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Short Communication

Sex differences in the single prolonged stress model

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HIGHLIGHTS

- Sex differences in the SPS model of PTSD were examined.
- SPS induces extinction retention deficits in male, but not female, rats.
- SPS enhanced GR expression in female, but not male, rats.
- Female rats are resilient to SPS.
- GR upregulation does not always coincide with extinction deficits in the SPS model.

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ABSTRACT

Post traumatic stress disorder (PTSD) is a debilitating anxiety disorder resulting from traumatic stress exposure. Females are more likely to develop PTSD than males, but neurobiological mechanisms underlying female susceptibility are lacking. This can be addressed by using nonhuman animal models. Single prolonged stress (SPS), a nonhuman animal model of PTSD, results in cued fear extinction retention deficits and hippocampal glucocorticoid receptor (GR) upregulation in male rats. These effects appear linked in the SPS model, as well as in PTSD. However, the effects of SPS on cued fear extinction retention and hippocampal GRs in female rats remain unknown. Thus, we examined sex differences in SPS-induced cued fear extinction retention deficits and hippocampal GR upregulation. SPS induced cued fear extinction retention deficits in male rats but not female rats. SPS enhanced GR levels in the dorsal hippocampus of female rats, but not male rats. SPS had no effects on ventral hippocampal GR levels, but ventral hippocampal GR levels were attenuated in female rats relative to males. These results suggest that female rats are more resilient to the effects of SPS. The results also suggest that GR upregulation and cued fear extinction retention deficits can be dissociated in the SPS model.

Post traumatic stress disorder (PTSD) is a debilitating anxiety disorder which results from traumatic stress exposure [1]. Females are twice as likely to suffer from PTSD relative to males [2] even though the probability of trauma exposure in females is lower [3]. This suggests that females are more susceptible to the effects of trauma. However, neurobiological mechanisms through which this increased susceptibility manifests have not been sufficiently explored. Non-human animal models of PTSD [4], such as the single prolonged stress (SPS) model, can be useful for exploring these mechanisms. Unfortunately, SPS has only been previously conducted in male rats.

Fear extinction retention deficits and enhanced glucocorticoid receptor (GR) expression are both symptoms observed in PTSD patients [5–7]. Fear extinction retention deficits refer to the failure to inhibit fear conditioned responding (e.g. conditioned freezing) to a previously extinguished fear conditioned stimulus (CS) [8]. Enhanced GR levels have been implicated in PTSD symptomatology [7], and changes in GR function have been suggested to contribute to trauma susceptibility in females [9]. Fear extinction retention deficits and enhanced hippocampal GR expression are also observed in the SPS model [10,11], and these two effects may be linked [12]. Thus, examining sex differences in the SPS model with regard to fear extinction retention and GR expression could lead to a better understanding of how GR function contributes to susceptibility to trauma in female humans. The current study examined the effects of SPS on cued fear extinction retention and GR expression in the dorsal hippocampus (dHipp) and ventral hippocampus (vHipp) of male and female rats.

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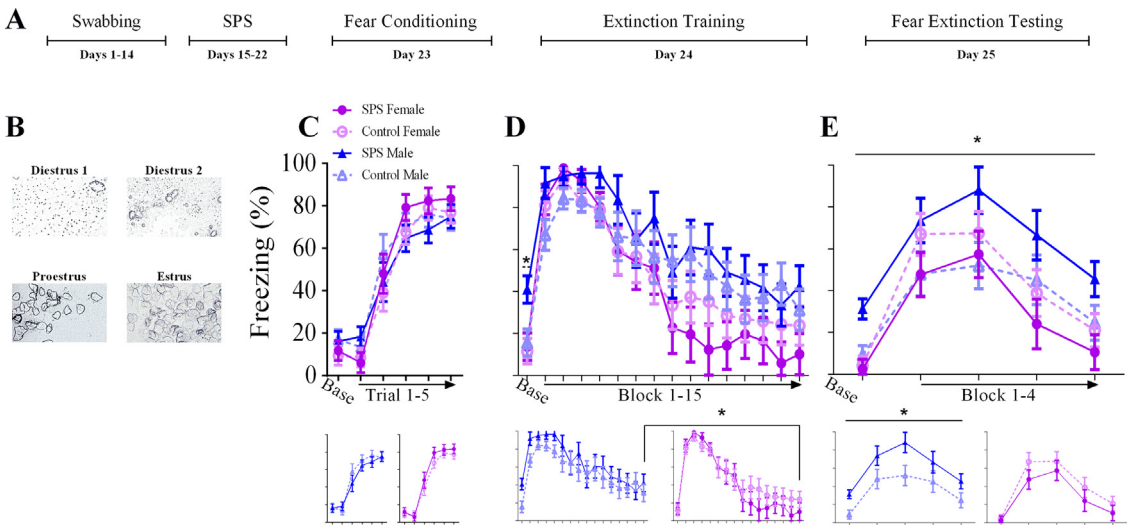

Fig. 1. (A) Denotes experimental design. (B) Representative cell types for each stage of the estrus cycle. (C) Acquisition of fear conditioning was not affected by stress or sex. (D, top panel) SPS/males exhibited enhanced freezing to the context during the baseline of extinction training, indicative of deficits in contextual fear memory discrimination. (D, bottom panel) Female rats showed enhanced acquisition of fear extinction relative to male rats as evidenced by lower freezing in the final fear extinction block. (E) Contextual fear discrimination deficits in SPS/males seen in extinction training persisted into the cued fear extinction retention test. SPS/males also showed cued fear extinction retention deficits. Neither of these effects were present in SPS/females. In (C), (D), and (E), the bottom panels show the data for males and females separately. (*) Denotes statistical significance.

