Sex and gender in psychoneuroimmunology research: Past, present and future

Beth D. Darnall[1] and **Edward C. Suarez**[2]

[1]Department of Anesthesiology & Perioperative Medicine, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd, UHN-2, Portland, Oregon 97239−3098. Email: darnallb@ohsu.edu

[2]Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, PO Box 3328, Durham, NC 27710. Email: suare001@mc.duke.edu

Abstract

To date, research suggests that sex and gender impact pathways central to the foci of psychoneuroimmunology (PNI). This review provides a historical perspective on the evolution of sex and gender in psychoneuroimmunology research. Gender and sexually dimorphic pathways may have synergistic effects on health differences in men and women. We provide an overview of the literature of sex and gender differences in brain structure and function, sex steroids, gender role identification, hypothalamic-pituitary-adrenal axis function, genetics, immunology and cytokine response. Specific examples shed light on the importance of attending to sex and gender methodology in PNI research and recommendations are provided.

Keywords

gender; sex differences; immune; hypothalamic-pituitary-adrenal axis; sex hormones; genes

1. Introduction to sex and gender research

In 1985 a report issued by the United States Public Health Services noted that outside the field of reproductive physiology, little was known about women's health. While the number of research studies examining sex- and gender differences has grown significantly in the past 25 years, our knowledge of such differences has been hampered by research designs that either overlook sex and gender or apply suboptimal methodology. Until recently, a lack of consensus on how to address methodological concerns and how to define sex and gender also limited progress in all areas of science (Wizemann, 2001) including psychoneuroimmunology (PNI).

This review offers a broad overview of the historical progress of sex and gender in PNI research and the influence that national policy had on promoting research in this area. We discuss scientific evidence that illustrate the relevance of sex and gender and provide examples that show how studies that examine these associations may yield rich and novel findings. Toward promoting rigorous science, we highlight common pitfalls and opportunities associated with sex and gender research, and provide directions for evaluating the effects of sex on brain,

Corresponding Authors: B. D. Darnall and E. C. Suarez.

behavior and immune system interactions. But first, it is important to address the question: what do we mean by sex and gender?

A. Sex and gender terminology

Sex and gender are terms that have often—and inaccurately—been used interchangeably. To maximize the distinction between these two terms the Institute of Medicine issued the following definitions; sex is the "classification of male or female according to their reproductive organs and function assigned by the chromosomal complement," whereas gender comprises "a person's self-representation as male or female and the social and cultural influences of the gender 'role' that influence cognition, emotion, behavior, and choices" (Wizemann, 2001). Providing specific definitions facilitates a more focused discussion of the effects attributed to sex, or the chromosomal fact of being male or female, and gender, a more complex construct that incorporates the notion of being male or female in his or her own culture. While seemingly discrete, these categories intersect and we recognize that both structural and functional sex differences mediate the influence of gender in science and in practice. Some have even suggested that gender is best viewed as a continuum with a person's sense of gender changing during a person's lifetime (Wizemann, 2001). Thus, parsing a purely sex-based difference in humans is more problematic than in animal studies. Throughout this article, when the context involves both sex and gender influences, the term sex/gender is used.

B. Early studies in psychoneuroimmunology

While it is now well recognized that sex, gender and levels of sex steroid hormones profoundly affect the immune system, our understanding of how sex and gender influence the immune system has grown exponentially in the last two decades. This growth is clearly evidenced by a content review of the initial studies published in *Brain, Behavior and Immunity*, and specifically, how researchers at that time handled sex and gender. Some researchers failed to state the sex of the animal (e.g., Kusnecov et al., 1987). In studies that included men and women, the researchers never discussed the possibility of sex and/or gender-differences (Glaser et al., 1987; Gorczynski and Kennedy, 1987), and in those studies that included only men or only women, the authors never addressed the generalizability of their findings (Gorczynski, 1987; Irwin et al., 1987). Foreshadowing things to come, the authors of one study that included both men and women suggested that sex steroids might play an important role in immunity. For the most part, these early-published studies only hinted at what is now more clearly evident in the pages of *Brain, Behavior and Immunity*, in PNI research, and in many other areas of science: sex and gender matter.

2. Historical and political progress

Important policy mandates called for inclusion of both sexes in research, and these policies spurred the increasing focus on sex/gender differences that has occurred in the past 15 years. In 1993, the National Institutes of Health (NIH) mandated that clinical trials funded by NIH include a female sample unless exclusion was scientifically defensible (e.g., testicular cancer research). In 1998, the Federal Drug Administration began requiring new drug applications to include data by sex for drug safety and efficacy. Critics have noted that so far there has been inadequate compliance to such policies, despite scientific evidence supporting sex/gender as significant determinants of health-related processes (Fish, 2008; Wizemann, 2001). In 1996 and 1997, the NIH Office of Research on Women's Health sponsored national meetings designed to foster an agenda for future women's health research; their 6-volume report was particularly relevant to PNI research as it noted the importance of sex differences in normal and abnormal immune function and proposed additional research to examine the effects of sex steroid hormones on the immune response (Whitacre, 2001). That same year sex/gender research received additional support when the National Academy of Sciences acknowledged

that "being male or female is an important basic human variable that should be considered when designing and analyzing studies in all areas and at all levels of biomedical and health-related research."

