SEX DIFFERENCES IN THE GENERALIZATION OF FEAR AS A FUNCTION OF

RETENTION INTERVALS

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By

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Introduction

Anxiety disorders are the most prevalent mental disorder in the United States (Kessler, Chiu, Demler, & Walters, 2005). More specifically, females are 60% more likely than males to be diagnosed with an anxiety disorder (Kessler et al., 1994; Wang et al., 2005), and the exact cause of this gender difference is unknown. Posttraumatic stress disorder (PTSD) is one specific anxiety disorder with a sex difference in prevalence rates with females again showing a higher prevalence rate than males (Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995). PTSD is marked by the persistence of fear and anxiety as well as the tendency to generalize fear to novel cues (Brewin, 2001). Although little basic research has focused on sex differences in the generalization of fear responses, patients with PTSD display deficits in differential conditioning and context conditioning. For instance, PTSD patients have worse performance compared to controls when asked to discriminate between two CSs where one (CS+) predicts an unconditioned stimulus (US) and the other represents a safety cue (CS-) predicting no US (Grillon & Morgan, 1999; Lissek et al., 2008). In addition, PTSD patients show heightened responding to contextual cues compared to healthy controls (Grillon & Morgan, 1999).

As the above descriptions suggest, PTSD includes many of the features characteristic of classical (Pavlovian) fear conditioning. In classical fear conditioning, an innocuous stimulus is paired with an aversive outcome (i.e. footshock), which produces an association between the stimulus and the outcome. Subsequent presentations of the

cue alone will come to elicit a fearful response, which can be assessed directly (e.g., freezing) or indirectly (avoidance).

Studies with nonhuman animals have demonstrated that this learned response is context dependent; the fear response is disrupted when the animal is tested in a novel context shortly after training. However, when testing occurs after a long delay, animals no longer discriminate between the contexts; rodents freeze equivalently to the training and the novel context (Biedenkapp & Rudy, 2007; Riccio, Richardson, & Ebner, 1984; Wiltgen & Silva, 2007; Zhou & Riccio, 1996). Animals forget the cues associated with the learning episode faster than they forget the learned response. This phenomenon, in which responding generalizes over time, has been explained in terms of forgetting and is often referred to as the forgetting of stimulus attributes (for review, see Jasnow, Cullen, & Riccio, 2012).

The first evidence for generalized responding over time came from a study by Perkins and Weyant (1958) in which rats were trained in an appetitive runway task. They found that altering runway color from training to test significantly impaired rats' performance during an immediate test, but had no effect on performance when introducing a longer retention interval between training and test.

Many studies have replicated these initial findings by Perkins and Weyant, demonstrating that a change in context at test significantly impairs performance when tested immediately and the detriment of context change is alleviated at a longer retention interval. For example, Zhou and Riccio (1996) trained male rats in passive avoidance and tested 1or 14 days later in the same context as training or a novel context. At 1 day,

rats tested in the novel context displayed a significant impairment of the fear response (avoidance) compared to the group tested in the same context (i.e. the context shift effect). However, after 14 days, the groups tested in a novel context had comparable performance to the group in the same (training) context (Zhou & Riccio, 1996).

Within the broad range of research on the loss of context specificity over time, no study to date has investigated sex differences in the generalization of fear responses. A serendipitous finding in our lab looking at sex differences on the effect of adolescent nicotine exposure on fear generalization found that control males exhibited a context shift effect at a 5 day retention interval whereas control females exhibited comparable avoidance scores in either context (Cullen, Pickens, Fountain, & Riccio, In preparation). This observation suggested faster fear generalization in females than in males.

Extensive research has demonstrated sex differences in basic anxiety and conditioned fear responses (Archer, 1975; Frye, Petralia, & Rhodes, 2000; Stark et al., 2006; Stewart, Taylor, & Baker, 1997; Zorawski, Cook, Kuhn, & LaBar, 2005). Many of these studies have demonstrated that estrogens play a prominent role in sex differences of fear and anxiety-like behaviors (Morgan & Pfaff, 2001; Morgan, Schulkin, & Pfaff, 2004). For example, evidence suggests that changes in circulating levels of estrogens are linked to alterations in mood and emotion as well as to the development of mental illness in women (Hendrick, Altshuler, & Burt, 1996; ÖStlund, Keller, & Hurd, 2003; Sherwin, 2003; Sichel, Cohen, Robertson, Ruttenberg, & Rosenbaum, 1995; Walf & Frye, 2006), and also play a significant role on several aspects of nonhuman behavior (Edwards & Burge, 1971; Kimura & Hagiwara, 1985; Wu & Shah, 2011). Sex differences have been

well established in fear-related responses. However, the role of sex differences in the generalization of fear remains unknown.

The current study investigates a temporal gradient for fear generalization for males and females across different retention intervals and attempts to determine what role estrogens play in fear generalization in female rats. We hypothesized that intact females would exhibit fear responses in a novel context sooner than males, implying more rapid forgetting of contextual cues. In addition, we hypothesized that ovariectomized females receiving exogenous estradiol would show behavior similar to intact females, whereas those receiving no estradiol replacement would display fear responses similar to males. To test these hypotheses, we used a passive avoidance procedure in which rats were trained in one context and were later tested at different retention intervals in either the same or a novel context.

