Males and Females Respond Differently to Controllability and Antidepressant Treatment

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Background: *Women are much more likely to suffer from stress-related mental illness than men; yet few, if any, animal models for such sex differences exist. Previously, we reported that exposure to an acute stressor enhances learning in male rats yet severely impairs learning in female rats. Here, we tested whether these opposite effects in males versus females could be prevented by establishing control over the stressor or by antidepressant treatment.*

Methods: *Learning was assessed using the hippocampal-dependent task of trace eyeblink conditioning. In the first experiment, groups of male and female rats were exposed to controllable or uncontrollable stress and trained. In a second experiment, they were exposed to an uncontrollable stressor after chronic treatment with the antidepressant fluoxetine (Prozac). In a final experiment, females were exposed to uncontrollable stress after acute treatment with fluoxetine.*

Results: *Establishing control over the stressful experience eliminated the detrimental effect of stress on learning in females as well as the enhancing effect of stress in males. Moreover, chronic but not acute treatment with fluoxetine prevented the learning deficit in females after exposure to stress. Treatment with fluoxetine did not alter the male response to stress.*

Conclusions: *These data indicate that males and females not only respond in opposite directions to the same stressful event but also respond differently to controllability and antidepressant treatments.*

Key Words: Stress, memory, fluoxetine, sex differences, anxiety, depression

Stressful life events can affect our abilities to learn and remember, as well as our emotional state. It has been proposed that establishing control over these stressors can ameliorate some of the effects on cognition and behavior. This idea evolved from studies conducted in laboratory animals demonstrating that exposure to inescapable shock interfered with subsequent learning of operant tasks (Overmier and Seligman 1967; Seligman and Maier 1967). The effect of stress was not evident following exposure to equivalent amounts of escapable shock, suggesting that the impaired learning was due to the psychological nature of the stressor, namely controllability, rather than the physical properties associated with the shocks. This deficit in operant conditioning was termed learned helplessness and is regarded as a behavioral model of stress-related mental illness such as posttraumatic stress disorder (PTSD) and major depression (Seligman 1975; Foa et al 1992; Nestler et al 2002). Although there has always been some controversy about whether or not helplessness in laboratory animals can model depression in humans (Willner 1990), the phenomenon does appear sensitive to treatment with antidepressants. Specifically, chronic treatment with serotonergic antidepressants alleviates cognitive and emotional deficits in most depressed individuals and reduces some of the behavioral symptoms of helplessness in laboratory animals (Martin et al 1990; Levkovitz et al 2002; Vythilingam et al 2004).

Although these findings support the idea of helplessness as a model of depression in humans, there are findings that do not. For example, it is well established that women are more likely to experience depression than men (Kendler 1998); yet female rats display little if any symptoms of helplessness. Thus, while males show performance deficits after exposure to an uncontrollable stressful event, females do not (Denti and Epstein 1972; Kirk and Blampied 1985; Steenbergen et al 1990). One of the problems with these findings is that female rats are generally more active than males and thus are more likely to move and "learn" to escape even after exposure to an inescapable stressful event. Simply put, the absence of a helplessness effect in females may reflect gender differences in performance and not learning, per se (Shors and Leuner 2003; Shors 2004). This problem can be minimized using classical eyeblink conditioning, in which the learned response is not dependent on gross motor activity. Using this task, we have observed sex differences in conditioning itself such that females acquire this task at a facilitated rate and overall emit more learned responses (Wood and Shors 1998; Shors and Miesegaes 2002). Moreover, females and males respond in opposite directions after exposure to inescapable tail shocks or swim stress (Shors et al 1992; Shors et al 1998; Wood et al 2001; Beylin and Shors 2003). While exposure to the stressful event enhances subsequent conditioning in male rats, exposure to the same event dramatically impairs conditioning in females. These effects appear indicative of learning, at least to the extent that stressor exposure does not alter the unconditioned motor response in either sex (Servatius et al 2001; Bangasser and Shors 2004). They do not appear dependent on sex differences in performance, since the effects persist even when performance is similar between unstressed males and females (Wood and Shors 1998).

