Sex, Hormones, and Genotype Interact to Influence Psychiatric Disease, Treatment and Behavioral Research

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Abstract

Sex differences exist in the vulnerability, incidence, manifestation, and treatment of numerous neurological and psychiatric diseases. Despite this prominent observation in the literature, there has been little consideration given to possible sex differences in outcome in both preclinical and clinical research. This review highlights evidence supporting why studying sex differences matter for advances in brain health as well as improving treatment for neurological and psychiatric disease. Additionally, we discuss some statistical and methodological considerations in evaluating sex differences, as well as, how differences in the physiology of the sexes can contribute to sex difference in disease incidence and manifestation. Furthermore, we review literature demonstrating that the reproductive experience in the female can render the female brain differentially vulnerable to disease across age. Finally, we discuss how genes interact with sex to influence disease risk and treatment and argue that sex must be considered in precision medicine. Together, the evidence reviewed here supports the inclusion of males and females at all levels of neuroscience research.

Significance

This review discusses the importance of sex differences in preclinical and clinical neuroscience. Despite the well-known biological differences between men and women, how these biological differences may influence the brain under normal and diseased conditions is not well understood. Sex differences are apparent in prevalence, manifestation, and treatment of psychiatric diseases. In this review, we address how age, experience, hormones, and genotype interact to influence disease risk and treatment. Collectively, the literature reviewed strengthens the argument that more research investigating sex differences in the brain will ultimately contribute to a better understanding of psychiatric diseases.

Introduction

Many neurological and psychiatric diseases, including the top diseases contributing to the global burden of disease (see Table 1), are characterized by a sex difference, favoring men or women, in incidence of disease. For example, women are more likely to present with depression (Angst et al., 2002) and Alzheimer's disease (Ruitenberg et al., 2001; Zhou et al., 2006) than men whereas boys/men are more likely to present with autism (Fombonne et al., 2009; Werling et al., 2013; Idring et al., 2015), attention deficit hyperactive disorder (Ramtekka et al., 2010; Rucklidge 2010), and Parkinson's disease than girls/women (Van Den Eeden et al., 2003; Wooten et al., 2004). Although both sexes can be diagnosed with each of these diseases, the fact that more members of one sex are more likely to be affected than the other, gives researchers some biological clues about the development of the disease. Importantly, even when there is no sex difference in overall incidence of disease, such as in schizophrenia, there may be sex differences in other aspects of the disease. As an illustration of this, although there is no sex difference in the overall incidence of schizophrenia, men are more likely to be diagnosed with schizophrenia earlier in life (late teens to twenties) whereas women are more likely to be diagnosed later in life (mid 40s; Ochoa et al., 2012). Furthermore, men with schizophrenia present with more cognitive disturbances (Han et al., 2012) and greater reductions in temporal lobe volume than women with schizophrenia (Bryant et al., 1999). Thus, biological sex can impact manifestation of psychiatric illness even in diseases that do not show a sex difference in prevalence.

Sex differences are present in the brain at the cellular, structural, and connectivity levels (Straface et al., 2012; Cahill 2014; Ingahalikar et al., 2014; Ruigrok et al., 2014). The presence of X and/or Y chromosomes are influential at the genetic level, and gonadal hormones interact with other hormone and immune systems within the central and peripheral nervous systems as well as regulate numerous genes (De Vries & Forger, 2015). It is therefore surprising that sex as a factor is not largely investigated in the literature (Beery & Zucker, 2011), especially in regards to research in diseases which show distinct sex differences favoring men or women in incidence and manifestation. Despite the evidence pointing to sex differences in a variety of diseases, advances in medicine have yet to fully capitalize on these differences to advance therapeutics and disease management. Indeed, most studies have either used exclusively men or male rodent subjects or have not reported the sex of the subjects observed (Beery and Zucker, 2011).

Moreover, poor experimental design which fails to consider sex as a factor can hinder the current progress of drug advancement. As an example, Addyi™ was developed to treat hypoactive sexual desire disorder in premenopausal women. Despite this drug targeting women's sexual behaviour, a study that evaluated the possible side effects of Addyi™, including hypotension, when consuming alcohol, was assessed with only 2 women in a subject pool of 25 participants (Sprout Pharmaceuticals, Inc.). This is a poor representation of the target population (women) and likely distorted the final interpretation of the side effect results. This lack of consideration for sex differences has been extensively found in both preclinical and clinical research, inspiring American, Canadian, and European funding agencies to call for more women in clinical studies and female subjects in both *in vivo* and *in vitro* research (Clayton and Collins, 2014; Tannenbaum et al., 2016). A better understanding of basic and clinical research examining sex as a factor will strengthen translation between preclinical and clinical research, and ultimately will provide more information in terms of advancing disease identification and treatment in both sexes. While sex is presently not considered a large part of precision medicine, we argue that sex of the subject is an important consideration to optimize precision medicine. This review will give examples of how: sex matters in neuroscience research, appropriate statistical analysis is crucial for studying sex differences, and how examining reproductive experience in females and genotype contribute to a better understanding of individual differences in disease.