Experiment 1

Experiment 1 used a systematic approach to determining differences in the rate of fear generalization among male and female Long Evans rats. Previous research has demonstrated that fear generalization to a novel context takes several days in males (Wiltgen & Silva, 2007; Winocur, Moscovitch, & Sekeres, 2007; Zhou & Riccio, 1996), and the previous experiment in our lab demonstrated a difference between male and female controls at 5 days. Therefore, Experiment 1 used a passive avoidance procedure in which animals were trained to avoid the black compartment of a black-white shuttlebox and were tested in either the same context as training or a novel context at 4 different retention intervals of 1, 3, 5, or 7 days. In the experiment, females were expected to demonstrate significant generalized responding across contexts by the 5 day test and show complete generalization by the 7 day test, whereas males would show context specificity in responding at all intervals.

METHOD

Animals

Male and female adult Long-Evans rats approximately 90 days old provided by the breeding colony in the Department of Psychology at Kent State University were used in the experiment. Each group contained $n = 8$ or larger (always indicated). Animals were maintained on a 14/10 hour light/dark cycle. Food and water were available *ad libtum* throughout the experiment. A week prior to beginning the experiment, all rats were individually housed. All animal procedures were carried out in accordance with Kent State University Institutional Animal Care and Use (IACUC) guidelines.

Apparatus and Contexts

The training and testing apparatus were two identical 43.18 X 17.78 X 17.78 cm shuttle boxes placed on grid floors. The boxes were comprised of two chambers of equal size—one black and one white—that were divided by a guillotine door. Each box was placed on a table in one of two distinct contexts. Context A was a 1.6 X 2.33 meter room with house fluorescent lights, bare white walls, and no artificial scents or sounds. Context B was a 1.83 X 2.74 meter room lit by a 25-w red light bulb with posters on the walls. Context B contained white noise (70db) provided by a GPX AM/FM digital clock radio as well as a residual artificial scent via a Glade Plug-Ins Scented Oil Country Berry air freshener. In each context, the experimenter wore a different glove (Rubber *dish* glove in

A; vinyl lab glove in B) to handle the rat. Therefore, the stimulus conditions in the two

contexts differed in olfactory, visual, auditory, and cutaneous cues.

Training

Prior to training, animals were handled for 5 minutes on two consecutive days.

For training, animals were brought to Context A, held on the experimenter's hand for 30

seconds to provide a brief exposure to the context before being placed on the white side

of the shuttle box facing away from the closed guillotine door. Following a 20 second

exposure, the guillotine door was raised and the initial latency to cross into the black

compartment (all four paws) was recorded. Upon crossing, the guillotine door was

lowered and 2 nonescapable, 1 second, 0.6 milliamp (mA) scrambled footshocks were

delivered 5 seconds apart. A constant current AC shock generator (Model 5806, Lafayette

Instruments, Lafayette, IN) provided footshocks. Ten seconds after receiving the second

footshock, the animal was removed from the passive avoidance chamber and returned to

the main colony. Animals with a latency to cross longer than 100 seconds during

training—<1% of the animals— were removed from the final analysis (e.g. Ahmadi,

Zarrindast, Haeri-Rohani, Rezayof, & Nouri, 2007; Ahmadi, Zarrindast, Nouri, Haeri-

Rohani, & Rezayof, 2007; Khakpai, Nasehi, Haeri-Rohani, Eidi, & Zarrindast, 2012;

Zarrindast, Eidi, Eidi, & Oryan, 2002; Zarrindast, Farajzadeh, Rostami, Rezayof, &

Nourjah, 2005)

Testing

For testing, animals were brought back into the experimental room after a specific retention interval (1, 3, 5, or 7 days) at the same time of day as training, which has been shown to provide optimal retention (Holloway & Wansley, 1973). Half of the animals were tested in Context A (same) and half in Context B (shift). The test procedure was identical to training, except that the guillotine door remained open for 600 seconds and no shocks were delivered. The initial latency to cross was recorded as the dependent measures. After a total of 600 seconds had elapsed, the rat was removed and returned to the main colony.

Results and Discussion

Prior to analyzing the data with an analysis of variance (ANOVA), assumptions of normality and homogeneity of variance were checked. The assumption of normality was tested for the latency to cross at test measure and found non-significant skewness (-0.525) and kurtosis measures (-1.397), suggesting that the data were normally distributed. However, a Kolmogorov-Smirnov test to determine the deviation from normality of the latency to cross scores was significant, $D_{(178)} = 0.234$, $p < .001$. Although this finding suggests that the data significantly deviated from normality, transformation of the data was not undertaken considering the robustness of ANOVA analyses on non-normal data (e.g. Schmider, Ziegler, Danay, Beyer, & Bühner, 2010). To test the assumption of homogeneity of variance, a Levene's test of Equality of Error Variances was conducted and found to be significant, $F_{(15, 162)} = 2.687$, $p <.001$, indicating a violation of the assumption. ANOVA analyses are robust even when a violation is present with only a small probability of Type I error (e.g. Refinetti, 1996). Despite the significant value for Levene's—violating the assumption of homogeneity of variance—the impact of the violation were determined to be insufficient to affect the use of ANOVA for data analysis.

