

1 Supplemental materials

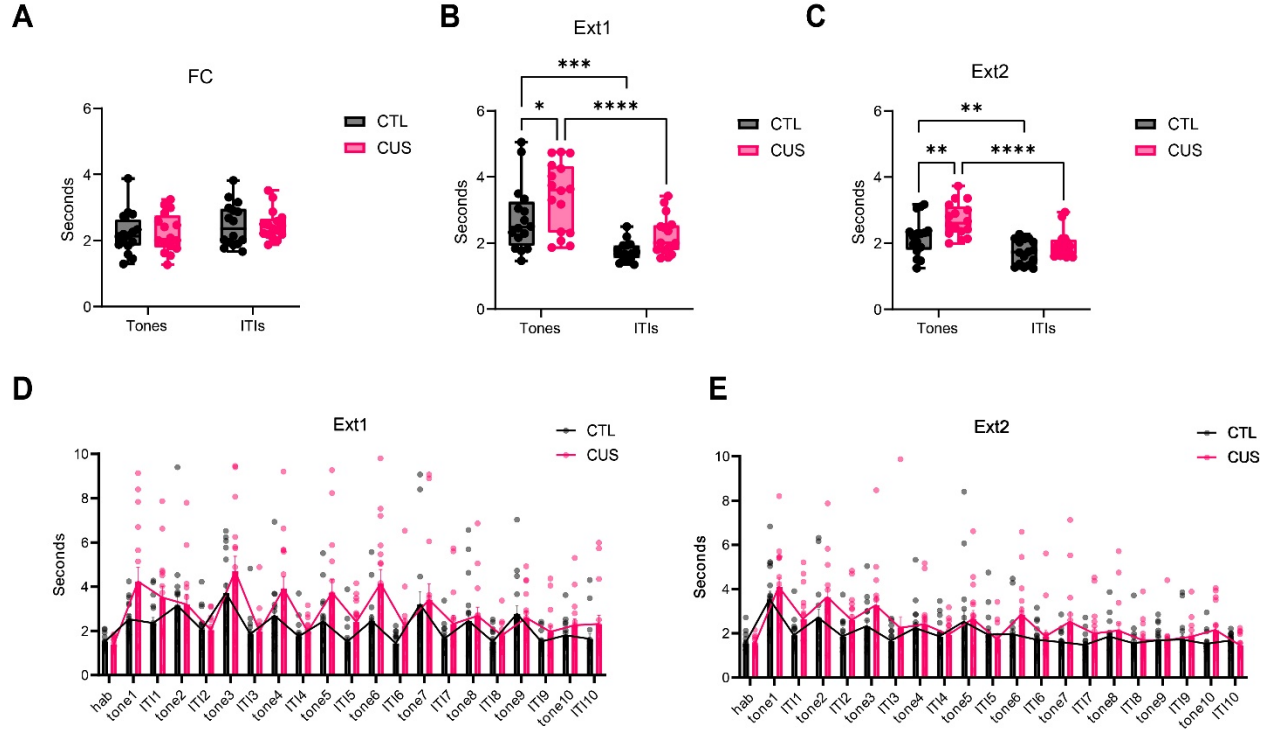


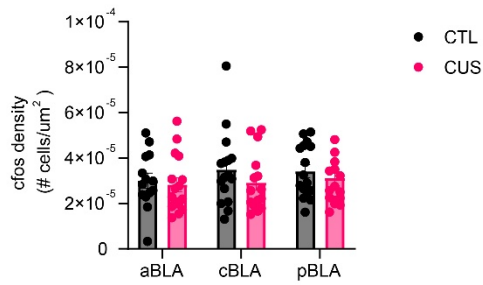
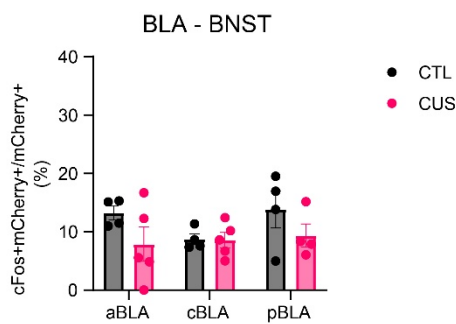
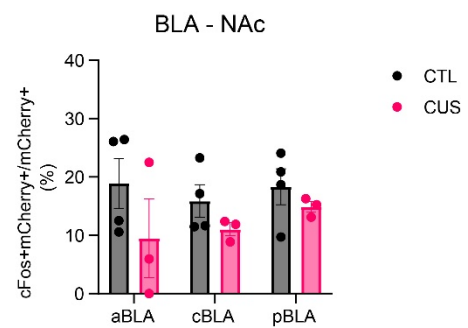
Figure S1: Freezing bout duration during fear conditioning, extinction day 1 and extinction day 2.