The Many Faces of Sex Differences

Researchers have argued convincingly that there are many types of sex differences that are seen qualitatively, quantitatively, across populations, and in mechanisms (McCarthy et al., 2012, Becker and Koob, 2016). Qualitative sex differences refer to traits that are observable in one sex but not the other. One example of this type of differences is that only females have ovaries, and only men have testes. Quantitative sex differences refer to a trait present in both sexes but the magnitude of that trait expression differs by sex. For example, specific brain areas show large sex differences favoring males as the size of the spinal nucleus of the bulbocavernosus, the central portion of the medial preoptic nucleus, and the bed nucleus of the stria terminalis are all larger in male compared to female rodents (Forger, 2006). In addition, during sexual interactions, female rodents exhibit lordosis whereas male rodents mount, and these behaviors occur predominantly in females and males, respectively (Veening et al., 2014)

although not exclusively (Dagg 1984). Population sex differences refer to sex differences in the incidence or presentation of certain traits. Along these lines, women suffering from depression are more likely to present with co-morbid anxiety than men with depression (Angst et al., 2002). Finally, even when there are no overt sex differences in behavior, there can be sex differences in the neural representation of the behavior (McCarthy et al., 2012; Becker and Koob, 2016; Gruene et al., 2015a; Oberlander and Woolley, 2016). Recent examples of this have been found in the recent literature (Gruene et al., 2015a; Oberlander and Woolley, 2016). Oberlander & Woolley (2016) found that *in vitro* application of estradiol to CA1 pyramidal neurons of the dorsal hippocampus of adult rats had similar excitatory effects in both sexes. However, the mechanism underlying this excitation differed by sex: presynaptic glutamate release was mediated via estrogen receptor (ER) β in females but via ER α in males. Additionally, postsynaptic sensitivity to glutamate was mediated via the G-coupled estrogen receptor (GPER) in females but via ER β in males. This is an example of how sex can influence differential outcome even at the cellular level for *in vitro* research, and highlights that the same hormone (in this case, estradiol) can have similar effects yet via different mechanistic pathways in males and females. Additionally, in an *in vivo* example, Gruene et al (2015a) found that in a large cohort of male and female rodents, there were no sex differences in the acquisition, extinction, or retrieval of cued fear conditioning. However, when categorizing the rodents as either ‘high freezing’ or ‘low freezing,’ the neural representation of this behavior diverged between the sexes. The ‘high freezing’ males exhibited shorter dendritic length and more thin spines than ‘low freezing’ males in the infralimbic neurons of the prefrontal cortex innervated by the basolateral amygdala. However, there were no morphological differences in this circuit between ‘high freezing’ or low freezing’ females. This is another example of how neural circuits underlying behavior can be distinct based on sex as well as highlights that individual differences (such as low or high freezing) can interact with sex in this regard.

Not all Sex Differences are Dichotomous

It should be noted that some researchers have equated sex differences to sexual dimorphism (Joel, 2011), meaning that to be considered a valid sex difference there must exist two distinct non-overlapping categories. Sexual dimorphism refers to two distinct forms between males and females such as in appearance or behavior that are apparent in one sex but never in the other. For some researchers a true sex difference must be dimorphic which would then suggest

that the brain should give rise to non-overlapping “male” or “female” brains. To this end, Joel et al (2015) found that there was poor internal consistency in whether MRI measures (grey matter volume, white matter volume, and connectivity) of 10 different brain regions could be dichotomized as either ‘male’ or ‘female’ within each subject. The researchers did not find a strict dichotomy but observed a great deal of variability and argued effectively that human brains are mosaic rather than male or female. However, their argument extended to the idea that because there was no true male or female brain, sex would, therefore, play a minimal role in the brain. While we agree with the authors that individual differences play a vital role in brain function and that our brains are mosaics of male and female-like characteristics, the authors’ conclusions regarding the importance of a lack of truly sexually dimorphic brain is challenged by ourselves and others (Del Giudice et al., 2016; Chekroud et al., 2016). First, this study measured sexual dimorphism on 10 separate measures within the brain as their endpoint. However, sexual dimorphism is only one type of (extreme) sex difference that can be detected. Few human organs can be considered truly sexually dimorphic (e.g. ovaries, prostate gland), and there is little reason to expect that sexual dimorphism would be found in the brain. The idea that sexual dimorphism in the brain is a necessary condition for a sex difference in the brain to be meaningful for neuroscience research appears unfounded. Indeed, the authors even point out quantitative sex differences in the brain are more influential than qualitative sex differences, so it is unclear why only qualitative sex differences were sought after in this study. Del Giudice et al (2016) further criticizes this by pointing out that the concept of internal consistencies is invalid in this case as there is little internal consistency even within species and that this definition is so extreme that the hypothesis would have to be proven. Chekroud et al (2016) used an analysis that integrated a pattern or mosaic of the morphological data from the 10 brain regions and was able to accurately predict the sex of the individual, indicating that it is the entire pattern of the brain that may generate “maleness” or “femaleness” as opposed to discretely dichotomizing each brain region. Second, the study exclusively analyzed measures of grey matter volume and white matter volume via fMRI and connectivity via diffusion tensor imaging. However, their observation that there were no sex differences in gross anatomical or connectivity differences does not preclude the possibility that there are sex differences in the function of the studied region which could yield sex differences in brain function under basal or disease states (for further critique of this conclusion, please see Glezerman 2016). As an example, there is limited evidence to suggest that

the volume of the hippocampus is different between men and women when corrected by total cranial volume (Tan et al., 2016). However, depression is characterized by a smaller hippocampal volume (McKinnon et al., 2009) particularly in men (Frodl et al., 2002), and antidepressants boost hippocampal volume and neurogenesis particularly in women (Vakili et al., 2000; Epp et al., 2013). Therefore, the tenuous argument that a lack of volumetric changes between the sexes is illustrative of a lack of sex differences in brain function and disease is not relevant when considering neural function. Finally, Joel et al (2015) also demonstrated that the majority of male brains had zero ‘female-end’ characteristics and the majority of female brains had zero ‘male-end characteristics’ (Joel et al., 2015). These findings indicate that a majority of men had few female-brain characteristics and the majority of women had few male-brain characteristics and would support the concept of men and women exhibiting differences in the brain. As noted in the previous section, there are many types of sex differences that are not always dimorphic in nature but yet can lead to valuable information regarding basic processes and potential disease outcome in men and women.

