Supplemental Information

Dependence of acoustic startle response (ASR) on exposure to situational reminders (SRs). To determine if there was a relationship between SR exposure and the development of the behavioral correlates of PTSD, we assessed the ASR in a group of Traumatic Experience with Reminders of Stress (TERS) protocol-exposed mice after the 3rd and the 6th SRs.

Methods: The ASR was tested as described in the main body of the paper. For these experiments, mice were tested as described in the TERS protocol (see Figure 1), but also the day after the third SR. Repeated measures analysis of variance (ANOVA) was utilized to assess the significance of the dose response effect of repeated SRs.

Results: To determine if the increase in ASR was due to repeated exposures to SRs, comparisons were made in ASR at baseline, after 3 SRs, and after 6 SRs (Figure S1). Repeated measures ANOVA of each group revealed that only in the TERS-susceptible group did the ASR change after 3 and 6 SRs (F (2,24) = 19.28, $p < 0.0001$). Post hoc analysis with Tukey's test revealed that the ASR in this group increased in a dose dependent manner following 3 and 6 SRs in the TERS-susceptible group (Baseline vs. 3 SR $p < 0.01$; 3 SR vs. 6 SR $p < 0.05$).

Distribution of startle data in control, TERS-susceptible, and TERS-resilient groups. In order to better illustrate the distribution of ASR scores in control, TERS-susceptible, TERS-resilient, and TERS-excluded groups, here we present scatter plots of the percent change in ASR and the raw ASR posttest scores for all mice included in the present study.

Methods: The ASR was tested as described in the manuscript.

Results: Results are shown in Figure S2.

cFos expression in control, TERS-susceptible, and TERS-resilient mice following a swim stress. Representative sections of cFos staining in the locus coruleus (LC), central nucleus of the amygdala (CeA), basal amygdala (BA), ventral bed nucleus of the stria terminalis (vBNST), and ventral tegmental area (VTA) are shown to demonstrate the patterns of cFos expression we observed in control, TERS-susceptible, and TERS-resilient mice.

Methods: cFos labeling was performed as described in the manuscript.

Results: Sections are shown in Figure S3.

TERS-resilient mice show a trend toward decreased responsiveness to the behavioral effects of increased noradrenergic outflow. Since increased noradrenergic outflow and receptor activation may contribute to the behavioral correlates of PTSD symptoms seen in TERS-susceptible mice, we posited that one mechanism that might confer resiliency to the TERS-resilient group is decreased sensitivity to noradrenergic outflow. To test this hypothesis, the drug yohimbine was administered to TERS-exposed and control mice. Yohimbine is an α_2-AR antagonist that induces anxiogenic effects by increasing central noradrenergic outflow (1, 2). In healthy human controls, yohimbine elicits an anxiety response (3), whereas in patients with PTSD, yohimbine can induce flashbacks and panic attacks (1, 2). In mice, yohimbine increases freezing behavior and thereby decreases locomotion (4).

Methods: Yohimbine Challenge. Locomotion during the yohimbine challenge test was quantified using the Flex-Field Cage Rack locomotor system (San Diego Instruments, San Diego, CA). Mice were placed in a clear Plexiglas container with clean bedding (47 × 25.4 × 20.3 cm) for two hours to acclimate. Mice were then given an injection of saline (ip) and their locomotion was recorded for 2 hours. Mice were then given an injection of yohimbine (2 mg/kg ip) and their locomotor activity was recorded for two hours. Periodic observation of the mice during both trials confirmed that decreased locomotor activation during the yohimbine trial was due to freezing behavior, defined as no movement except for breathing. Data were summarized by subtracting each mouse's yohimbine locomotor score from its saline locomotor score, then analyzed by a 1-way analysis of covariance (5). The dependent variable was change in locomotor activity across treatment (yohimbine minus saline), the independent variable was TERS group, and saline locomotor count was a covariate.

Results: TERS-resilient mice showed a trend (F (2,78) = 2.696, $p < 0.0737$) toward less of a decrease in locomotor activation following yohimbine treatment compared to controls. TERS-susceptible mice did not differ from control mice. These data suggest the possibility that TERS-resilient mice may be less sensitive to the effects of yohimbine-induced increased noradrenergic outflow than controls.