(A) Average bout duration during fear conditioning did not differ between CTL and CUS (2-Way repeated-measure ANOVA; stress: $F_{(1,30)} = 0.003879$, $P = 0.9507$) and was comparable between tones and ITIs (cue: $F_{(1,30)} = 3.475$, $P = 0.0721$; cue x stress interaction: $F_{(1,30)} = 0.008169$, $P = 0.9286$).

(B) Average bout duration during extinction day 1 was greater to tones than ITIs (2-Way repeated-measure ANOVA; cue: $F_{(1,30)} = 49.88$, $P < 0.0001$) and elevated in CUS mice (stress: $F_{(1,30)} = 5.955$, $P = 0.0208$). Although there was no significant cue and stress interaction ($F_{(1,30)} = 0.3985$, $P = 0.5326$), post-hoc Sidak's tests were significant to Tones CTL v.s. CUS ($P = 0.0400$), CTL Tones v.s. ITIs ($P = 0.0002$) and CUS Tones v.s. ITIs ($P < 0.0001$). CUS mice (Tones: median = 3.609, IQR = 2.310-4.328; ITI: median = 2.003, IQR = 1.781-2.544) exhibited not only longer median freezing bout durations but also a broader interquartile range, indicating increased variability in freezing behaviors than CTL (Tones: median = 2.476, IQR = 1.916-3.259; ITI: median = 1.677, IQR = 1.539-1.930).

(C) Average bout duration during extinction day 2 was greater to tones than ITIs (2-Way repeated-measure ANOVA; cue: $F_{(1,30)} = 37.50$, $P < 0.0001$) and elevated in CUS mice (stress: $F_{(1,30)} = 8.037$, $P = 0.0081$; cue x stress interaction: $F_{(1,30)} = 2.011$, $P = 0.1665$), post-hoc Sidak's tests were significant in Tones CTL v.s. CUS ($P = 0.0055$), CTL Tones v.s. ITIs ($P = 0.0047$) and CUS Tones v.s. ITIs ($P < 0.0001$). CUS mice (Tones: median = 2.609, IQR = 2.314-3.118; ITI: median = 1.881, IQR = 1.640-2.111) exhibited longer median freezing bouts and broader interquartile range during tones than CTL (Tones: median = 2.243, IQR = 1.796-2.369; ITI: median = 1.730, IQR = 1.338-2.106).

(D-E) Individual freezing bout durations for each mouse across sessions on extinction day 1 (D) and extinction day 2 (E) were shown, with the line graph indicating the mean bout duration across all animals within each group. Data are presented as mean $\pm$ SEM.

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31 **Figure S2: Brain activity across the BLA AP-axis.**

32 (A) BLA cFos density did not vary across AP-axis (Mixed-effects model; AP-axis: $F_{(1.467, 41.82)} =$
 33 1.068, $P = 0.3342$; Stress: $F_{(1, 30)} = 1.146$, $P = 0.2929$; AP-axis x stress: $F_{(1.467, 41.82)} = 0.4343$, $P =$
 34 0.5892). # data points = 46-47; # outliers = 1-2/groups).

35 (B) The BLA-BNST pathway did not show differences in the percentage of mCherry neurons co-
 36 expressing c-Fos along the AP axis (Mixed-effects model: AP-axis: $F_{(1.828, 11.88)} = 0.9493$, $P =$
 37 0.4066; Stress: $F_{(1, 7)} = 3.490$, $P = 0.1040$; AP-axis x stress: $F_{(1.828, 11.88)} = 0.8572$, $P = 0.4395$, #
 38 data points = 12-14; # outliers = 0-1/group).

