

## ORIGINAL RESEARCH ARTICLE

# Estrogen mediates sex differences in stress-induced prefrontal cortex dysfunction

RM Shansky<sup>1</sup>, C Glavis-Bloom<sup>1</sup>, D Lerman<sup>1</sup>, P McRae<sup>1</sup>, C Benson<sup>1</sup>, K Miller<sup>1</sup>, L Cosand<sup>1</sup>, TL Horvath<sup>1</sup> and AFT Arnsten<sup>1</sup>

<sup>1</sup>Department of Neurobiology, Yale University School of Medicine, New Haven, CT, USA

**Many anxiety disorders, as well as major depressive disorder (MDD), are at least twice as prevalent in women as in men, but the neurobiological basis of this discrepancy has not been well studied. MDD is often precipitated by exposure to uncontrollable stress, and is frequently characterized by abnormal or disrupted prefrontal cortex (PFC) function. In animals, exposure to stress has been shown to cause PFC dysfunction, but sex differences in this effect have not been investigated. The present study tested male and female rats on a PFC-dependent working memory task after administration of FG7142, a benzodiazepine inverse agonist that activates stress systems in the brain. Female rats were impaired by lower doses than males during proestrus (high estrogen), but not during estrus (low estrogen). Similarly, ovariectomized females showed increased stress sensitivity only after estrogen replacement. These results suggest that estrogen amplifies the stress response in PFC, which may increase susceptibility to stress-related disorders.**

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**Keywords:** sex differences; estrogen; stress; prefrontal cortex; working memory; depression

## Introduction

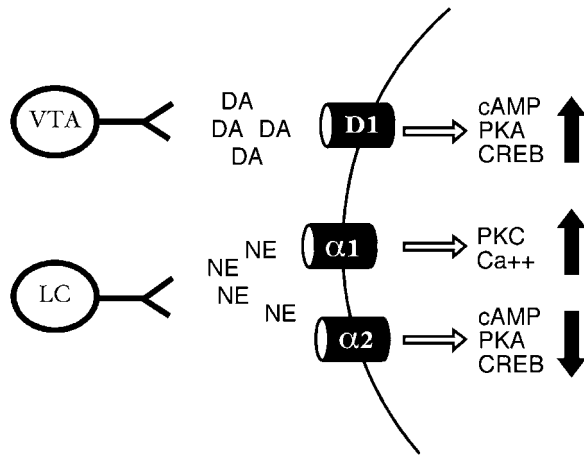
Major depressive disorder (MDD) is a debilitating and common affliction, affecting at least 15% of the population. However, its prevalence is twice as high in women as in men<sup>1</sup>, a discrepancy that is also found with respect to assault-related post-traumatic stress disorder<sup>2</sup> and most anxiety disorders.<sup>3</sup> The reasons for this imbalance, however, are unknown. While the disparity may be due in part to social and cultural factors, neurobiological differences likely contribute as well. Recently, Zubenko *et al.*<sup>4</sup> reported linkage of MDD to a chromosomal region that includes the gene for CREB1 (cAMP response element-binding protein 1), a transcription factor that can be activated or suppressed through the protein kinase A (PKA) intracellular signaling cascades coupled to neurotransmitter receptors. Sequence variations in this gene were found to cosegregate according to the presence or absence of MDD in women and not men or healthy subjects, suggesting that this gene could contribute to or be a marker for women's heightened vulnerability to MDD.<sup>5</sup> This finding is of particular interest, as recent studies implicate CREB as an important factor in the expression of learned helplessness (a model of depression) in animals, as well as an indirect target of many antidepressants.<sup>6</sup> To date, however, there is no known explanation for how

variations in CREB activity might produce gender differences in the prevalence of MDD. A better understanding of the mechanisms underlying depressive behavior in males and females will likely provide insight into this problem.

Functional imaging and neuropathological studies have identified the ventromedial prefrontal cortex (PFC) as a site of abnormality in depressed patients, for example, Baxter *et al.*,<sup>7</sup> Drevets *et al.*,<sup>8</sup> Elliott *et al.*,<sup>9</sup> and Mayberg *et al.*<sup>10</sup> The PFC regulates behavior, thought, and affect using working memory<sup>11</sup>, allowing us to plan and organize our behavior effectively.<sup>12</sup> Lesions of the PFC in humans commonly produce poor concentration, impaired initiative and word fluency, and perseverative thoughts—symptoms associated with depression. Moreover, lesions of the left PFC have been shown to induce depressive states.