The trajectory of PNI research has largely developed in tandem with these policies. The past 15 years have seen the expansion of our understanding of the interactions among the neural, behavioral, endocrine and immune systems. The field of PNI has been both challenged and strengthened by the resulting diversity of ideas, paradigms, and programs. Sex and gender research have been products and beneficiaries of this diversity, and while these areas have moved closer to the forefront of PNI research, there are still many gaps in our knowledge. The following sections provide an overview of the progress of sex/gender research in the pathways central to the foci of PNI.

3. Primary pathways associated with sex and gender differences in PNI research

Sex/gender influence factors inherent to PNI research through various pathways, and we focus on a few of the more prominent pathways. The study of sex/gender dimorphism is arguably in its infancy in some domains, but important findings in the current literature underscore the need to consider such differences in research at all stages, from design to interpretation of findings.

A. Brain structure, function, and neurochemistry

Between sexes, structural differences in the brain have been identified using morphometric analysis of magnetic resonance imaging (MRI). For example, although men tend to have greater brain volume and higher white matter volume (Gur et al., 1999), women tend to have higher grey matter tissue and greater cortical complexity in the frontal and parietal regions (Luders et al., 2004). Cortical complexity is a measure that quantifies the spatial frequency of gyrification and fissuration of the brain surface. Increased complexity implies more cortical surface area, essentially suggesting that women's brains are equipped to make good use of a smaller space. Sex-specific cortical complexities may contribute to sex-specific abilities and/ or behavioral differences. Increasingly, scientific findings are suggesting that sex differences in structure influence brain function. For instance, Goldstein and colleagues (2005) have shown that parts of the orbital frontal cortex region are relatively larger in women than in men, as are parts of the limbic cortex, areas involved in emotional functioning, social behavior, and higher order cognitive skills such as reasoning and decision making (Fuster, 1991).

In addition to these structural differences, sexual dimorphism in brain function is evident from functional magnetic resonance imaging (fMRI) studies showing women access areas of the brain associated with pain, verbal fluency and imagination more robustly than men (Andreano and Cahill, 2006; Cahill, 2003; Cahill et al., 2004; Cahill and van Stegeren, 2003; Canli et al., 2002), although it should be noted that sociocultural and gender influences were not controlled in these studies. One fMRI study used analysis of ovarian hormones in a within-subjects design to examine brain activation patterns in women during affective response inhibition comparing low estrogen and progesterone (early follicular phase) and high estrogen and progesterone (mid-luteal phase) groups (Amin et al., 2006). Luteal phase estradiol levels were found to positively correlate with activation in the anterior cingulate and dorsolateral prefrontal cortex (brain regions associated with affective processing) while inhibiting response to positive words; a similar relationship was not found for the follicular phase. Luteal phase estradiol was also found to negatively correlate with activation while inhibiting response to negative words. The authors concluded that their results supported the role of estrogen as a significant modulator of affective processing.

Although estrogen is commonly thought to be the primary mediator of emotional response differences between sexes, brain morphology studies suggest that structural brain differences between men and women may also play a role in emotion regulation. At the cellular level, women have greater neuron density in parts of the temporal lobe cortex associated with language processing and comprehension. While evidence suggests that females have superior verbal ability and males have superior spatial ability (Sherwin and Henry, 2008), the topic remains one of debate and warrants further research (Wallentin, 2008). Interestingly, men and women process emotional memories by using different hemispheres of the amygdala (Amin et al., 2006; Cahill et al., 2004; Cahill and van Stegeren, 2003; Canli et al., 2002). With women having a higher percentage of grey matter, lower volume of white matter, and larger posterior corpus callosum, the collective evidence suggests that these prominent anatomical dimorphisms may account for sex differences in behaviors that require varying amounts of interhemispheric communication relative to intrahemispheric communication. Along with hormonal, psychological, social/environmental and cultural factors, morphological differences may help explain differences between men and women with respect to emotional behaviors and subsequent immune responses.

B. Sex steroid hormones

Sex steroid hormones include androgens, such as testosterone; estrogens, such as estradiol; and progestins, such as progesterone. These hormones are present in both sexes with higher levels of circulating androgens in males and higher levels of estrogens and progestins in females. Sex steroid hormones are known to be potent mediators for sex differences across disciplines, including the fields of neuroscience, psychiatry, and immunology; for this reason sex steroid hormones are frequently given first consideration when unanticipated dimorphic findings emerge. Not surprisingly, the effects of sex steroid hormones on immune activities are heterogeneous. For example, sex-steroid effects may be immunosuppressive, as is the case for testosterone's depressing effect on macrophage immune function (Wichmann et al., 1997), or sex-steroid effects may be immunostimulatory, as exemplified by estrogen-mediated improvements in macrophages cytokine production following trauma-hemorrhage (Suzuki et al., 2008). Sex-steroid effects are also known to depend upon steroid levels. Cellular responses to sex steroids also dependent upon the expression of hormone receptors that include androgen and progesterone receptors and two estrogen receptors (ER), ER-α and ER-β. Receptor-mediated effects on immune responses are not well elucidated but it is generally thought that the actions of androgens and progestins are immunosuppressive whereas estrogens are generally regarded as enhancers of immune response (Cutolo and Wilder, 2000; Lahita, 2008).

