly prevalent among patients with psychiatric disorders, this study aims to investigate the association between cannabis use and suicide attempts in this patient population. We hypothesize that cannabis use will be associated with an increased risk of attempting suicide in this already high-risk population, and that women would be at a particularly elevated risk. A multi-variable logistic regression model was employed to determine the strength of the relationship between cannabis use and suicide attempts while adjusting for sex, age, marital status and employment status. Data from 907 patients with psychiatric comorbidities was analyzed. The mean age of participants included in our study was 40.2 years (SD=12.4), 273 of which reported attempting suicide in their lifetime. Our findings revealed that being a woman is associated with an increased risk of suicide attempt (OR=1.59, 95% CI 1.18, 2.14, p=0.002), while having a job is protective against suicide attempts (OR=0.36, 95% CI 0.26, 0.52, p<0.001). No significant association was found, however, between cannabis use and suicide attempts among psychiatric patients. The impact of cannabis use, although common, on people with psychiatric disorders is therefore different than that in the general population and may not be associated with an increased risk of suicidal behavior. This study, however, found that women and unemployed individuals with psychiatric comorbidities are at higher risk of suicide attempts in keeping with previous research. Taken together, our findings provide data upon which we may base decisions when striving towards achieving the WHO's Mental Health Action Plan to reduce the rate of suicide by 10% by 2020.

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Title: Relationship between white matter lesions and the regional cerebral blood flow changes during longitudinal follow-up in Alzheimer's disease

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Abstract: Aging and vascular risk factors, especially hypertension, are related to the presence and severity of white matter lesions (WMLs) with elderly people. We previously evaluated the effect of WMLs on cognitive function and cerebral perfusion at baseline in AD patients. AD patients with WMLs had significantly decreased regional cerebral blood flow (rCBF) in regions associated with memory and learning, and a rapid progression of cognitive decline compared to those without WMLs. These findings suggested that baseline WMLs is one of the factors associated with progression of cognitive and brain functional impairment in Alzheimer's disease (AD) patients. Thus, the aim of the present study was to evaluate the relationship between baseline white matter lesions (WMLs) and changes in regional cerebral blood flow (rCBF) during longitudinal follow-up of patients with Alzheimer's disease. Thirty-eight patients with AD were included in the study (16 men, 22 women; mean age, 77.8). All patients were evaluated using the Mini-Mental State Examination (MMSE) and brain perfusion single-photon emission computed tomography (SPECT) at baseline with an approximately 2-year follow-up. The patients were divided into two subgroups according to the presence of WMLs on magnetic resonance imaging. SPECT data were analyzed using a voxel-by-voxel group analysis with SPM8 and region of interest (ROI) analysis using FineSRT. Mean MMSE scores in AD patients with WMLs significantly decreased from 19.4 ± 4.8 to 15.5 ± 6.5 ($p= 0.003$). SPM8 and FineSRT analysis revealed more severe and widespread rCBF reduction, mainly in the frontal and mesial temporal regions in AD patients with WMLs compared to those without WMLs. No sex difference was observed between with WMLs and without WMLs groups.

Conclusion: Baseline WMLs may predict a rapid progression of cognitive and brain functional impairment during longitudinal follow-up in AD.

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Title: A five-species, multi-tissue survey of sex differences in gene expression and splicing

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Abstract: Sex differences are common in human development, health, and disease, and are increasingly modeled in laboratory or domesticated mammals. However, the extent to which molecular sex differences are conserved across both tissues and mammalian species remains unclear. Therefore, we have undertaken a multi-tissue, five-species survey of sex differences in mRNA expression and splicing by high-throughput sequencing of RNA (RNA-seq), with the goal of assessing the evolutionary conservation of human sex bias in gene expression and splicing in a tissue-specific manner. To assess human sex differences, we analyzed RNA-seq data from 14 tissues generated as part of the Genotype-Tissue Expression (GTEx) Consortium. We identify substantial sex differences in both gene expression and exon usage that show minimal overlap between human tissues, suggesting a tissue-specific contribution of hormones or sex chromosome complement to sex-specific gene regulation. In order to generalize these findings across evolutionary time, we are generating RNA-seq data from male and female tissue samples from 4 non-human mammals (cynomolgus macaque, mouse, rat, and dog). By performing deep, long-read sequencing on a subset of samples, we uncovered a female bias in the existing dog transcriptome, and discovered thousands of novel exons in each species, allowing for improved accuracy of gene abundance estimation. Using this dataset in combination with our analysis of human sex bias in the GTEx data will provide insight into the suitability of various tissues and organ systems for modeling sex biases.

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Title: Blood glucose normalization reduces the enhanced rewarding effects of nicotine in diabetic rats.

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Abstract: Diabetes is characterized by compromised processing of insulin (decrease in insulin levels in type 1 and insulin resistance in type 2) that leads to a concomitant increase in blood glucose levels. In turn, diabetes enhances the motivational and rewarding effects of nicotine. In the present study we sought to determine whether the diabetes induced enhancement in nicotine reward is due to direct effects of insulin, or a result of increased glucose levels. Male and female Sprague Dawley rats were treated with streptozotocin (STZ), a pancreatic beta cell toxin, to produce a model of type 1 diabetes or advanced stages of type 2 diabetes. Animals were then placed in a nicotine conditioned place preference (CPP) procedure during which the hyperglycemic state of rats were reduced by means of: a) insulin supplementation, which increases insulin levels and leads to a decrease in blood glucose levels, or b) daily injections of the sodium/glucose cotransporter type 2 (SGLT2) inhibitor dapagliflozin (10 mg/Kg) to decrease blood glucose levels without altering insulin levels. The results revealed an enhanced preference for the nicotine-paired compartment in the CPP paradigm in STZ-treated male rats, as compared to non-diabetic control rats. Insulin supplementation or dapagliflozin administration normalized the enhanced nicotine CPP in STZ-treated rats. STZ-treated female rats did not exhibit the enhanced preference for the nicotine-paired compartment observed in male rats. In general, CPP scores for female rats were lower than for male rats at the dose of nicotine used in this study. These results suggest that hyperglycemia, rather than direct effects of insulin are responsible for the enhanced rewarding effects of nicotine found in diabetic rats.

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Title: Responsiveness to the bronchodilator ipratropium bromide in male and female patients with chronic obstructive pulmonary disease

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Abstract: Although the prevalence of chronic obstructive pulmonary disease (COPD) is similar between men and women, current evidence used to support bronchodilator therapy has been generated in therapeutic trials that have predominately enrolled male patients. Here, we determined whether there is any significant sex-related differences in FEV_1 responses to ipratropium bromide. Data from the Lung Health Study (n=5,887; 37% females) were used to determine changes in lung function (forced expiratory volume in 1 second [FEV_1]) with ipratropium or placebo in male and female subjects with mild to moderate COPD over 5 years. Lung tissue gene expression dataset (n=1,111) was used to determine whether there were any sex-related differences in gene expression for the muscarinic (M2 and M3) receptors (encoding the receptors for the bronchodilator ipratropium) in lungs of male and female patients. After 4 months, ipratropium therapy increased FEV_1 by 6.0% in female and 2.9% in male subjects from baseline values ($p=2.42x10^{-16}$). This effect was modified by body mass index (BMI) such that the biggest improvements in FEV_1 with ipratropium were observed in thin female subjects (p for BMI*sex interaction=0.044). The sex-related changes in FEV_1 related to ipratropium persisted for 2 years (p=0.0134). Female compared with male lungs had greater gene expression for M3 relative to M2 receptors ($p=2.99x10^{-8}$). Ipratropium induces a larger bronchodilator response in female than in male patients and the benefits are particularly notable in non-obese females. Female lungs have greater gene expression for the M3 muscarinic receptor relative to M2 receptors than male lungs. Female patients are thus more likely to benefit from ipratropium than male COPD patients.

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Title: Neurobiological and hormonal regulation of risky decision making in male and female rats

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Abstract: Many psychiatric diseases characterized by altered risk taking are differentially represented in males and females. Progress towards understanding this relationship is constrained, however, by our limited knowledge regarding sex differences in risk taking. To begin to address this, I recently showed that females are more risk averse than males in a rat model of risky decision making. I hypothesized that these sex differences were due to differences in hormonal modulation of risk taking. To test this, males and females rats were trained in a risky decision making task. Subsequently, half of the females were ovariectomized (OVX) and half of the males were castrated and were then re-tested. There were no differences between CAST and sham male rats; however, there was an increase in risky choice in OVX rats relative to sham females. Chronic testosterone (T) administration can modulate risk taking in intact male rats; thus, the effects of acute T injections on risk taking were assessed in male CAST and sham rats. Similarly, two months after OVX, both OVX and sham female rats received acute injections of estradiol (E) before testing. Acute T did not alter risk taking in either CAST or sham rats. Acute E also did not alter risk taking in OVX rats; however, the highest dose of E increased risky choice in sham females. To determine if effects of E in OVX rats depended on the duration of hormone deprivation, separate OVX and sham females received E 3 weeks after OVX. Effects of E in OVX and sham female rats on risk taking were identical to those seen after 2 months. These data show that T is not essential for maintaining risk taking in males. Further, the data show that ovarian hormones are critical for normal risk taking in females and that E in females may have both acute and chronic effects: while chronic E (i.e., the gonadally intact state) may suppress risk taking, acute E may enhance risk taking. This hypothesis will be tested with chronic E treatment in OVX females.

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Title: Neonatal infection produces sex-specific changes in neuroimmune function with no associated deficits in spatial learning in juvenile rats

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Abstract: Immune activation during early development can have profound effects on later immune function, cognition and behavior. Epidemiological data indicate a strong correlation between early-life immune activation and later diagnosis of disorders such as autism, schizophrenia, and depression. Despite this, there has been little investigation into the impact of sex or early-life immune activation on the development of the immune system or the ontogeny of cognitive processes, such as learning. Microglia are the resident immune cells of the brain and can affect neural function and behavior. Male rats have more microglia than female rats in the hippocampus (HP), cortex, and amygdala on postnatal day four (P4), suggesting that males may be more vulnerable to the long-term consequences of early-life immune activation than females. Thus, we examined the effect of neonatal immune activation (E.coli; $1x10^6$ CFU/0.1mL/kg) alone, or in concert with a second immune challenge of LPS (Lipopolysaccharide; 25μg/mL/kg), during late juvenile development on behavior and microglia function in males and females. Neither neonatal infection nor a second immune activation produced spatial learning deficits in juveniles. However, neonatal infection produced sex-specific alterations in the marker of microglia activation CD11b, and the cytokines IL-1β, and IL-6 in the HP, prefrontal cortex (PFC), and cerebellum in juveniles. Additionally, we found sex-specific alterations in microglial density in CA3, dentate gyrus, and the PFC. These data suggest that neonatal infection produces sex-specific changes in cytokine expression and microglia activation in juveniles, but the consequences of these changes may have a delayed effect on behavioral outcomes.