Exposure to uncontrollable stress is a major risk factor for MDD, and studies have found that the cognitive functioning of the PFC becomes markedly impaired under conditions of uncontrollable stress, such as exposure to very loud noise or administration of FG7142, a benzodiazepine inverse agonist that activates stress systems.<sup>13</sup> FG7142 is a well-documented anxiogenic drug that is frequently used as a model for stress, given its reliability in producing the biochemical and physiological effects of stress: increased corticosterone release, increased catecholamine turnover, elevated heart rate, and increased blood pressure.<sup>14</sup> Studies in animals have shown that acute administration of FG7142 rapidly impairs PFC cognitive function via high levels of catecholamine release in the PFC, which activate intracellular

Correspondence: RM Shansky, Yale University School of Medicine, Department of Neurobiology, PO Box 208001 New Haven, CT 06520-8001, USA. E-mail: rebecca.shansky@yale.edu  
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**Figure 1** Effects of stress in the prefrontal cortex—during stress, excess catecholamines are released into the PFC. The PFC becomes impaired when dopamine D1 receptors or norepinephrine  $\alpha$ -1 receptors are bound, activating the PKA and PKC intracellular signaling cascades, respectively. Stimulation of the norepinephrine  $\alpha$ -2 receptor can reverse stress-induced PFC impairment, likely due to a decrease in PKA signaling.

signaling cascades (summarized in Figure 1) High levels of norepinephrine (NE) impair working memory through stimulation of  $\alpha$ -1 adrenoceptors that activate protein kinase C, while high levels of dopamine (DA) impair through D1 receptors that activate protein kinase A (PKA).<sup>15</sup> Activated PKA can in turn translocate to the nucleus and phosphorylate CREB; however, the role of CREB in stress-induced PFC function is not currently known. In contrast to  $\alpha$ -1 and D1 receptors, stimulation of  $\alpha$ -2 adrenoceptors can ameliorate stress-induced working memory impairment,<sup>16</sup> likely due to inhibition of PKA via Gi proteins. However, all of these studies have been performed in male rats, and it is not known if these mechanisms vary between the sexes.

Estrogen is capable of modulating many of the factors involved in stress-induced PFC dysfunction. In both rodents and primates, estrogen has been shown to facilitate DA tone.<sup>17,18</sup> Extracellular catecholamine levels may also be prolonged by estrogen's promotion of corticosterone release,<sup>19</sup> as corticosterone potently blocks extraneuronal catecholamine transporters.<sup>20</sup> As mentioned above, high levels of catecholamines can lead to PFC dysfunction. Estrogen may also amplify the stress response by altering NE adrenoceptor expression. When bound to its receptor, estrogen can act as a transcription factor for many genes, including the NE  $\alpha$ -1a receptor,<sup>21</sup> whose stimulation contributes to stress-induced PFC dysfunction. In addition to upregulating the expression of this receptor, estrogen has been shown to modulate its signaling levels.<sup>22</sup> It has further been demonstrated that estrogen administration leads to a decrease in NE  $\alpha$ -2a receptor binding in cortex<sup>23</sup>, whose stimulation protects the PFC from stress-induced dysfunction.

Thus, a reduction in this protective factor could exacerbate the PFC impairment associated with stress.

Taken together, this evidence suggests that estrogen is in a position to upregulate factors that lead to PFC dysfunction, and downregulate factors that can protect PFC function. Thus, it is plausible that females with high estrogen levels might exhibit greater PFC dysfunction with stress, which may leave them more vulnerable to depressive and PTSD symptomatology. An understanding of how the PFC might respond differently to stress in males and females is a crucial step in the development of new treatment strategies for stress-related disorders. The present study offers the first evidence that females are more impaired than males during stress in performing a PFC-mediated task, and that this effect can be modulated by the presence of high levels of estrogen.

## Materials and methods

**Subjects** Male ( $n=10$ ) and female ( $n=31$ ) Sprague-Dawley rats (Camm, Wayne, NJ, USA) were single-housed in a 12 h light/dark cycle with all testing conducted during the light phase. The animals were fed Purina rat chow (15 g/rat/day) immediately following behavioral testing and water was available *ad libitum*. Rat weights increased from an average of 240 g at the beginning of the study to 390 g (males) or 300 g (females) by the end of each study (approximately 10 months). Food rewards during cognitive testing were highly palatable miniature chocolate chips, thus minimizing the need for dietary restriction.