Since females under these training conditions are particularly vulnerable to the negative consequences of stressful events, we questioned whether these effects might model or in some way inform us about sex differences in stress-related illness. In the first experiment, we tested whether the effects of stress on learning in males and females could be prevented if animals established control over the stressor. In a second experiment, we tested whether chronic treatment with the antidepressant fluoxetine (Prozac) would prevent the effects of stress on learning. As an indirect measure of anxiety associated with stress and antidepressant treatment, we also measured exploratory behavior in the elevated plus maze. Lastly, we tested whether acute treatment with fluoxetine would prevent the effects of stress on

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learning and anxiety, since it is well known that humans only respond to these drugs after weeks of treatment (Wong and Licinio 2001).

Methods and Materials

Experiment 1

Subjects and Surgery. Adult (~2–4 months) female (250–350 g) and male (300–450 g) Sprague-Dawley rats were individually housed, had unlimited access to chow and water, and were maintained on a 12-hour light/dark cycle. To implant electrodes for measuring the eyeblink response, rats were anesthetized with sodium pentobarbital anesthesia supplemented with Isoflurane and oxygen. A headstage attached to four stainless steel electrodes was secured to the skull with screws and acrylic. Electrodes were threaded through the eyelid: two electrodes recorded electromyographic (EMG) activity associated with an eyeblink and two electrodes delivered eyelid stimulation to elicit the eyeblink reflex. Rats were allowed to recover for at least 3 days before escape training and classical conditioning. Stages of estrus were monitored with daily vaginal smears, as previously described (Shors et al 1998). Only female rats with normal 4- to 5-day cycles were tested.

Acclimation and Escape Training. On the first day of escape training, rats were taken from their home cages and acclimated for 1 hour to the chamber in which they would later undergo classical eyeblink conditioning. Headstages were connected to a shielded grounded cable that allowed free movement within the conditioning chamber. The chamber consisted of an illuminated (7.5 W bulb) inner chamber (22 cm × 26 cm × 35 cm) with metal walls and a grounded grid floor located within a sound-attenuated outer chamber (51 cm × 52 cm × 35 cm). After acclimation, unstressed males ($n = 10$) and females ($n = 10$) were returned to their home cages. Separate groups of males and females ($n = 8$ per group) were taken into a different room and placed in one of two identical shuttle boxes (46 cm × 19 cm × 18 cm) located within a sound-attenuated illuminated (7.5 W bulb) chamber (69 cm × 69 cm × 53 cm). A scrambled shock generator delivered 1 mA shocks through the grid floor of the apparatus every minute for 30 minutes with an intertrial interval (ITI) of 60 seconds. The shuttle boxes were electrically linked so that when one rat received shock, the yoked animal received the same amount of shock. Rats in the controllable stress condition learned to escape the shock by running through a doorway (7.5 cm) and tripping a balance switch that shut off current to both boxes simultaneously. The time to escape the footshock was recorded. Yoked animals could not escape and thus had no control. Training occurred between 10:00 AM and 2:00 PM for 7 consecutive days. Females in the same stage of estrus were yoked on the first day of escape training.

Conditioning Procedure. Twenty-four hours after the last day of escape training, rats were returned to the classical eyeblink conditioning chamber. Spontaneous blinks and responses to 10 white noise stimuli (83 dB, 250 milliseconds, 5 millisecond rise/fall time) were recorded. Rats were then exposed to 300 trials of eyeblink conditioning per day for 2 consecutive days. We used the hippocampal-dependent version of this task known as trace conditioning, which is sensitive to sex differences and stressor exposure (Beylin et al 2001; Wood et al 2001). In the paradigm, a 250-millisecond burst of white noise conditioned stimulus (CS) (83 dB, 5 millisecond rise/fall time) was followed by a 100-millisecond, .7 mA periorbital shock unconditioned stimulus (US). The two stimuli were separated by a 500-millisecond stimulus-free interval, and eyeblinks during this period and before the US were considered conditioned responses (CRs). Every 10-trial sequence consisted of 1 CS alone presentation, 4 paired presentations of the CS and US, 1 US alone presentation, and 4 paired presentations of the CS and US. The ITI was randomized between 20 and 30 seconds. To detect eyeblinks, the maximum EMG response occurring during a 250-millisecond prestimulus baseline recording period was added to four times its SD. Responses that exceeded that value and had a width of at least 3 milliseconds were considered eyeblinks.

Statistical Analysis. Analysis of variance (ANOVA) with repeated measures was used to analyze escape latency data and the percentage of CRs emitted during 600 trials of trace eyeblink conditioning. Newman-Keuls post hoc analysis was applied to significant main effects.