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Title: Sex/gender differences in muscular and exertion responses during a neck/shoulder fatiguing task

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Abstract: Work-related musculoskeletal disorders (WMSDs) of the neck/shoulder are common and affect many workers. Research demonstrates a clear sex/gender (s/g) difference in the prevalence of WMSDs; women report injuries of the neck/shoulder more often than men. Furthermore, muscle fatigue is a known risk factor for the development of WMSDs. Perceived exertion can be considered as a concept at the intersection of perceptual and physical aspects of fatigue. S/G differences have been identified in ratings of perceived exertion (RPE) and in electromyography (EMG) signals during fatiguing contractions of the neck/shoulder. However it is unknown whether there are s/g differences in the relationship between muscular and perceptual characteristics of muscle fatigue development. The purpose was to validate the Borg CR10 scale as a sex/gender-sensitive fatigue indicator through the comparison of EMG signals and RPE. We hypothesized that a significant correlation would be observed for RPE and EMG during the fatiguing task for both men and women, with a difference in the relationships between men and women. 28 healthy young adults completed a shoulder fatiguing task by working with that arm held horizontal at shoulder height. EMG amplitude data and RPE were collected at the end of each work minute during the task. Men showed significant positive correlations between RPE and both upper trapezius EMG [r_s = 0.436, p = 0.000] and anterior deltoid EMG [r_s = 0.370, p = 0.001], with a stronger relationship between RPE and anterior deltoid EMG, whereas women showed a weaker relationship between RPE and upper trapezius EMG [r_s = 0.223, p=0.034], and no relationship between RPE and anterior deltoid EMG [r_s = 0.105, p = 0.293]. Findings suggest that men and women differ in their patterns of shoulder stabilization and mobilization execution, which most likely contributes to s/g differences in coping with muscular fatigue and subsequently, neck/shoulder pain development and prevalence.

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Title: Rapid Effects of 17β-Estradiol in the Paraventricular Nucleus on Social Recognition in Female Mice

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Abstract: Social species, like humans and mice, need to be able to identify others based on information received from previous encounters. This ability, social recognition, is important for the development of social bonds, dominance hierarchies, and various other aspects of social life. Both estrogens and oxytocin (OT) influence social recognition in mice. Both estrogens and OT regulate social recognition, suggesting that they may interact. We hypothesize that estrogens may mediate the production and release of OT in the paraventricular nucleus (PVN) of the hypothalamus ERβ and/or the G protein-coupled ER (GPER). OT binding to its receptor (OTR) in the medial amygdala then facilitates social recognition. In the medial amygdala ERα, ERβ, and GPER all rapidly mediate social recognition which may occur via the regulation of OTR activity. To test this hypothesis, we first determined whether 17β-estradiol infused into the PVN can facilitate social recognition in ovariectomized female, CD-1 mice. Social recognition was tested 15 minutes after the infusion of 17β-estradiol, at doses of 25, 50, or 100nM. First experimental mice were presented with two ovariectomized stimulus mice (habituation) and, after a delay, they were presented with one mouse from the habituation phase and a novel stimulus mouse. This paradigm is designed to be difficult so that the control mice do not show social recognition, so that any enhancing effects of the treatment might be observed. In addition, the paradigm takes place within 40 minutes of treatment to test rapid effects of estrogens. The infusion of 17β-estradiol into the PVN at doses of 25nM and 50nM facilitated social recognition whereas control mice were impaired. Thus, we demonstrated that estrogens in the PVN facilitates social recognition and we can next determine if this facilitation occurs through an interaction with OT and OTR in the medial amygdala.

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Title: Trans men's performance on spatial and verbal tasks depends on the activational effects of androgens

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Abstract: A key theory in the origins of transgenderism is that brain circuits mediating gender identity (GI) are formed prior to birth. Sexual differentiation of some cognitive circuits is also thought to occur prior to birth, suggesting that as cognitive circuits differentiate so does GI. However, some literature reports changes due to exogenous androgens in cognitive circuits of female-to-male trans individuals (FTM). To determine whether GI and sex-specific cognition are separable, we asked whether visuospatial and verbal ability vary with GI or the activating effects of hormone therapy (HT). We compared mental rotation and verbal memory performance—two tasks showing sex differences—in + and -HT FTM, males, and females, including a subanalysis of follicular and luteal phases in all who cycled. We observed that H+ FTM performed the same as natal males and that cycling natal females in the follicular (but not luteal) phase performed as strongly on mental rotation as natal males, suggesting that visuospatial ability of +HT FTM is linked to hormonal activation by HT, not gender identity. It also demonstrates that the "male-typical" advantage in visuospatial ability is shared by natal females, contingent on menstrual phase. +HT FTM also showed greater verbal memory decay—similar to natal females with hormone deprivation, further supporting the role of ovarian steroids in the differentiation of cognitive tasks thought to have a "male" or "female" advantage. Taken together, these data suggest (1) GI for FTM is not an organizational effect and (2) ovarian cycling in all natal females is central to both visuospatial and verbal memory performance. Our findings challenge organizational theories of transgender development, and underscore the centrality of ovarian effects in establishing sex "typical" cognitions.

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Title: Knockout of TLR5 in mice results in sex-dependent changes to neural vasopressin

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Abstract: Toll-like receptor 5 (TLR5) is a pattern recognition receptor expressed on intestinal epithelial cells and immune cell populations. TLR5 recognize bacterial flagellin and play a central role in maintaining intestinal homeostasis. TLR5 knockouts (T5KO) animals harbor an altered intestinal microbiota composition, with a small subset of T5KO mice developing spontaneous colitis, while the other animals develop low-grade intestinal inflammation resulting in symptoms of metabolic disease. We have recently observed that T5KO mice have increased anxiety-like and depressive-like behaviors, as well as a trend towards an increase in sociability compared to wild type (WT) mice. Interestingly, this phenotype is more pronounced in the female T5KO mice. However, the mechanisms underlying those behavioral changes in T5KO mice are unknown. We hypothesized here that these behavioral changes could be due to alterations in the expression of vasopressin (AVP), a neuropeptide implicated in the control of anxiety-like and social behaviors. To investigate this, the brains of adult (14 weeks) male and female C57Bl/6 WT and T5KO mice were processed for AVP immunoreactivity (AVP-ir). We observed that male and female T5KO mice have increased AVP-ir in the paraventricular nucleus of the thalamus. Moreover, female T5KO mice have increased AVP-ir in the suprachiasmatic nucleus and a trend towards an increase in the paraventricular nucleus of the hypothalamus, compared to male T5KO or WT mice. In addition, sex and genotype interact on AVP-ir in the lateral septum. As all of these regions process signals from and communicate to the rest of the body to regulate behavior, behavioral alterations seen from a lack of TLR5 may be due, in part, to changes in AVP expression. These results suggest that the vasopressin system is a target of the bidirectional communication between the intestine and the brain, and begin to elucidate the mechanisms of how intestinal inflammation affects behavior.

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Title: Sex differences in a thalamo-limbic circuit regulating alcohol drinking and anxiety

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Abstract: Binge alcohol drinking is the most common form of excessive alcohol consumption and a leading risk factor for the development of alcohol addiction, anxiety, and other stress-related mood disorders. The comorbid expression of these neuropsychiatric diseases is higher in women than men, however the reason for this increased vulnerability is unknown. Female mice binge drink more and have greater basal anxiety than males, and these behaviors are driven by neurons that synthesize the neuropeptide corticotropin-releasing factor (CRF) in the bed nucleus of the stria terminalis (BNST), a sexually-dimorphic limbic brain region. In order to determine whether the excitability of BNST CRF neurons provide a mechanism for the observed sex difference in behavior, we performed *ex vivo* slice electrophysiology in CRF-reporter mice following three cycles of binge alcohol (or water control) drinking. We show that BNST CRF neurons were more likely to be tonically active at baseline in females than males and that repeated binge drinking produced a "female-like" phenotype in BNST CRF neurons of males (increased intrinsic excitability), at a time point when escalation in alcohol intake to baseline female levels emerges. Using neuronal tracing and *ex vivo* optogenetics + slice electrophysiology, we also mapped a dense, direct projection of glutamatergic neurons from the paraventricular nucleus of the thalamus (PVT) to the BNST that produced a monosynaptic excitatory and larger polysynaptic inhibitory input to BNST CRF neurons. These BNST-projecting PVT neurons were hyperpolarized at baseline in females compared to males, and again a history of binge drinking induced a female-like phenotype in males. We hypothesize that decreased excitability of BNST-projecting PVT neurons disinhibits BNST CRF neurons to drive binge drinking and anxiety behavior, conferring increased risk of comorbid addiction and anxiety disorders in females and increased susceptibility following alcohol exposure in males.

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Title: LDLR activity and subcellular distribution is sensitive to estrogen in PCSK9 deficient mice

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Abstract: Plasma PCSK9 is secreted by hepatocytes and binds to the LDL receptor (LDLR) or similar receptors. After internalization, PCSK9-LDLR complexes are sent to lysosomes for degradation, preventing LDLR recycling to the cell surface. Individuals lacking functional PCSK9 have extremely low levels of LDL-cholesterol, while those carrying PCSK9 mutations that reinforce the PCSK9-LDLR interaction develop familial autosomal hypercholesterolemia. Since 2015, therapeutical mAb against PCSK9 are prescribed and achieve an unprecedented 60% drop in LDL-cholesterol, never achieved by statins. Male or female mice lacking PCSK9 (KO) exhibit 3-fold higher LDLR levels in the liver. However, the subcellular distribution of the LDLR differs in a sex-dependent manner: KO male mice show an accumulation of the LDLR at the cell surface, while KO female mice do not. This was shown by immunohistochemistry and WB analyses of membrane preparations from liver homogenates. By performing ovariectomy and subsequent supplementation with placebo (OvxP) or 17 -estradiol (OvxE2), we demonstrated that physiological doses of E2 were responsible for the absence of LDLR accumulation of the cell surface of female KO hepatocytes.We are now showing that LDLR surface levels are correlated with LDLR internalization activity. Indeed, the injection and clearance analysis by ELISA in mice of human PCSK9, an excellent LDLR ligand, or human lipoprotein (a) reveals that KO males (vs KO females or WT males) or KO OvxP females (versus KO OvxE2) exhibit the highest clearance efficiencies. If LDLR subcellular distribution is also sensitive to E2 in human, hypercholesterolemic women that receive PCSK9 mAb, mimicking a PCSK9 KO phenotype, are expected to respond less efficiently than men due to reduced LDL clearance activity. Because the latest analyses indicate that this is the case (30% lower efficiency), it becomes important to understand how E2 reduces the presence of the LDLR at the hepatocyte cell surface.