**Estrus phase monitoring** After testing each day, intact females were vaginally lavaged, and the cells were spread on a microscope slide. Cells were stained with Cresyl Violet, covered, and examined under a light microscope in order to determine estrus cycle phase. Proestrus cells are irregular in shape, have small nuclei, and often are found in organized clumps. Estrus cells are also irregular in shape but are non-nucleated.

**Ovariectomy surgery** In total, 19 of the female rats were anesthetized with isoflurane, and bilaterally ovariectomized; 10 were implanted subcutaneously with a capsule of 10% 17- $\beta$  estradiol, 90% cholesterol (to mimic estrogen levels of proestrus, see McGinnis *et al*, 1981),<sup>24</sup> while the remaining nine received a placebo capsule, containing cholesterol. These capsules provide steady estrogen release, thus animals have been exposed to chronic estrogen when tested. Animals began habituation 2 weeks after surgery.

**Habituation** Delayed alternation training and testing were performed in a T-maze (90  $\times$  65 cm). Rats were

habituated to the T-maze until they were readily eating chocolate chips from the experimenter's hand. In each of the three phases of habituation, the animal reached criterion when it ate 10 chips in less than 6 min. In the first phase, the animal was placed in the maze, and allowed to explore and eat the 10 chips that were placed at the end of both arms. In the second phase, the animal was allowed to explore, and received a chocolate chip from the experimenter's hand when it entered an arm. In the third phase, the animal was picked up and placed back in the start box after eating a chip from the experimenter's hand. Adaptation to handling is particularly important, as it can be stressful to some animals.

**Cognitive testing** PFC cognitive function was measured by the spatial working memory task, delayed alternation. This task requires working memory, behavioral inhibition, and sustained attention, and has been shown to be impaired in animals with ventromedial PFC lesions,<sup>25</sup> as well as in male rats administered FG7142.<sup>16</sup>

Following habituation, rats were trained on the delayed alternation task. A rat was placed in the start box of the T-maze and the gate was opened, allowing the rat to run to the choice point in the maze. On the first trial each day, animals were rewarded for entering either arm. The rat was then picked up and returned to the start box of the maze for the intertrial delay. On all subsequent trials, the rat was rewarded only if it entered the maze arm, which was not chosen on the immediately preceding trial. If the correct choice was made, the rat was given a reward and returned to the start box for the intertrial delay. Following an incorrect choice, the rat was immediately returned to the start box for the intertrial delay without reward. During the intertrial delay, the choice point was wiped with alcohol to remove any olfactory cues. Each test session consisted of 10 trials. Rats were scored for accuracy of response and response time. Response time was measured from the time the start gate was lifted until the animal made its choice. Rats were tested once daily, at the same time of day, five times per week. Please note that impairment on this task is reflected by performance of ~50% correct, or chance level. A score lower than this indicates perseverance towards one arm of the maze.

The intertrial delay was initially ~2 s, the minimal time needed to clean the choice point. Delays were raised by 5 s increments as needed in order to stabilize each rat's performance at approximately 70% correct. Training continued until a rat scored between 60 and 80% correct for two consecutive days. If an animal scored above 80% for two consecutive days, its delay was raised by 5 s. After 60 days of testing, animals did not differ between groups in level of delay (males: mean 2.7 s, females: mean 1.1 s,  $P > 0.05$ . OVX + Pbo: 4.4 s, OVX + Est: 1.9 s,  $P > 0.05$ ). Drug treatment was administered on the third day, with a minimum of 1 week between drug treatments for each rat.

**Drug treatment** The stress response was activated by the benzodiazepine inverse agonist, FG7142, a well-documented anxiogenic that reduces GABAergic transmission through the GABA<sub>A</sub> receptor. Animals were habituated to the intraperitoneal (i.p.) injection procedure, so as to avoid confounding effects due to the stress of receiving an injection. Animals whose performance was <70% correct after saline injection continued to receive injections prior to testing until they reached criterion performance, after which drug testing began. FG7142 (Tocris Cookson, St Louis, MO, USA) was suspended in a saline vehicle containing Tween 80, hydroxybetacyclodextrin and ethanol. FG7142 was ground to a maximal particle size of <180 μm in order to ensure consistent suspension. In all experiments, the order of drug treatments was counterbalanced between rats.