Experiment 1 utilized three independent variables. Therefore, several ANOVAs were conducted to examine the effects of a context change at different retention intervals across sex. The latency to cross into the black compartment during training was recorded for each animal and analyzed to determine any differences in cross-through latency prior

to fear conditioning. ANOVA analyses revealed no significant differences between groups, $F_{(15, 162)} = 1.725$, p > .05.

Following training, separate groups of rats were tested in either the same context as training or a novel context after retention intervals of 1, 3, 5, or 7 days. At test, males (Fig. 1) and females (Fig. 2) discriminated between contexts at equivalent levels after one day, but females exhibited fear generalization after a shorter retention interval compared to males. Specifically, males discriminated between contexts at all retention intervals, whereas females displayed comparable levels of fear, regardless of the testing context after a 5-day retention interval. (Fig. 1, 2).

A three-way ANOVA analysis revealed the interaction of sex*context was significant, $F_{(1,162)} = 7.047$, p <.01, demonstrating that females displayed significant generalization compared to males. The interaction of sex*retention intervals was also significant, $F_{(1,162)} = 4.341$, p <.01, indicating initial cross differences for males and females at different retention intervals. The main effect for sex was significant $F_{(1,162)} = 10.042$, p <.01, suggesting that overall, females had higher latency to cross scores indicative of higher levels of fear regardless of the context of testing or the retention interval. The main effect for context was also significant, $F_{(1,162)} = 110.224$, p <.001, indicating higher latency to cross scores in the same condition compared to the novel condition. Simple effect analyses were run to parse apart the significant interactions.

Simple effect analyses confirmed the prediction that males would maintain memory precision across the retention intervals. Males displayed a context shift effect at 1 day, same: $n = 11$; novel: $n = 11$; $F_{(1,162)} = 24.730$, p <.001, 3 days, same: $n = 10$; novel:

$n = 11$; $F_{(1,162)} = 25.463$, p <.001, 5 days, same: $n = 11$; novel: $n = 8$; $F_{(1,162)} = 16.407$, p <

.001, and 7 days, same: $n = 11$; novel: $n = 11$; $F_{(1,162)} = 16.315$, p <.001.

The expectation that females would exhibit fear generalization by day 7 was also

confirmed, same: $n = 10$; novel: $n = 10$; F $F_{(1,162)} = 2.609$, ns. In fact, females were

unable to discriminate between contexts by the 5-day test, same: $n = 10$; novel: $n = 10$;

$F_{(1,162)} = 3.117$, ns. Overall, females took significantly longer than males to cross into the

black compartment in the novel context at the 5-day and 7-day retention interval, 5 Day:

$F_{(1,162)} = 10.970$, p < .001; 7 Day: $F_{(1,162)} = 13.498$, p < .001, but not 3 days, $F_{(1,162)} =$

2.440, ns. At 1 day, females display a nearly identical context shift effect as seen in

males, same: $n = 10$; novel: $n = 12$; $F_{(1,162)} = 28.045$, p <.001, and displayed significant

memory precision for contextual cues at 3 days, same: $n = 11$; novel: $n = 11$; $F_{(1,162)} =$

7.957, p <.01. Thus, females discriminated between contexts 1 and 3 days after training,

but exhibited generalized fear to a novel context when testing occurred 5 days or more

after training.

Overall, these data suggest that, although females can perceive the contextual

differences (one day test), they tend to generalize fear to novel contextual cues at a

shorter retention interval than males. Females spent comparable amounts of time on the

safe side—regardless of the context—when testing occurred 5 or 7 days after training.

This outcome indicates a rapid loss of the precision of memory for contextual

characteristics in female rats.

Experiment 2

Experiment 1 provided evidence that females display a distinct pattern of fear generalization to novel contextual cues compared to males. In order to better determine potential factors involved in the sex difference of fear generalization, Experiment 2 utilized a more systematic investigation of the role of estrogens in fear generalization. In Experiment 1, no control over the estrus cycle was exerted. Therefore, Experiment 2 used ovariectomized female rats to control for the levels of estrogens present during the experiment.

Estrogens are a type of gonadal steroid hormone that consists of several different subtypes. Of those subtypes, 17β-estradiol is considered to be the most active subtype of the estrogen family (Fiocchetti, Ascenzi, & Marino, 2012). 17β-estradiol has been shown to influence memory formation and cognitive processes by affecting areas of the brain not associated with reproductive behaviors, such as the hippocampus and cortex (Barha & Galea, 2010; McEwen, 2002; McEwen & Alves, 1999). Therefore, Experiment 2 was designed to examine the role of estrogens in the generalization of fear by manipulating the normal function of estrogens in females via 17β-estradiol capsule implantations in ovariectomized females.

METHOD

Animals

86 female Long Evans rats approximately 71 days old were obtained from the breeding colony in the Department of Psychology at Kent State University. All animals were individually housed following ovariectomy surgery and were maintained on a 14/10 hour light:dark cycle. Food and water were available *ad libtum* throughout the experiment. All animal procedures were carried out in accordance with Kent State University Institutional Animal Care and Use (IACUC) guidelines.