Experiment 2

Next, we tested whether the effects of uncontrollable stress on trace conditioning in male and female rats could be alleviated with antidepressant treatment. Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), was chosen because of its efficacy in treating humans with stress-related mental illness (Wong and Licinio 2001; Cryan et al 2002). Adult male (300–450 g) and female (250–350 g) rats received daily injections (intraperitoneal [IP]) of fluoxetine (5 mg/kg) or vehicle (.9% saline) for a minimum of 14 days while monitoring estrous cycles in females. When females had received at least 14 days of fluoxetine or vehicle and were in the diestrus-2 stage of their cycle, they were acclimated to the classical conditioning chamber and then exposed to an acute uncontrollable stressor consisting of restraint and 1 mA, 1-second shocks applied to the tail at a rate of one per minute for 30 minutes or returned to their home cage (no stress). Similarly, males were acclimated to the classical conditioning chamber and exposed to the same uncontrollable stressor or returned to their home cage. Groups consisted of no stress males ($n = 9$) and females ($n = 9$) injected with vehicle; stressed males ($n = 9$) and females ($n = 9$) injected with vehicle; no stress males ($n = 10$) and females ($n = 9$) injected with fluoxetine; and stressed males ($n = 8$) and females ($n = 8$) injected with fluoxetine.

Twenty-four hours after the stressor, animals were returned to the conditioning chamber and trace conditioned as in Experiment 1. Twenty-four hours later, rats were placed in the elevated plus maze, which consisted of a cross-shaped platform made of black Plexiglas elevated 50 cm from the floor. The apparatus was located in a dimly lit room and consisted of four arms each 50 cm in length: two were open and two were enclosed by walls 40 cm high. The rat was placed into the central area facing one of the open arms and allowed to explore for 10 minutes. Time spent and entries into open versus closed arms were recorded. To measure gross motor activity, rats were placed in a 30 cm^2 Plexiglas chamber equipped with eight photo beams 4 cm apart. Beam breaks were used to detect activity.

Analysis of variance followed by Newman-Keuls post hoc analysis were used to analyze percentage of CRs during trace conditioning, time and entries in the plus maze, and beam breaks for locomotor activity.

Experiment 3

Fluoxetine's effectiveness in treating human mental disorders emerges only after weeks of continuous administration (Wong and Licinio 2001). To determine whether treatment with fluoxetine would only prevent the effects of stress if delivered

chronically, we next tested the effects of acute fluoxetine treatment. Females in diestrus-2 were acclimated to the classical conditioning chamber and then given a single injection of either fluoxetine or vehicle and returned to their home cage. One hour later, half of each group was exposed to the stressor of restraint and brief tailshocks or remained in their home cage (no stress). Groups consisted of no stress females injected with vehicle ($n = 7$); stressed females injected with vehicle ($n = 7$); no stress females injected with fluoxetine ($n = 10$); and stressed females injected with fluoxetine ($n = 7$). Twenty-four hours later, animals were trace conditioned. Twenty-four hours after trace conditioning, anxiety behavior in the plus maze and gross motor activity were measured.

Results

Experiment 1

No Sex Differences in Escape Performance. During escape training, latency to escape the footshock and enter the other side of the shuttlebox was recorded and used as the measure of performance. As expected, latencies decreased dramatically over the 7 days of training. In males, the mean latency decreased from 2.2 seconds on the first day to .64 second on day 7 [$F(6,42) = 7.41$; $p = .00002$] (Figure 1A). In female rats, the mean latency decreased from 1.7 seconds on the first day to .61 second on day 7 [$F(6,42) = 4.46$; $p = .001$] (Figure 1A). We observed no difference in escape latencies between male and female rats over the 7 days of training ($p = .17$).

Sex Differences in Trace Conditioning. Analyzing performance (percentage of CRs) in unstressed animals alone, there was a main effect of sex (male or female) on the percentage of CRs emitted during trace conditioning [$F(1,18) = 10.85$, $p = .004$]. As shown previously, females emitted a greater percentage of CRs than males (Wood and Shors 1998; Wood et al 2001; Shors and Miesegaes 2002).

