# SUPPLEMENTARY INFORMATION

Fear Reduced Through Unconditional Stimulus Deflation Is Behaviorally Distinct From Extinction and Differentially Engages the Amygdala

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Male and female rats were used in the present experiments to create experimental groups. Here, we stratify the data from each experiment by sex. While in some cases there was overall more freezing in males, the general patterns observed in overall group means held when sex was accounted for as a biological variable. However, it should be noted that in every experiment, we were underpowered to fully detect sex differences. Therefore, while our data suggest there were no meaningful sex differences in the UCS deflation paradigm or associated molecular changes, this does not mean that a sufficiently-powered experiment designed to more explicitly examine sex as a biological variable would not detect sex differences. The present results suggest that inclusion of male and female subjects within experiments will help uncover basic learning processes that hold between sexes and provide important steps forward in the treatment of anxiety disorders which are common in both men and women.
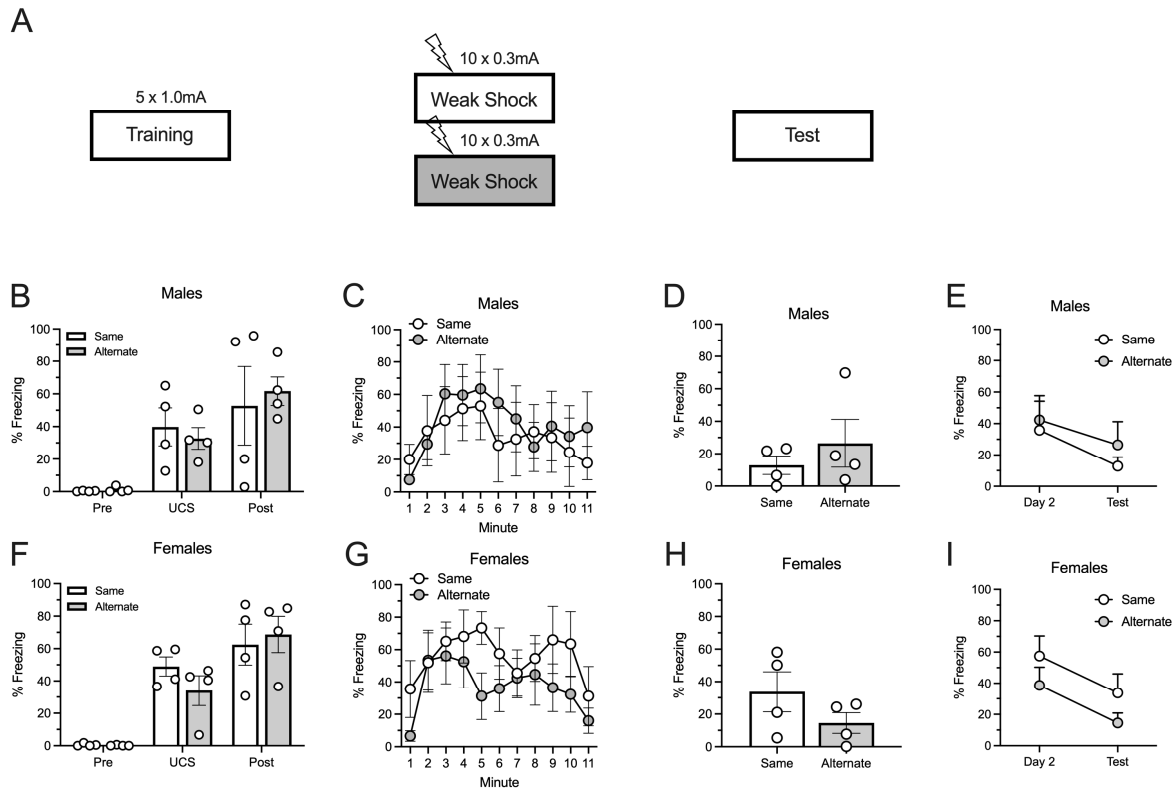
Supplementary Figure 1. Results from the top half of Figure 1 stratified by sex. **A)** Experimental design. Data from male subjects are presented in Panels B-E, data from female subjects are presented in Panels F-I. **B, F)** Sex did not impact acquisition of responding in a meaningful way. This was confirmed by a 3(Group) x 2(Sex) x 3(Time Period) ANOVA conducted to assess responding during this session. This found a main effect of time, $F_{(2,46)}=251.95$, $p<0.001$, but no other main effects or interactions, largest $F=1.13$, $p=0.333$. **C, G)** Sex did not interact with Day 2 responding in a meaningful way. A 2(Group) x 2(Sex) x 11(Minute) ANOVA found a main effect of time, $F_{(10,50)}=8.81$, $p<0.001$, sex, $F_{(1,15)}=7.14$, $p<0.017$, and group, $F_{(1,15)}=7.89$, $p<0.013$, but no interactions, largest $F=1.08$, $p=0.385$. This suggests that while males and weak shock groups froze more on average, this did not interact in a meaningful way and all groups decreased responding throughout the session. **D, H)** Sex did not interact with testing. A 3(Group) x 2(Sex) ANOVA assessed responding during the test and found a main effect of group, $F_{(2,23)}=7.51$, $p=0.003$, and a marginal effect of sex, $F_{(1,23)}=4.11$, $p=0.054$, but no interaction, $F=1.54$, $p=0.237$, again suggesting that males in general froze more than females, even if marginally. **E, I)** A 2(Group) x 2(Sex) x 2(Session) assessed responding from Day 2 to testing and found a main effect of session, $F_{(1,15)}=29.83$, $p<0.001$, and a session by group interaction, $F_{(1,15)}=6.99$, $p=0.018$, but no other main effects or interactions, largest $F=4.22$, $p=0.058$.