Apparatus and Contexts

The passive avoidance chambers and contexts were the same as in Experiment 1.

Training and Testing

Passive avoidance training and testing were conducted as described in Experiment 1. Animals were only tested at the 1 and 5 day intervals, which was the only difference in the procedure. Animals with a latency to cross longer than 100 seconds during training—<1% of the animals— were removed from the final analysis.

Ovariectomy

Female rats were anesthetized with isoflurane and received a 5 mg/kg dose of Ketoprofen 5 minutes before bilateral ovariectomy through a dorsal incision. After

removal of the ovaries, the incision was sutured using surgical staples and either an empty silastic capsule or a silastic capsule containing 17β-estradiol was inserted behind the shoulder blades of the animal. The silastic (polydimethylsiloxane) implants were constructed from silastic tubing (i.d. 0.078 inches, o.d. 0.125 inches) cut to a 5 mm length. Each end was filled with Factor II medical adhesive 1 mm in length.   The hormone was packed into the remaining 3 mm length, which has been shown to produce levels of about 30-40 pg/ml of estradiol (Bridges, 1984; Hiroi & Neumaier, 2006). Before implantation, all capsules were incubated in saline solution for 24 hours at 37°C. Animals received another injection of Ketoprofen 24 hours post-surgery at the same dosage as the first injection. Animals were handled 7 days following surgery and began training 9 days after surgery.

Results and Discussion

Assumptions of normality and homogeneity of variance were analyzed prior to using ANOVA analyses. Normality analyses revealed non-significant skewness (-0.641) and kurtosis (-1.437) measures, but a significant Kolmogorov-Smirnov test, indicating that the latency to cross scores at test significantly deviated from a normal distribution, $D_{(85)} = 0.298$, $p < .001$. To test homogeneity of variance, a Levene's test of Equality of Error Variances was conducted and found to be significant, $F_{(7, 77)} = 2.738$, $p < .05$, indicating a violation of the assumption. These findings are similar to those found in Experiment 1, therefore, ANOVA analyses were determined to be the most effective approach to analyzing the data, despite the violations of assumptions of normality and homogeneity of variance.

ANOVA analyses of latency to cross at training revealed no significant differences between the groups, $F_{(7, 77)} = 1.430$, $p > .05$. This finding demonstrates that surgeries and capsule implantation did not adversely affect one group over another. Testing occurred 1 or 5 days after training in either the same or novel context. The 5-day interval was chosen because that was when intact females displayed increased fear generalization. OVX Control females (OVX-C) with no estradiol replacement (Fig. 3), and OVX Estradiol (OVX-ES) females (Fig. 4) discriminated between contexts at 1 day at equivalent levels; but OVX-ES females exhibited fear generalization at the 5-day retention interval, whereas OVX-C females exhibited context discrimination through 5 days.

The three-way ANOVA analysis revealed a non-significant three-way interaction, $F_{(1, 77)} = 0.430$, ns, and no significant two-way interactions, Context*Hormone: $F_{(1, 77)} = 0.115$, ns; Context*Interval $F_{(1, 77)} = 0.623$, ns; Hormone*Interval $F_{(1, 77)} = 0.215$, ns. Despite no statistical significance in our interaction terms, the lack of a significant interaction is not evident by examination of Figure 4. The lack of a significant interaction appears to be driven by the stability of the OVX-C females in both the same and shifted context at 1 and 5 days. Therefore, to investigate the impression of a significant interaction as demonstrated by Figure 4, we conducted additional analyses to investigate whether differences among groups were present.

Simple effect analyses revealed that both ovariectomized groups displayed a context shift effect at 1 day. OVX-C, same: $n = 10$; novel: $n = 9$; $F_{(1,77)} = 4.471$, p $<.05$, and OVX-ES, same: $n = 12$; novel: $n = 12$; $F_{(1,77)} = 7.393$, p $<.01$, spent significantly more time in the same context compared to the novel context when tested 1 day after training. Removal of the ovaries with no estrogen replacement resulted in male-like memory precision for the OVX-C animals at 5 days, same: $n = 10$; novel: $n = 9$; $F_{(1,77)} = 3.949$, p $< .05$. However, 17β –estradiol capsule implantation resulted in fear generalization similar to that seen in intact females. OVX-ES animals were unable to discriminate between contexts at the 5 day interval, same: $n = 12$; novel: $n = 11$; $F_{(1,77)} = 1.316$, ns. These results suggest that estrogens have an impact on fear generalization to novel contextual cues in female rats.

General Discussion

We have demonstrated sex differences in fear generalization driven by estrogen concentrations. In the current study, the longest interval was not long enough to decrease memory precision in males (Wiltgen & Silva, 2007; Zhou & Riccio, 1996). In contrast, intact females—who could discriminate between contexts at 1 day—showed comparable levels of fear in either context by day 5. After ovariectomy, females with no hormone replacement behaved in a similar manner to males, whereas those implanted with 17β-estradiol capsules behaved the same as intact females, demonstrating that the tendency to generalize fear faster in females is driven, in part, by estrogens—specifically 17β-estradiol.