Controllability Prevents the Effects of Stress on Trace Conditioning in Males and Females. One day after escape training, animals underwent trace conditioning using an eyeblink response. Performance (percentage of CRs) was analyzed with type of stressor (controllable, uncontrollable, no stress) as the independent variable. In males, there was a main effect of stressor type on the percentage of CRs [$F(2,23) = 3.66$; $p = .04$] (Figure 1B). Males exposed to uncontrollable shock emitted more CRs than those exposed to the same number and amount of controllable shock ($p = .03$). There was no difference in the percentage of CRs between unstressed males left in their home cage before trace conditioning and those exposed to controllable shock ($p = .96$). Therefore, exposure to controllable but not uncontrollable stress enhanced trace conditioning in males. Neither stressor affected the numbers of spontaneous blinks ($p = .65$) or sensitized responses to the white noise stimulus ($p = .66$).

In females, we also observed that uncontrollable but not controllable stress affected subsequent trace conditioning; however, the effect was in the opposite direction to that in males. There was an overall effect of stressor type on trace conditioning [$F(2,23) = 5.5$, $p = .01$] (Figure 1B). Females exposed to the uncontrollable shock emitted fewer CRs when compared with females that were exposed to the same number and amount of controllable shock ($p = .01$). The percentage of CRs did not differ between unstressed females left in their home cage before trace conditioning and those exposed to controllable shock ($p = .84$). Therefore, exposure to uncontrollable but not controllable stress reduced trace conditioning in females with no detectable effect of stress on spontaneous blinking ($p = .54$) or responses to the white noise stimulus ($p = .11$).

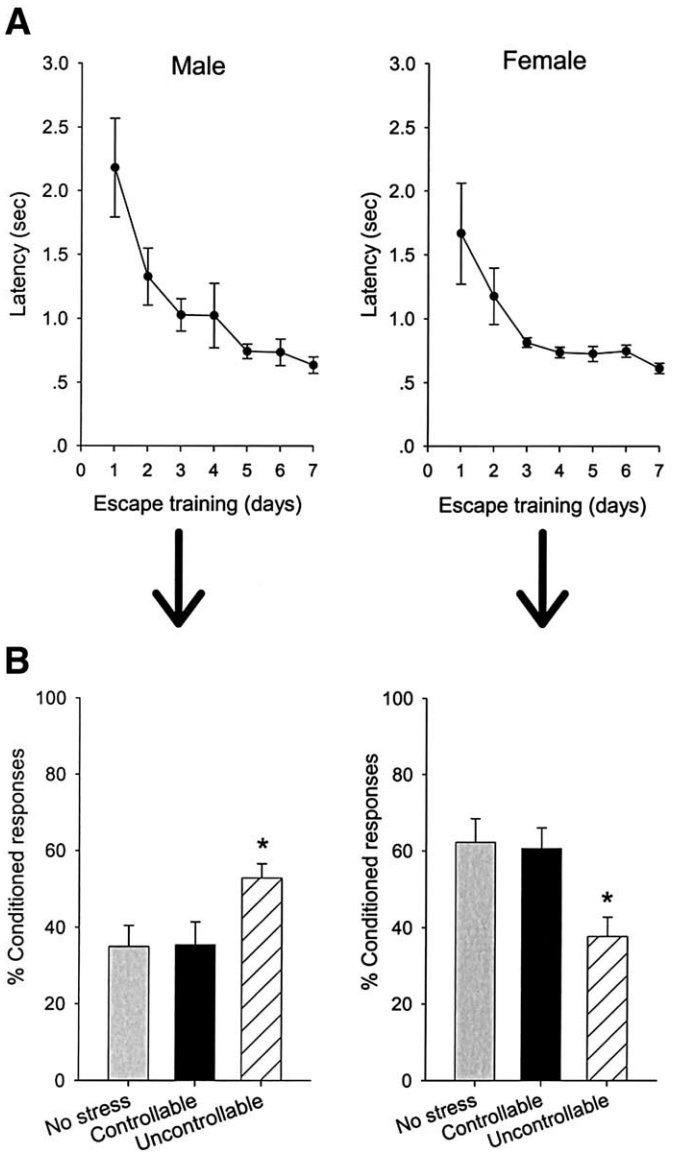

Figure 1. Controllability prevents the effects of stress on learning in males and females. Groups of male and female rats were trained to escape a footshock in a shuttle box apparatus in which one rat had to transverse the cage to escape footshock (controllable). Yoked controls were exposed to the same numbers and amount of stress but could not escape (uncontrollable). **(A)** Latencies to escape the shock decreased over the 7 days of training and did not differ between males and females. **(B)** Learning to escape prevented the effects of stress on subsequent trace eyeblink conditioning in both males and females. Thus, conditioning was enhanced in males exposed to uncontrollable stress but not in those exposed to the same amount of controllable stress. Conditioning was impaired in females that were exposed to uncontrollable stress but not in those exposed to the same amount of controllable stress. Values represent mean ± SE. Significant differences are noted with asterisks, $p < .05$.