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Title: Sex-specific differences in a mouse model of CNS autoimmunity.

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Abstract: Multiple Sclerosis (MS) is a chronic neurodegenerative disease in which T cells mount an autoimmune attack against central nervous system (CNS) myelin. Although women have a 3-fold greater risk of developing MS, disease progresses more rapidly in men. Understanding sex specific differences in MS is important to develop more effective treatments. Experimental autoimmune encephalomyelitis (EAE) is a mouse disease that recapitulates the immune aspects of MS. In our lab, we exploit transgenic 1C6 T cells that have specificity for the myelin antigen $MOG_{[35-55]}$. We culture 1C6 $CD4^+$ T cells *ex vivo* into effector subsets that make either the pathogenic cytokines IFN-γ (Th1 cells) or IL-17 (Th17). We then adoptively transfer effector 1C6 T cells to NOD.Scid mice, which we assess for signs of paralytic disease using a semi-quantitative 0-5 scale. Importantly, recipients develop a series of relapses and remissions followed by a secondary progressive phase, mimicking the most common MS course seen in human. Here, we hypothesized that female sex hormones may increase the frequency, but decrease the severity, of EAE. We first generated male and female effector $CD4^+$ 1C6 T cells and adoptively transferred them to sex-matched recipients. We observed no differences in disease incidence between males and females. However, recipients of male Th1 cells or Th17 cells showed heightened disease severity relative to female counterparts ($p<0.0001$). CNS-infiltrating male Th1 cells produced more IFN-γ than female Th1 cells ($p<0.05$), however there was no significant difference in IL-17 production between male and female Th17 cells. Next, we investigated the role of 17-β-estradiol on the pathogenicity of female Th1 cells. Treating female 1C6 Th1 cells with 17-β-estradiol prior to adoptive transfer significantly decreased EAE severity in recipients relative to controls ($p<0.0001$). These data suggest that female sex ameliorates the severity of disease in a preclinical MS model.

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Title: Streptozotocin-induced diabetes exerts depressive-like behavior in male and naturally cycling female rats. A comparative study.

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Abstract: Depression and diabetes (DM) is a highly prevalent comorbidity that affects more frequently women than men. A depressive-like profile in streptozotocin (STZ)-treated rodents (a very popular animal model of DM) has been reported in males, but scarce and controversial results have been found in females. These studies ignore the putative variations depending on the estrous-cycle (EC). In this study we hypothesized that sex and the endocrine condition within the female´s EC would affect the depressive-like behavior of diabetic male (DMR) and naturally cycling diabetic rats (NCDR) observed in the Forced Swim Test (FST, a paradigm to study depressive-like behaviors, which is sensitive to hormonal fluctuations). STZ (50 mg/kg, i.p., in 2 consecutive days) was injected to males and females; control rats received citrate buffer vehicle.10 days after STZ rats were evaluated in the FST and in an actimeter. EC-phases were determined by vaginal smears during 25 days. DMR lost more weight and were more hyperglycemic and polyphagic than NCDR. DMR and NCDR showed similar polydipsia. STZ altered the normal sequence of the EC and drastically reduced the number of proestrus at an expense of an increase in the number of diestrus. Behaviorally, a similar depressive-like profile in the FST (higher immobility and reduced swimming, without changes in climbing) was observed in DMR and NCDR tested in Proestrus/Estrus. However, NCDR in Metestrus/Diestrus (M/D) did not show significant changes when compared to DMR, and their respective buffer control. In the actimeter, DMR moved more than their controls, but moved less than control females and NCDR. Diabetic females in M/D showed more spontaneous locomotion and stereotyped movements than DMR. These findings support the idea that, to avoid false negatives and to homogenate data, sex and female´s hormonal fluctuations should be considered when screening for new treatments for the DM and depression comorbidity.

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Title: Increased default mode network connectivity in girls compared to boys prior to gonadarche

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Abstract: Sex differences in the prevalence of several behavioral disorders emerge during adolescence and are thought to be related, at least in part, to the (re)activation of the hypothalamic pituitary gonadal axis (i.e., gonadarche). One hypothesis is that the rise of gonadal steroids during this time affects neurodevelopment such that boys and girls differentially become more (or less) vulnerable to specific disorders. As a first step in addressing this hypothesis, we tested for sex differences in resting-state functional connectivity (FC) in healthy, typically developing children prior to gonadarche. Forty-one participants (8.7$\pm$0.3yrs, 18F) were categorized as pre-gonadarchal based on the absence of secondary sex characteristics by clinician-rated Tanner staging. Resting-state fMRI scans were collected with a 3T scanner, preprocessed with AFNI, and normalized to MNI space with ANTs. Signals associated with motion parameters and physiological noise were removed. First, data were analyzed using a connectome-wide association study (CWAS) approach to identify clusters of voxels with significant sex differences in overall, whole-brain connectivity. Next, these clusters were used as seed regions in a voxel-wise analysis to localize specific neural circuits driving these sex differences in FC. CWAS identified a sex difference ($p<.001$, uncorrected) in overall FC of the medial prefrontal cortex (mPFC), a key region in the default mode network (DMN). Seed-based post hoc tests localized seven regions where FC with mPFC differed between the sexes (all p's<.05, FDR-corrected): two clusters within the DMN (right middle temporal gyrus and posterior cingulate) and five within the executive control network (bilateral inferior parietal cortex and inferior/middle frontal gyrus). In each case, girls showed greater connectivity than boys, both within- and between-networks. These data suggest that sex differences in resting state FC, particularly involving the DMN, exist prior to gonadarche.

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Title: Sex-dependent effects of stress on immobility behavior and VTA dopamine neuron activity: modulation by ketamine

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Abstract: Stress constitutes a risk factor for psychiatric disorders. Females are more susceptible to stress-related disorders, such as depression, than males. Although dopamine (DA) system underactivation is implicated in the pathophysiology of depression, little is known about the female DA system at baseline and post-stress. Male and female DA neuron activity was compared at baseline and post-chronic mild stress (CMS). Moreover, we assessed effects of sex and CMS on forced swim test (FST) immobility and ventral tegmental area (VTA) DA neuron activity post-FST and tested whether a single dose of ketamine (10mg/kg, i.p.), a fast-acting antidepressant, could reverse stress-induced behavioral deficits and exert long-lasting effects on VTA DA neurons. Baseline VTA DA activity was comparable in both sexes. CMS females exhibited lower population activity compared with CMS males. Females exhibited roughly double the FST immobility duration than males, which corresponded to ~50% decrease in VTA DA population activity compared with similarly treated (i.e. post-FST) males. CMS induced greater immobility duration in both sexes and reduced VTA DA neuron activity by approximately 50% in males and nearly 75% in females. Ketamine restored behavior and post-FST VTA DA activity for up to 7 days in females and CMS rats. These data suggest increased female susceptibility to depression-like phenotypes (i.e. greater immobility, VTA hypofunction) is associated with higher DA system sensitivity to stress relative to males. Understanding the neural underpinnings of sex differences in stress vulnerability will provide insight into mechanisms of disease and optimizing therapeutic approaches in both sexes.

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Title: **Chronic stress and sexual functioning among African American women with at-risk partners in South Los Angeles**

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Abstract: The biopsychosocial model posits that chronic stress can influence female sexual functioning. We examined potential associations of various sources of chronic stress on sexual function using baseline data from a behavioral intervention study of 219 African American women with at-risk partners in South Los Angeles. Data were collected on: 1) self-reported measures of chronic burden, lifetime history of trauma, sexual abuse, perceived racism, sexism and psychological distress; 2) female sexual function domains: desire, arousal and satisfaction using the Female Sexual Function Index (FSFI); and 3) potential moderators: social support and spirituality. This largely low-income population experienced significant chronic and acute stressors. Most (61.6%) reported experiencing moderate-to-high chronic burden (score>29); however, just 9.6% of the sample had scores indicating moderate-to-high psychological distress symptoms (score>50). Participants reported moderate levels of social support. Sexual functioning showed a median score of 2.4 for desire, 4.5 for arousal, and 4.8 for satisfaction (reference scores of healthy controls: desire = 2.1; arousal = 5.08; satisfaction = 5.04). Multiple logistic regression found that chronic burden was a significant predictor of decreased overall sexual function, sexual arousal and satisfaction (OR = 0.52; 95% CI 0.28 - 0.99, OR = 0.51; 95% CI 0.27 – 0.96 and OR = 0.44; 95% CI 0.21 – 0.91, respectively). In addition, having a main partner was associated with sexual satisfaction, while age greater than 48 was associated with decreased sexual arousal. These data indicate that ongoing stressors can have a greater impact on sexual functioning than acute traumatic events.

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Title: Decreased Morphine Analgesia in T-cell Deficient Mice

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Abstract: It is now known that neurons are not the only cell type involved in pain processing, which involves cell of the immune system, such as T-cells. Many pain researchers have adopted the use of T-cell deficient mice in their experimental methods to elucidate the role of T-cells in neuropathic pain (Yu et al., 2004; Costigan et al., 2009), and T cells have been shown to release endogenous opioids (Bouè et al., 2011). While it is well known that opioids have varying effects on T-cell populations (Wang et al., 2003), very little attention has been given to how T-cells may affect opioid regulation. Here, we observe that T-cell deficient mice (CD-1 *nude* and *Rag1* null mutant) exhibit pronounced deficiencies in morphine analgesia, measured using the tail withdrawal or formalin test. In addition, T-cell deficient mice do not exhibit stress-induced analgesia after restraint. The adoptive transfer of CD4+ T-cells into *nude* mice rescues the morphine analgesia. Furthermore, there are reported sex differences in morphine analgesia, with females requiring 2-3 times more morphine than males to produce equal analgesia. We observe an equivalent sex difference in CD1 mice, however T-cell deficient mice do not exhibit a sex difference in morphine analgesia. The sex difference in morphine analgesia is restored in *nude* male and female mice receiving CD4+ T-cells from their respective sex. These results suggest that CD4+ T-cells play a role in opioid analgesia, and may be a driver of the observed sex differences in morphine analgesia.