Other explanations for the sex differences in fear generalization—such as differences in exploratory behavior (Archer, 1974), differential rates of acquisition in passive avoidance (Denti & Epstein, 1972), and shock sensitivity (Beatty & Beatty, 1970; Blizard, 1971; Snowdon, Bell, & Henderson, 1964)—can be ruled out due to intact males and females displaying equivalent behavioral responses at 1 day, as indicated by the context shift effect. Similarly, the context shift effect exhibited by both OVX-C and OVX-ES females suggests that neither surgery nor capsule implantation had a negative impact on passive avoidance acquisition or the ability to discriminate contextual cues at a short interval.

In general, estrogens appear to increase arousal either by increasing overall activity levels in a safe environment or increasing fear or anxiety in an uncertain

environment (For review, see Morgan et al., 2004). However, the effect of estrogens on fear learning has yielded some conflicting results. Some experiments have demonstrated decreased fear in rodent models after estrogen treatment (Gupta, Sen, Diepenhorst, Rudick, & Maren, 2001; Markus & Zecevic, 1997), whereas others have reported an increase in fear (Jasnow, Schulkin, & Pfaff, 2006; Morgan & Pfaff, 2001).

Although not specifically examined, estrogens did not increase or decrease overall avoidance levels. Rather, estrogens affected the ability to discriminate between contexts at a longer retention interval (i.e. 5 days). Therefore, the results may be best discussed in terms of selective attention. Estrogens in females—either endogenous or exogenous— appear to affect selective attention. For instance, estradiol benzoate given to ovariectomized rats results in decreases latent inhibition (Nofrey, Ben-Shahar, & Brake, 2008) and also disrupts fear inhibition in a discrimination learning task (Toufexis, Myers, Bowser, & Davis, 2007). Thus, estrogens seem to enhance attention capacity, resulting in negative consequences when the task in question requires focus on specific stimuli or ignoring specific stimuli. Animals respond fearfully to novel contextual cues instead of inhibiting the fear response in the presence of those irrelevant stimuli.

In the present study, we demonstrated that estrogens mediate, in part, the generalization of fear responses to novel contexts in females. Questions still remain about the mechanisms of this effect. One potential mechanism is an interaction of estrogens with the pituitary adenylate cyclase activating peptide (PACAP) system, which has been implicated in anxiety-like behaviors (e.g. Hammack et al., 2010). For instance, Ressler et al. (2011) found that levels of PACAP were associated with greater PTSD symptoms and

greater fear responses in a fear discrimination task. Moreover, a polymorphism in the receptor for PACAP (PAC1), which contains an estrogen response element, was highly associated with PTSD only in females (Ressler et al., 2011). The interaction of estrogens with PACAP and downstream elements of the stress response require further study in order to determine a more specific role of estrogens in the generalization of fear and in disorders like PTSD.

In addition to the stress response system, estrogens may be acting upon memory precision within two theoretical frameworks of memory storage: systems consolidation and the transformation hypothesis. The systems consolidation approach to memory storage states that a memory is consolidated and preserved in original form in neocortical sites. However, evidence suggests that the transformation hypothesis of memory storage may explain the loss of memory precision over time. The transformation hypothesis states that a memory is transformed over time to a more schematic representation that is stored in neocortical sites (Nadel, Samsonovich, Ryan, & Moscovitch, 2000; Rosenbaum, Winocur, & Moscovitch, 2001; Winocur et al., 2007; see Moscovitch, Nadel, Winocur, Gilboa, & Rosenbaum, 2006 for review). Brain scans during memory retrieval show differential activation of the hippocampus and prefrontal cortex for newly acquired memories versus older memories. New memories have high hippocampal activation and low activation of the prefrontal cortex (PFC), whereas older memories have low hippocampal activation and high PFC activation (Bontempi, Laurent-Demir, Destrade, & Jaffard, 1999; Frankland, Bontempi, Talton, Kaczmarek, & Silva, 2004; Maviel, Durkin, Menzaghi, & Bontempi, 2004). The differences in activation are mirrored in studies

assessing the effects of lesions of the hippocampus; lesions directly after training eliminate the context shift effect at short intervals. However, when the hippocampal lesions are delayed, performance is not affected in either context, suggesting the hippocampus is only required when the memory is still context-dependent or precise (Wiltgen et al., 2010; Winocur, Frankland, Sekeres, Fogel, & Moscovitch, 2009; Winocur et al., 2007). Therefore, the hippocampus may be involved in processing a memory as long as that memory remains context-dependent.

Hippocampal function is important for the acquisition of new memories and is a site for estrogenic effects. For instance, synaptic remodeling of the CA1 region of the hippocampus occurs during the estrous cycle in female rats (McEwen, Gould, Orchinik, Weiland, & Woolley, 1995; Woolley, Gould, Frankfurt, & McEwen, 1990). Ovariectomy results in a decrease of spine density in the CA1 region and estrogen replacement prevents such a decrease (Gould, Woolley, Frankfurt, & McEwen, 1990; Woolley, Weiland, McEwen, & Schwartzkroin, 1997). Furthermore, treatment with estradiol benzoate in females facilitates synaptic plasticity in the CA1 (Córdoba Montoya & Carrer, 1997) and dentate gyrus of the hippocampus (McClure, Barha, & Galea, 2013).