Studying Sex Differences: Important Statistical & Methodological Considerations

Headlines from studies such as Joel et al (2015) may inadvertently undermine the recent call by multiple funding agencies to include a consideration for sex differences in research and perhaps contribute to the general reluctance of the scientific community to study sex as a biological variable and/or add confusion about whether sex actually influences the outcomes of neuroscience research. This reluctance and/or confusion within the scientific community may also be partly attributed to complications at the level of statistical analyses. It is not merely the addition of females to research that will improve the present state of scientific understanding, but rather every study using males and females needs to involve a careful statistical examination of any sex differences. One statistical concern is that some argue for examining sex differences using the same overall sample size but then to split the overall sample size into an equal ratio of males and females (McCarthy, 2015). However, such studies would be underpowered to detect sex differences and would preclude the examination of different types of sex differences as described by Becker and Koob (2016). Under-powered studies are a major problem in neuroscience (Button et al., 2013) and lead to a lack of reproducibility due to a low probability of finding a true effect. Even if a true effect is detected, low power can lead to an overestimate of

the size of that effect (Button et al., 2013). Thus we recommend when both sexes are to be included in a study that the study needs to be correctly powered to study possible sex differences (Button et al., 2013).

Another statistical concern is the popular use of sex as a covariate rather than a factor. Beery and Zucker (2011) surveyed the animal and human literature from 2009 and found that those studies that identified that they had conducted research on both sexes had only analyzed sex as an independent factor 20% of the time. This suggests that 80% of studies that used both sexes either combined the sexes in their analyses or used sex as a covariate. However, important information is lost when using sex as a covariate because whether or not there is a significant effect of the covariate does not indicate whether there are non-linear variations with sex and/or interactions of sex with treatment (Mefford and Witte, 2012). Thus, using sex as a covariate rather than an independent factor loses important information, and studies detailed in the following sections will highlight examples of this loss of information. It is important to note that the type of statistical approach for studying sex differences will be determined by the question being addressed in any one study. It is also important to keep in mind that some statistical approaches (e.g., linear regression or correlations) are optimal for exploring linear relationships. However, others approaches are optimal for exploring non-linear relationships (McDonald 2014) and need to be considered because hormones often have curvilinear (inverted U) associations with other variables. Thus, it is important to use statistical approaches that can capture those relationships (McDonald, 2014).

Methodological considerations are also important when studying both sexes. For example, studies using restraint stress in rats need to account for the size of a restraint tube in order for the stressor to be perceived equally between the sexes due to the simple fact that female rats are typically smaller than male rats. Furthermore, the sexes may differ in expression of a behavior, and behavioral tests may need different parameters or metrics to measure this. Recent examples of different expressions of behavior between the sexes are darting behaviors in females seen in response to conditioned fear (Gruene et al., 2015b) and head shakes in males seen in the forced swim test (Kokras et al., 2016). Additionally, the vast majority of studies examining aggression has focused on the “overt aggression” (i.e. attacks) males show when responding to a male intruder placed in their cage (so called “territorial aggression, e.g. Parmigiani et al., 1998). The male bias in aggression research was due to the known male predominance in human

aggression (de Almeida et al., 2015, see also the most recent (2014) U.S. Department of Justice—Federal Bureau of Investigation data showing a large male predominance (73.3%) in overall arrests, especially for violent crimes such as murder (88.5%; U.S. Department of Justice – Federal Bureau of Investigation, 2015) and the observation that attacks in females are very uncommon. Hence females were long considered non-aggressive, except when they were defending their pups called maternal or postpartum aggression (e.g. Palanza et al., 1994). However, with detailed, ethological, observation of behavior, it can be demonstrated that non-nursing females also display aggression (e.g., Scott and Fredericson, 1951; Ferrari et al., 1996). More than attacks, laboratory female rodents perform other agonistic behaviors aimed at establishing dominance over other females, such as following or chasing, pinning down, or aggressively grooming an intruder (e.g. Clipperton-Allen et al., 2010, 2011; Grant and Mackintosh, 1963). Similarly, in humans it well established that women tend to show predominantly indirect or verbal aggression, whereas males show more physical and direct aggression (Vaillancourt, 2013; Björkqvist, 1994; de Almeida et al., 2015). Hence, the proper behaviors need to be assessed when studying a phenomenon with known sex differences in its behavioral manifestation. With an ethological behavioral assessment female CD1 mice were shown to spend at least as much (Clipperton-Allen et al., 2010), if not more (Clipperton-Allen et al., 2011), time as males in intrasexual agonistic interactions against a same sex intruder to their cage (Clipperton-Allen et al., 2010, 2011). Although, males did indeed attack a same-sex intruder much more than females, females performed more dominance-related agonistic behavior than males (Clipperton-Allen et al., 2011). As such the overall time the females were engaged in agonistic interactions was not less than the overall time in males. In addition, when the role of the ERs was investigated, selective activation of ER α or ER β increased sex-typical agonistic behavior in mice, i.e. attacks in males and dominance-related behaviors in females (Clipperton-Allen et al., 2011). These results lead to the conclusion that female mice do not actually show less aggression than males, but they engage in different types of agonistic behaviors and that when one observes the appropriate behavior, the underlying hormonal regulation is actually similar in the two sexes. Similarly, when male-typical (physical/direct) and female-typical (verbal/indirect) agonistic behaviors are evaluated in humans, elevated levels of sex hormones or hormone precursors are associated with enhanced competitiveness in both men and women (de

Almeida et al., 2015). Collectively, these studies exemplify the diverse ways that sex differences can be present in many levels of neuroscience research.