Sex steroids may also influence immune activity indirectly. For example, estrogen has also been shown to modulate both anxiety (Bodo and Rissman, 2006; Lund et al., 2005) and depression (via neurotransmitters 5HT [serotonin] and dopamine) (Zhou et al., 2002) whereas testosterone has been more frequently linked with sexual behavior and behavioral aggression in male animals (Kiyokawa et al., 2004; Pinna et al., 2005). Animal studies using brain imaging have suggested that estrogen is associated with amplified sensitivity to stress and impaired cognitive processing (Shansky et al., 2006). By extension, animal models also have shown that estrogen modulates behavior by altering neurochemistry (Zhou et al., 2002). The relation of sex steroids to anxiety, depression and aggression raises the possibility that the relation of depression and other indices of negative affects to immune markers differ between men and women as a consequence of indirect and direct effects of sex steroids. This possibility is particularly salient for depression given that women are disproportionately affected by depression. In light of the number of published studies that have examined the relation of depression to immune function, it is important that PNI researchers address the questions; Are there gender specific associations between depression and immune markers and are these

differences between men and women due to sex differences in the effects of sex steroid hormones on immune markers? We stress that differences in the prevalence of depression between men and women do not address these questions.

In addition to depression and depressive symptoms, gender acts as a moderator between behavior and immune activity in other areas. For example, Suarez showed that sleep problems, more often reported by women, show gender specific associations with markers of inflammation and that these differences may reflect sex differences in underlying mechanisms influenced by sex steroids (Suarez, 2008). Thus, it may also be the case that depression and severity of depressive symptoms have more deleterious effects on immune activity in women than in men. Unlike androgens in men that decline over decades, the potential for estrogens to affect immune function is further complicated by variability across the monthly menstrual cycle. In premenopausal women, estrogen has been shown to affect mood and anxiety similarly by influencing serotonergic, dopaminergic, γ-aminobutric acid (GABAnergic) and adrenergic pathways (e.g., Amin et al., 2005; Epperson et al., 2002; Pandaranandaka et al., 2006). Consistent with estrogen's effects on various pathways implicated in mood regulation, negative mood is more often experienced during the late-luteal phase (Amin et al., 2005; Payne, 2003). More directly, functional MRI studies have shown that estrogen levels moderate neural patterns associated with affective processing (Amin et al., 2006). Therefore, the effects of estrogen on immune function may be both direct and indirect via estrogen's influence on affect, emotion, brain function, and behavior. Combined, these findings highlight estrogen as a conspicuous player across the spectrum of biological processes including immune activities such as inflammation. However, it is not yet possible to categorize the valence of estrogen's role because it is known to vary. Here we provide evidence showing the seemingly binary role estrogen plays in health-related outcome.

On the whole, preclinical and clinical evidence supports estrogen as a neuroprotectant (Brann et al., 2007; Noppens et al., 2008; Vagnerova et al., 2008). In addition, estrogen enhances dendritic spine density in the prefrontal cortex of young and old monkeys and in young rats (Brann et al., 2007). Following neuronal injury, neuronal regeneration and plasticity have been demonstrated, suggesting that estrogen facilitates the brain to repair and remodel itself (Suzuki et al., 2007). Other studies have suggested that estrogen hampers microglial action, thus suppressing inflammatory factors that would increase neural damage [for a review see Brann et al (2007)]. The presence of estrogen also promotes neuroprotection from neurodegenerative disorders, such as Parkinson's and Alzheimer's diseases (Brann et al., 2007; Weiss, 2007).

While estrogen offers neuroprotection on the one hand, its effects are complex and we see it also affords amplification of stress responses and conditioning processes on the other hand. Preclinical research has shown that estrogen mediates stress-related dysfunction in the prefrontal cortex (Shansky et al., 2004). The influence of estrogen on brain function may be responsible for women's heightened vulnerability to major depressive disorder and anxiety disorders (Shansky et al., 2004). Thus far research in this area has shown how estrogen's amplification qualities are detrimental to the health of female animals and women, although it will be intriguing to discover novel ways in which the amplification properties of sex steroids might be used to promote health in *both* sexes.

While estrogens have received much of the attention of sex-steroid researchers, recent work has focused on androgens as mediators for health and disease processes. Similar to estrogen in women, testosterone appears to confer neuroprotective benefit for men (Pike et al., 2008) with evidence suggesting that these benefits may be sex specific (Spritzer and Galea, 2007). Animal studies have shown injections of higher doses of testosterone, but not estradiol, results in a significant increase in hippocampal neurogenesis. These latter observations have led researchers to suggest that testosterone enhances hippocampal neurogenesis via increased cell

survival in the dentate gyrus through an androgen-dependent mechanism (Spritzer and Galea, 2007). Interestingly, estrodial in these animals failed to alter hippocampal neurogenesis suggesting that the enhancing effects of testosterone in this region occur through androgen-dependent, but not estrogen dependent, mechanisms. Other studies have shown similar neuroprotective effects of testosterone following neural injury. Barreto et al (2007) showed that, following a stab wound brain injury, administration of testosterone induced a reduction in the volume fraction of major histocompatibility complex-II (MHC-II) immunoreactive microglia suggesting that regulation of gliosis may be part of the neuroprotective mechanism of testosterone.

