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Gender bias in the preclinical psychopharmacology of anxiety: male models for (predominantly) female disorders

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Overrepresentation of females in anxiety disorders

The recently published Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV, 1994) acknowledges that women are diagnosed with particular anxiety disorders far more frequently than are men. For the specific codable disorders for which gender features are noted, the following overrepresentation of women is noted: panic disorder (PD) without agoraphobia is diagnosed twice as often, and PD with agoraphobia three times as often as in men, while agoraphobia without a history of PD "is diagnosed far more often in females than in males" (DSM-IV, p. 403). Women receive from 55 to 90% of the diagnoses for specific phobias, including the animal and natural environment type, the situational type and the blood-injection-injury type. Generalized anxiety disorder is diagnosed slightly more often in women (55–60% of diagnoses) but epidemiological studies show about twice as many females. The only codable disorders for which approximately equal ratios of men and women are noted are obsessive–compulsive disorder and social phobia. For the latter, however, it is also noted that epidemiological and community-based studies suggest that it is more common in women. Also, although DSM-IV does not provide information on gender factors in post-traumatic stress disorder (PTSD), one study utilizing data from the Epidemiological Catchment Area study found that >70% of those with PTSD in its large sample were women (Helzer, Robins and McEvoy, 1987).

Taken in conjunction with the long acknowledged (Silverman, 1968; Nolen-Hoeksema, 1987) overrepresentation of women in depression, these findings for anxiety suggest a striking gender difference in reactivity to stressful or threatening situations. Because the gender discrepancy for anxiety disorders has only recently been recognized (Cameron and Hill, 1989), there have been comparatively few studies analyzing the mechanisms that may be involved. However, Thyer and co-workers (Thyer, Tomlin and Curtis, 1985) reported that clinical severity of phobic symptoms is not different for phobic men and women, suggesting that the gender difference for phobic disorder cannot be attributed to a lower threshold for reporting a phobic response or presenting at a clinic, by women. That such an explanation is inadequate is also suggested by the Cameron and Hill (1989) report that overrepresentation of women among those suffering from simple phobia was very similar for an epidemiological study, compared to one involving a clinic population: 80–85% of phobics were female. Studies of possible mechanisms of gender discrepancy in depression have strongly suggested that the difference is not an epiphenomenon, and that it remains after factors such as gender-specific diagnostic biases and differential experience with aversive events (Amenson and Lewinsohn, 1981), income, education level and occupation (Radloff, 1975; Ensel, 1982), and particular response biases (Clancy and Gove, 1974) have been considered and controlled for. These factors are reviewed in Nolen-Hoeksema (1987).

Gender differences in behavioral and physiological mechanisms of response to stress or threat

These findings suggest gender modulation of the biological mechanisms of responsivity to stress and threat. Gender differences or gonadal hormone effects have been reported for anatomic or functional characteristics of several neurotransmitter and neuromodulatory systems, including γ-aminobutyric acid (GABA)/benzodiazepines (Carey, Billing and Fry, 1992; Meng and Drugan, 1993), dopamine (Morissette and Di Paolo, 1993), noradrenalin (Liaw *et al.*, 1992; Shellenberger, 1982) and acetylcholine (Overstreet, 1979). Such differences have been reported for both laboratory rodents (Carter and Lightman, 1987) and for humans (Arato *et al.*, 1991; Claustre *et al.*, 1980). They are particularly notable with regard to serotonin (5-HT) systems (Simerly, Swansson and Gorski, 1984; Biegon and Israeli, 1987). The brain 5-HT system appears to be more highly expressed in females, with higher levels of the precursor tryptophan, of 5-HT itself, and of its metabolite 5-hydroxyindoleacetic acid (5-HIAA), in a variety of brainstem and limbic forebrain sites, and with higher 5-HIAA/5-HT ratios in hypothalamus/pre-optic area and in limbic forebrain (Carlsson and Carlsson, 1988). Moreover, 5-HT systems are influenced by the estrus cycle in female rats (Biegon, Bercovitz and Samuel, 1980) and the menstrual cycle in women (Halbreich, 1990) as well as by the administration of estradiol (Biegon and McEwen, 1982).

While each of these neurotransmitter systems may be involved in response to threat or stress, again, this relationship is particularly notable for 5-HT systems. Thus, Heinsbroek and collaborators (1988) found that *p*-chloroamphetamine (PCA)

injections which severely reduced both 5-HT and its metabolite 5-HIAA in the frontal cortex of male and female rats abolished the substantial sex differences found in controls, on a passive avoidance task. Fernandez-Guasti and Picazo (1990) noted that the anxiolytic action of a number of 5-HT_{1A} receptor agonists appears to be different for male and female rats and, within females with different phases of the estrus cycle. Following restraint stress, binding at 5-HT_{1A} receptors in particular layers of the CA1 region of hippocampus was higher in female than male rats (Mendelson and McEwen, 1991). When compounds active at a variety of 5-HT receptor subtypes were tested in an anxiety/defense test battery (Shepherd *et al.*, 1992, 1993), or for analysis of the '5-HT syndrome' (Blanchard *et al.*, 1993) frequent gender × drug interactions attest to their differential effects on defense-related behaviors for females. Steiner (1993) recently summarized sex differences in serotonergic regulation, suggesting that 5-HT systems should be reconceptualized as a gender-differentiated psychobiological interface system mediating response to internal and external change.