Finally, it is also important to consider how endogenous changes in ovarian hormones in females (further discussed below) may account for discrepancies in preclinical research translating to clinical research. As mentioned above, depression is more prevalent in women than men. However, when rodent models of depression that have been validated in male subjects were used with females, they yielded mixed results with some studies indicating that female subjects are actually resilient rather than vulnerable to depressive-like behaviour (Dalla et al., 2005; Dalla et al., 2010). This may be because behavioral tests of depressive phenotypes, such as sucrose anhedonia, have been optimized in male subjects but not female subjects (also see discussion of sex differences in aggression expression above). Sucrose palatability can fluctuate with sex and the estrous cycle with low palatability reported during low estradiol conditions (Valenstein et al., 1967; Ossenkopp et al., 1996). Thus, if estrous cycle confounds outcome in these tests, the parameters must be optimized in females in order to appropriately assess behavioral phenotypes. Furthermore, if cycling female mice or rats are used, the experimental design should consider whether it is likely to influence the results if one particular phase of the estrous cycle is to be tested or if a categorization of proestrus (higher ovarian hormones)/non-proestrus (lower ovarian hormones) is sufficient. Statistically, estrous cycle phase can be handled by using it as a covariate and or reanalysis females alone with phase of cycle as a separate factor. Generally, in measures of hippocampus-dependent learning or spine density in the hippocampus, it is the proestrous phase that is statistically different from the other phases. Thus, we would suggest for hippocampal measurements that a proestrus/non-proestrus distinction be made and could be used as a covariate as a first step. However, it is likely that when tested at random, not many females will be in proestrus, and thus different approaches need to be used if specifically targeting estrous phase in the study. Hence, a better understanding of what contributes to modulations in performance in females is needed, including monitoring estrous cycle, for improving preclinical models of disease.

Studying Sex Differences: Treatment and Treatment Outcomes

An area of research that is often neglected is assessing sex differences in treatment efficacy of pharmaceutical drugs (Gartlehner et al., 2010). It is particularly imperative for sex

effects in efficacy be examined in diseases that already exhibit a sex difference in the etiology or manifestation of the disease, as this may indicate that treatment may also need to be different in males and females. Given that males and females have different physiological states that will ultimately influence how a drug acts and is metabolized, it is necessary to take sex and physiological state into account when evaluating the pharmacological efficacy of a drug. Indeed, there are sex differences in drug absorption, distribution, and elimination (Soldin & Mattison, 2009) which have important implications for evaluating the clinical efficacy and safety of drugs. As an example, zolpidem (trade name: Ambien, Intermezzo) is prescribed for insomnia and had been prescribed at equal doses for both men and women until women began reporting adverse reactions (Inagaki et al., 2010). Greenblatt et al (2014) reported that when administered the same dose of zolpidem as men, women had a slower clearance rate than men perhaps due to an inherent sensitivity to zolpidem in women. For this reason, the U.S. Food and Drug Administration (2013) now recommends that women be prescribed a lower dose than men. Given that dose of drug is sensitive to factors that differ by sex such as body weight and body fat composition, clinicians need to consider sex differences when considering doses for different drugs for the safety and efficacy of patient outcome.

As in the case of doses, efficacy may also be altered based on male and female physiology, because certain drugs may be more efficacious in one sex than the other or only efficacious in one sex but not the other. One review found that antiemetics were less efficacious in women than men and that sexual dysfunction as a side effect of the antidepressant paroxetine affected more men than women (Gartlehner et al., 2010). Furthermore, in the case of antipsychotics, the effects of clozapine and olanzapine are more pronounced in women than men (Marazziti et al., 2013; Crawford & DeLisi, 2016). Furthermore, age and hormone levels interact with antidepressant efficacy as SSRIS have different efficacy and metabolism in older women with and without hormone treatment in postmenopausal women (Thase et al., 2005; Ferguson & Hill, 2006). These findings suggest that sex may influence efficacy of certain treatments and side effects of treatments, perhaps due to differences in metabolism. In preclinical research, fluoxetine is metabolized faster in female mice than in male mice (Hodes et al., 2010). Female rats are also more sensitive to lower levels of fluoxetine as they show lower levels of immobility in the forced swim test (less depressive-like behavior) at lower doses (5 mg/kg) than male rats (Fernandez-Guasti et al., 2015). Furthermore, gonadal hormones can facilitate some of

the effects of fluoxetine to offset stress-induced depressive phenotypes in either male or female rats (Mahmoud et al., 2016; Wainwright et al., 2016). However, to date, preclinical research has not yet directly compared males and females in the same experiment within a valid model of depression and antidepressant exposure.