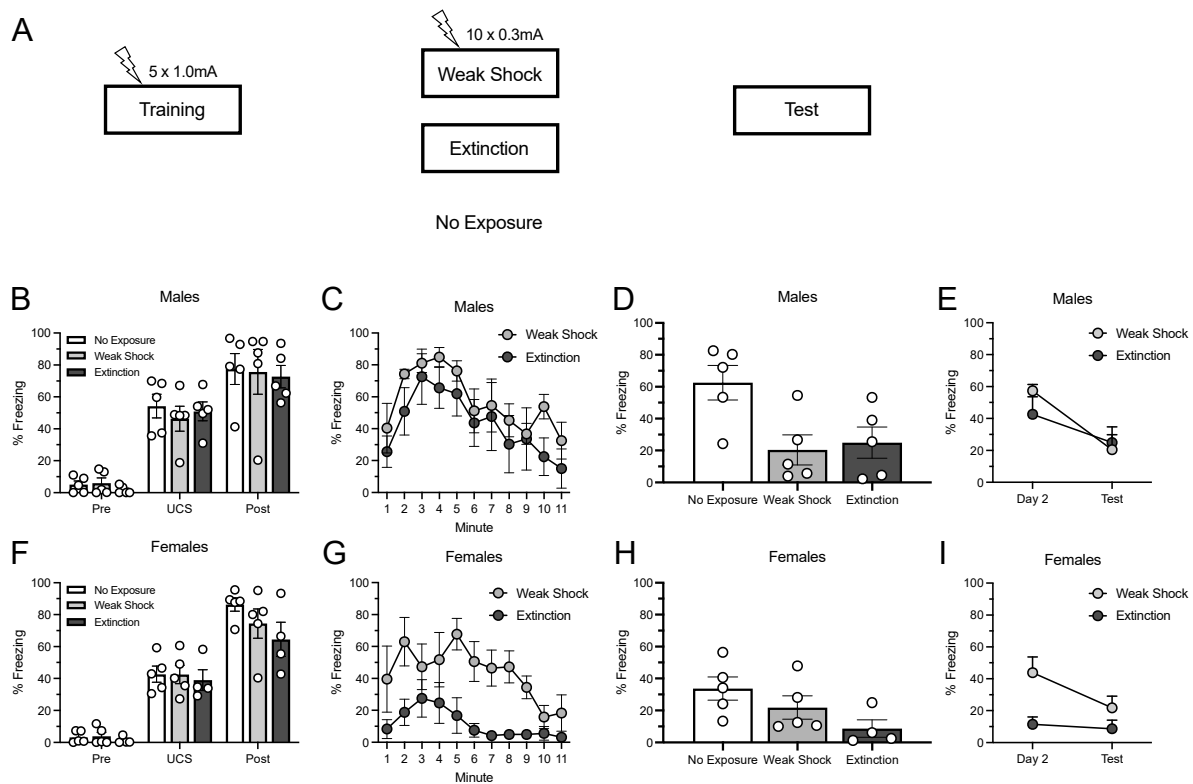
Supplementary Figure 2. Results from the bottom half of Figure 1 stratified by sex. **A)** Experimental design. Data from male subjects are presented in Panels B-E, data from female subjects are presented in Panels F-I. **B, F)** Sex did not interact during acquisition of responding. A 3(Group) x 2(Sex) x 3(Time Period) ANOVA conducted to assess responding during this session found a main effect of time, $F_{(2,24)}=114.15, p<0.001$, and a main effect of sex, $F_{(1,12)}=4.75, p=0.050$, but no other main effects or interactions, largest $F=2.52, p=0.102$. The effect of sex seemed to be driven by the fact that males froze more during the UCS period but both groups increased their responding during the session. **C, G)** Sex did not impact Day 2 in a meaningful way, as confirmed by a 2(Group) x 2(Sex) x 11(Minute) ANOVA that found a main effect of time, $F_{(10,80)}=4.90, p<0.001$, a marginal time by group interaction $F_{(10,80)}=1.94$, $p=0.051$, and no other main effects or interactions, Fs<1. **D, H)** Sex did not interact with testing. A 3(Group) x 2(Sex) ANOVA assessed responding during the test and found no main effects or interactions, largest $F=1.31$, $p=0.274$. **E, I)** A 2(Group) x 2(Sex) x 2(Session) assessed responding from Day 2 to testing and found a main effect of session, $F_{(1,8)}=14.19$, $p=0.005$, but no other main effects or interactions, Fs<1.