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Title: Sex differences in psychiatric comorbidity among patients receiving methadone maintenance treatment

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Abstract: Canada is in the midst of an opioid epidemic, with unprecedented increase in the prevalence of opioid use disorder (OUD). The demographic of opioid users is also changing, and a rising number of women are using opioids and developing OUD. Identifying sex-specific factors associated with outcomes in the most common treatment for OUD, methadone maintenance treatment (MMT), is sorely needed. Psychiatric comorbidity merits investigation as it places opioid users at an increased risk for developing OUD, yet little is known about sex differences in psychiatric comorbidity in this population. Our prospective cohort study examined 652 patients receiving MMT for OUD, with the hypothesis that there is sex-specific impact of psychiatric comorbidity on continued illicit opioid use (CIOU) during treatment. The main outcome measure, CIOU, is percentage of opioid-positive urine screens for 6 months. We examined sex differences in psychiatric comorbidity and clinical variables using linear regression analysis, we evaluated the sex-specific impact of comorbid psychiatric disorders on CIOU during MMT. Psychiatric comorbidity was identified in 83% of females and 75% of males. Mood and anxiety disorders were more prevalent in females (52.17% vs 32.29%, $p<0.001$, and 51.17% vs 35.41%, $p<0.001$, respectively), whereas antisocial personality disorder was more prevalent in males (25.78% vs 18.39%, $p<0.05$). Males and females showed other substance use disorders equally ($p=0.1$). In males, cocaine use disorder was associated with increased CIOU ($\beta=11.345$, $p<0.01$), whereas comorbid anxiety disorders were associated with the opposite ($\beta=-9.051$, $p<0.01$). Comorbid tranquilizer use disorder was associated with increased CIOU in females ($\beta=28.112$, $p<0.01$). These data suggest important sex differences in prevalence of psychiatric comorbidities in OUD, and their association with MMT outcome, which may inform future interventions and MMT protocols.

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Title: Blood pRessure and vAscular hEalth around menopause (BRAVE) Study: Pilot Results

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Abstract: The incidence of hypertension and vascular risk sharply increases in the post-menopausal period in women. Whether other hemodynamic markers such as blood pressure variability (BPV) correlate with increased vascular risk during this transition from pre- to post-menopausal status is unknown. We evaluated 24hr and visit-to-visit BPV, mean 24hr BP and vascular function in pre- and post-menopausal women. The BRAVE pilot study is a cross-sectional study of healthy, non-hypertensive, pre- and post-menopausal women recruited. BP was recorded in all participants using BPTru, 24hr ambulatory monitoring, and 7-day home monitoring. Each woman completed vascular function assessments; carotid intima-media thickness (cIMT), carotid-femoral pulse wave velocity (cfPWV), and peripheral arterial tone (EndoPAT). A total of 29 women (11 pre-menopausal (50±2 years), 18 post-menopausal (54±3 years); $p<0.002$) were recruited. No significant differences were noted for other demographic variables nor for cardiovascular risk factors between pre- and post-menopausal women. There were no significant differences in mean BP, 24hr BP, BPV or vascular function measures between pre- and post-menopausal women. However, we noted statistically significant correlations between increased 24hr systolic BPV and increased average systolic BP (correlation coefficient: 0.4, $p=0.04$), cfPWV (correlation coefficient: 0.4, $p=0.03$), and cIMT (correlation coefficient: 0.59, $p=0.001$). No statistically significant correlation was noted with the EndoPAT results (correlation coefficient: -0.07, $p=0.75$). The findings from our pilot data show that higher BPV is associated with higher systolic BP in women with normal BP; however, no association was found with menopausal status. Additionally, higher BPV is associated with increased vascular disease as measured by cIMT and cfPWV in this healthy sample. Further study is needed to establish whether higher BPV may be an early indicator of vascular dysfunction during menopause.

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Title: The effects of lipopolysaccharide (LPS) on central cytokine expression in CD1 mice

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Abstract: Puberty is a critical period of development characterized by sexual maturation, development of the immune system, and brain remodeling. Pubertal exposure to lipopolysaccharide (LPS), a bacterial endotoxin, alters brain functioning & behavior in an enduring manner. Acutely, there are age & sex differences in LPS-induced immune response. More specifically, pubertal mice treated with LPS display a hypo-responsive immune response relative to adult mice. Female mice display fewer sickness symptoms and less fluctuation in body temperature than male counterparts. The mechanism underlying the age & sex difference in immune response remains to be investigated. It is also unclear whether there are age & sex differences in LPS-induced central cytokine production. The objective of the current study was to examine whether there are age & sex differences in central vs. peripheral cytokine levels following LPS exposure. We hypothesized that there will be age & sex differences in LPS-induced cytokine expression in the brain. To test this hypothesis, male and female mice received LPS or saline treatment at 6 (puberty) or 10 (adulthood) weeks of age. All brains were collected 2 hours post treatment. Brain tissues containing the prefrontal cortex and hippocampus underwent RNA extraction, reverse transcription polymerase chain reaction (RT-PCR), and quantitative PCR to amplify and quantify IL-1β, TNF-α, and IL-6 expression. Preliminary results show that pubertal males display the greatest expression of IL-1β in the prefrontal cortex, while both pubertal & adult males display higher expression of IL-1β in the hippocampus; however, the expression is slightly higher for the adult males. We expected greater expression of TNF-α and IL-6 among the pubertal males for both the prefrontal cortex and hippocampus. These findings provide a better understanding of age & sex differences in immune response to a bacterial infection and the effect of circulating gonadal hormones in these differences.

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Title: Hypocretin modulation of accumbal dopamine and motivated behavior in females.

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Abstract: Hypocretin (HCRT; also called orexin) is a hypothalamic neuropeptide that is widely implicated in motivational components of addiction and, thus, has been posited as a target for pharmacotherapies for addiction. Under normal conditions, HCRT provides excitatory input to the dopamine (DA) neurons of the ventral tegmental area. Inhibition of HCRT signaling at the HCRT receptor 1 (HCRTr1) in the VTA has been demonstrated to reduce DA release in the nucleus accumbens (NAc) under baseline conditions and in response to cocaine and also reduces the motivation to obtain drugs of abuse. The majority of previous research directed towards understanding HCRT's role in addiction, however, comes from work conducted exclusively in males. The risk and course of addiction in females differs considerably from males; females progress to dependence more rapidly, have greater negative symptoms associated with addiction, and expend more effort to obtain cocaine reinforcement. Additionally, previous work suggests that DA neurons in females may fire more under baseline conditions than males. Thus, we hypothesized that HCRTr1 antagonists would reduce DA release in the NAc and concomitant motivated behavior to a greater extent in females. Surprisingly, we instead found increased DA release in the NAc of female rats following administration of a HCRTr1 antagonist—a finding that was recapitulated in mice lacking the HCRT peptide—and preliminary evidence suggests that HCRT antagonism may be less effective in reducing the motivation for cocaine. This suggests that HCRT may differentially regulate DA and cocaine seeking in males and females, with significant implications for the development of hypocretin-based pharmacotherapies.

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Title: Impact of sex and aortic valve morphology on the relationship between aortic valve calcification and mean transvalvular gradient in aortic stenosis patients

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Abstract: A previous study in patients with aortic stenosis (AS) showed that the relationship between aortic valve calcification (AVC) and mean transvalvular gradient (MG) was different between bicuspid (BAV) and tricuspid aortic valve (TAV) patients, especially in younger patients. While TAV and older BAV patients had good AVC-MG correlation, no association was found in young BAV patients. This difference in young BAV patients was particularly notable in women with a significant MG but no AVC. We hypothesized that they might be a sex difference of AVC impact on AS hemodynamic severity (MG) according to the aortic valve morphology (BAV vs TAV). Forty BAV and 40 TAV patients with AS were matched for sex and for AVC indexed to the aortic annulus area to obtain the AVC density (AVCd). AVC was measured by computed tomography and patients also underwent a Doppler-echocardiography to assess the AS hemodynamic severity. The cohort consisted of 75% of men. As expected, TAV patients were older (70±11 vs 53±11) and had more risk factors than BAV patients. AS hemodynamic severity and AVC were similar in BAV and TAV patients ($p>0.21$). For a same AVCd, men showed similar MG whether they had a BAV (19±7mmHg) or TAV (19±7mmHg) whereas MG was higher in BAV women (21±11mmHg) than in TAV women (16±7mmHg). After adjusting for AVCd, the aortic valve morphology did not have an impact on the MG in men ($p=0.82$) contrary to the women ($p=0.04$). AVCd assessed by computed tomography could underestimate AS severity in BAV women and should then be interpreted cautiously as it might result in misdiagnosis in women and thus less referral to treatment. However, further studies with more patients, particularly women, are needed to better understand this sex difference between BAV and TAV aortic stenosis.

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Title: The effects of MEK/ERK inhibition on rapid estrogen receptor alpha and G-protein coupled estrogen receptor facilitated social recognition

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Abstract: Estrogens affect learning and memory through classical, long-term (hours-days), genomic and nonclassical, rapid (within minutes) mechanisms. Social recognition learning, in which an animal becomes capable of distinguishing between conspecifics, is rapidly facilitated with systemic administration of 17β-estradiol (E2), as well as specific estrogen receptor (ER) agonists for the ERα, ERβ, and the G-protein coupled estrogen receptor (GPER), 15 min prior to a 25 min combined training-and-testing paradigm (Phan et al., 2011, 2012; Gabor et al., 2015). The dorsal hippocampus can mediate these effects as infusion of E2, ERα agonist PPT, and GPER agonist G-1, but not ERβ agonist DPN, facilitated social recognition in the same paradigm (Phan et al., 2015; Lymer et al., 2017). These rapid effects may be elicited via membrane-bound ERs activating cell-signaling cascades, such as the MEK/ERK pathway. Through MEK/ERK, estrogens rapidly affect object and spatial memory consolidation (Fernandez et al., 2008; Fan et al., 2010; Fortress et al., 2013), dendritic spine density both *in vitro* (Sellers et al., 2015) and *in vivo* (Tuscher et al., 2016), as well as social recognition (Sheppard et al., 2016). Which ERs mediate estrogen/MEK/ERK rapid social recognition learning is unknown. In this study, young adult, ovx female mice receive bilateral infusions of MEK/ERK inhibitor U0126 into the dorsal hippocampus followed by (5 min) an ERα or GPER1 agonist. Fifteen min later, subjects perform a social recognition task in which they are presented with two conspecifics for two 5 min sample phases and one 5 min test phase in which one of the previously encountered conspecifics is replaced by a novel conspecific. This paradigm takes only 40 min from the time of ER agonist/control treatment to completion; likely too little time for genomic actions to account for observed results. Animals who preferentially explore the novel conspecific during test phase are said to have good social recognition.