Gender affects behavioral, as well as physiological, systems involved in response to threatening stimuli or situations. Crepeau and Newman (1991) have reviewed gender-specific reactivity profiles to threatening stimuli in a variety of primate species under natural conditions, and suggest that these differences may be related to variations in adaptive outcome of specific defensive behaviors for males and females, given their different social and reproductive roles. In laboratory rodents, a recent series of experiments using an anxiety/defense test battery measuring a wide range of defensive behaviors to a present or cued predator have demonstrated consistent gender differences in the behavior of vehicle controls (Blanchard *et al.*, 1991). Control females showed consistently higher levels than males of many specific defensive behaviors, particularly in situations involving cued or anticipatory threat. In particular, they tend to show more risk assessment and inhibition of non-defensive behavior (Johnson and File, 1991) but less freezing (Fanselow, Maren and De Oca, 1994) than males. This increased risk assessment/behavioral inhibition tendency fits well with previous findings that although social interaction scores and punished licking rates of female rats were lower than those of males, females showed a reduced aversion to the open arms of an elevated plus maze (Johnson and File, 1991).

These studies suggest an emerging consensus that there are important gender differences in defensive behaviors, very probably linked to gender variation in neurotransmitter and neuromodulatory systems, at least one of which involves 5-HT. This clearly suggests that studies of the biology of these behaviors ought to include female animals as well as males. When defense-related behaviors are used as animal models of anxiety in preclinical psychopharmacology, the need for female representation is even more salient.

Use of females in preclinical studies of response to stress and threat: the case of 5-HT and anxiety

To what extent is the need for utilization of female animals in preclinical research of the psychopharmacology of anxiety being met? A database (Griebel, 1995) has recently become available that includes essentially all experiments using compounds influencing 5-HT neurotransmission in conjunction with tests designed to provide a model of anxiety. This database is based on examination of some 1600 experiments published between 1960 and early 1994 (see Griebel, 1995, for description of the methodologies leading to identification and selection of these articles for inclusion). Because the database includes information on the gender of the (typically rat and mouse) subjects used in each experiment, it was possible to extract information on trends involving the use of male and female subjects in such research over this time period. In this context the term 'experiment' refers to a single procedure using a single compound, a unit necessary for the further analyses made in that review (Griebel, 1995). Thus a published article may include only one, or it may include many, separate experiments.

Fig. 1 provides an overview of the number of 5-HT/anxiety experiments in published articles during 3-year time blocks over this period, and it clearly demonstrates the dramatic increase in such experiments in recent years. Fig. 2 presents the proportions of these experiments in which only males were used as subjects; in which only females were used as subjects; or in which both males and females were used as subjects, and the results of measures on the two sexes were compared, during 3-year time blocks between 1973 and 1994.

This figure is as clear as the one preceding it. Only an extremely small proportion of the experiments on the relationship between 5-HT systems and situations/behaviors potentially linked to anxiety involved female animals. Even fewer included both male and female animals as subjects, such that gender comparisons, either under control conditions or in response to the drugs used, could be made.

Nonetheless, some slight trend toward increasing proportions of experiments using females may be suggested by the data of the last time block (1991–1994). Fig. 3 therefore presents the same data on proportions of experiments using male only, female only, or male + female subjects, for each of the last 7 years (note that 1994 is incomplete). The data in this figure indicate that even the slight trend toward increasing proportions of female experiments suggested in Fig. 2 is an illusion. It reflects only two articles, albeit two containing a considerable number

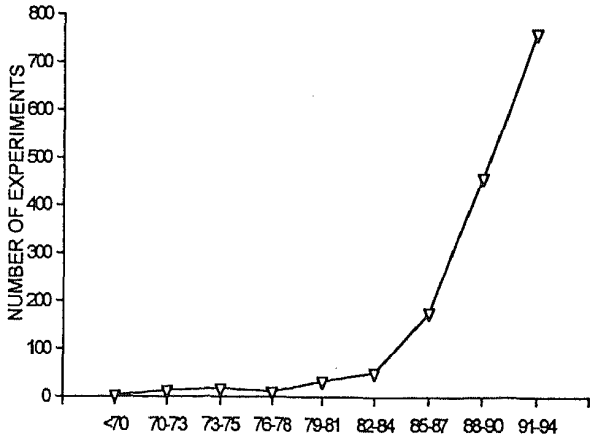

Figure 1 The number of serotonin/anxiety experiments in published articles during 3-year time blocks from 1960 to 1993. Experiments published during early 1994 are included in the last 3-year block