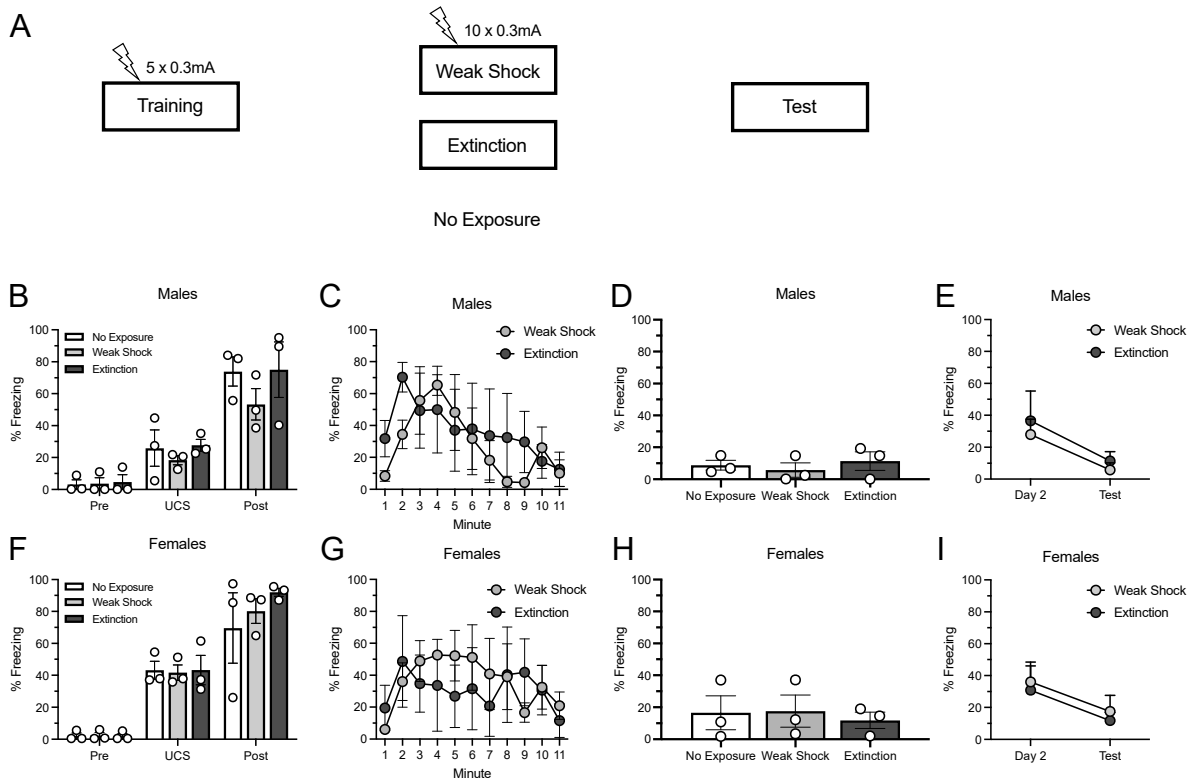
**Supplementary Figure 3.** Results from the top half of Figure 2 stratified by sex. **A)** Experimental design. Data from male subjects are presented in Panels B-E, data from female subjects are presented in Panels F-I. **B, F)** Sex did not interact during acquisition of responding. A 2(Group) x 2(Sex) x 3(Time Period) ANOVA conducted to assess responding during this session found a main effect of time, $F_{(2,24)}=53.44, p<0.001$, but no other main effects or interactions, largest $F=1.25, p=0.305$. **C, G)** Sex did not impact Day 2 in a meaningful way, as confirmed by a 2(Group) x 2(Sex) x 11(Minute) ANOVA that found a main effect of time, $F_{(10,120)}=6.25, p<0.001$, but no other main effects or interactions, Fs<1. **D, H)** Sex did not interact with testing. A 2(Group) x 2(Sex) ANOVA assessed responding during the test and found no main effects or interactions, largest $F=2.42, p=0.146$. **E, I)** A 2(Group) x 2(Sex) x 2(Session) assessed responding from Day 2 to testing and found a main effect of session, $F_{(1,12)}=10.14, p=0.008$, but no other main effects or interactions, largest $F=1.77, p=0.208$, again suggesting sex had no impact.