Although genetic factors are most certainly involved, one may wonder if the enigma lies at the heart of the sex differences in longevity. While women tend to have greater rates of physical disease, they enjoy greater longevity than men. Indeed, for many of the most frequent causes of death, males die at a higher rate across the lifespan (Austad, 2006). Future studies in psychoneuroimmunology may benefit from hormonal profiling of both sexes and examining gender specific effects of sex steroid hormones on immunological parameters implicated in health and disease. The stark sex differences evident in life and in death shed light on the truism that sexual dimorphism in biology, phenomenology, pathophysiology, and longevity are too important and too vast to overlook in PNI research.

C. Gender

In the previous sections our focus was on the role of sex steroid hormones and brain function. We described the distinction between the sexes and how these distinctions could illuminate observed sex differences in measures of immune function. Now we turn our attention to gender, a much more complex construct, and gender differences. As noted in the introduction, gender refers to "a person's self-representation as male or female" or "how that person is responded to by social institutions on the basis of the individual's gender presentation" (Wizemann, 2001). Given the relevance of culture and social environments in defining gender, it is not surprising that a large body of research has focused on examining how men and women differ on measures of risk taking behavior (Byrnes et al., 1999), income/salary (e.g., Blau and Kahn, 2000) and educational performance (e.g., Brown and Joseph, 1999; Haist et al., 2000; Llabre and Suarez, 1985). For the most part, these studies have shown that, in contrast to women, men exhibit more risky behaviors, receive higher salary for the same job and or educational attainment, and perform better on tests of mathematics and spatial ability but not on tests of verbal ability. These reported gender differences, and specifically differences in income and education, are relevant to PNI research given recent evidence linking low socioeconomic status (SES), as indexed by years of education and income, and elevations in markers of inflammation (Rathmann et al., 2006).

Some research has examined the influence of gender role on the appraisal of stressors and how such differences impact physiological responses to stressful tasks (Lash et al., 1995; Stroud et al., 2002). In such gender role studies, the gender orientation of a stress task is manipulated by tailoring the language of the introduction to the task to reference either masculine role, feminine role, or gender neutral role. One study examined the effect of gender role matching on cardiovascular stress responses following two tasks, the cold pressor test and a task that included memory testing, in 108 men and women (van Well et al., 2008). Gender relevance was manipulated by tailoring the introduction of the stressors in the following ways. The masculine-relevant introduction discussed a research-related association between ability to tolerate the cold pressor test and good physical and mental condition. The feminine-relevant introduction implied the tests were linked to one's ability to form meaningful relationships and be emotionally supportive. The gender-neutral introduction discussed the goal of the experiment as being accuracy of physiological measurement. The researchers found that, in

accordance with prior work, cardiovascular stress responses were significantly influenced by the interaction between subjects' gender role identification and the gender relevance of the stressor in gender-matched fashion for the cold pressor test. Women showed significantly greater cardiovascular responses to the stress task following female-relevant introductions only (and not following masculine-relevant or gender-neutral introductions). Men showed significantly greater cardiovascular responses following the masculine-relevant introductions only (and not following female-relevant or gender-neutral introductions). However, whether a gender-match or gender-mismatch introduction increased cardiovascular responses depended on the stressor type. In contrast to the gender-match effect found for the cold pressor test, the memory task evidenced stronger cardiovascular responses for a gender-mismatch introduction. The investigators hypothesized that in accordance with prior theory on the topic (Tomaka 1993) the active nature of the memory task tapped challenge appraisal from participants, whereas the passive nature of the cold pressor test is more likely to elicit threat appraisal (van Well et al., 2008). Their results highlight the influence of gender on situational appraisal and subsequent physiological responses, and also highlight the need to either control for this confound in stress research or to conduct specific gender analyses.

Gender is also a factor that impacts treatment approaches in medical settings. Consistent with the complexities of defining gender, a number of studies have suggested that social and behavioral factors underlie gender differences in therapeutic adherence and treatment discontinuation of such medical conditions as HIV/AIDS (Floridia et al., 2008) and coronary heart disease (Kattainen et al., 2005). Thus, revascularization is less frequently performed in women than men and fewer women use antithrombotic medications (Kattainen et al., 2005). While medical conditions differ across studies, the overall conclusions are that gender is a significant predictor of primary and secondary treatment with women receiving less than optimal care in all areas of the world. Gender differences in treatment approach and delivery are important pathways that exemplify how gender influences health outcomes.