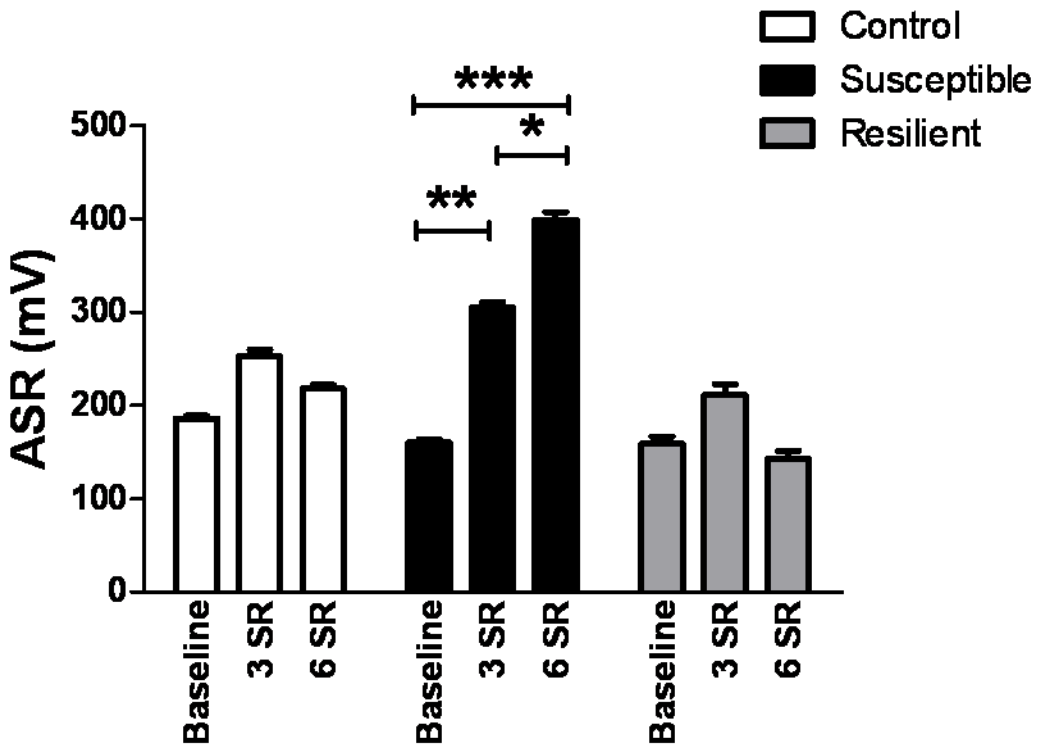

Figure S1. Acoustic startle response (ASR) increases in a situational reminder (SR)-dependent manner in TERS-susceptible, but not TERS-resilient or control mice. To determine if exposure to SRs contributed to increased ASR in TERS-susceptible mice, we assessed ASR after three and six SRs. TERS-susceptible mice (n = 25, black bars) showed a progressive increase in ASR after three and six SRs. TERS-resilient (n = 13, gray bars) and no-shock control mice (n = 30, white bars) did not show increases in ASR after either 3 or 6 SRs.
**p* < 0.05, ***p* < 0.01, ****p* < 0.001