FG7142 (0.5, 2.0, 5, 10, 15 mg/kg) or an equal volume of vehicle was administered (i.p.) 30 min prior to behavioral testing in the T-maze. The experimenter testing the animal was blind to drug treatment conditions.

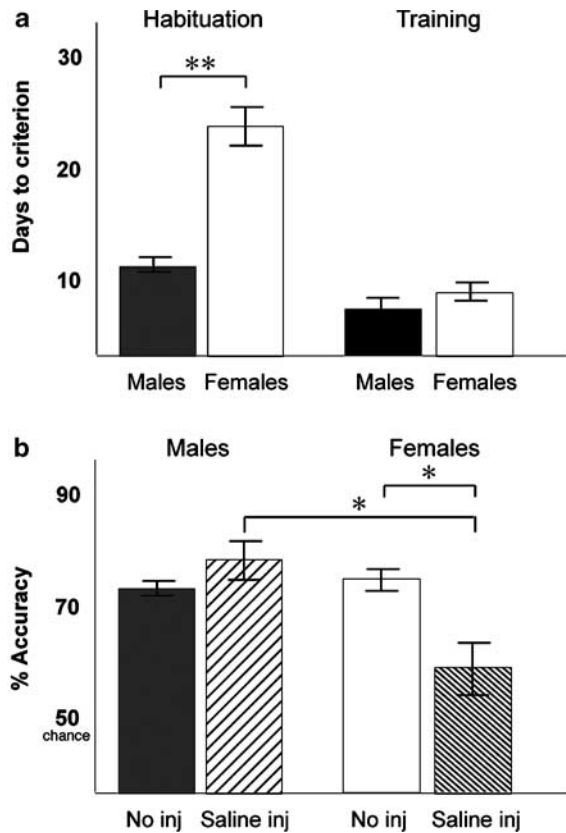
FG7142, similar to nonpharmacological stressors, increases catecholamine turnover in the PFC, and results in increased cFos labeling in the PFC, consistent with stress-activating CREB.<sup>26</sup> Furthermore, the effects of natural stressors can be prevented by blocking this site. FG7142 has advantages over more commonly used 'natural' stressors (such as tailshock, swim stress, and restraint stress) in that animals do not habituate to it, and there can be precise control over dosing. Thus, there is better control over the level of stress to which the animal is exposed, allowing a dose-response curve to be generated. Furthermore, the animal performs the task while experiencing the stress, rather than after being released from stress, as in most other paradigms. Our research has shown that FG7142 produces a profile identical to loud noise stress, and that all agents which reversed the FG7142 response similarly reversed the effects of noise stress.<sup>27,28</sup>

## Results

Animals were habituated and trained on the delayed alternation task until they reached a stable performance of ~70% correct. Animals were then acclimated to the i.p. injection procedure. After injection acclimation, animals received an i.p. injection of vehicle or FG7142 (0, 0.5, 2.0, 5, 10, 15 mg/kg) 30 min prior to testing. All animals ultimately received all treatments.

### *Males vs intact females*

**Habituation and training** Females ( $n=12$ ) took significantly more days than males ( $n=12$ ) to reach criterion for habituation to the maze, food reward, and handling ( $P < 0.002$ ) (Figure 2a). This pattern of results is consistent with females exhibiting a heightened stress response to novelty. After criterion