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Title: Sex differences and role of the estrous cycle in the lung inflammatory response to acute ozone exposure

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Abstract: Studies have shown that women are at increased risk of adverse health outcomes from air pollution (e.g. ozone) than men. Emerging data indicate that hormones could play a role in the regulation of lung inflammation in response to environmental challenges, but the mechanisms involved remain unknown. The goal of this project was to investigate whether fluctuations in circulating hormone levels could modulate the inflammatory response to ambient ozone. For this, we exposed adult male and female C57BL/6 mice to ozone (2ppm) or filtered air (FA) for 3 hours (n=6/group). Females were exposed to ozone or FA in different stages of the estrous cycle, which was determined by vaginal smear. Four hours after exposure, lung function testing was performed using a rodent ventilator, and blood and lung tissue were collected. Total RNA was purified using Trizol, and the mRNA expression of inflammatory cytokines and chemokines was analyzed by the RT² Profiler™ PCR Array Mouse Inflammatory Response & Autoimmunity (QIAGEN). Serum levels of LH, estradiol, and progesterone were measured by ELISA to confirm the cycle stage. Results were analyzed with the limma package on R. We found that exposure to ozone significantly increased expression of multiple inflammatory markers including Cxcl2, Ccl20, and Il6 in both male and female mice. Females expressed higher expression levels than males in all stages of the estrous cycle. However, females exposed in the follicular phase of the cycle (proestrus, estrus) exhibited a stronger inflammatory response to ozone than animals exposed in the luteal phase (diestrus, metestrus). Our results indicate sex differences and differences in the female lung inflammatory response to ozone at different stages of the estrous cycle. These observations suggest that the negative effects of air pollution in women's lung health may be affected by their hormonal status, and have important implications for diseases such as premenstrual asthma.

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Title: Sex differences in firefighter physiological response and task performance strategies: Implications for injury prevention

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Abstract: Female firefighters (FF) represent 3% of professional firefighters and report 33% more injuries than male firefighters (MF). Understanding the impact of workplace injury risk factors on task performance might elucidate the differential injury frequency in FF and MF towards injury prevention. Active duty, professional firefighters (n=109; female, n=5) from a Southwestern Ontario fire service performed two tasks: a hose drag task and a stair-climbing task that included lifting a high-rise pack (HRP). Heart-rate and blood pressure were measured pre- and post-task completion. Grip strength was also measured. During a separate data collection with the same fire service, upper and lower body kinematics during the HRP lift were measured using Dartfish software (n=12; female, n=6). FF seemed to perform the hose drag task faster (42.5s ± 8.3s) than MF (50.0s ± 13.8s); however, FF performed the stair climb task slower (FF = 72.1s ± 12.4s; MF = 64.5s ± 14.0s). FF demonstrated less change in heart rate (35% vs. 47% increase) and blood pressure (diastolic: 2.0% vs. 5.3% increase; systolic: 13.7% vs. 22.0% increase). FF also demonstrated less grip strength than male firefighters (39.2 kg ± 4.1 kg vs. 54.1 kg ± 11.2 kg). There was no difference between FF and MF lower extremity kinematics; upper extremity kinematic analysis suggested that FF performed the task with less forward reach compared to MF (147% ± 26% vs. 115% ± 25%, $t=3.034, p<0.05$). Although a small sample of FF was available for study, preliminary findings suggest that FF might have lower cardiovascular stress and greater strength challenges performing firefighting tasks compared to MF. FF and MF used a similar lower extremity lifting strategy however upper extremity analysis suggests FF use a shorter forward reach. FF may move their torso closer to the HRP before initiating the lift to adopt a more biomechanically advantageous lift strategy and accommodate for reduced strength.

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Title: New neuron functions in memory are dependent on stress and sex.

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Abstract: New neurons are added to the mammalian hippocampus throughout adult life. They have greater synaptic plasticity and neuronal excitability than their mature counterparts, suggesting a potentially critical role in the memory functions of the hippocampus. Indeed, there is evidence that new neurons are important for learning and memory but their precise role remains unclear. Independently, a series of studies have discovered a function for new neurons in emotion. Inhibiting adult neurogenesis leads to a depressive/anxiogenic response to acute stressors and an exaggerated neuroendocrine stress response. Whether the cognitive and emotional functions of adult neurogenesis intersect is unclear, but these observations suggest that newborn neurons may be particularly important for learning in emotional/stressful situations. Since males and females differ in the stress response and susceptibility to stress-related disorders such as anxiety and depression, these findings also raise the possibility of sex differences in functions of new neurons. To test these possibilities we tested male and female transgenic neurogenesis-deficient rats on a spatial water maze task. The emotional component of the task was manipulated by changing the temperature of the water (16ºC = high stress, 25ºC = low stress). Consistent with our prediction, we found that male rats that lacked neurogenesis were impaired on learning the task selectively in the high stress condition. Surprisingly, in females that lacked neurogenesis, learning was enhanced, also in the high-stress condition. These sex differences in memory function parallel previous findings that chronic stress impairs spatial memory in males but enhances spatial memory in females. Since chronic stress reduces neurogenesis, our data suggest that the sexually dimorphic effects of chronic stress on spatial memory may be mediated by newborn neurons.

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Title: The Paradox of Pregnancy: Past Parity is Protective Following Ischemia

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Abstract: Females show a fluctuating degree of ischemic sensitivity throughout various stages of life. Though we know that stroke is a sexually dimorphic disease, these changes in ischemic sensitivity are not fully explained by hormonal or genetic factors. Epidemiological data suggests that sex-specific life experiences such as pregnancy can increase the risk of stroke. We developed an experimental design that would evaluate the role of parity and parturition on neurovascular function and behavior in both normal female mice, and those exposed to stroke. The mice were evaluated at baseline as well as during the post-stroke critical window. We expected to find multiparous mice to have a higher risk as well as a poorer stroke outcome compared to their virgin counterparts. Age-matched nulliparous and multiparous mice were subjected to 60 minutes of reversible middle cerebral artery occlusion (MCAO) and evaluated for infarct volume and behavioral recovery. Behavioral assessments of locomotor activity, motor coordination, forelimb strength, and depressive phenotype were performed. We discovered a paradoxical finding, parity was associated with sedentary behavior and weight gain; however, the multiparous brain also exhibited features such as smaller infarct and behavioral improvement after stroke. Reproductive experience has a profound effect on neurovascular health and disease, which leads us to believe that it's translational value is very high and a parity model should be further explored. Moreover, inclusion of female mice with reproductive experience in pre-clinical studies may better reflect the life-long changing patterns of ischemic stroke risk in women.

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Title: Hypotestosteronemia-induced hypertension in male Sprague-Dawley rats is renin-angiotensin system-dependent

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Abstract: Acutely, testosterone (TES) and other androgens are efficacious vasodilators, both *in vitro* and *in vivo*; however, long-term effects of the androgens on arterial blood pressure (BP) are unclear. Thus, long-term effects of endogenous and exogenous TES on BP were studied in male Sprague-Dawley rats. 12-13 wk old rats remained intact (InT) or were castrated (CsX). Weekly measurements of systolic BP (tail cuff plethysmography) revealed a progressive rise in BP over 10 wks in CsX (108 ± 0.9 *vs.* 139 ± 2 mmHg), while BP remained stable in InT (109 ± 3 *vs.* 113 ± 0.3). During the next 5 weeks, half of the CsX received TES replacement therapy (CsX+TES-enanthate-replaced; 1.75 mg/kg 2x/wk). BP gradually declined to normal in CsX+TES replaced rats (118 ± 1), while BP remained elevated in CsX (141 ± 1) and normal in InT (112 ± 3). Seminal vesicle and body weights of InT (1.212 ± 0.038 g, 441 ± 9 g), CsX (0.065 ± 0.006 g, 420 ± 4 g) and CsX+TES (1.203 ± 0.080 g, 433 ± 3 g) revealed that TES replacement was physiological. In a separate group of CsX rats, treatment with Losartan (angiotensin II receptor antagonist, LST; 250 mg/L drinking water) prevented development of hypertension at 10 wks (94 ± 2 CsX+LST *vs.* 131 ± 3 CsX). During the next 5 weeks with TES replacement therapy, BP declined in CsX+TES (114 ± 2) and remained lower in CsX+LST (101 ± 1). These data suggest that: 1) endogenous androgens (TES) exert antihypertensive effects in male SD rats; and 2) these antihypertensive effects may involve TES-induced reductions in renin-angiotensin system function, through downregulation of angiotensin II-mediated increases in vascular tone and/or blood volume.

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Title: Heterochronic regulation of the sex-specific maturation of the *C. elegans* nervous system

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Abstract: Sexually dimorphic remodeling of the nervous system during adolescence is critical for enabling adult-specific sex differences in behaviors, such as mate-searching and parental care. This remodeling involves neurogenesis, changes in connectivity, and altered gene expression in existing neural circuits. Sex hormones released from the gonad are important for shaping sexually dimorphic remodeling but the genetic sex of the nervous system also plays a role in this process. We are using the nematode *C. elegans* to investigate how genetic sex regulates neuronal sex differences during the juvenile-to-adult transition. In *C. elegans,* several existing neural circuits undergo gene expression changes during this transition, particularly in males. For example, juveniles of both sexes have similar levels of the food-associated chemoreceptor *odr-10* in the sensory neuron AWA; however, upon maturation males downregulate expression of this chemoreceptor, reducing food sensitivity and facilitating mate-searching behavior. *tra-1*, the master regulator of *C. elegans* sex-determination, acts cell-autonomously to repress male development, allowing us to alter the sexual identity of single cells by manipulating its expression. Feminizing AWA by inducing *tra-1* is sufficient to generate high, hermaphrodite-like *odr-10* expression in adult males, demonstrating that dimorphic expression of *odr-10* is dependent on the sex of AWA. We find that *tra-1* is expressed in the nervous system of juvenile males and is downregulated during the juvenile-to-adult transition. This suggests that the male nervous system may default to a female state before sexual maturation. Further, we find the heterochronic pathway, known to mediate timing of hypodermal development in *C. elegans*, regulates the timing of neural maturation by controlling *tra-1* expression. Together our results suggest that the sexual identity of the nervous system is dynamic and controlled by a molecular timer.