In clinical research, sex differences in fluoxetine are apparent when considering how to improve fluoxetine efficacy. Folate deficiency has been implicated in the etiology of major depression (Young & Ghadirian, 1989), and the therapeutic value of folate has garnered attention in neuroscience research. While folate supplementation alone does not appear to have substantial antidepressant properties, folate supplementation is effective as an adjunct therapy to fluoxetine (Coppen & Bailey, 2000). However, when analyzing the sexes separately, this effect was seen selectively in women and not in men. Specifically, 94% of women taking folic acid in addition to fluoxetine showed a significant reduction in Hamilton Depression Scale scores in comparison to 61% of women taking placebo and fluoxetine. Conversely, there was no significant difference in reduction in depression scores in men taking fluoxetine with adjunct folate (61% of men showed reduction in depression scores) or placebo and fluoxetine (64% of men showed reduction in depression scores). These findings illustrate how important sex as a factor can be when determining influence of treatment and how a treatment can be more valuable to one sex than the other. This study followed the recent recommendations from the U.S. Food and Drug Administration (2014) which states that the first set of statistical analyses should determine if there is an overall treatment effect, and if positive, only then analyze by sex. However, this order of statistical events is not effective. In fact, this may miss instances where a drug or treatment may be efficacious in one sex but not the other. For example, in a recent randomized control trial evaluating the efficacy of progesterone after traumatic brain injury (TBI), progesterone was found to not be effective despite previous positive Phase II trials (Wright et al., 2007; Xiao et al., 2008). However, the secondary analyses indicated that men were more likely to benefit from progesterone than women although this did not reach significance ($p=0.07$; Wright et al., 2014). The effect of progesterone to be more favorable in men on TBI outcome may interact with other factors such as severity of TBI or age of patients. Based on the present FDA recommendations, because there is no overall treatment effect, then sex differences would not be analyzed. This approach is a problem if treatment effectiveness proves to be different in one sex over another. Additionally, work from the Galea laboratory discovered that in postmortem tissue, depressed

individuals taking antidepressants exhibited greater levels of neurogenesis (ratio of immature to mature neurons). Although sex was not a significant factor in main or interaction effects, an *a priori* analysis revealed that this antidepressant effect on immature neurons was selective to women but not to men (Epp et al., 2013). Thus, as we mentioned above, we caution against making conclusions based solely on analyses done with both sexes collapsed into the same subject pool as vital information for one sex may be lost in this way. We recommend that sex differences be included in the statistical analyses and in situations where the biological differences between men and women are a reasonable *a priori* justification should be used with appropriate corrections for type I error to investigate differences between the sexes.

Studying Women (and Men): The Influence of Puberty, Aging and Reproductive Experience

Thus far, we have discussed the necessity of treating men and women as distinct subject groups. However, it is important to consider that physiology can dramatically change across the lifespan with puberty onset, reproductive experience and aging. For example, the pubertal rise in gonadal hormones coincides with an increased risk for anxiety and impulse-control disorders (Kessler et al., 2005) as well as the emergence of sex differences in depression (Ge et al., 2001). In post-pubertal women, there are cyclical fluctuations in estrogens and progesterone over the course of 28 days. These hormonal changes over the menstrual cycle correspond with changes in how psychiatric illnesses can present. As an example of this, in women suffering from schizophrenia, symptoms are exacerbated during low estradiol phase, implying that higher estradiol levels may protect against schizophrenia symptoms (Bergemann et al., 2002). However, the efficacy of estrogens as an adjunct therapy to prescribed antipsychotics is limited (Bergmann et al., 2005). Similarly, fluctuations in ovarian hormones are implicated in mood changes related to premenstrual dysphoric disorder (Steiner et al., 2003). Interestingly, use of oral contraceptives is associated with reduced incidence of mood disorders (Keyes et al., 2013) although there is a stronger association with oral contraceptives based on estrogens rather than progestins (Böttcher et al., 2012). Oral contraceptives, themselves, can lead to reduced activity of CYP1A2, an important enzyme for clearing drugs from the system (Hilli et al., 2008). In addition to CYP1A2, there are numerous cytochrome enzymes such as CYP3A4, CYP2D6, and CYP3C19 that are also heavily impacted by oral contraceptive use and influence drug action (Kokras et al., 2011). For this reason, women prescribed antidepressants like fluoxetine require a lower dose while taking

oral contraceptives as higher doses result in drug accumulation in the body (Damoiseaux et al., 2014). Menstrual cycle phase has also been an important consideration for treating breast cancer as survival after curative surgery is most effective in the luteal phase of the menstrual cycle (Kucuk & Atalay, 2012). Manipulation of ovarian hormones, such as ovarian hormones withdrawal from hormones simulated pregnancy can also be used to induce depressive-like phenotypes in female rats (Galea et al., 2001). Thus, both endogenous and/or exogenous changes in ovarian hormones can impact disease manifestation as well as treatment.

Mice and rats typically experience a 4-5 day long estrous cycle, and these cyclical fluctuations can influence neural and behavioral outcomes. Over the course of the estrous cycle, there are fluctuations in densities of CA1 hippocampal dendritic spines with more spines present during proestrus and estrus (high estradiol levels) but fewer during diestrus (low estradiol levels; Woolley et al 1990.) Additionally, estrous cycle fluctuations can interact with other systems in the brain such as the mesolimbic dopamine system (Thompson & Moss, 1997). Female rats in proestrus were more motivated to seek cocaine as well as enhanced cocaine self-administration in comparison to rats in diestrus, likely due to the potentiating role of estradiol on stimulant-induced dopamine release in the striatum (Becker & Cha, 1989). In addition, when an estrous cycle effect is observed, this can indicate that sex hormones are involved and inform further research. For example, the estrous cycle was found to affect social learning, or the acquisition of adaptive information from conspecifics. Female mice displayed a socially acquired food preference for a longer time when they were in proestrus than when they were in diestrus at the time of learning and testing (Choleris et al., 2011). Similarly, only females who were in proestrus at the time of learning still showed a socially acquired food preference when tested 24 hr later (as reported in Sánchez-Andrade et al., 2005). Because proestrus is the phase of the estrous cycle with high circulating estradiol levels, these results suggest estrogens promote social learning. This was indeed demonstrated, with 17 β -estradiol rapidly enhancing the social transmission of food preferences (Ervin et al., 2015). This effect of estrogens appears mediated by the GPER for rapid effects (Ervin et al., 2015) and ER β for long-term effects of estrogens (Clipperton et al., 2008). One concern is that the additional workload of daily monitoring estrous cycle may deter researchers from including female subjects. We emphasize that monitoring estrous cycle when relevant, and using it as a factor when it is appropriate for the original research question, is important rather than losing valuable information by simply not including female subjects. Thus,

it may not be as necessary to monitor estrous cycle daily during a six-week chronic paradigm because the animal will cycle through many stages during the experiment presumably, but it will be necessary to determine phase of estrous cycle in females in acute studies, during behavioural testing, and at the time of tissue collection.