D. HPA axis functioning

Scientists in the field of psychoneuroendocrinology have offered compelling data that show marked sex/gender differences in HPA functioning (Binder et al., 2008; Kirschbaum et al., 1992; Uhart et al., 2006). To begin with, pre-pubertal differences in the HPA stress response are absent (Romeo, 2005; Romeo et al., 2008), as are differences in the incidence of many forms of psychopathology, such as anxiety and major depression. However, after puberty and onset of sex-steroid differentiation, gender differences in HPA axis reactivity emerges (Uhart et al., 2006), as does a female preponderance for the prevalence of depression and anxiety disorders (Wilhelm et al., 2002). While some conflicting data prevents wholly clear-cut distinctions between men and women, lack of concordance also suggests an influence of other unconsidered factors.

As was noted in the previous section, it is difficult to discuss stress experiments in humans without accounting for the influence of gender. Kajantie and Phillips (2006) recently reviewed the effects of sex/gender and hormonal status on the physiological response to acute psychological stress. These authors' summary of the literature led them to conclude that men and women show differences in HPA axis function. Among women, HPA axis responses increase following menopause (Seeman et al., 2001) and administration of hormone replacement therapy mitigates such increases (Lindheim et al., 1992). In addition to menopausal status, studies show women's HPA responses vary by phase of the menstrual cycle (Tersman et al., 1991), usage of oral contraceptives (Kirschbaum et al., 1999b), and pregnancy status (de Weerth and Buitelaar, 2005). On the whole, pre-menopausal women evidence lower HPA axis responses than men. However, the magnitude of HPA axis responses of women in the luteal phase approaches that of men (Kirschbaum et al., 1999b; Rohleder et al., 2001;

Tersman et al., 1991), and this variance within the cycle serves to warn researchers against classifying subjects based on sex only. It is recommended that the differential influence of the ovarian cycle be considered in study designs. An excellent review and guide for methodological concerns in research on HPA functioning is detailed elsewhere (Kudielka and Kirschbaum, 2005).

Although it is tempting to conclude that differences in HPA responses based on sex-steroid modulation indicates a purely sex-based difference, other factors may be involved. For instance, it has been suggested that m en and women evidence differential stress responses in HPA functioning and immune parameters based on the *type* of stressor employed (Uhart et al., 2006), indicating potential gender influences may be in play. Lastly, evaluating the effects of sex and gender is also dependent upon methodological issues in assessing HPA axis function and specifically whether assessing total or bioavailable cortisol and whether measuring salivary or plasma indices of HPA axis responses. For instance, an analysis of five independent studies that used the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993) and samples of health older adults (mean age: 67.3 y), younger adults (mean age: 23.5 y), and children (mean age: 12.1 y) revealed an elevated overall free salivary cortisol response in elderly men compared to elderly women while no gender differences emerged in young adults or children (Kudielka et al., 2004). While no gender differences appeared in the young age group, a follow-up study by the same group of researchers showed that adrenocorticotropic hormone (ACTH) responses to the TSST were elevated in men compared to women, regardless of menstrual cycle phase or use of oral contraceptives (Kirschbaum et al., 1999a). However, differences by menstrual phase were evidenced in salivary cortisol response, such that women in the luteal phase had comparable salivary cortisol stress responses to men whereas women in the follicular phase or women taking oral contraceptives showed significantly lower free cortisol responses (Kirschbaum et al., 1999a). While men were not age-matched to women in this study, there were no significant differences in age between genders or within groups of women. Beyond the importance of menstrual cycle phase and oral contraceptives, the authors stressed the necessity of distinguishing between the total cortisol secretion and the levels of bioavailable free cortisol when addressing gender differences.

Other stress experiments that have focused on physiologic challenge (e.g., naloxone) or cognitive-emotional challenge (e.g., anger recall) have also reported gender effects on measures of HPA axis function (Darnall, 2008; Suarez et al., 2004; Uhart et al., 2006). Similarly, one study of 50 young adults (26 women, random menstrual phase) found that women evidenced greater salivary cortisol response to social rejection, while men evidenced greater cortisol responses following performance-oriented tasks (Stroud et al., 2002). These findings offer additional evidence to show that stress responses are impacted by an interaction between gender and the type of stressor. Interestingly, negative emotional expression has been found to mediate the magnitude of cytokine response in women (Darnall, 2008) and women who have high anger expression are at increased risk for glucose dysregulation (Suarez, 2006). The aforementioned sex/gender differences are rather unsurprising, given the structural and functional brain differences that have been documented, as well as the influence of sex/ gender on appraisal (Rhudy and Williams, 2005; van Well et al., 2008), cognition (Berman et al., 1997), emotion (Rhudy and Williams, 2005), and behavior. However, while further study is needed, the current literature suggests women may have a biological and environmental diathesis for increased physiologic stress responses to negatively-valenced emotional expression.

As discussed above, sex/gender are known to influence glucocorticoid responses following acute psychological stress and this is one important pathway known to mediate inflammation (Kirschbaum et al., 1992; Wiegers and Reul, 1998). Indeed, an important feedback loop exists between the HPA axis and the immune system whereby pro-inflammatory cytokines activate

the HPA axis (Turnbull and Rivier, 1995; Turnbull and Rivier, 1999). Glucocorticoids then downregulate cytokines and thus quell inflammatory processes (Wiegers and Reul, 1998).