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Title: **DNA methylation promotes hippocampal cell genesis in newborn males while histone acetylation suppresses it in females**

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Abstract: Embryonic neurogenesis is recognized as a key process in brain formation and maturation. Discovery of ongoing neurogenesis in the adult brain was a transformative observation and has garnered much investigation. However, the nexus between embryonic and adult phases of neurogenesis have largely gone ignored. During the period immediately following birth, male rats generate more new cells than female littermates (Bowers et al., 2010). This is also a time when hippocampal androgen or estrogen content does not differ between males and females (Konkle and McCarthy, 2011), suggesting steroids do not mediate the observed sex difference. Instead, we hypothesized that sexually differentiated epigenetic regulation is responsible for the sex difference in cell proliferation. Administration of the DNMT inhibitor and demethylating agent, Zebularine (Zeb; 300ng i.c.v.), or the histone deacetylase (HDAC) inhibitor, Trichostatin A (TSA; 0.5 mg/Kg i.p.) on PN0 and PN1 exerted sex specific effects on proliferation within the dentate gyrus (DG) of the hippocampus as assessed by BrdU quantification ($F[3,20] = 6.792$, $p = 0.0024$). Zeb treatment reduced proliferation in males, but had no effect in females (Newman-Keuls, $p<0.05$). Conversely, administration of TSA increased cell genesis in females but not males (Newman-Keuls, $p<0.05$). Males also had higher levels of global DNA methylation in the DG compared to females ($t[10] = 2.728$, $p=0.0213$), whereas HDAC activity was relatively higher in the DG of females ($t[12] = 3.057$; $p=0.010$). Together this data suggests that sexually dimorphic epigenetic regulation mediates the sex difference in neonatal cell genesis within the DG whereby elevated methylation promotes proliferation in males and HDAC activity represses proliferation in females. Ongoing research will focus on identification of genes that are differentially regulated by these epigenetic mechanisms to coordinate the sex difference in cellular proliferation within the DG.

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Title: High fat diet impairs hippocampal function and memory: Sex differences in energy metabolism and insulin signaling.

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Abstract: Modern western diet with excess energy-dense fats disturb metabolic homeostasis promoting obesity and type-2 diabetes. Cognitive deficits have been clinically observed in humans suffering from obesity and diabetes; and intensely studied in animal models fed high fat diet (HFD) from weaning. However, sex-differences are rarely systematically analyzed and compared. Previous studies from our lab on an outbred rat HFD model showed impaired spatial memory in both the sexes, correlating with significantly reduced intrinsic excitability of the CA1 neurons. Notable sex-differences in hormone signaling were observed, wherein HFD induced clinically relevant symptoms of type 2 diabetes in males (obesity, loss of blood glucose control, elevated insulin), but not in females (normal weight and blood glucose control, reduced insulin). Sex-differences were also found in insulin-dependent intrinsic excitability of CA1 neurons; HFD male neurons lost all insulin sensitivity while HFD female neurons had increased sensitivity. We hypothesize that there is an underlying sex-difference in hormones regulating energy balance (ghrelin and leptin) and insulin signaling pathway in HFD rats. Blood plasma was collected for ghrelin and leptin ELISAs. Rat brains were either flash frozen or perfused to analyze protein expression using western blotting or IHC/IFC, respectively. Diet-dependent increase in SK channel and hippocalcin expression correlates with the decrease in intrinsic excitability of HFD neurons. Only HFD female rats showed increased circulating ghrelin levels; along with upregulated insulin receptor expression and Akt activity in CA1 region. The cognitive deficits in males—also exhibiting clinical symptoms— would be readily assessed and treated, while covert female deficits (no clinical symptoms) would likely remain undiagnosed. Considering the profound differences, a critical public health issue is design of sex-specific treatment strategies.

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Title: Sex differences in pain ratings and proxemics; A translational study investigating how pain and illness influence interpersonal distance and pain ratings

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Abstract: Seeing others in pain is known to elicit empathy and may have a specific purpose in motivating pro-social and other oriented behaviours, This process may also not be limited to humans, and has been shown in mice. Our experiment aims to seek out whether or not pain behaviours and sickness behaviours will elicit approach or avoidance in both humans and rodents. We also aim to see as to whether or not these pain and illness behaviours will change participants' pain ratings. Indeed, we have found that when females are exposed to a female confederate who acts in pain, they tend to sit closer to the confederate ($n=18$, $p<0.05$). Females pain ratings also increase, compared to baseline measures. ($n=18$,$p<0.05$) When the female confederate acts sick, female participants tend to sit further away compared to the control ($n=17$,$p<0.05$), and their pain ratings decrease ($n=17$,$p<0.05$). In male participant/male confederate conditions, we do not see any difference in conditions. This appears to be a sex-specific effect, wherein females have approach behaviours when another female is in pain, and have an increase in pain ratings themselves when seeing other females in pain.

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Title: Sex differences in hemodynamic progression of calcific aortic stenosis: Impact of aortic valve calcification

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Abstract: Aortic valve calcification (AVC) is the main culprit lesion in calcific aortic stenosis (AS) and it is readily measurable by multidetector computed tomography (MDCT). Recent studies showed that AVC by MDCT is a strong determinant of hemodynamic severity and clinical outcomes in AS, with sex-specific thresholds. However, it remains unclear whether the contribution of AVC load to hemodynamic progression of AS might differ in women versus men. 323 patients (68±13 yrs, 30% women) with AS were prospectively enrolled in 2 academic centers. Hemodynamic AS progression was assessed by annualized increase in mean gradient (MG) measured by echocardiography. AVC was measured by MDCT using the Agatston method and expressed in arbitrary unit (AU). As expected AVC was significantly lower in women vs men (531[224-1098] vs 1019[571-1921]AU; p<0.0001), whereas baseline hemodynamic severity was similar in both sexes (MG women 24±13 vs MG men 22±10 mmHg; p=0.48). Mean echocardiographic follow-up was 2.3±1.6 yrs. Despite a comparable hemodynamic progression rate of AS in women vs men (MG: 3.4[0.7-6.4] vs 2.5[0.8-5.7]mmHg/yr; p=0.48), the slope of correlation between MG progression and AVC was steeper in women than in men (p_{ancova}=0.003). When compared according to previously proposed sex-specific threshold to define severe AVC (women ≥1200AU and men ≥2000AU), women and men with severe AVC at baseline have similar hemodynamic progression rate of AS (MG women 6.4[3.4-16.3] vs MG men 5.6[2.4-8.8]mmHg/yr; p=0.38). Of note, in comprehensive multivariable adjustment, baseline AVC remained an independent predictor of AS hemodynamic progression. Aortic valve calcification load by MDCT is an independent predictor of faster AS progression. For a given degree of hemodynamic AS severity, women have less calcium load than men, thus for a given degree of calcification, they have faster AS hemodynamic progression. Sex-specific thresholds of valve calcium must be used to predict valve stenosis progression.

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Your exTitle: Proteomic analysis of androgen-regulated sexually dimorphic protein expression in the mouse hypothalamus

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Abstract: One of the most important factors responsible for brain sexual differentiation is testosterone secreted by the developing testes. During late gestation and on the day of birth, testosterone and its metabolites cause male brains to develop differently, which can be mediated by androgen receptors, and after aromatization to 17β-estradiol, by estrogen receptors, α and β. However, the molecular mechanisms downstream to sex steroid hormones and their receptors to control the sexual dimorphic development remain unclear. The hypothalamus is the brain region implicated in regulation of various sexually dimorphic reproductive, parental, and aggressive behaviors, and it contains abundant androgen receptors (AR). The aim of the present study was to identify the androgen-regulated sexually dimorphic proteins in the developing mouse hypothalamus using proteomic approach. The proteins were extracted from the hypothalamus of testicular feminized (Tfm) mice and their wild-type littermates (n=3 per group) 21 days after birth. Tfm mice lack functional AR due to a frame-shift mutation. Two-dimensional (2-D) SDS-PAGE was used to separate the total proteins of each sample, followed by Commossie Blue staining and then imaging analysis. We identified 12 protein spots differentially expressed on 2-D gels among the three groups. Among them, 8 spots showed AR-dependent, sex-based changes, including five male-based and three female-based. One AR-dependent, female-based spot (more abundant in female and Tfm mice than males) was selected and excised, subjected to in-gel digestion, and analyzed by MALDI-TOF/TOF. Using Mascot™ search, we identified myelin basic protein (MBP) from the selected protein spot with the matching molecular weight and pI. In summary, our preliminary data demonstrate the AR-dependent downregulation of MBP expression in the developing male hypothalamus, suggesting that myelination might be critical for creating sexual dimorphism in hypothalamic structures and functions.

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Title: Systematic review: Sex and gender differences in prevalence of diastolic dysfunction in diabetics.

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Abstract: Women who develop heart failure suffer more from heart failure with preserved ejection fraction (HFpEF) than heart failure with reserved ejection fraction (HFrEF). However, before signs and symptoms of heart failure occur, the diastolic function of the heart can already be compromised. One of the comorbidities contributing to the development of diastolic dysfunction (DD) is diabetes. The associated chronic, systemic inflammation predisposes to endothelial dysfunction and microvascular inflammation. This can, in turn, lead to development of DD. Prevalence numbers on DD in diabetics vary considerably, but are important as worsening of DD towards HFpEF may be prevented in an early stageWe performed a systematic search for studies reporting prevalence numbers of DD, based on echo criteria, in men and women with diabetes. We included both hospital and population studies. Risk of bias was assessed by an adapted questionnaire. Included studies with prevalence numbers were combined for a pooled estimate of prevalences. We included 24 studies, of which 15 were hospital based and 5 were general population studies. We found pooled prevalence estimates of 47% of women with diabetes developing DD as compared to 46% of men with diabetes. Prevalence numbers on DD, in the studies, vary from 19% to 81%, with a pooled estimate of 35% prevalence of DD in the general population and 48% in the hospital population. It seems both men and women have a similar high prevalence of DD, especially in the hospital population. This finding, in combination with the knowledge that women suffer more from HFpEF than men, points towards sex-differences in the progression of DD to HFpEF.

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Title: Endocannabinoid-induced phagocytosis by microglia determines a sex difference in cell genesis in developing rat amygdala.

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Abstract: The amygdala is a sexually dimorphic brain region with relevance to social behaviors including play by juveniles. During early postnatal development, the male amygdala contains fewer newborn cells than females. This sex difference inversely correlates to the expression of juvenile social play, a process we previously demonstrated to be the result of a higher developmental endocannabinoid (ECB) tone in the male amygdala (Krebs-Kraft *et al. PNAS* 107(47), 2010). We now report that microglia, the resident immune cells of the brain, are more phagocytic in the amygdala of males during this postnatal window, suggesting a possible mechanism by which ECBs affect the number of newborn cells. Increasing ECB tone in female pups by treatment with specific agonists of both the CB1 (ACEA) and CB2 (Gp1a) receptors increases the number of phagocytic microglia to that of males and results in a corresponding decrease in the number of newborn cells indicated by BrdU labeling. We hypothesize that microglia control the number of newborn cells in the postnatal rat amygdala by phagoptosing (targeted phagocytosis of viable cells) newborn cells in an ECB-dependent manner. We find that males have more phagocytic microglia between postnatal day 0 and 4, during which time they also have higher ECB tone than females. Masculinizing female pups with testosterone increases the number of phagocytic microglia and decreases the number of BrdU+ cells. Immunohistochemical analysis together with confocal microscopy indicates phagocytic microglia contain markers of DNA as well as newly proliferated cells in their phagocytic cups. To directly implicate microglia phagoptosis we utilized a complement receptor 3 (CR3) function-blocking antibody to inhibit phagocytosis, which increased the number of BrdU+ cells in both males and females. Thus microglia actively control developmental sex differences in cell genesis and this correlates with sex differences in later life social behavior.