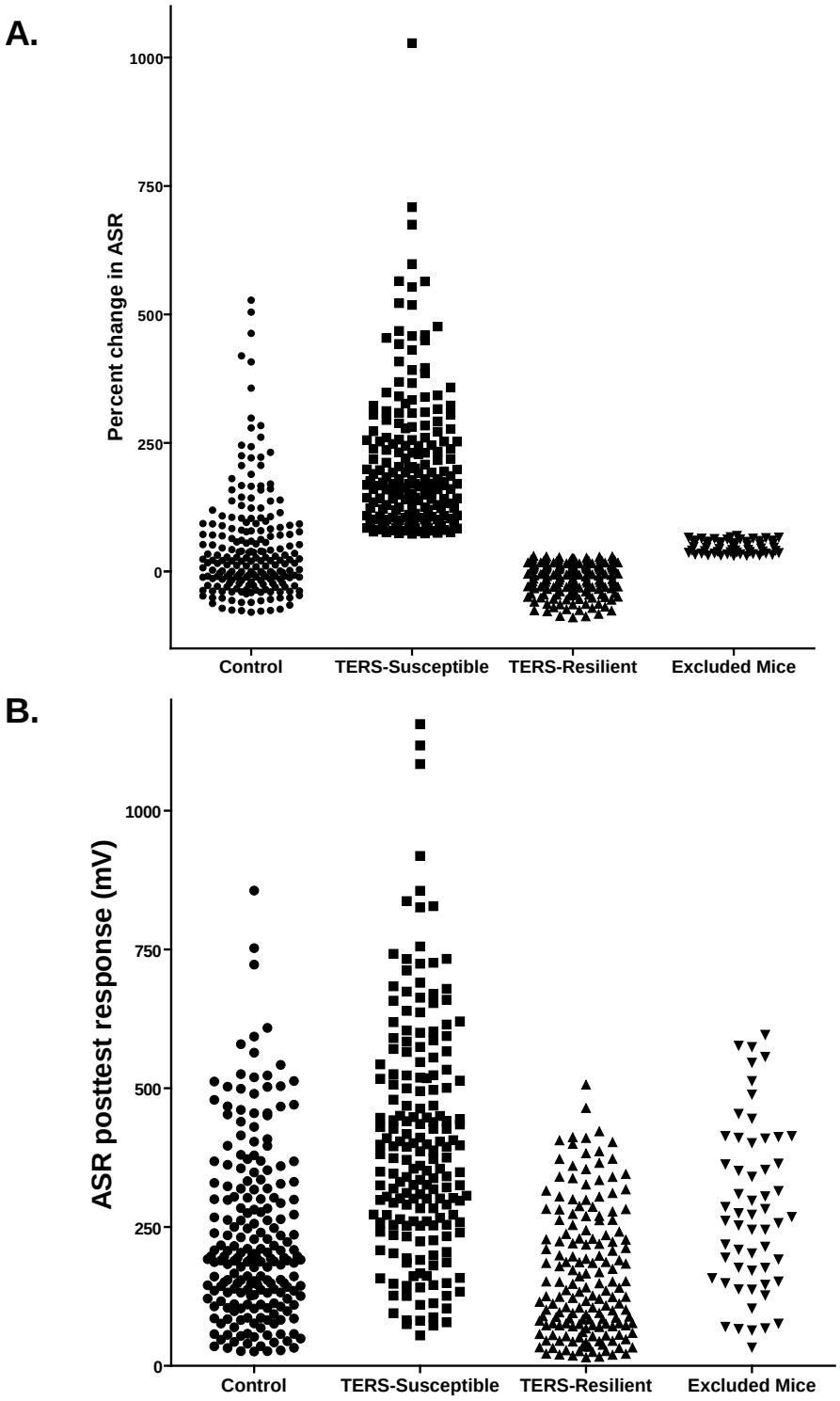

Figure S2. Acoustic startle response (ASR) in TERS-susceptible, TERS-resilient, TERS-excluded and control mice. **(A)** Scatter plot of percent change in ASR scores of control (n = 208), TERS-susceptible (n = 186), TERS-resilient (n = 164), and TERS-excluded mice (n = 57). Mice were separated into TERS susceptible, resilient, and excluded cohorts using these data. **(B)** Scatter plot of raw posttest ASR scores of all mice.