Another biological event that is uniquely female, includes pregnancy and the postpartum, which are periods characterized by distinct physiological changes as well as greater vulnerability to psychiatric illness. During pregnancy, high concentrations of steroid and peptide hormones are secreted from the placenta, an endocrine gland generated during pregnancy (Hendrick et al., 1998). After parturition and into the postpartum period, steroid and peptide hormone levels plummet with the expulsion of the placenta and remain low throughout most of the lactation period (Brett and Baxendale, 2001). Along with changes to levels of estrogens and progesterone in women, there are dramatic alterations to the stress hormone system. Basal levels of glucocorticoids gradually increase over the course of pregnancy and remain high during lactation (due to its high metabolic burden) while the HPA axis becomes unresponsive to acute stressors (Brunton and Russell, 2008). Given these dramatic hormone alterations, it is perhaps not surprising that the female brain changes during pregnancy and postpartum. In fact, by parturition, the maternal brain shrinks by 8% and reverts to its original volume by 6 months postpartum (Oatridge et al., 2002). Thus, pregnancy and postpartum can impact the brain, and this may have functional implications for psychiatric illness at this time. These reproductive events need to be highlighted in neuroscience research to advance women's health. For example, a majority of women (approximately 80%) experience mild and transient mood changes or "baby blues" after childbirth. However, more severe mood disruptions can also arise at this time. Prenatal depression affects approximately 12% of pregnant women (Bennett et al., 2004), and postnatal depression affects approximately 10-15% of postpartum women (Gavin et al., 2005). Even more concerning, 40% of mothers experiencing postnatal depression are developing their first bout of depression (Wisner et al., 2013). While depression during pregnancy is a strong predictor of postnatal depression, prenatal and postnatal depression can occur in isolation (O'Hara and McCabe, 2013). The aforementioned physiological changes that differ between pregnancy and postpartum may then suggest that the biological circuits underlying depression occurring solely in pregnancy are different from those underlying depression occurring solely in the postpartum. More research directed towards understanding the diverging and converging mechanisms of

depression outside of pregnancy, during pregnancy, and during the postpartum period (O'Hara and McCabe, 2013) will better inform our understanding of maternal depression and given that there are marked physiological changes during these periods it is entirely possible that treatment may need to be altered.

Treating maternal depression has been complicated by a poor understanding of depression at this time as well as the unique physiology of women during this time. Maternal depression is classified as depression occurring during pregnancy and within the first four weeks of the postpartum period (DSM 5), although the greatest incidence of depression occurs 2-3 months postpartum (Gavin et al., 2005). As noted above, there are marked changes in steroid and peptide hormones from pregnancy throughout the postpartum which may complicate treatment and diagnosis. Indeed, treating maternal depression is challenging as antidepressant efficacy is limited in pregnancy and postpartum (DeCrescenzo et al., 2014). Generally, mothers suffering from depression are prescribed the same antidepressants that are used to treat major depression in men and women. Research from the Galea laboratory found that maternal fluoxetine during the postpartum period was unable to prevent glucocorticoid-induced depressive-like behaviour and reduction in hippocampal neurogenesis in dams, unlike in nulliparous rats (Workman et al., 2016). Thus differences in drug efficacy during the postpartum may not be surprising given that steroid and peptide hormones, metabolism, and the brain change during pregnancy and the postpartum (Brett & Baxendale, 2001).

Finally, motherhood can moderate disease risk and, perhaps, treatment well beyond the time of reproduction. Untreated postpartum depression results in a four-fold increased risk for depression after the postpartum period, resulting in a life-long burden of disease (Joseffson & Sydsko, 2007). Even much later in life, reproductive experience increases risk for Alzheimer's disease in women but not men (Ptok et al., 2002). Interestingly, research from the Galea laboratory found that in middle age female rats with no reproductive experience, hippocampal cell proliferation is not significantly affected by acute estradiol or estrone administration (Barha et al., 2011). However, in multiparous, middle age female rats, exposure to either estradiol or estrone increased hippocampal cell proliferation. This highlights that even long after mothers are pregnant or actively raising pups, the experience of motherhood can render the brain more sensitive to the effects of estrogens. This has important implications for preclinical research in terms of taking reproductive experience into account as this may play a moderating role in neural

processes. This also has important clinical implications in terms of how reproductive experience may be an additional factor in the relationship between sex, genotype, and disease outcome.