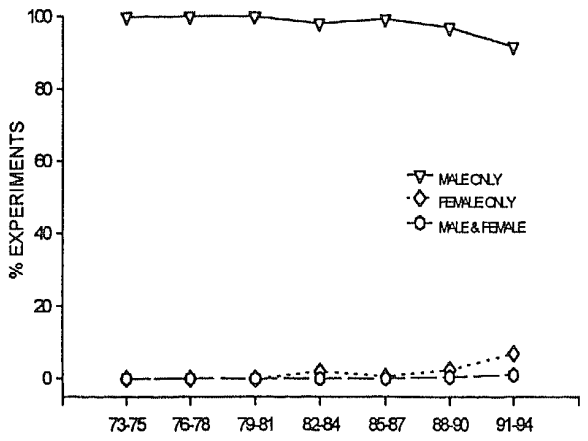

Figure 2 Proportions of serotonin/anxiety experiments using males (alone), females (alone) or both males and females as subjects, during 3-year time blocks between 1973 and 1993. Experiments published during early 1994 are included in the last 3-year block

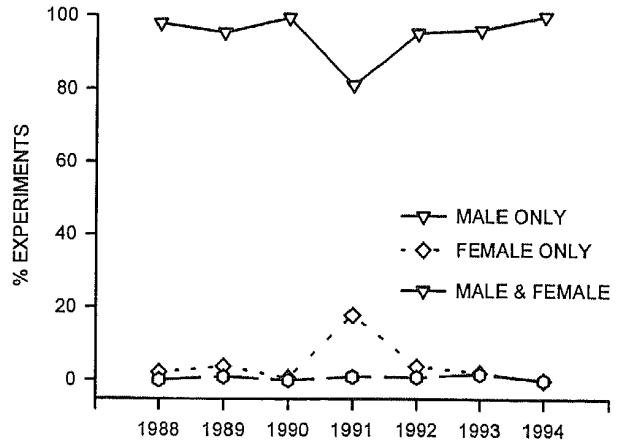

Figure 3 Proportions of serotonin/anxiety experiments using males (alone), females (alone) or both males and females as subjects, for each year between 1988 and 1994. Data on 1994 is incomplete

of separate experiments each using females, that were published in 1991. Given the paucity of female experiments, these articles (Njung'e and Handley, 1991a,b) created a striking anomaly in 1991, which was also reflected in the last block of 3+ years (which also included early months of 1994) for Fig. 2. This more detailed figure provides little indication of any trend toward use of females, and certainly not toward use of both females and males in particular experiments. In fact, of the 80 or so studies in which females were used, < 10 ran males also. Thus the entire literature providing gender comparisons of the effects of compounds influencing the activity of 5-HT receptors in animal models of anxiety consists of this handful of studies.

Consequences of the failure to use females in preclinical studies of response to stress and threat

If anxiety, and perhaps other disorders involving response to stressful or threatening stimuli or situations, does reflect gender-related mechanisms, then the consequences of failing to use female subjects in studies of the preclinical psychopharmacology of these disorders may be serious. At a minimum, this failure will necessarily result in a more shallow and incomplete understanding of the dynamics of these neurobehavioral systems, since such understanding will not include consideration of the gender-related mechanisms involved in obtained sex differences. The result of this incomplete analysis may well be inferior progress toward treatment for men as well as for women.

However, women suffering from emotional psychopathologies are likely to pay a disproportionate price for this failure of design and analysis. Significant drug × gender interactions, such as were consistently obtained in a high proportion of those rare studies in which male and female subjects were compared, indicate differential response to drug treatment for females confronted by stressful or threatening situations. Thus dose–response curves for women may be different from those for men, or it may even be that some drugs are more useful or appropriate for subjects of one gender than for the other. If the effect of a compound is affected by (for example) the hormonal characteristics of the subject, this information might be used to improve treatment in both males and females, but it is likely to have a greater impact in the latter case.

In the United States, new federal initiatives have been aimed at enhanced representation of female subjects in clinical research. While this initiative is useful and praiseworthy, it does not obviate the use of females as subjects in preclinical research, the normal avenue by which new treatments progress to the point of evaluation in clinical trials. Such omission, in the case of anxiety (and depression?) research, might well result in failure to identify compounds effective against these disorders but acting through mechanisms more important in women than in men.

The underlying rationale for a failure to use females in preclinical research on stress- or defense-related psychopathology is simple: it is easier and cheaper to use only males. However, the price for such lazy science is paid not only by the scientist but also by those for whom more effective treatments will not be available, and by the society that must therefore make other adjustments for these individuals. Moreover, the need for elaborate gender comparisons in studies of the preclinical psychopharmacology of anxiety/depression may well be a temporary one. If the effects of gender on the neurobehavioral systems underlying these conditions were thoroughly understood, male–female comparisons in specific studies might be unnecessary and the use of only one gender, perhaps male, entirely appropriate. However, until this degree of understanding is reached, it appears counterproductive for preclinical research to employ subjects of only the gender that, on the human level, shows so sharply reduced a tendency to display these disorders.

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