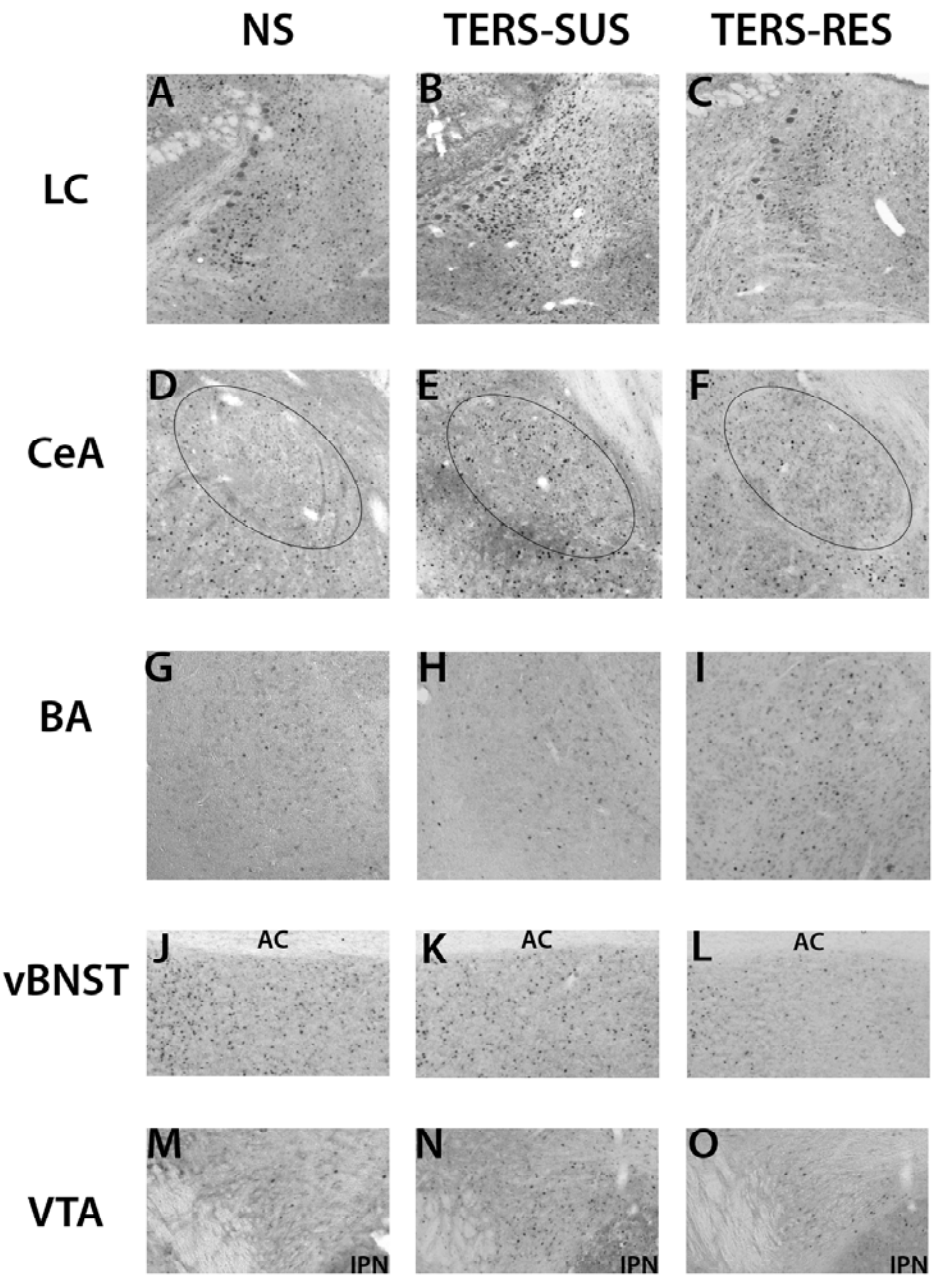

Figure S3. Representative sections of cFos staining in TERS-exposed and control mice. **(A-C)** Representative sections from the locus coeruleus (LC) of no-shock control mice (NS), TERS-susceptible mice (TERS-SUS), and TERS-resilient mice (TERS-RES). Note the preponderance of staining in the TERS-susceptible LC compared to the control. **(D-F)** Representative sections from the central nucleus of the amygdala (CeA) of control and TERS-exposed mice. Oval shapes represent approximate area where cFos labeled cells were counted. **(G-I)** Representative sections from the basal amygdala (BA) of control and TERS-exposed mice. **(J-L)** Representative sections from the ventral bed nucleus of the stria terminalis (vBNST) of control and TERS-exposed mice. Note the relative paucity of staining in the TERS-resilient brain compared to the control. **(M-O)** Representative sections from the ventral tegmental area (VTA) of control and TERS-exposed mice. AC, anterior commissure; IPN, interpeduncular nucleus.