Studying sex differences: genotype interactions and individual differences

Men and women differ in genotypes which is expressed in every cell in the body (de Vries and Forger, 2015). There are sex differences in how the genome is read (Bellott et al., 2014; Ellegren and Parsch, 2007) and in the epigenetic modification of gene expression (Shen et al., 2015). Sex interacts with Val66Met polymorphisms of the brain derived neurotrophic factor (BDNF) gene to influence loneliness in adolescence (Verhagen et al., 2014), stroke rehabilitation (Mirowska-Guzel et al., 2014) and regional cerebral blood flow in the prefrontal cortex and hippocampus (Wei et al., 2012). These sex by genotype interactions may be especially relevant for diseases like major depression which are complicated by heterogeneous presentation.). Women are more likely than men to present with major depressive disorder (Schuch et al., 2014). However, the presentation of major depressive disorder diverges between the sexes. Women with major depression are more likely to endorse symptoms such as depressed mood, sleep disturbances, somatic and gastrointestinal complaints and changes in weight than men (Angst et al., 2002; Schuch et al., 2014). Furthermore, women with depression are more likely to present with either co-morbid anxiety or have greater lifetime prevalence of anxiety than men (Young et al., 2009; Schuch et al., 2014). However, men with major depression are more likely to present with co-morbid alcohol or substance abuse and endorse symptoms related to anhedonia (Angst et al., 2002; Schuch et al., 2014). Women are more likely than men to present with either atypical and melancholic depression, although a greater proportion of men have melancholic depression whereas a greater proportion of women to have atypical depression (Hildebrandt et al., 2003). Subtypes of depression differ in their biological, inflammatory, and metabolic correlates (Lamers et al., 2013). While both sexes may still be diagnosed with either of these subtypes of depression, melancholic depression was associated with the long allele of the serotonin-transporter-linked polymorphic region (5-HTTLPR) of *SLC6A4* (serotonin transporter gene) particularly in women not men (Baune et al., 2008). Even within women, the AA variant of *ESR2* gene for ER β was associated with increased risk for lifetime depression compared to other polymorphisms of the same gene (Keyes et al., 2015). Polymorphisms of the *ESR1* gene for ER α also interacted with postmenopausal hormone treatment to increase depression risk selectively among homozygous T

allele carriers (Keyes et al., 2015). More studies such as these will improve our understanding of how major depression manifest differently in men and women in the clinical population.

If individual differences related to sex and genotype are apparent in the presentation of disease, then treating these diseases will need to take individual differences into account. For example, in continuing to examine the case of major depression, prescribed antidepressants initially provide relief for only a third of patients (Trivedi et al., 2006) and cumulatively for 67% of patients (Rush et al., 2006), and of those who respond to treatment, 57% will experience relapse due to a failure in continual drug efficacy (Byrne and Rothschild, 1998). The fact that only a subset of the clinically depressed population responds to these treatments that specifically target the monoamine system suggests that perhaps this system is not the primary site of pathology in all depressed patients. There are sex differences in serotonin synthesis and content (Carlsson & Carlsson, 1988; Nishizawa et al., 1997). Additionally, men have more serotonin reuptake binding sites (which antidepressants specifically target) than women in the thalamus, insula, and hippocampus (Jovanovic et al., 2008), which may impact antidepressant efficacy. Collectively, these studies indicate that sex and genotype may be contributing factors in antidepressant inefficacy, particularly for selective serotonin reuptake inhibitors, and also highlight that sex may play an important role in the field of pharmacogenetics. These findings represent an opportunity to connect sex, genotype, and even multiple theories describing depression (serotonin, stress, glutamate, etc.) into a novel way of viewing the disease and how we can better understand treatment of depression and likely even other psychiatric diseases.

Conclusions

The evidence we reviewed here points to a crucial role for considering the role of sex differences in disease manifestation and treatment in both preclinical and clinical research. Prioritizing sex differences in neuroscience research will require specific alterations to current research methods. For instance, we reviewed how fluctuations in ovarian hormones can contribute to behavioural and neural changes which may be important for treating brain disease. There is a misleading conception that only female subjects experience these hormone fluctuations unlike male subjects. However, men show marked alterations in testosterone levels across the day (Diver et al., 2003), with increasing age (Zirkin and Tenover, 2012), and seasonally (Moffat & Hampson, 2000). These alterations in levels of testosterone may be associated with variations in cognitive performance in men (Holland et al., 2011) and are related

to changes in fMRI activity patterns (Schöning et al., 2007). It is currently not known whether circulating levels of testosterone in men or estradiol in women show similar or different effect sizes on brain activation and/or cognition. Other endogenous ovarian hormone changes such as those observed in pregnancy and postpartum result in permanent changes to the maternal brain. This has important implications for factoring reproductive experience for disease risk. Moreover, exogenous hormone changes via hormone replacement therapy may interact with these changes and influence disease outcome. Thus, fluctuating hormone levels are an important consideration for male and female research.

Consideration of sex differences, sex hormones, reproductive experience, and genetic differences will better inform precision medicine options. As neuroscience research advances and prioritizes individual differences, the current ‘one size fits all’ treatment approach for all psychiatric diseases seems naïve. Studies investigating how sex and genotype interact will have important implications for understanding disease manifestation and treatment over the lifespan for both males and females. Ultimately, unraveling the complex etiology of psychiatric diseases as well as optimizing treatments will be improved by including sex and genotype in understanding physiology, treatment ramifications, and disease susceptibility.

Conflict of Interest

The authors have no conflict of interest to declare.

Authors’ Contributions

AG and LAMG conceived of the review. AG, LAMG, and EC wrote and contributed to the synthesis of the review paper equally.

Box 1. Important Considerations for Science Policy

- Limited consideration for sex differences is contributing to the poor translation between preclinical and clinical research. Given that many diseases are characterized by a sex difference in prevalence and/or manifestation, there is a need to include a consideration for sex in the etiology, and treatment of disease.
- Men and women are biologically different, and research needs to appropriately and accurately consider sex differences. The present set of FDA recommendations to only analyze for sex differences if an overall treatment effect is problematic as sex differences may be erroneously neglected via this statistical method (U.S. Food and Drug Administration, 2014). We recommend that when including both sexes in the

experiment, every statistical analysis must use sex as a factor and not as a covariate throughout all statistical analyses.