Experiment 2

Sex Differences in Trace Conditioning Are Not Affected by Fluoxetine. As in the first experiment, there were sex differences in trace conditioning itself. Examining only the unstressed animals, females trained during proestrus emitted a greater

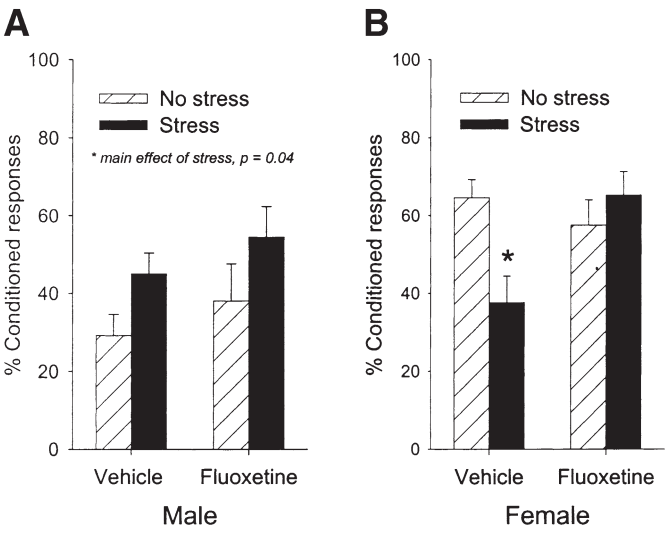

Figure 2. Chronic treatment (≥ 2 weeks) with the antidepressant fluoxetine prevents the effect of stress on learning in females but not males. **(A)** Exposure to the acute stressor of brief uncontrollable tailshocks enhanced conditioning in males, irrespective of chronic treatment with fluoxetine. **(B)** Exposure to the tailshock stressor impaired conditioning in vehicle-treated females but not in those that received chronic fluoxetine. Values represent mean ± SE. Significant differences are noted with asterisks, $p < .05$.

percentage of CRs than males [$F(1,33) = 15.50$, $p = .0004$]. The enhanced performance of females during trace conditioning occurred irrespective of fluoxetine treatment.

Fluoxetine Prevents the Effect of Stress in Females but Not Males. The percentage of CRs was used as the dependent measure of performance. Stressor exposure (stress, no stress) and antidepressant treatment (fluoxetine, vehicle) were independent variables. In males, there was a main effect of stress on the percentage of CRs [$F(1,32) = 4.7$, $p = .04$] (Figure 2A). Irrespective of treatment with fluoxetine, males emitted more CRs after stressor exposure when compared with unstressed males. Chronic treatment with fluoxetine did not affect conditioning itself, since the percentage of CRs emitted by unstressed males treated with fluoxetine did not differ from that of unstressed males injected with vehicle ($p = .22$). There was no effect of stress or antidepressant treatment on spontaneous blinking ($p = .07$; $p = .36$, respectively) or responses to the white noise stimulus ($p = .56$; $p = .64$, respectively).

In females, there was an interaction between antidepressant treatment and stressor exposure on the percentage of CRs [$F(1,31) = 8.16$, $p = .008$] (Figure 2B). Exposure to the stressor reduced the percentage of CRs in females injected with vehicle ($p = .01$); however, stressor exposure did not alter the percentage of CRs in females treated with fluoxetine ($p = .64$). Importantly, treatment with fluoxetine prevented the effect of stress on trace conditioning in females, since those injected with fluoxetine and stressed emitted more CRs than those injected with vehicle and stressed ($p = .02$). As in males, there was no detectable effect of fluoxetine on trace conditioning itself, since the percentage of CRs emitted by unstressed females treated with fluoxetine was not different from that of unstressed females injected with vehicle ($p = .42$). There was no effect of stress or antidepressant treatment on spontaneous blinking ($p = .33$; $p = .55$, respectively) or responses to the white noise stimulus ($p = .49$; $p = .93$, respectively).