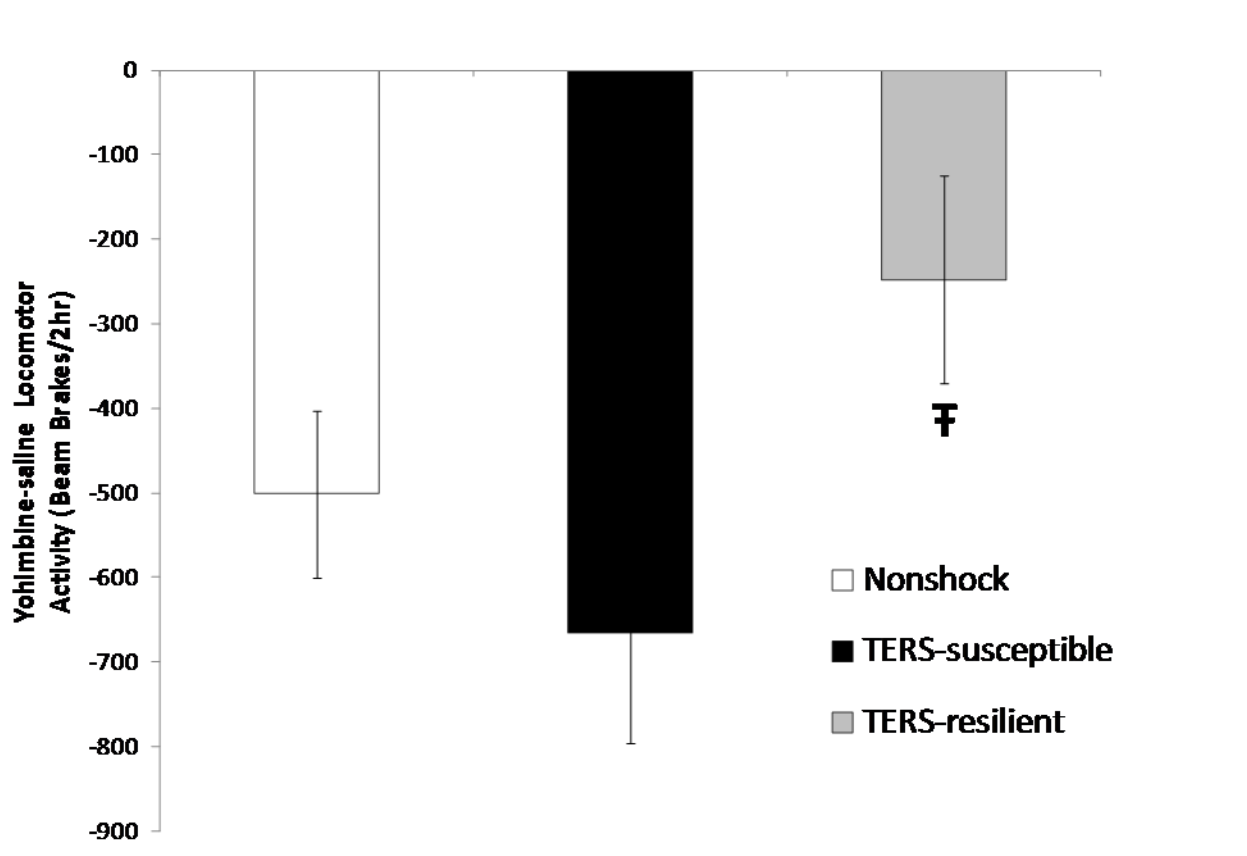

Figure S4. TERS-resilient mice show a trend toward decreased responsiveness to yohimbine. Data were summarized by subtracting each mouse's yohimbine locomotor score from its saline locomotor score, then analyzed by a 1-way analysis of covariance (5). When compared to control mice (white bars), TERS-resilient mice (gray bars) showed a trend ($^{Ŧ}p = 0.0737$) toward less of a decrease in locomotor activation following yohimbine injection (2 mg/kg, ip). TERS-susceptible mice (black bars) did not differ from controls.

Supplemental References

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