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Title: Repeated mating does not modify Mu, kappa and delta opioid gene expression in the preoptic area, the ventromedial hypothalamus and amygdala in the female rat

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Abstract: Reports about rewarding properties of mating in both sexes indicate that the endogenous opioid system is a relevant mediator, effecting their actions through three main receptors: μ, κ and δ, in brain areas that regulate sexual behavior and sexual reward such as, the medial preoptic area (mPOA), the ventromedial hypothalamus (VMH), and the amygdala (AMG) in both sexes. For example, the administration of the μ-receptor antagonist, naloxone in the aforementioned brain areas, blocked the sexual reward state after paced-mating (PM) in female rats, suggesting that there is an endogenous opioid release during PM. Sexual reward can induce plastic changes on the brain that might be more robust with repeated mating. For female rats, sexual studies are under two experimental conditions: PM or non-PM, depending on the female's ability to control the sexual interaction. After repeated PM, neurogenesis in the olfactory bulb is more than the occurred with one session of PM or non-PM. Our hypothesis was that repeated mating modifies the expression of the μ, κ and δ receptor genes in the mPOA, VMH and/or AMG in the female rat, these changes would be more robust if female is able to regulate sexual contact (PM). The aim of this work is to determine if repeated mating is sufficient to induce changes in the expression of μ, κ, and δ receptor genes in the mPOA, the VMH and the AMG. We formed three groups of female ovariectomized rats: control, PM and non-PM (n=8/group). Females were sexually receptive by the administration of hormonal replacement. Females mated four times, with a sexually experimented male during 1 h every 5 days. Twenty-four hours after the last mating session, females were sacrificed by decapitation and immediately, dissected the mPOA, VMH and AMG. The mRNA levels of μ, κ, and δ receptor genes were measured by q-RT- PCR. We found no differences between groups; it is possible that sexual stimuli during mating are not enough to induce robust changes in gene expression and/or posttranscriptional mechanisms occur.

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Title: Adolescent social stress results in sex-specific transcriptional reprogramming of the medial amygdala, a critical region for sex differences in reward.

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Abstract: Adolescence is a time of heightened sensitivity to rewarding stimuli and is associated with vulnerability to psychiatric disorders. Social isolation stress (SI) during this period increases preference for drugs of abuse in male rodents. However, little is known about how females respond to SI. The medial amygdala (meAMY) is sexually dimorphic, develops during adolescence and is sensitive to SI at this time. Our preliminary data suggest that SI reverses sex differences in reward behaviors and permanently reduces baseline sex differences (M>F) in neuronal projections from meAMY to ventral tegmental area (VTA). We tested the hypothesis that SI results in persistent transcriptional changes in the meAMY that underlie such sex differences. Mice were isolated or group housed (GH) from postnatal day (P) 22 - P42, then GH until ~P90. Transcriptome-wide changes in meAMY and VTA were investigated by RNA-seq after acute or chronic cocaine or saline. Sexually dimorphic genes in both regions were disproportionately affected by SI with the greatest number of transcripts affected in the meAMY (Sex X SI: 869 genes). Hierarchical clustering revealed that SI reversed baseline sex differences in expression in both regions, similar to observed behavioral effects. Specifically, GH males cluster with SI females and vice versa for those sex X SI genes in the meAMY and VTA (276 genes). Cluster analysis also revealed that sex differences in response to chronic but not acute cocaine were reversed by SI, suggesting that meAMY to VTA projections are important for the regulation of sex differences in reward. Serum hormones were measured across SI (P22, P32, P42 & P72) to identify peripheral factors influencing alterations in reward-circuitry. SI females show a male-typical developmental pattern in corticosterone. In adults (M & F), progesterone is reduced by SI. Therefore, SI disrupts sex-specific adolescent development of brain connectivity, transcription and endocrinology.

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Title: Sex differences in the effects of heightened prenatal testosterone on social and object recognition

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Abstract: Sex hormones Testosterone (T) & Estradiol (E) are crucial for sexual differentiation of the brain, resulting in sexually dimorphic morphology and behavioral phenotypes. Recognition memory (RM), such as object recognition (OR) and social recognition (SR), involve sexually dimorphic hormone-sensitive regions of the brain. However, how gonadal hormones affect RM remains unclear. In this study, we treated pregnant CD1 mice with 10μg of testosterone propionate (TP) or the oil injection vehicle, on embryonic days 12, 14 and 16. Prior to puberty (PD 34-42), mice were tested in (A) SR: measuring the ability for an individual to discern a novel conspecific from previously encountered mouse; and (B) OR: measuring item declarative/recognition memory. Mice were then gonadectomized (GDX) or sham operated and given hormone replacement (12.5μg E for females or T for males). Mice were re-tested in SR and OR as adults. Pre-puberty we found that increased T exposure in-utero had an enhancing effect on males in OR and in both males and females in SR. In adulthood, we found that males, but not females, who received T replacement post-GDX and were exposed to prenatal TP performed worse in the OR test than T-replaced males who were not prenatally TP exposed. Prenatal control T replacement males outperformed prenatal control female E replacement in OR. SR, by contrast, was impaired in males. In GDX males that did not receive T replacement, prenatal TP exposure worsened performance compared to males that did not receive prenatal T. Thus, prenatal T interacts with later T exposure, impairing OR in adulthood and predisposing mice to impairing effects of GDX on SR. These effects were specific to males, suggesting important sex differences in hormonal regulation of RM.

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Title: Reporting of sex and gender in randomized controlled trials in Canada: a cross-sectional methods study.

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Abstract: Accurate reporting on sex and gender in health research is integral to ensuring that health interventions are safe and effective. In Canada and internationally, governments, research organizations, journal editors and health agencies have called for more inclusive research, provision of sex-disaggregated data, and the integration of sex and gender analysis throughout the research process. Sex and gender analysis is generally defined as an approach for considering how and why different subpopulations (e.g., of diverse genders, ages, and social locations) may experience health conditions and interventions in different or similar ways. The objective of this study was to assess the extent and nature of reporting about sex and/or gender, including whether sex and gender analysis (SGA) was carried out in a sample of Canadian randomized controlled trials (RCTs) with human participants. Two reviewers screened 256 records of 1,433 records from a MEDLINE search limited to January 2013 to July 2014, to identify the first 100 RCTs that were either identified in the trial publication as funded by a Canadian organization or which had a first or last author based in Canada. Data were extracted in duplicate during an initial training period for 10% of the RCTs; once agreement was reached, the remainder of the data was extracted by one person and verified by a second. The median sample size of the RCTs was 107 participants (range 12 - 6085). While 98% of studies described the demographic composition of their participants by sex, only 6% conducted a subgroup analysis across sex and 4% reported sex-disaggregated data. No article defined "sex" and/or "gender." No publication carried out a comprehensive sex and gender analysis. Findings highlight poor uptake of sex and gender considerations in the Canadian RCT context and underscore the need for better articulated guidance on sex and gender analysis to improve reporting of evidence, inform policy development and guide future research.

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Title: Genetic sex regulates feeding behavior in *C. elegans* through modulation of the food chemoreceptor ODR-10

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Abstract: While nearly all animals display sex differences in neural function and behavior, the biological mechanism through which neural circuits are modulated by sex are poorly understood. Hormonal signaling from the gonad is critical for the development of sex differences in vertebrates; however, recent work has shown that some differences are controlled by the genetic sex of the nervous system. Using the nematode *C. elegans*, in which sex-determination relies exclusively on genetic sex, we can study the mechanisms by which genetic sex modulates neurophysiology and behavior. Our lab has developed a system by which the nervous system can be "sex reversed" genetically enabling us to study the mechanisms by which behavior and gene expression in the nervous system are tuned by sex. In *C. elegans* feeding behavior is sexually dimorphic with hermaphrodites much more strongly attracted to food than males. This sex difference in behavior is governed in part by the expression of *odr-10*, a chemoreceptor which senses the food-associated odorant diacetyl. In males, *odr-10* expression in the sensory neuron AWA is significantly reduced compared to hermaphrodites, promoting the prioritization of exploratory behavior over feeding. By sex reversing the AWA neuron we found that the genetic sex AWA regulates *odr-10* expression and feeding behavior. We have identified an element in the *odr-10* promoter necessary for the regulation of *odr-10* by genetic sex which suggests an unknown hermaphrodites-specific activator generates high *odr-10* expression. We are working towards identifying this sex-specific regulator of gene expression. In pursuit of identifying specific molecular regulators of neural sex difference we have identified TGFβ signaling as a male-specific regulator of *odr-10*. Loss of TGFβ signaling in males results in upregulation of *odr-10* in males. These findings provide insight into the possible molecular mechanism through which genetic sex modulates the nervous system.

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rsTitle: Effects of developmental exposure to an environmentally relevant combination of phthalates on apoptosis in the medial prefrontal cortex of male and female rats.

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Abstract: Phthalates are a class of endocrine disrupting compounds which exert actions on androgenic and estrogenic systems, and are found ubiquitously throughout the environment in a variety of consumer goods. The developing fetus can exposed to phthalates through the placenta and lactation during critical periods of neural development. There are indications that developmental exposure to the phthalate DEHP can decrease cell number and increase apoptosis. Here we examine the effects of combined exposure to the phthalates found in pregnant human females on the development of the medial prefrontal cortex (mPFC) in rats. Pregnant and lactating rats were orally dosed with a 0mg/kg, 1mg/kg or 5mg/kg phthalate solution containing six different phthalates at environmentally relevant levels. Phthalate mixtures (and oil vehicle) were pipetted onto a cookie daily from embryonic day 2 through postnatal day (P)10. Brain and body weights were collected from male and female pups at P10 and P25. We additionally examined markers for apoptosis in the medial mPFC at P10, and total mPFC volume at P25. Data show that pups dosed with 5mg/kg phthalates had a higher density of TUNEL positive cells in the mPFC, indicating higher levels of cell death, while the 1mg/kg dose caused a reduction in mPFC volume at P25. Additionally, there was a sex difference in the amount of apoptosis in the 1mg/kg group at P10 with effects in males, but not females. Collectively, these results imply that combinations of common phthalates found in the environment alter the development of the mPFC in a dose and a sex-dependent manner in a brain region critical for executive function and attention.