A finding by Ruediger et al. (2011) implicated the involvement of feed-forward inhibition within the CA3 hippocampus in precise contextual memories. Ruediger et al. (2011) demonstrated that feed-forward inhibition via increased filopodial contacts on neurons in the CA3 region of the dorsal hippocampus was associated with context fear conditioning. After training, these filopodial contacts decreased over time and were associated with the generalization of fear to a novel context. Immunoreactivity and *in*

situ hybridization studies have shown the presence of the two major estrogen receptor subtypes—ERα and ERβ—in the CA3 region of the hippocampus (Azcoitia, Sierra, & Miguel Garcia-Segura, 1999; Milner et al., 2005; Shughrue & Merchenthaler, 2000). Estrogens may act upon these receptors to modulate the connections made between the CA3 and CA1 region of the hippocampus by increasing the ability of CA3 neurons to synchronize with CA1 targets (Woolley, Wenzel, & Schwartzkroin, 1998; Yankova, Hart, & Woolley, 2001) and interact with the filopodial contacts from the CA3 region. Taken together, these data suggest that estrogens may lead to a quicker transfer of a memory to neocortical sites from the hippocampus, resulting in a less precise memory at earlier time points in females compared to males, although much remains unknown about the specific role of estrogens in memory precision. Future studies should focus on the role of estrogens in the hippocampus to help elucidate the role of estrogens in the transformation of memory from the hippocampus to neocortical sites.