To be sure, sex/gender differences have important clinical implications; unfortunately, many unknowns remain, and thus development of sex/gender-specific treatment is difficult. While sex steroids are considered the 'usual suspects' in much research on sexual dimorphism, other mechanistic pathways exist. For example, one study measured HPA axis function in men and women being hospitalized for depression (Binder et al., 2008). HPA-axis dysregulation at admission predicted response to antidepressant treatment for men but not for women. Interestingly, the authors did not find sex-steroids to mediate their findings. Taken together with concordant findings from pre-clinical research, the authors stated that there is a "yet unresolved effect of gender on the HPA-axis which is not determined by circulating levels of sex hormones" (Binder et al., 2008).

Given that sex/gender influences the HPA axis it is unsurprising to discover it also affects subsequent immune cascades. Below we describe literature that documents sex/gender differences in immunology and cytokine responses.

E. Immunology and cytokine response

Women have more vigorous cellular and more vigorous humoral immune reactions than men (Bouman et al., 2005), and this confers an advantage to women for trauma (Angele et al., 1998), hemorrhage and sepsis (Schroder et al., 1998). Testosterone has been shown to suppress immune function, and this is demonstrated in delayed cutaneous wound healing in males (Fimmel and Zouboulis, 2005). However, women are disadvantaged by an increased incidence of autoimmune conditions. For instance, women are 7−10 times more likely than men to have systemic lupus erythmatosus, Graves' disease, or Sjogren's syndrome. Similarly, other autoimmune conditions, such as rheumatoid arthritis and multiple sclerosis, are 2−3 times more common in women than men. Genetic factors appear to play a large role in the differences found for immune system function. Of the more than 1,100 genes identified on the X-chromosome, many factors have been identified as likely being responsible for sexually dimorphic immune responses, including numerous receptors and associated proteins, immune-response related proteins, and transcriptional and translational control effectors (Fish, 2008). Furthermore, estrogen and progesterone are known to influence immune responses, and fluctuations in factors of immunoregulation (e.g., T helper 1 and T helper 2 levels) are seen across the menstrual cycle (see section B of this article for more on this topic). In persons with rheumatoid arthritis, levels of pro-inflammatory cytokines are related to aromatase activity and concomitant increased estrogen synthesis (Cutolo et al., 2004). Estrogen receptors (ERα and ERβ) are expressed in many types of immune cells, including B cells, T cells, dendritic cells, and natural killer cells. Estrogens affect innate immune responses (Harkonen and Vaananen, 2006) and enhance the expression of adhesion molecules and chemokines (Murphy et al., 2004). For a comprehensive review of estrogens and inflammation, the reader is directed to Straub (Straub, 2007).

Despite the prominent effects of sex on immunity, PNI research that examines immune response by sex is limited. For instance, in the focal area of stress and immune responsivity, surprisingly few studies include an examination of sex effects. The authors of a comprehensive meta-analysis of psychological stress and the immune system noted that the majority of studies failed to group data by sex (Segerstrom and Miller, 2004). As a result, Segerstrom and Miller were unable to conduct rigorous analyses of sex effects due to lack of data; instead, they correlated the sex ratio of the studies with the effect size only. The authors' conundrum nicely illustrates the fact that inclusion of women in research is insufficient; data *must* be examined by sex. It should be noted that simply examining data by sex is not sufficient due to the potentially confounding effects of sex-steroid fluctuations in women. Few studies have

controlled for the effects of the ovarian cycle, and in certain cases we must ask whether effect sizes are diminished by this omission.

While the extant literature offers mixed data regarding sex/gender dimorphism for stress-related inflammatory responses, future studies may enjoy greater clarity of results by attending to sex/gender methodology. Following is a description of study that highlights the importance of considering the influence of the ovarian cycle in stress and immunity research. Rohleder, Kirschbaum and colleagues (Rohleder et al., 2001) examined the relationship between HPA responses and pro-inflammatory cytokine production following administration of the TSST in 45 healthy adults. They aimed to test whether gender dimorphic cytokine responses remained when glucocorticoid levels are similar between genders (Kirschbaum et al., 1999b). Results showed that HPA responsivity following the psychosocial stressor was similar between genders, as the researchers expected. Cytokine production decreased in men but significantly increased in women, indicating a gender dimorphic inflammatory response. Importantly, they found increased glucocorticoid sensitivity for men one hour post-stressor, while women's glucocorticoid sensitivity decreased, thus placing them at risk for unchecked systemic inflammation. Heuristically, it may be beneficial to never assume sex/gender responses will be similar. Men and women may evidence differential immune responses, and some work shows the timing of the responses differs by gender, with women showing later cytokine peaking (Darnall, 2008; Edwards et al., 2006). Finally, there is some support for men and women to exhibit differential stress and inflammatory responses based on the type of stressor, and the types of *emotions* elicited by that stress (Darnall, 2008; Suarez, 2008; Suarez and Krishnan, 2006).