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Title: The role of X chromosome inactivation in ovarian cancer

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Abstract: In females, X-chromosome inactivation (XCI) randomly silences one copy of the X chromosome. While some genes are known to escape XCI in normal tissue, aberrant XCI patterns are thought to occur in female-specific cancers. We hypothesize that XCI patterns may impact ovarian tumor development or progression. Integration of gene expression, DNA methylation, and copy number data can inform the XCI status of individual genes and chromosome-wide patterns for individual patients. By integrating these data types, we evaluated gene- and chromosome-level XCI patterns in tumor samples from 99 ovarian cancer patients. We measured allele-specific expression (ASE) for 397 X-linked genes to identify the active alleles for each tumor, and used a Bayesian beta-binomial mixture model to estimate which genes escaped XCI for each patient. To assess global XCI patterns, we performed cluster analyses on the ASE and methylation data, adjusted for loss of heterozygosity, and examined the relationship between the clusters and clinical factors. Cluster analyses demonstrated two tumor clusters, representing normal XCI and global XCI dysregulation. The dysregulated XCI cluster was associated with lower X-inactive specific transcript expression ($p<0.01$). Patients with XCI dysregulated tumors were higher grade, stage, serous histology and were sub-optimally debulked ($p<0.05$), and had shorter overall and progression free survival (HR=2.34, $p<0.01$). We observed escape gene patterns consistent with previous reports of multiple tissue types; however, compared to normal tissue, eight genes (*CXorf23, CXorf36, BRWD3, ELF4, SLITRK4, GABRE, CLCN4, SH3BGRL*) showed putative escape in the tumor and two genes (*RBBP7, OFD1*) showed discrepant tumor inactivation. The discrepant gene-level XCI tumor classifications compared to normal tissue and the group of patients with chromosome-wide XCI dysregulation associated with worse clinical prognosis provide converging evidence of the role of XCI in ovarian cancer.

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T Title: Prohibitin has sex dimorphic role in adipose and immune functions

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Abstract: Prohibitin (PHB) is a pleiotropic protein with cell compartment specific functions such as mitochondria and the plasma membrane. It can be regulated through mechanisms such as insulin induced phosphorylation at tyrosine 114 (Tyr 114), which subsequently dampens the insulin signaling pathway. Recently, new evidence suggests that PHB has roles in adipocyte and immune cells. To discern the cell compartment specific functions of PHB in adipocyte and immune cell, we developed two novel transgenic mice by expressing wild type PHB and a phospho mutant PHB (Y114F or m-PHB) from the adipocyte protein-2 (*aP2*) gene promoter, separately. The *aP2* is primarily expressed in adipocytes, but also selectively in monocytic macrophages and dendritic cells among various immune cell types. Thus, the *aP2* gene promoter provides an opportunity to simultaneously manipulate adipose and immune functions in a transgenic animal model. Both transgenic mice develop obesity in a sex-neutral manner suggesting that m-PHB retains mitochondria-related adipogenic function. However, both transgenic mice develop obesity-related metabolic dysregulation in a male sex-specific manner. Interestingly, the male PHB mice spontaneously developed T2D and liver cancer, whereas the male m-PHB mice developed lymph node tumors, or T1D in a context-dependent manner. This would imply that plasma membrane-related phosphorylation of PHB at Tyr-114 has a role in immune cell functions. Collectively, our data suggests that PHB has sex dimorphic roles in adipose and immune functions, which are mediated in a cell compartment specific manner. Also, we provide evidence that sex differences in adipose and immune functions contribute to sex differences in diabetes and cancer, which warrant further investigations.

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Title: Sex-specific differences in neutrophil death

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Abstract: Females with cystic fibrosis (CF) have a lower predicted survival than males with CF. Although the underlying cause is currently not well-understood, multiple studies have shown a correlation between the female sex and worsening of CF lung disease. Also, linked to the severity of CF lung disease is the increase in neutrophil immune response in the lung. The excessive release of DNA as extracellular traps by neutrophils (NETs) increases mucus viscosity in the airways and damages lung tissue. Not only in CF, females show increased inflammatory response during several other disease condition. Therefore, we hypothesized that neutrophils from females many undergo increased NET formation (NETosis). As the first step of the study, we isolated neutrophils from healthy male and female participants and compared the level of NETosis between the two groups. NETosis was induced by both pharmacological and biological agents, including phorbol 12-myristate 13-acetate (PMA), bacterial lipopolysaccharide (LPS), the calcium ionophores A23187 and ionomycin, and *Pseudomonas aeruginosa*, the most prevalent bacteria in the CF lung. Sytox Green is a cell impermeable dye and emits intense green fluorescence when the dye binds extracellular DNA. Hence, we used Sytox Green as an indicator of NET release. We also measured the level of production of reactive oxygen species (ROS) by the neutrophils using dihydrorhadamine 123 (DHR 123) and detected the presence of NET associated proteins using spinning disk confocal microscopy. Our preliminary data show similar levels of ROS-mediated NETosis in male and female neutrophils when stimulated with PMA, but lower NETosis in female neutrophils stimulated with A23187 and ionomycin ($p=0.05$ and $p=0.002$, respectively). Therefore, neutrophil death induced by calcium influx is potentially different in males and females. These findings could help to explain sex differences in neutrophil death and inflammation in general, and may be relevant to cystic fibrosis airway disease.

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Title: Effect of sex on hemodynamic recovery after transcatheter Valve-in-Valve implantation for the treatment of degenerated surgical valves

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Abstract: Transcatheter aortic Valve-in-Valve (ViV) implantation is an increasingly performed procedure to treat failing surgical bioprostheses (BP). The major concern remains the presence of high residual gradients and patient-prosthesis mismatch (PPM) or non-recovery of the initial valve (BP) performance: effect of russian dolls. Size-related factors such as BP size and degeneration of BP by stenosis have been related to the presence of high gradients post-ViV and to increased mortality respectively. As women are smaller, thus have smaller aortic root and cardiac cavities, we wonder if ViV is well-suited to women. Early post-surgical aortic valve replacement (SAVR), pre-VinV, and post-VinV echocardiographic data of 42 consecutive patients who underwent aortic ViV at our institution were retrospectively analyzed. To characterize the hemodynamic recovery we measured the change in mean transvalvular pressure gradient (ΔmTPG) and in effective orifice area indexed by the body surface area (ΔEOA_i) between post-surgical valve replacement (SAVR) and post-ViV procedure. Men and women had similar clinical (age, indexed stroke volume) and prosthetic characteristics (BP type, mode of failure, mTPG, EOA_i, rate of severe PPM at time of SAVR [$sPPM_{SAVR}$], transcatheter valve type) except size-related characteristics as expected: BP size, any EOA but similar when indexed. Women had larger $|\Delta EOA_i|$ (0.34±0.25 vs. 0.21±0.15 cm^2/m^2; p=0.044) and ΔmTPG (14.9±16.6 vs. 7.5±5.3 mmHg; p=0.030) as compared to men. After comprehensive adjustment, female sex remained a predictor for worse outcome of ViV, i.e. increase in $|\Delta EOA_i|$ (β=0.34; p=0.007) and ΔmTPG (β=0.37; p=0.021). The ViV procedure was associated with significant deterioration in valve hemodynamic performance compared to the first SAVR and the magnitude of this deterioration was worse in women than in men. Further studies are needed to elucidate the mechanisms underlying these differences.

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Title: A novel sex difference in corticotropin releasing factor receptor 1 containing cells in the rostral periventricular hypothalamus

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Abstract: Corticotropin releasing factor (CRF) signaling through CRF receptor 1 (CRFR1) regulates autonomic, endocrine and behavioral responses to stress and has been implicated in pathophysiology of several disorders including anxiety, depression, and addiction. Using a CRFR1 reporter mouse line (bacterial artificial chromosome identified green fluorescence protein (BAC GFP-CRFR1)), we report a novel sex difference in CRFR1 expressing cells within the anteroventral/rostral periventricular nucleus of the hypothalamus (AVPV/PeN) which is exclusively expressed in female mice. The AVPV/PeN is interconnected with many forebrain structures involved with endocrine functions, stress, and reproductive behaviors (e.g., preoptic area, arcuate nucleus, and paraventricular nucleus of the hypothalamus). Our studies indicate that this nucleus is sexually dimorphic at birth, and the difference persists into adulthood. Gonadectomy of adult male and female mice fails to alter CRFR1-GFP cell number. However, older female mice (16mo) display significantly reduced CRFR1-GFP cell number. Dual-label fluorescent immunohistochemistry shows nearly all CRFR1 cells co-express estrogen receptor alpha (ERα). ERα is necessary for masculinization of the rodent brain, and may serve a functional role in the AVPV/PeN CRFR1 nucleus. The CRFR1 AVPV/PeN cell population is largely distinct from the sexually dimorphic tyrosine hydroxylase (TH) in the same region with only ~17% of TH cells co-localizing with CRFR1-GFP. AVPV/PeN CRFR1-GFP cells also respond to psychological stress, showing increased co-localization with the neural activation marker phosphorylated CREB after a 30-minute restraint stress. Overall, these findings demonstrate the presence of a novel sexually dimorphic clustering of CRFR1 cells that is functionally engaged during stress and may contribute to sex differences observed in anxiety and reproductive disorders.

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Title: Sex differences in changes in the vasculature of the subventricular zone niche with age.

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Abstract: In the adult brain, neurogenesis occurs in the subventricular zone (SVZ) lining the lateral ventricles, but declines markedly with aging. Blood vessels within the SVZ play a vital role in the neural stem cell niche by promoting proliferation, neurogenesis and guiding the chains of migrating neuroblasts. Here we sought to determine how the structure of this vascular stem cell niche differs between sexes and how it changes with age. Mouse SVZ wholemounts at 2, 18 and 22 months were immunostained for laminin to label blood vessels and doublecortin to label neuroblasts. Using 3D computer-based image analysis, we assessed changes in vessel diameter, vessel tortuosity and organization of the neuroblast chains. In addition, we used uptake of EdU, a thymidine analog, to assess progenitor cell proliferation. We found that vessel tortuosity increases slightly with age, yet sex differences do not emerge until 22 months of age, when males have more torturous vessels than females. Vessel diameter in the SVZ changes with age in a sex-dependent manner. Young females have smaller vessel diameters than males, however, their vessel diameters increase between 18 and 22 months of age. Conversely, in males, vessel diameters steadily decrease with age. Thus, although young males start with larger vessels than females, by 18 months the males have smaller vessels, a difference that is exacerbated by 22 months. With regard to the progenitor cells, the chains of migrating neuroblasts become disorganized and less linear at 18 months but surprisingly this organization improves by 22 months, especially for the females. While both sexes show a decline in proliferation with age, as expected, females have significantly less proliferation than males at 18 months. In conclusion, we have found complex, sex-dependent changes in the SVZ niche vasculature with age that correlate with changes in proliferation of progenitor cells.

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