PTSD is marked by the expression of inappropriate fear behaviors in normally safe environments. Given the relevance of fear generalization in PTSD and other anxiety disorders, the current study attempted to determine if differences in fear generalization might help explain differential prevalence rates among males and females. As hypothesized, female rats responded fearfully in a novel context at a shorter retention interval than male rats, and the finding is due, at least in part, to circulating estrogens in female rats. Research into sex differences in fear generalization may provide a new direction for developing effective treatments for PTSD and other anxiety disorders with discrepancies in prevalence rates.
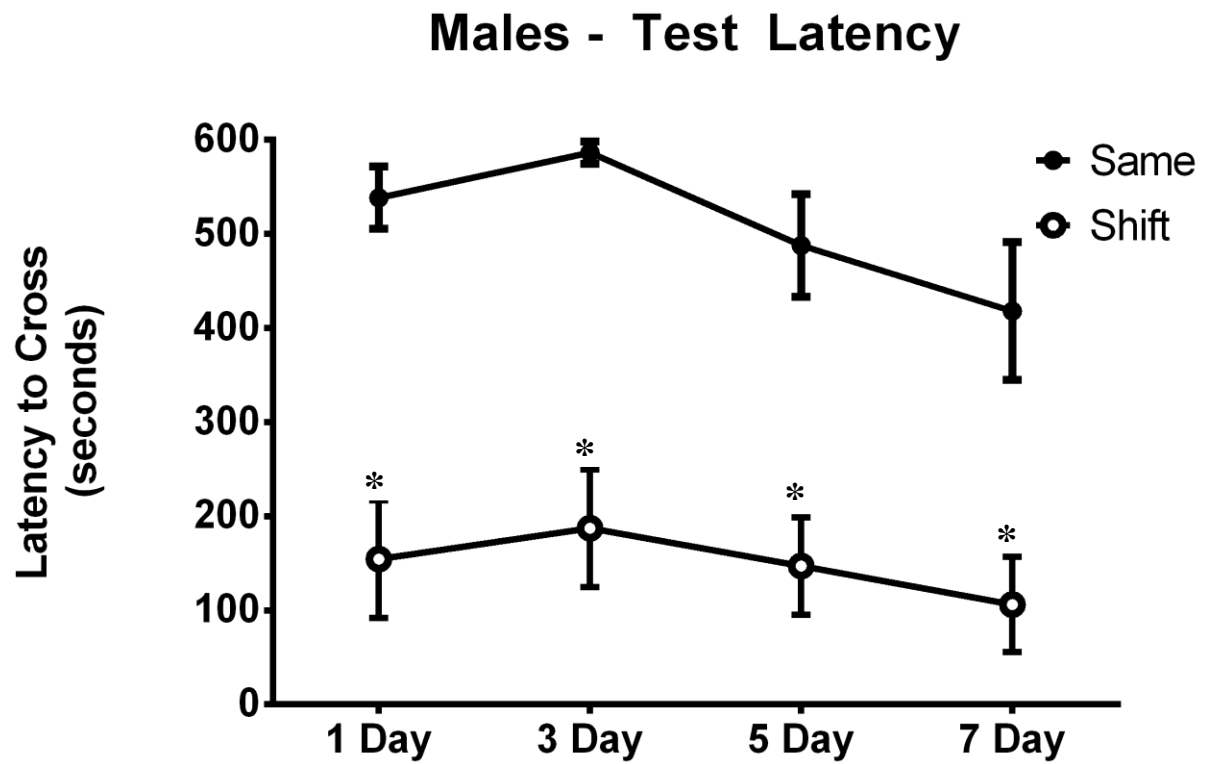
Figure 1: Grand mean (±SEM) latency to cross in seconds for males. The novel context groups were tested in context B. Males exhibit a significant context shift effect at all retention intervals.
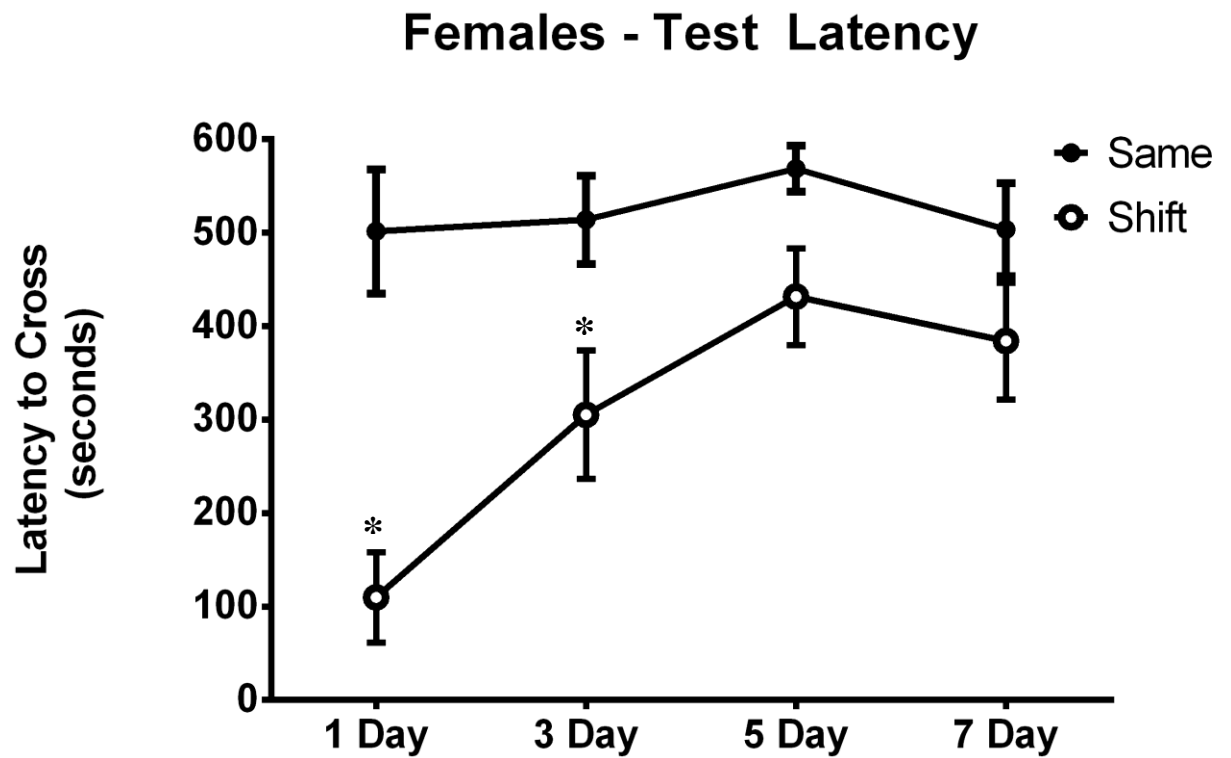
Figure 2: Grand mean (±SEM) latency to cross in seconds for females. The novel context groups were tested in context B. Females exhibit a significant context shift effect at a 1-day and 3-day retention interval. The shift groups at 5 and 7 days demonstrate significant generalization of fear to the novel context (context B).
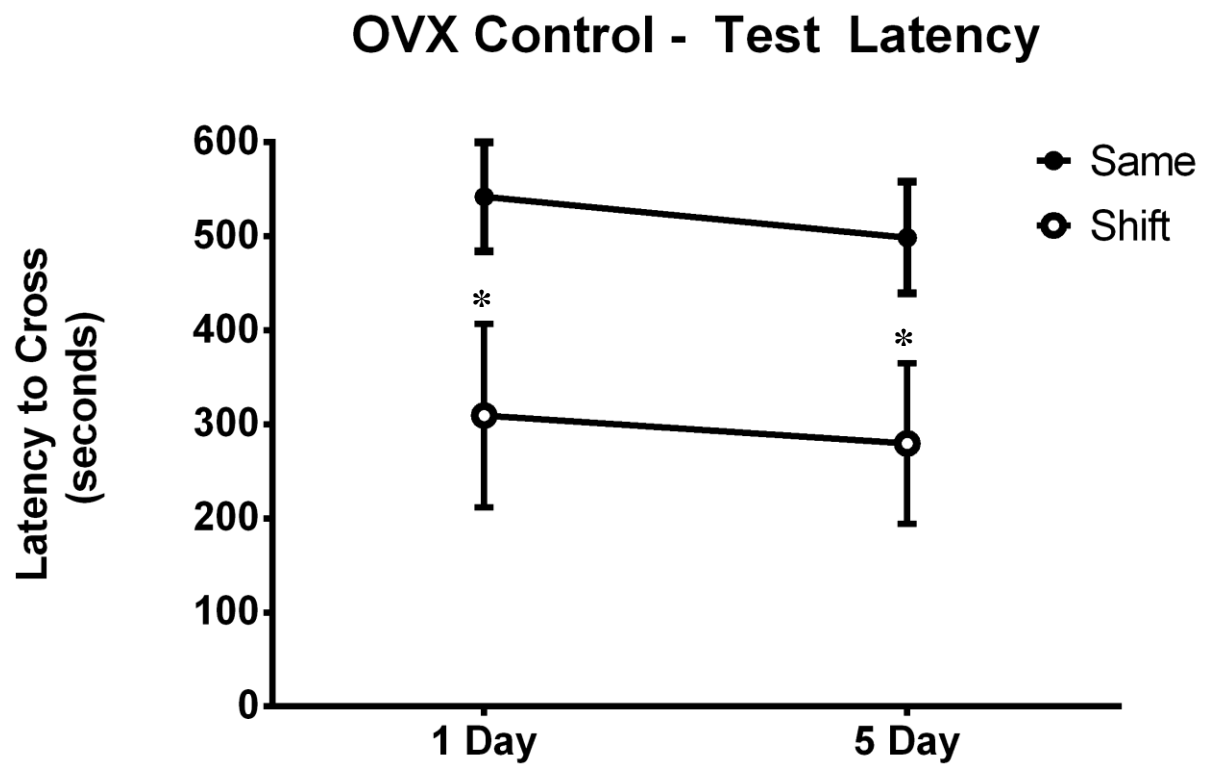
OVX Control - Test Latency