The Protective Effect of Fluoxetine in Females Is Not Necessarily Associated with Anxiety. After trace conditioning, we assessed the effects of stressor exposure with and without antidepressant treatment on anxiety-related behavior in the elevated plus maze. This test creates a conflict between the exploratory drive of the rat and its innate fear of open spaces. Thus, increased open arm exploration is thought to reflect a decrease in anxiety. In males, neither stressor exposure nor antidepressant treatment affected percent time in the open arms of the elevated plus maze ($p = .64$; $p = .28$, respectively) (Table 1); however, in females, there was a main effect of stress as well as a main effect of antidepressant treatment on percent time in the open arms (Table 1). Specifically, both stressor exposure [$F(1,31) = 4.45$, $p = .04$] and fluoxetine [$F(1,31) = 8.08$, $p = .008$] decreased percent time in the open arms (Table 1). These effects were not attributable to changes in activity in the plus maze, since neither stress nor fluoxetine affected the number of closed arm entries ($p = .12$; $p = .13$, respectively). Also, there was no effect of stress or fluoxetine on gross motor activity ($p = .51$; $p = .81$, respectively).

Experiment 3

Acute Treatment with Fluoxetine Is Ineffective. A single injection of fluoxetine before the stressful experience did not lessen the impact of stress on trace conditioning. With or without fluoxetine, females exposed to the stressor emitted fewer CRs than unstressed females [$F(1,27) = 6.28$, $p = .02$] (Figure 3). There was no main effect of fluoxetine ($p = .92$) or interaction between stress and fluoxetine ($p = .75$) on the percentage of CRs. A single injection with fluoxetine did not affect anxiety behavior in the elevated plus maze ($p = .21$). As in the previous experiment, exposure to the acute stressor decreased percent time in the open arms [$F(1,27) = 7.10$, $p = .01$] (Vehicle no stress = 14 ± 2; Vehicle stress = 7 ± 3; Fluoxetine no stress = 22 ± 4; Fluoxetine stress = 9 ± 4). The number of closed arm entries was also decreased in response to stress ($p = .002$), but gross motor activity was unaffected ($p = .37$). Overall, these data indicate that acute exposure to fluoxetine does not prevent the effects of stress on trace conditioning or anxiety-related behaviors in females.

Discussion

Previous studies have shown that exposure to an uncontrollable stressful event impairs associative learning in females, but enhances learning in males (Shors et al 1992; Wood and Shors 1998; Wood et al 2001). Thus, females appear especially sensitive to the detrimental consequences of stressful experience. This observation is consistent with the clinical literature in which women are at higher risk for stress-related illness such as depression and posttraumatic stress disorder (Kendler 1998). In

Table 1. Anxiety Behavior in the Elevated Plus Maze

	% Time in Open Arms	
	Male	Female
Vehicle No Stress	23 ± 8 ($n = 9$)	33 ± 8 ($n = 9$)
Vehicle Stress	22 ± 8 ($n = 8$)	22 ± 6 ($n = 9$)
Fluoxetine No Stress	17 ± 8 ($n = 10$)	18 ± 5 ($n = 9$)
Fluoxetine Stress	12 ± 3 ($n = 8$)	7 ± 2 ($n = 8$)

In males, neither stressor exposure ($p = .64$) nor fluoxetine treatment ($p = .28$) affected percent time in the open arms. In females, exposure to the stressor ($p = .04$) and fluoxetine ($p = .008$) decreased percent time in the open arms, reflecting increased anxiety. Values represent mean ± SEM.