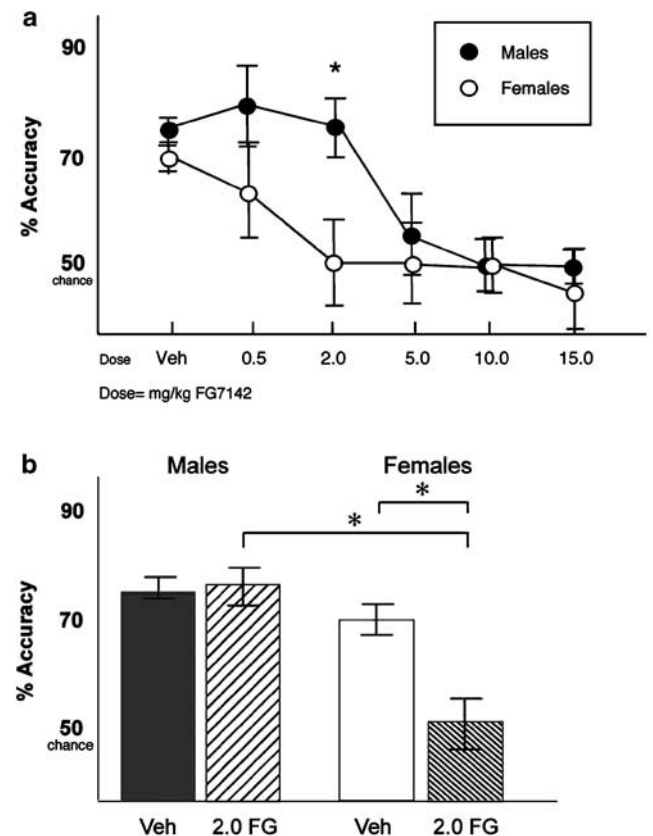
**Figure 2** Cycling female rats are more sensitive than males to mild natural stressors. (a) Males and cycling females were adapted to handling, the maze, and to testing procedures (habituation), then trained on the delayed alternation task. Females took significantly more days to reach criterion for habituation, but not for training, suggesting a greater sensitivity to the stress of novel situations, but equal learning ability. Results represent mean + SEM number of days to reach criterion (see text). \* $P < 0.002$ , two-tailed test. (b) To acclimate animals to injection procedures, males and females were given a saline injection 30 min prior to delayed alternation testing. Performance after injection (saline inj) is shown compared to average performance on the 2 days prior to injection day (no inj). Females were significantly impaired following injection, while males were not, again suggesting an increased sensitivity to mild stress. Note that impaired performance on the delayed alternation task is 50% correct, or chance. Results represent mean + SEM percent correct on the delayed alternation task. \* $P = 0.015$ , ANOVA.

was reached for habituation, animals were trained on the delayed alternation task. Criterion for training completion was achieved when animals tested two consecutive days at >70% accuracy. There was no significant effect of sex in days to criterion for training ( $P = 0.13$ ).

**First exposure to injection** A two-way analysis of variance with repeated measures (2-ANOVA-R) revealed that females responded differently to the novel i.p. injection procedure than did males, with females' performance significantly impaired, while

males remained unimpaired (sex  $\times$  injection interaction,  $F(1,20) = 7.2$ ,  $P = 0.015$ ; Figure 2b). Animals that were impaired were given saline injections until their performance returned to >70%, after which drug testing began.

**FG7142 administration** Dose-response curves for both males and females can be seen in Figure 3a. 2-ANOVA-R revealed a significant main effect of FG7142:  $F(5,40) = 16.2$ ,  $P < 0.0001$ , indicating that both males and females were impaired by the drug. However, females were more sensitive to the drug than males, as indicated by a significant sex  $\times$  FG7142 interaction:  $F(5,40) = 2.4$ ,  $P = 0.05$ . *Post hoc* analysis (test of effects) showed the 2.0 mg/kg dose to have the most prominent sex difference, where the females were impaired, but the males were not



**Figure 3** Cycling female rats are more sensitive than males to low doses of FG7142, a pharmacological stressor. (a) Dose-response curves are shown for male and female rats administered FG7142 30 min prior to testing. Both sexes demonstrated significant decreases in performance as FG7142 dose was increased. Results represent mean + SEM SEM percent correct on the delayed alternation task. \* $P < 0.0001$ , ANOVA. ANOVA also revealed a significant gender  $\times$  dose interaction,  $P = 0.05$ . (b) Female, but not male, rats were impaired at the 2.0 mg/kg dose, suggesting a sensitivity to mild levels of stress. No sex differences were seen with vehicle administration. Results represent mean + SEM percent correct on the delayed alternation task. \* $P = 0.037$ , 2-tailed *t*-test.

$F(1,8)=6.2$ ,  $P=0.037$  (Figure 3b). No gender differences in performance were observed after vehicle administration ( $p=0.51$ ). There was no difference in response time between sexes ( $5.2 \pm 0.82$  vs  $5.25 \pm 1.66$  min,  $P=0.97$ ). Both sexes' response times increased at higher doses, as freezing behavior became prevalent with increasing levels of stress response activation.