39 (C) The BLA-NAC pathway did not show differences in the percentage of mCherry neurons co-
 40 expressing c-Fos along the AP axis (two-way repeated-measures ANOVA: AP-axis: $F_{(1.382, 6.911)} =$
 41 0.4485, $P = 0.5870$; Stress: $F_{(1, 5)} = 3.032$, $P = 0.1421$; AP-axis x stress: $F_{(1.382, 6.911)} = 0.4029$, $P =$
 42 0.6112, # data points = 9-12, # outliers = 0/group). Data are presented as mean $\pm$ SEM; * $p <$
 43 0.05.
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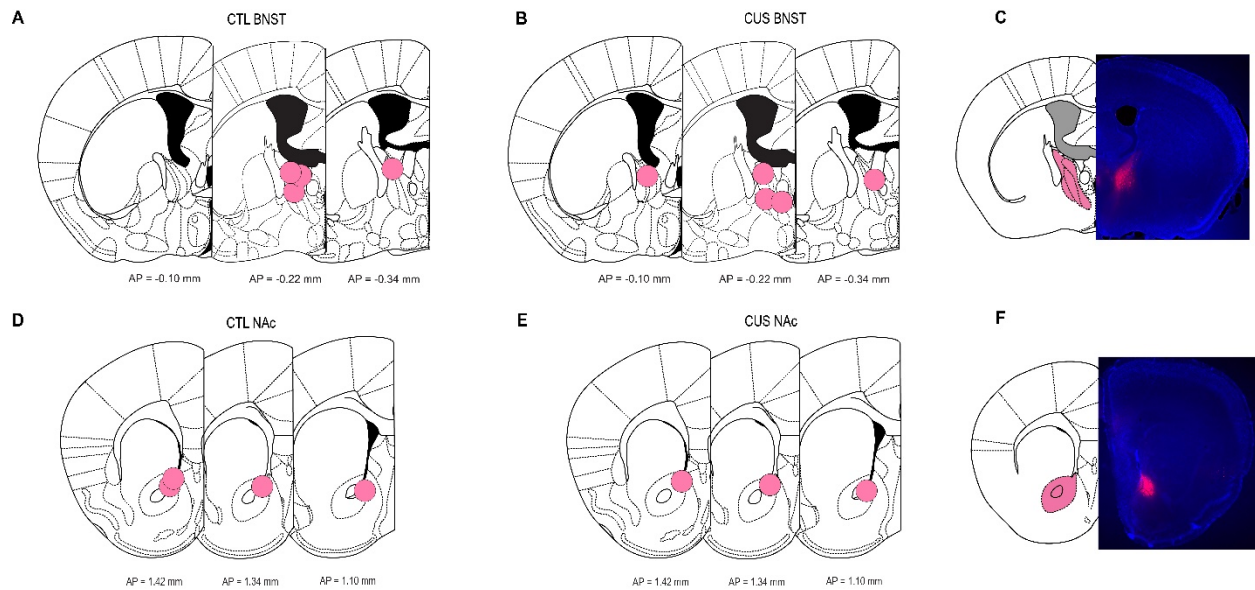


Figure S3: Retrograde virus hit map.

(A-B) Subgroups of CTL (N = 4) and CUS (N = 5) mice, indicated as pink circles, received retrograde virus injection targeting BNST. In some cases, the virus spreads into neighboring regions.

(C) Representative image of BNST injection site.

(D-E) Subgroups of CTL (N = 4) and CUS (N = 3) mice, indicated by pink circles, received retrograde virus injection targeting NAc. In some cases, the virus spreads into neighboring regions.

(F) Representative image of NAc injection site.

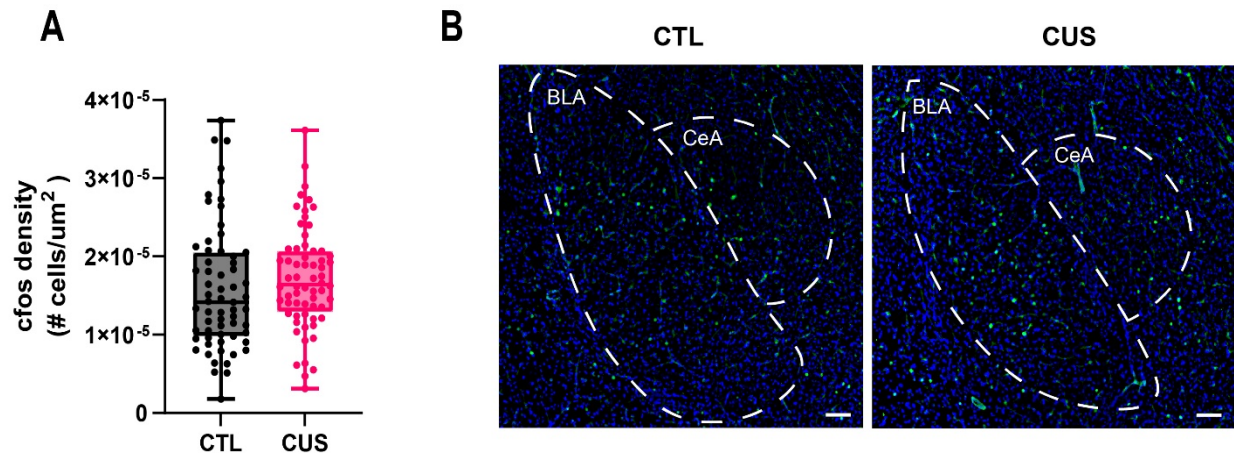


Figure S4: CeA c-Fos expression after extinction day 2.

(A) CUS and CTL had comparable level of c-Fos expression in CeA (Mann-Whitney test, $U = 1664$, $P = 0.0901$, # data points = 63-64/group, # outliers = 0-2/group).

(B) Representative images of c-Fos expression in the CeA. Data are presented as mean $\pm$ SEM; Scale bar = 100 μm .