F. Genetics

At first glance, the primary genetic difference between men and women is likely found on the 1100 diverse genes on the X chromosome, the majority of those not being expressed on the Y chromosome. Many of these genes are involved in immune activities, blood coagulation and metabolic functions (Migeon, 2007). Given this, sex differences in the expression of those genes likely underlie the differential expression of diseases affected by those genes. It was eloquently stated that this genetic biology should be considered for any disease or phenotype that occurs in one sex more than the other, because the disease mechanism may be influenced directly by an X-linked gene or indirectly through the consequences of X inactivation (Migeon, 2007). Advances in the field of genetic medicine have already shown the importance of respecting, at minimum, the phenotypic differences between sexes as a proxy for the underlying genetic differences. This is exemplified by one recent study that showed that a genetic variation found on the X-chromosome is associated with an increased risk of Alzheimer's disease in women but not men (Carrasquillo et al., 2009). The aforementioned observation has particular relevance to PNI researchers given the fact that patients with Alzheimer's disease show a pro-inflammatory phenotype (Remarque et al., 2001). Genetic differences between men and women provide one rationale for the inclusion of sex-specific arms in research designs if we aspire to tell a meaningful story through our findings. Going further, sex/gender should be included in our research toward the end of effectively translating our work to enhance health for both males and females.

G. Illustrations of the importance of sex and gender analysis in PNI research

i. The example of pain—The area of pain research demonstrates how multiple factors underlying sex and gender may synergize to create dimorphism in health outcomes. Sex/gender differences in pain are pronounced and disparity in the incidence and prevalence of pain is well documented (Hurley and Adams, 2008). Epidemiological studies have shown women are 40 percent more likely to suffer from chronic neuropathic pain than men (Torrance et al., 2006). Moreover, women are 2−9 times more likely to acquire varied painful conditions such as

complex regional pain syndrome (de Mos et al., 2007), migraine (Stewart et al., 1994), fibromyalgia (White et al., 1999; Wolfe et al., 1995), rheumatoid arthritis (Symmons et al., 2002), and systemic lupus erythmatosus (Lahita, 2008) . Women's *experience* of pain is different than men's (Fillingim and Ness, 2000): women experience greater pain severity, frequency and duration (Unruh, 1996). Sexual dimorphism in pain mechanics is supported with evidence showing that modulation of the central nervous system processing of nociceptive input is different for men and women (Cairns, 2007). While clear sex differences in pain exist, the differences in the reporting of pain may also be influenced by gender on psychological factors (Fillingim et al., 2000; Fillingim et al., 2005; Rollman and Gillespie, 2000), gender role expectations (Levine and De Simone, 1991), situational factors (Levine and De Simone, 1991), the type of pain stimulation used (Lautenbacher and Rollman, 1993) and history of sexual abuse (Leserman, 2005).

Functional MRI research has shown sex differences in brain functioning in response to pain (Henderson et al., 2008). The authors of this study hypothesized that sexual dimorphism in brain functioning may reflect differences in emotional processing of noxious information in men and women and may underlie the gender bias that exists in many chronic pain conditions. Data from Darnall's lab supports this hypothesis (Darnall, 2008). Briefly, this pilot study examined pro-inflammatory cytokine response following a pain catastrophizing induction in persons with chronic pain. Men and women focused on their pain, imagined it worsening, and described the expected negative consequences their increased pain would have on their life. Women were observed to display more negative affect and emotion than men, and they evidenced greater increases in interleukin (IL)-6 and higher levels of tumor necrosis factor alpha (TNF-α) than men following the catastrophizing induction. Cytokine response was predicted by participants' level of negative affect. Given that IL-6 and TNF-α are implicated in pain processes, these results suggest that women's cognition, emotion, and expectation for pain may synergistically impact pain outcome at the molecular level.

Not only is the phenomenology of pain sexually dimorphic, differences in pharmacologic treatment response is known to vary by sex (Aubrun et al., 2005; Fillingim and Gear, 2004; Gear et al., 1996; Gear et al., 1999). Numerous animal and human studies have shown patterns of differential responses to classes of opioids (mu vs. kappa) based on sex (Binder et al., 2000; Holtman and Wala, 2006). In both rats and humans, males respond better to opioid treatment than females, and estrogen appears to mediate this effect by decreasing the number of opioid binding sites in the brain (Diaz et al., 2006; Ji et al., 2006). One study reported that women require 11 percent more morphine than men for post-operative pain control (Aubrun et al., 2005). Differences were non-significant in the elderly, suggesting that there may be an estrogen-driven sex effect for the efficacy of morphine treatment, although other pharmacokinetic factors associated with aging would need to be ruled out before this conclusion could be drawn. Undoubtedly, pain patients will benefit from research focusing on the pharmacokinetic and pharmacodynamic aspects of varied pain drugs. In addition to sexual dimorphism for pharmacology, some evidence supports sex/gender differences in response to behavioral treatment for pain (Hooten et al., 2007). Given these collective data, it is possible that pain management and treatment may evolve toward sex/gender-specialized care.