- Studying sex differences is not the same as improving women's health research. There is a need for funding and researching how physiology over the lifespan interacts with the brain. For instance, maternal mental health is under-recognized and needs to be prioritized. Pregnancy and postpartum are sensitive and vulnerable times for women to present with psychiatric illnesses. The physiological changes of pregnancy and postpartum in women is dramatically different from cycling women which likely affects drug action and metabolism. Thus, there is a need for funding research to study disease at this time in order to better inform mothers as well as clinicians for how best to treat these illnesses.

Box 2. Important Considerations for Scientists

- Data demonstrating an absence of a sex difference is as important as data demonstrating a sex difference. If an experimental manipulation results in male and female subjects being equally affected, that is a vital contribution to neuroscience research as it may suggest converging mechanisms for both sexes. Alternatively, a lack of sex difference in behavior may not reflect a lack of sex differences at the mechanistic level or in neural manifestations. Thus, we caution researchers from discarding data or withholding reporting a lack of sex differences.
- Monitoring estrous cycle is an important contribution to better understanding female subjects. We urge researchers to monitor estrous cycle when necessary.
- For preclinical research modeling diseases, if the disease has a sex difference, then the model should also consider incorporating both sexes into the research unless a proper justification can be provided for using one sex instead of the other.
- Using sufficient sample sizes of men and women as well as using sex as a factor instead of a covariate is important for better understanding disease. This does not necessitate that all research must use both sexes. Rather, if both sexes are being utilized, published research must specify how many males and how many females were used. This is necessary for *in vitro*, preclinical, and clinical research.
- Presentation and treatment of disease are altered by reproductive experience. There is a need for more research informing how the brain changes during and after reproductive experience as well as how to improve treatments targeting maternal mental health.
- We argue that evaluating sex differences in preclinical models will need to prioritize this aspect in the methods of the experiment. As an example of this, in evaluating aggression in rodents, the territorial aggressive behaviors that males exhibit are unlike the agnostic behavior that females exhibit. Thus, studies attempting to delineate sex differences in aggression incorporate these differences into the methods of the experiment in addition to maintain sufficient sample size of both sexes.

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	Worldwide Healthy Life Years Lost (in millions)	Is there a Sex Difference in PREVALENCE?	Is there a Sex Difference in CLINICAL PRESENTATION?	Is there a Sex Difference in TREATMENT?
Unipolar depressive disorder	65	✓ F > M (Angst et al., 2002)	Women exhibit more symptoms of depression than men and seek emotional support (Angst et al., 2002). Atypical depression affects a greater proportion of women than men (Schuch et al., 2014).	Men may respond better to tricyclic antidepressants than women (Keers & Aitchison, 2010)
			Men exhibit fewer symptoms and seek physical exercise or alcohol as an outlet (Angst et al., 2002). Melancholic depression affects a greater proportion of men than women (Hildebrandt et al., 2003).	Women may respond better to selective serotonin reuptake inhibitors (Keers & Aitchison, 2010).
Alcohol Use Disorders	24	✓ M > F (Ley-Ran et al, 2013)	Women are more likely to be diagnosed with depression and co-morbid alcohol use disorder (Goldstein et al., 2012). Alcohol abuse progresses more rapidly in women than in men (Randall et al., 1999).	Women are more likely to face financial, familial, and social pressures, posing obstacles to accessing treatment (Beckman & Amaro, 1986). These obstacles are also detrimental for pregnant women and can lead to fetal alcohol spectrum disorder. Better understanding of alcoholism in both sexes can improve the social barriers to treatment.
			Men are more likely to be diagnosed with bipolar II and co-morbid alcohol use disorder. (Goldstein et al., 2012)	
Schizophrenia	17	=	Women are diagnosed primarily between 25-35 yo and in peri-menopausal period (Ochoa et al., 2012).	Haloperidol (typical antipsychotic) or olanzapine (atypical antipsychotic) work equally well in reducing symptoms in men (Goldstein et al., 2002).
			Men are more likely to be diagnosed in adolescence (15-25 yo; Ochoa et al., 2012) with schizophrenia. Men with schizophrenia show more cognitive deficits (attention, verbal memory; Goldstein et al., 1998) and smaller temporal lobes than women with schizophrenia (Bryant et al., 1999).	Olanzapine but not haloperidol reduced symptoms in women (Goldstein et al., 2002). Another report indicates that women overall show a greater improvement in response to antipsychotic medication than men (Usall et al., 2007). Further research is needed to understand this.
Bipolar affective disorder	14	=	Women with bipolar I disorder are more likely to present with depression as their first mood episode. Women also report greater changes in weight and insomnia during the depression episode than men (Kawa et al., 2005).	Current mood stabilizers work equally well in men and women. However, treatment options are limited for pregnant and lactating women. (Arnold 2003).
			Men with bipolar I disorder are more likely to present with mania as their first mood episode (Kawa et al., 2005).	
Alzheimer's Disease	11*	✓ F > M	Women are more likely to present with co-morbid anxiety and/or depression and verbal agitation (Zuidema et al., 2009). Women exhibit more neurofibrillary tangles in the brain than men). Women with Alzheimer's disease are more likely to exhibit greater impairments in cognition than men (Laws et al., 2009; Chapman et al., 2011; Irvine et al., 2012).	Women with Alzheimer's disease exhibit greater improvement to the cholinesterase inhibitors donepezil and rivastigmine than men with Alzheimer's Disease (Scacchi et al., 2014).
			Men are more likely to present with aggressive behaviour and diurnal disturbances (Kitamura et al., 2012).	However, there are presently few options for treating Alzheimer's disease for either sex. Including sex differences and genotype in drug development could greatly improve our understanding of how to treat this disease.