### The role of estrogen

#### OVX + estrogen vs OVX + placebo

To explore the potential role of estrogen in manifesting the above effects, 19 animals were ovariectomized and implanted with either a 10% estradiol silastic capsule, which mimics the estrogen levels of proestrus (OVX+Est,  $n=10$ ), or a placebo capsule (OVX+Pbo,  $n=9$ ). After recovery from surgery, animals went through habituation and training procedures as described in the methods.

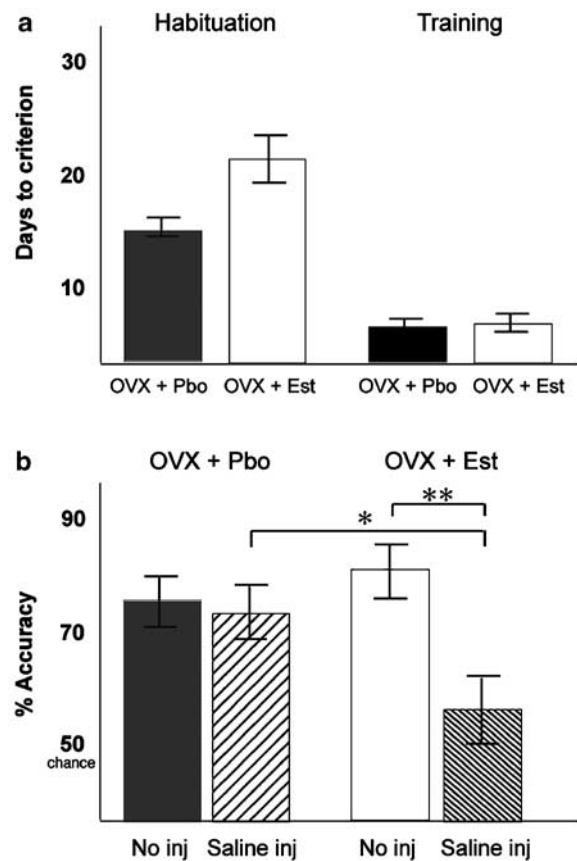
**Habituation and training** There were no significant differences between groups in days to criterion for either habituation or training (Figure 4a,  $P=0.12$ ). Interestingly, both groups required significantly more days to criterion than males (OVX+Est  $P<0.004$ ; OVX+Pbo  $P<0.04$ ), and OVX+Pbo required significantly fewer days to criterion than intact females ( $P<0.05$ ). OVX+Est did not differ from intact females ( $P>0.05$ )

**First exposure to injection** After receiving the first saline injection, a two-tailed  $t$ -test revealed that OVX+Est were significantly impaired, while OVX+Pbo remained unimpaired ( $P<0.03$ , Figure 4b), mimicking the effect seen when males and females were compared. These data suggest that the presence of estrogen promotes a sensitivity to mild natural stress.

### Pharmacological stress

#### Proestrus vs Estrus

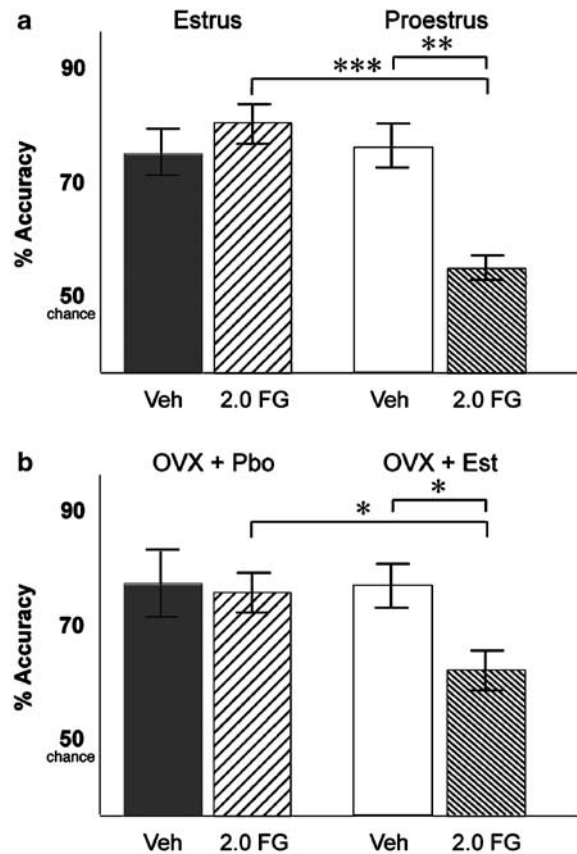
**FG7142 (2.0 mg/kg)** To further elucidate the role of estrogen, estrus cycles were monitored in a subgroup of the intact females from the first set of experiments, and either vehicle or 2.0 mg/kg FG7142 was administered before testing during proestrus (highest estrogen levels) or estrus (lowest estrogen levels). All animals ultimately received all four possible conditions, allowing a within-subjects comparison. As can be seen in Figure 5b, animals in proestrus were significantly impaired by administration of 2.0 mg/kg FG7142, while those in estrus were not ( $P<0.0002$ ). There was no difference between groups in performance after vehicle treatment ( $P=0.89$ ). These results suggest that high levels of estrogen may be contributing to the sex effects seen in the first set of experiments.