ii. The Example of Sleep—Although it is well recognized that women report more sleep problems and related complaints (Walsleben et al., 2004), emerging evidence from large population studies suggests that poor sleep and short sleep duration incur a greater risk of cardiovascular disease (CVD) in women than in men (Cappuccio et al., 2007; Ikehara et al., in press; Meisinger et al., 2007; Newman et al., 2000). Given those findings and the current perspective that inflammation plays a key role in the onset and progression of CVD, Suarez examined the relation of sleep quality to immune biomarkers in a sample of 210 healthy adult men and women with no clinical diagnosis of sleep disorders (Suarez, 2008). Studies of

premenopausal women were conducted during the follicular phase of the menstrual cycle to control for menstrual cycle variations in sex steroid hormones and sleep quality. Using the Pittsburgh Sleep Quality Index (Buysse et al., 1989) to assess sleep quality and components of sleep, Suarez (2008) showed that elevations in peripheral markers of inflammation and coagulation were associated with overall poor ratings of sleep and frequency of problems falling asleep but these associations were found for women only and not for men. The observed gender specific associations were quantified in multivariate analysis that included age, race, body mass index (BMI), and exercise frequency as covariates. In contrast, analysis that examined only main effects, that is a model that did not include the interaction between sleep quality and gender, failed to detect an effect of sleep quality on biomarkers of inflammation and coagulation. Although novel, the observed gender specific associations may explain the epidemiological evidence suggesting that poor sleep is associated with heightened risk of CVD morbidity and mortality in women than in men (Cappuccio et al., 2007; Ikehara et al., in press; Meisinger et al., 2007; Newman et al., 2000). Suarez postulated that the observed gender differences in the relation of poor sleep to elevations in inflammatory biomarkers could be reflect sex-differences in the serotonergic system and other mechanisms known to have direct effect s on immune activity. For example, one possible mechanisms is the peroxisome proliferators-activated receptor (PPAR)-α (Barbier et al., 2002; Daynes and Jones, 2002). Increased PPAR-α expression is associated with decreased nuclear factor (NF)-κB and c-jun, both transcription factors that are implicated in inflammation (Barnes and Karin, 1997). It has been shown that PPAR-α is expressed at higher levels in males than in females with higher testosterone associated with greater expression (Dunn et al., 2007) and higher testosterone has also been linked with poor sleep (Penev, 2007). In the Suarez study, higher testosterone was associated with decreasing sleep quality, thus raising the possibility that in men, higher levels of testosterone blunt the effect of poor sleep on markers of inflammation via up-regulation of PPAR-α. The approach taken by the Suarez study illustrates the wealth of information that can result when examining both gender- and sex-differences, in this case sleep behavior and sex steroid hormones respectively; one that yielded a more comprehensive understanding of the role sex and gender in moderating the relation of poor sleep to immune function.

H. PNI sex and gender research: pitfalls and opportunities

For researchers considering investigating the effect of sex and gender in PNI studies, the hurdles may seem high. Some barriers to conducting such science may include the costs associated with increasing sample size to achieve power adequate to detect group differences; another barrier may be the increased design complexity. Becker and colleagues offer an excellent overview of methods for research on sex differences in brain and behavior (Becker et al., 2005). Common pitfalls in clinical and preclinical research are illuminated, and researchers are guided to consider factors that are known to "mask" effects. Not only should females be included in research but reproductive status and ovarian cycle should be considered when studying sex/gender differences in health related outcomes, disease processes, or in the pharmacokinetic and pharmacodynamic properties of drugs. Other issues to consider are less expensive but require more complex statistical models. In order to test gender differences the analytic approach the investigators may consider including appropriate interaction terms between the independent variables and sex or gender. It is not enough for researchers to report significant main effects after "controlling for sex" and conclude that the effect is similar for men and women. On the contrary, controlling for sex only suggests that the independent variable accounts for a significant percentage of the variance beyond the percentage of variance accounted by sex or gender. To test the moderating effect of sex or gender, the researcher should include relevant interaction terms between sex and the variables of interest. Of course, these types of analyses are incumbent on power, an issue that is beyond the scope of this review but one that is important in testing these interactions.

We hope this article has shed light on the benefits of factoring sex/gender into research design and analysis. For every study that has not considered the impact of sex and gender, we ask whether the true story is yet known. A sex/gender analysis may unmask effects, thus fostering efficient science and the progression of one's research and of the field of knowledge. Results will aid the development of improved treatment and thus will promote more specialized care at the clinical level. Finally, the results of work that considers these factors will contribute to the broader understanding of human biology, health, disease, and thus this stands to benefit all.

4. Summary, future directions, and a call to action

Historically, preclinical and clinical researchers tended to avoid including females and women in studies because of the complexity involved. It is true that complexity in research design may accompany sex/gender research. However, the science to date implores researchers to consider that in the larger view, the notion of complexity may be misguided. Sex/gender-inclusive research stands to bring forth results that inherently control for what arguably may be one of the most important confounding variables in biomedical research. The perceived price of inclusivity diminishes somewhat when one considers that such designs may lead to discovery of effects not yet considered. Indeed, it was eloquently stated that, "Sex does matter. It matters in ways that we did not expect. Undoubtedly it matters in ways that we have not yet begun to imagine." (Wizemann, 2001).

Acknowledgments

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