Figure 3: Grand mean (±SEM) latency to cross in seconds for OVX-Controls. The novel context groups were tested in context B. Control animals exhibited a significant context shift effect at a 1-day and 5-day retention interval.
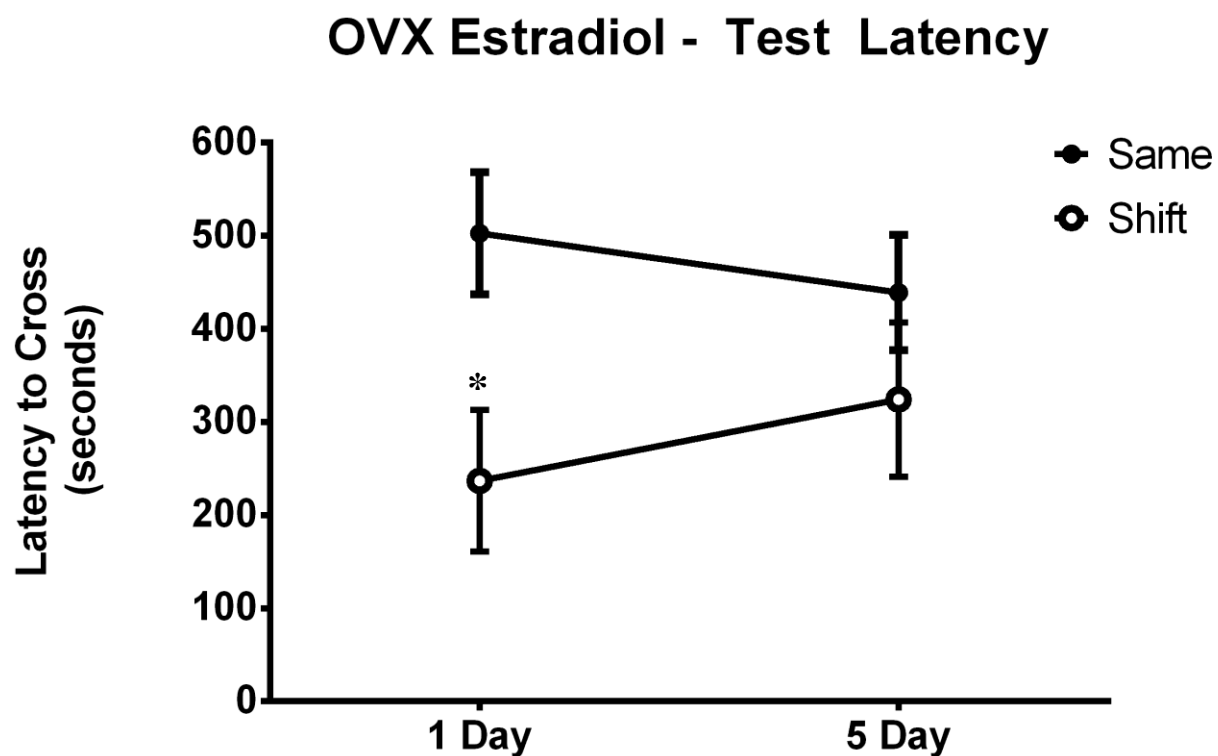
Figure 4: Grand mean (±SEM) latency to cross in seconds for OVX-Estradiol females. The novel context groups were tested in context B. OVX-Estradiol females exhibit a significant context shift effect at a 1-day retention interval. They demonstrated significant generalization of fear to the novel context by the 5-day retention interval.

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