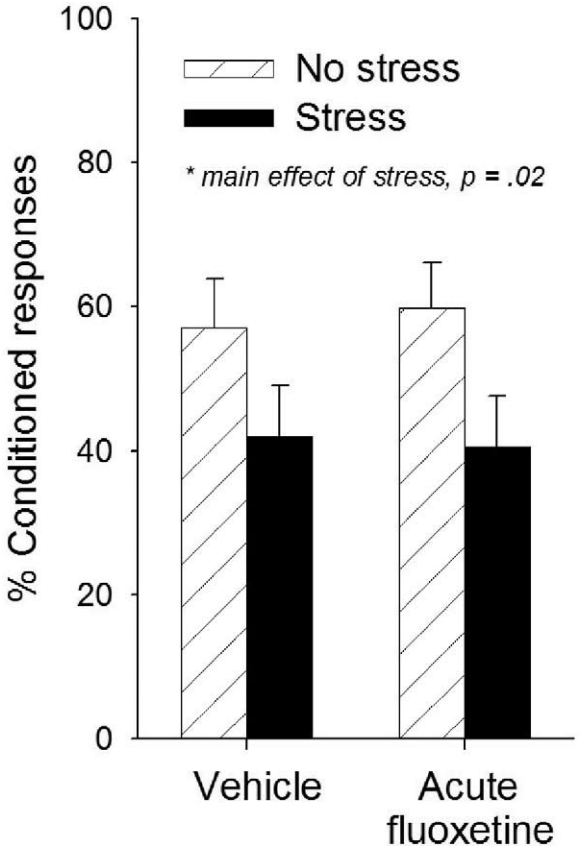

Figure 3. Acute (1 day) fluoxetine treatment did not alter the stress effect on learning in females. Females injected with vehicle and exposed to the acute tailshock stressor produced fewer conditioned responses as did those injected once with fluoxetine. Values represent mean ± SE. Significant difference noted with asterisk.

the present set of experiments, we asked whether these effects of stress on learning in rats were sensitive to treatment strategies used in these patient populations, namely controllability and antidepressant treatment. There are a number of findings to report from these studies but two stand out. The first is that the effects of stress on learning in both males and females were completely eliminated if the animals could establish control over the stress, even though they were exposed to the very same numbers and amounts of shock. Thus, the opposite effects of stress on conditioning in males versus females are mediated by psychological aspects of the stressful experience, namely controllability, and not the physical nature of the manipulation. The second notable finding reported here is that chronic treatment with the commonly prescribed antidepressant fluoxetine prevented the negative effect of stress on learning in females but had no effect in males. These data reveal sex differences in an animal's response to antidepressant treatment, effects that may be important for understanding the prevalence of stress-related mental disorders such as depression and PTSD in women. Each of these findings is discussed in turn below.

Controllability and Learned Helplessness in Males Versus Females

The present findings are generally consistent with most findings using "learned helplessness" procedures, that is, controllability prevents the effects of stress from being expressed (Maier and Jackson 1979; Shors et al 1989; Minor et al 1991; Jenkins et al 2001). However, the effect in males was unexpected since a "positive" response (that of enhanced learning) was prevented by establishing control. To our knowledge, this is the first demonstration that controllability can prevent an enhancement of subsequent learning. As noted, learned helplessness and the selective effects of uncontrollable stress on subsequent learning have been promoted as an animal model of depression. Since males without control were not helpless but were instead facilitated, these findings raise questions about the use of learned helplessness as an animal model for depression in humans. In fact, one might have expected the opposite result from that presented here–that "learning" how to control the stressor would further enhance their subsequent ability to learn. However, learning in males was essentially unaffected by controllable stress and not different from animals that were not exposed to any stressor experience. It could be argued that animals exposed to the uncontrollable stress experience the stressor differently than those exposed to the controllable stress. This is difficult to refute, although it has been shown that stress hormones such as corticosterone are not different in animals exposed to uncontrollable versus controllable stress (Maier et al 1986; Shors et al 1989).

In females, the effect of controllability was more expected in that controllability prevented the detrimental effect of stress on new learning. Helplessness effects have been notoriously difficult to observe in female animals (Kirk and Blampied 1985; Steenbergen et al 1990). A recent study did report helplessness effects during specific stages of the estrous cycle, although they likely reflect changes in performance rather than learning (Jenkins et al 2001). The detrimental effect of stress on classical conditioning, on the other hand, appears to reflect a learning deficit, at least to the extent that exposure to uncontrollable stress does not decrease the animal's sensitivity to the CS or the US (Bangasser and Shors 2004) nor alter pain sensitivity or general activity at the time of eyeblink conditioning (Wood and Shors 1998). Like those of Jenkins et al (2001), the effect of stress on learning is sensitive to stages of estrus and most evident when estrogen levels are increasing (Shors et al 1998; Wood et al 2001). Whether estrogen mediates the controllability effect reported here is unknown, since females were exposed to the escape training procedures each day for 1 week and would have been stressed at least once during each stage of their cycle.

Antidepressants and Learning in Males Versus Females

In a second experiment, we observed that daily treatment with the serotonergic antidepressant fluoxetine (i.e., Prozac) prevented the effect of stress on trace conditioning in females but not males. Treatment did not alter the overall rate of learning in either males or females, despite the presence of sex differences in learning itself. Together, these findings may inform us about the mechanisms whereby stress reduces performance in females (Wood et al 2001; Shors 2004). The effects of stress on learning can be interpreted in one of two ways; first, that stress directly impairs conditioning or second, that stress prevents the enhancement in learning that normally occurs during proestrus. The present data are consistent with the first explanation, since unstressed females (trained in proestrus) as well as those that were stressed in the presence of fluoxetine responded more than stressed females. Thus, treatment with fluoxetine did not prevent the enhanced learning that occurs in females during proestrus but rather prevented the effects of stress on learning.