**Figure 4** OVX + estrogen animals are more sensitive than OVX + placebo animals to mild natural stressors. (a) OVX + Est and OVX + Pbo were habituated to testing procedures, then trained on the delayed alternation task. OVX + Est animals showed a nonsignificant trend of taking more days to reach criterion for habituation than OVX + Pbo. The groups did not differ in days to criterion for training. Results represent mean + SEM days to criterion. (b) To acclimate animals to injection procedures, both groups were given a saline injection 30 min prior to delayed alternation testing. Performance after injection (saline inj) is shown compared to average performance on the 2 days prior to injection day (no inj). OVX + Est were significantly impaired following injection, while OVX + Pbo were not, suggesting a sensitivity to mild stress. Results represent mean + SEM percent correct on the delayed alternation task. \* $P<0.03$ , 2-tailed  $t$ -test.

#### OVX + Est vs OVX + Pbo

**FG7142 (2.0 mg/kg)** OVX + Est and OVX + Pbo animals were administered with either vehicle or 2.0 mg/kg FG7142 before testing. All animals ultimately received both treatments, allowing for within-subjects comparison. A two-tailed  $t$ -test revealed that OVX + Est animals were significantly impaired at this dose, while OVX + Pbo animals remained unimpaired ( $P<0.03$ , Figure 5a). There was no significant difference in performance after vehicle treatment ( $P=0.98$ ), nor were there any differences in response times ( $4.3 \pm 1.1$  vs  $7 \pm 1.2$  min,  $P=0.299$ ).



**Figure 5** Animals with high estrogen levels are impaired by low doses of the pharmacological stressor, FG7142, while animals with low estrogen levels are not. (a) Cycling females were administered 2.0 mg/kg FG7142 30 min prior to testing. Estrus phase was determined immediately after testing. Animals in proestrus (high estrogen levels), but not estrus (low estrogen levels), were impaired by this dose. There were no differences in performance between groups with vehicle treatment. Results represent mean + SEM percent correct on the delayed alternation task. \* $P < 0.0002$ , 2-tailed  $t$ -test. (b) OVX + estrogen animals and OVX + placebo animals were administered 2.0 mg/kg FG7142 30 min prior to testing. OVX + Est, but not OVX + Pbo, were impaired by this dose. Results represent mean + SEM percent correct on the delayed alternation task. \* $P < 0.03$ , 2-tailed  $t$ -test.

## Discussion

The present findings indicate that female rats are more sensitive to stress-induced PFC dysfunction, and that this effect is mediated by the presence of high levels of estrogen. Mild levels of natural and pharmacological stress that had no effect on male rats impaired female rats' performance, but only when estrogen levels were high. This effect did not appear to result from differences in motivation, as test times did not differ between groups. It could be argued that the observed sex difference might be due to females metabolizing the FG7142 more slowly than males. However, the finding that females were also more

sensitive to nonpharmacological stressors (novel maze and handling, i.p. injections) argues against this interpretation. It is also unlikely that the effect is due to a greater impairment in spatial ability rather than working memory, as estrogen has been shown to enhance spatial ability in rats under stressful conditions.<sup>28</sup>

Given that our results indicate a role for estrogen in promoting stress-induced PFC dysfunction, it is intriguing that a significant sex effect was observed when females' estrus phase was not monitored. One would assume there to be an equal distribution of animals in both proestrus and estrus, in which case their scores would balance, obscuring any main sex effect. However, we have not investigated the potential contribution of the two remaining estrus phases, metaestrus and diestrus. It is possible that the moderate levels of circulating estrogen present in these phases were sufficient to amplify the stress response, which when analyzed with proestrus and estrus produced a significant sex effect. This issue will be the focus of future experiments.