Supplementary Figure 4. Results from the bottom half of Figure 2 stratified by sex. **A)** Experimental design. Data from male subjects are presented in Panels B-E, data from female subjects are presented in Panels F-I. **B, F)** Sex did not interact during acquisition of responding. A 2(Group) x 2(Sex) x 3(Time Period) ANOVA conducted to assess responding during this session found a main effect of time, $F_{(2,22)}=87.02$, $p<0.001$, but no other main effects or interactions, largest $F=3.66$, $p=0.082$. **C, G)** Sex did seem to impact extinction, largely driven by females in the same context extinction group not showing declines throughout the session. This was confirmed by 2(Group) x 2(Sex) x 11(Minute) ANOVA that found a main effect of time, $F_{(10,110)}=5.23$, $p<0.001$, a marginal effect of sex, $F_{(1,11)}=4.41$, $p=0.060$, as well as a sex by group interaction, $F_{(1,11)}=5.96$, $p=0.033$, and a time by sex interaction, , $F_{(10,110)}=1.93$, $p=0.048$. **D, H)** Sex did not interact with testing. A 2(Group) x 2(Sex) ANOVA assessed responding during the test and found no main effects or interactions, largest $F=1.56$, $p=0.234$. **E, I)** A 2(Group) x 2(Sex) x 2(Session) assessed responding from Day 2 to testing and found a main effect of group, $F_{(1,11)}=7.66$, $p=0.018$, a marginal effect of sex, $F_{(1,11)}=4.68$, $p=0.053$, as well as a session by group interaction, $F_{(1,11)}=12.52$, $p=0.005$. No other main effects or interactions were significant, largest $F=2.10$, $p=0.176$.
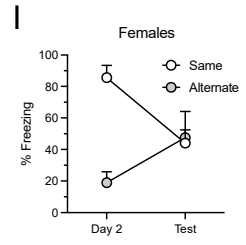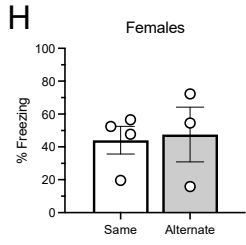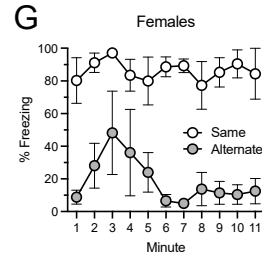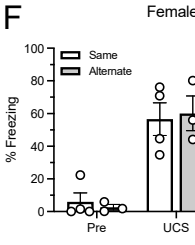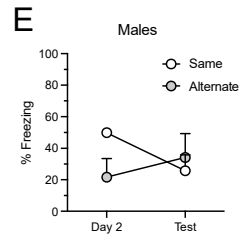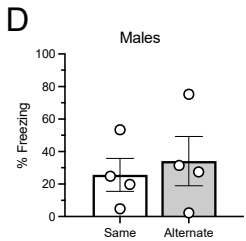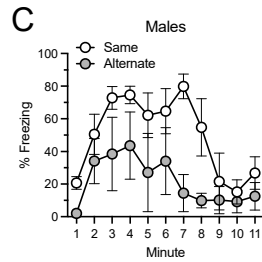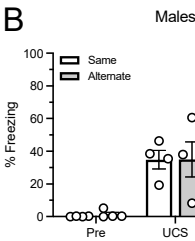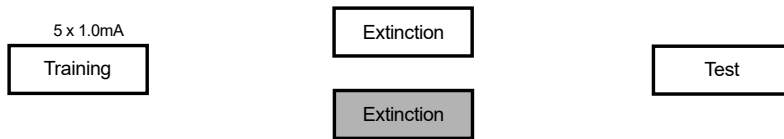
Supplementary Figure 5. Molecular data stratified by sex. **A, B)** Sex did not severely impact DH activity assessed through zif268 expression. This was confirmed by a 4(Group) x 2(Sex) ANOVA that found a main effect of group, $F_{(3,281)}$=4.34, p=0.005, but no effect of sex, F<1, and a marginal interaction, $F_{(3,281)}$=2.58,p=0.054. **C, D)** Sex did not impact BLA activity assessed through zif268 expression. This was confirmed by a 4(Group) x 2(Sex) ANOVA that found a main effect of group, $F_{(3,304)}$=8.04, p<0.001, but no effect of sex or interaction, largest F=1.22, p=0.302. **E, F)** Synaptic K48 within the BLA did not interact meaningfully with sex. This was confirmed by a 4(Group) x 2(Sex) ANOVA that found a main effect of group, $F_{(1,25)}$ =3.37, p=0.034, but no effect of sex or interaction, largest F=1.87, p=0.161.