The sex differences reported here are likely mediated by hormonal substrates that mediate the effects of stress on learning.

For example, glucocorticoids are necessary for the enhancing effect of stress on conditioning in males, yet do not contribute significantly to the impairment in females, which is instead dependent on the ovarian hormone estrogen (Wood et al 2001; Beylin and Shors 2003). Estrogen is considered a contributing factor to the high incidence of stress-related mental illness in women (Shors and Leuner 2003) and interacts with serotonergic processes (Bethea et al 1999). These interactions may explain why fluoxetine prevented the effects of stress in females but not males. It is also possible that fluoxetine selectively targets negative consequences of stress that just happen to be more prevalent in females. One of the predictions from these results is that women should be more responsive to SSRIs than men; this is indeed the case in depressed patients (Martenyi et al 2001; Joyce et al 2003).

Antidepressants and Anxiety in Males Versus Females

As an indirect measure of anxiety, we evaluated behavior in the elevated plus maze (Wall and Messier 2001). Three days after stressor exposure, females were more anxious whereas males were not, suggesting a sustained effect of stress in females. The increase occurred irrespective of treatment with fluoxetine and was not attributable to alterations in motor activity. Thus, the effect of stress on anxiety appears dissociated from that on learning and is generally consistent with studies in humans (Grillon and Hill 2003). For example, fluoxetine reportedly enhances memory performance in depressed patients independent of their emotional response or mood (Levkovitz et al 2002). Interestingly, chronic treatment ($\geq$ 2 weeks) with fluoxetine alone produced an increase in anxiety in females. One might assume that antidepressants would reduce anxiety, but humans and laboratory animals are often more anxious during the first few weeks of treatment (Jenike et al 1989; Silva et al 1999). In conclusion, the data on stress and anxiety add to the evidence that females are especially vulnerable to the detrimental consequences of stressful experience.

Potential Neural Mechanisms of Antidepressant Effects

Fluoxetine was only effective in preventing the detrimental effect of stress on learning in females if it was administered for weeks. These data are consistent with the well-established therapeutic delay seen in humans, an effect that has not been shown very often in the laboratory (Wong and Licinio 2001; Cryan et al 2002). Such a delayed response suggests that the effects of antidepressants are mediated by long-term changes in neuronal plasticity and/or anatomy (Brown et al 1999). One brain region that may be involved is the hippocampal formation. Clinical brain imaging studies have reported that hippocampal volume is decreased in depressed patients (Sheline et al 1996; Bremner et al 2000). In rats, dendritic spines in the female hippocampus decrease in number after stressor exposure and thus correlate with the decrease in learning after stress (Shors et al 2001a, 2004; Leuner and Shors 2004). Dendritic spines are sources of synaptic connectivity that are associated with the formation of new memories, including those acquired during classical eyeblink conditioning (Leuner et al 2003). Since these structures are sensitive to antidepressant treatment and manipulations of serotonin (Norrholm and Ouimet 2001; Alves et al 2002), fluoxetine may prevent the effects of stress on learning by its effects on spine density. Antidepressants are also known to affect the production of new neurons in the hippocampus (Malberg et al 2000), which are sensitive to stress and anxiety-related behaviors (Gould and Tanapat 1999; Santarelli et al 2003) and associated with the acquisition of trace memories (Gould et al 1999; Shors et al 2001b; Leuner et al 2004).

Women and Stress-Related Mental Illness

Although certainly not definitive, the present findings may model some aspects of depression and stress-related mental illness in women. Not only are women twice as likely to experience depression as men, they are also most vulnerable after stressful life events (Kendler et al 2000). Their depression is often accompanied by problems with declarative learning and memory, which is responsive to antidepressant treatment (Austin et al 2001; Levkovitz et al 2002; Vythilingam et al 2004). Since trace conditioning is considered a declarative memory task (Clark and Squire 1998), our results may reflect aspects of these learning deficits in humans (Kendler 1998; Shors and Leuner 2003). Minimally, they indicate major sex differences in the response to uncontrollable stress and in response to antidepressants. These differences may be important for understanding why women are so susceptible to stress-related mental illness such as depression, PTSD, and generalized anxiety disorder.

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