Another noteworthy consideration is the potential involvement of progesterone in these processes. OVX surgery not only removes circulating estrogen, but circulating progesterone as well, yet our animals received only estrogen replacement. Furthermore, progesterone levels fluctuate in cycling animals, with peak levels during the estrus phase. Progesterone has been shown to have antidepressant-like effects,<sup>30</sup> and it is possible that its presence (in intact animals) or absence (in OVX animals) could influence the data reported here. That said, it appears that estrogen plays a more primary role, as the replacement of estrogen alone was sufficient to amplify stress-induced PFC dysfunction. It should be noted, however, that the effects seen in OVX animals were not as robust as those in intact females. This may be due to the fact that while intact animals' estrogen levels fluctuate, OVX + Est animals experience a steady state of elevated estrogen levels. This constancy could potentially desensitize estrogen receptors, thus producing a more subtle effect. Future experiments will examine the relative effects of constant vs fluctuating levels of estrogen, as well as the role of progesterone in the mediation of stress-induced PFC dysfunction.

### *Estrogen influences on non-PFC functions during stress*

The current results are in concert with evidence that estrogen can mediate stress-related sex differences in tasks that rely on brain areas other than the PFC. It has been shown that acute stress exposure can impair classical eyeblink conditioning (a cerebellum-dependent task) in females, while in fact facilitating learning in males. Similar sex effects were seen in animals performing trace conditioning (a hippocampus-mediated task) after acute stress.<sup>31</sup> These studies also reported that estrogen mediated the effects; while stress impaired classic eyeblink conditioning in intact females, ovariectomized females were not affected.

Furthermore, the estrogen  $\alpha$  receptor antagonist tamoxifen blocked the impairment seen in intact females. It has also been demonstrated that the most severe stress-induced impairment occurred during proestrus, the estrus phase in which estrogen levels are highest.<sup>32</sup> In contrast, estrogen appears to facilitate spatial processing during stress<sup>29</sup>, indicating that estrogen's role in the stress response likely varies throughout the brain, enhancing function in some areas, while debilitating others.

#### Clinical relevance

Many studies use a chronic stress paradigm, such as learned helplessness, as an animal model for depression. The present study examined PFC performance during acute stress, and it is not to be inferred that these animals are 'depressed'. However, the brain region and mechanisms involved in the effects presented are still of direct relevance to stress-related clinical disorders. Melancholic depression, PTSD, and anxiety are all worsened by exposure to stress and share symptoms of PFC dysfunction such as poor concentration and perseverative thoughts. Interestingly, these disorders are also associated with decreased GABA and increased NE turnover, similar to the effects of FG7142 in animals. These disorders are more common in women than in men, and some evidence indicates that estrogen may contribute to this discrepancy. Specifically, the higher prevalence of depression in women first arises at puberty<sup>33,34</sup>, maintains through the child-bearing years, and then declines, such that it is equally likely to occur in postmenopausal women as in men of the same age.<sup>35</sup> Furthermore, administration of estrogen can exacerbate depressive symptoms in young women<sup>36</sup>, but not in menopausal women.<sup>37</sup> As discussed above, estrogen may have this effect through enhancement of DA tone (leading to greater stimulation of D1 receptors), upregulation of NE  $\alpha$ -1 receptors, and downregulation of NE  $\alpha$ -2 receptors, all of which can cause PFC dysfunction (see Introduction). Intriguingly, the intracellular consequences of these receptor changes would include an increased activation of CREB, a polymorphism of which was found to correlate with the presence of MDD in young women, but not men.<sup>4</sup> However, it is not known if or how estrogen might interact directly with CREB to produce the gender disparity in the incidence of MDD.

There are undoubtedly many contributing factors to the development of MDD in women, and much work remains before they can be well understood. What is becoming increasingly clear, however, is that the gender discrepancy in its prevalence has biological roots, and that estrogen likely plays a major role in its manifestation. In the present study, female animals exhibited greater stress-induced PFC impairment under conditions of high estrogen levels, complementing and supporting work from several fields. While this heightened stress response may confer survival value during danger, it may also increase susceptibility for stress-related disorders such as

depression. A better understanding of the neurobiology underlying sex differences in the cognitive response to stress may lead to more appropriate treatment targets and methods.

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