Twenty four male and female Sprague-Dawley rats were obtained from Charles River (Portage, MI) as subjects. Upon arrival, male (postnatal day (PD) 43–45, 151–175 g) and female rats (PD 40–44, 126–150 g) were housed in same-sex pairs. Rats were placed on a 12-h light/dark cycle and allowed ad libitum access to water and 23 g/day of standard rat chow per the manufacturer's recommendation after a five-day acclimation period with ad libitum access to food. All experimental procedures were performed in compliance with approval from The University of Delaware Institutional Animal Care and Use Committee following guidelines established by the NIH.

Vaginal smears were collected from female rats daily using cotton swabs dipped in sterile saline for 14 days prior to any experimental procedures to determine stage of estrus cycle. Loose epithelial cells gathered from swabbing were mounted onto slides to enable visualization under a light microscope. Male rats were swabbed in the anogenital region daily as a control procedure. Females were pseudo-randomly assigned to SPS or control condition based upon stage of estrus cycle such that equal numbers of females in each stage would be present in the SPS and control groups.

Female (PD 58–62) and male (PD 61–63) rats underwent SPS and control procedures as previously described [13]. The SPS procedure was comprised of 120 min of restraint, 20 min of forced swimming, and ether exposure (70 mL) until general anesthesia was induced. Control animals were left in their home cages in a novel room for the duration of the SPS procedure. Following these procedures, rats were housed individually and allowed an undisturbed post-stress incubation period of seven days because this is necessary to observe SPS effects [11,14].

Fear conditioning, extinction training, and retention testing protocols were conducted as previously described [12,14]. Fear conditioning was conducted in Context A and was comprised of five CS-unconditioned stimulus (US) pairings. The CS was a tone (2 kHz, 10 s, 80 dB) which co-terminated with the US footshock (1 mA, 1 s). Extinction training was conducted 24 h later in Context B and involved 30 CS-only presentations. Extinction retention testing was conducted in Context B (i.e. the extinction training context) 24 h after extinction training and involved eight CS-only presentations. This context-shift procedure minimizes the effects of contextual fear conditioning on cued fear and extinction memory phenomena [15]. All behavioral sessions employed a 210 s baseline period and 60 s inter-trial intervals (ITI). Cameras located on the boxes' ceilings recorded behavioral videos using Any-maze software (Stoelting Inc.). Videos were scored offline.

One day after cessation of fear extinction retention testing, all rats were sacrificed via rapid decapitation. Western blot electrophoresis was used to assay GR levels as previously described [12]. The hippocampus was divided into the dHipp and vHipp and GR content was analyzed in these brain regions separately. Homogenates from brain samples were electrophoresed on Tris–HCl gels and transferred onto nitrocellulose membranes. Rabbit polyclonal antibody (1:500 (Santa Cruz biotechnology Inc., Santa Cruz, CA, USA), M-20) was used to visualize GRs, while mouse monoclonal antibody (1:250–1000 (Santa Cruz biotechnology Inc., Santa Cruz, CA, USA), C4) was used to visualize the reference protein, β-actin. Fluorescent tagged goat anti-rabbit and anti-mouse antibodies were used to visualize primary antibodies (Li-COR, 1:2000). Membranes were scanned on a LI-COR Odyssey CLx scanner and Image Studio software was used to score protein bands. Samples for each subject were run across multiple gels. For each subject, data from all gels were averaged.

Any-maze was used to score freezing in behavioral videos as previously described [12]. Freezing during the CS presentation and the following ITI were blocked into one trial and converted into percentages for statistical analyses. For extinction training and testing, cued freezing during two trials was averaged into one block. All behavioral data was subjected to a stress (SPS vs. control) × sex (male vs. female) × trial or block ($1-n$) factor design. Main and simple effects were analyzed using analysis of variance (ANOVA), while main and simple comparisons were analyzed using a *t*-test with a Bonferroni correction where necessary.

GR levels were expressed relative to β-actin and subjected to a stress × sex factor design. A second analysis was performed on GR levels, whereby GR levels were normalized relative to the respective control group separately for males and females and then analyzed with a one-sample *t*-test. $p < 05$ was set as the threshold to define statistical significance. If data from an animal was at least three standard deviations from its corresponding group mean, the data from this animal was removed from the study. This resulted