Supplementary Materials for

Social Reactivation of Fear Engrams Enhances Recall

Abby Basya Finkelstein, Héloïse Leblanc, Rebecca Cole, Troy Gallerani, Anahita Vieira, Yosif
Zaki, Steve Ramirez

Correspondence to: afinkelstein@fas.harvard.edu and dvsteve@bu.edu

This PDF file includes:

Supplementary Text
Figs. S1 to S5
Captions for Movies S1 to S2
Captions for Datasets S1 to S7

Other Supplementary Materials for this manuscript include the following:

Movies S1 to S2
Datasets S1 to S7

Supplementary Text

Statistical Analysis Details

<u>**Figure 2**: Socially salient but not directly stressful experience reactivate pre-existing fear ensembles</u>

Fig. 2d: Males, 1-sample t-test: DG Restraint t=0.0667, df=4, p=0.95; Shocked Cagemate t=0.085, df=3, p=0.937. BLA 1-Way Mirror t=2.101, df=7, p=0.0737; Shocked Cagemate t=3.492, df=2, p=0.0731.

Fig. 2e: Females, 1-Way ANOVA: DG $F_{2, 18}$=0.4501, p=0.6445. BLA $F_{2,13}$=0.4962, p=0.6199.

Fig. 2f: 1-sample t-test for females, DG Restraint t=1.022, df=7, p=0.3406; 1-Way Mirror t=0.9085, df=8, p=0.3902; Shocked Cagemate t=0.3829, df=3, p=0.7273. BLA Restraint t=1.496, df=6, p=0.1852; Shocked Cagemate t=7.348, df=1, p=0.0861.

Correlations of DG to BLA reactivation, Pearson Correlations: Males, 1-Way: n=8, Pearson r = 0.116, p=0.784; Restraint: n=4, Pearson r = 0.869, p=0.13; Juvenile Intruder: n=9, Pearson r=0.530, p=0.142. Females, 1-Way: n=6, Pearson r=-0.266, p=0.610; Restraint: n=5, Pearson r=-0.139, p=0.823.

<u>**Figure 4**: DG ensembles active during social stress drive fear only in previously fear conditioned mice</u>

Fig. 4e: 2-Way RM ANOVA light x group $F_{6, 57}$=1.628, p=0.1560; light $F_{1.917, 36.43}$=0.9090, p=0.4081; group $F_{62, 19}$=3.959, p=0.0365.

Fig. 4f: 2-Way RM ANOVA light x group $F_{6, 57}$=3.071, p=0.0113; light $F_{3, 57}$=0.3763, p=0.7704; group $F_{2, 19}$=1.645, p=0.2193.

Fig. 4g: 2-Way RM ANOVA light x group $F_{2, 20}$=5.120, p=0.0160; group $F_{2, 20}$=1.801, p=0.1909; light $F_{1, 20}$=5.315, p=0.0320.

Fig. 4h: 2-Way RM ANOVA light x group $F_{2, 19}$=7.889, p=0.0032; group $F_{2, 19}$=2.007, p=0.1619; light $F_{1, 19}$=0.1406, p=0.7118.

Fig. 4i: 2-Way RM ANOVA light x group $F_{6, 36}$=0.3690, p=0.8938; light $F_{3, 36}$=0.1871, p=0.9045; group $F_{2, 12}$=0.7903, p=0.4760.

Fig. 4j: 2-Way RM ANOVA light x group $F_{6, 36}$=1.266, p=0.2971; light $F_{2.559, 30.71}$=0.6596, p=0.5602; group $F_{2, 12}$=3.119, p=0.0812.

Fig. 4k: 2-Way RM ANOVA light x group $F_{2, 12}$=0.5180, p=0.6084; group $F_{2, 12}$=1.062, p=0.3762; light $F_{1, 12}$=0.8643, p=0.3709.

Fig. 4l: 2-Way RM ANOVA light x group $F_{2, 12}$=2.285, p=0.1443; group $F_{2, 12}$=2.503, p=0.1235; light $F_{1, 12}$=0.2462, p=0.6287.
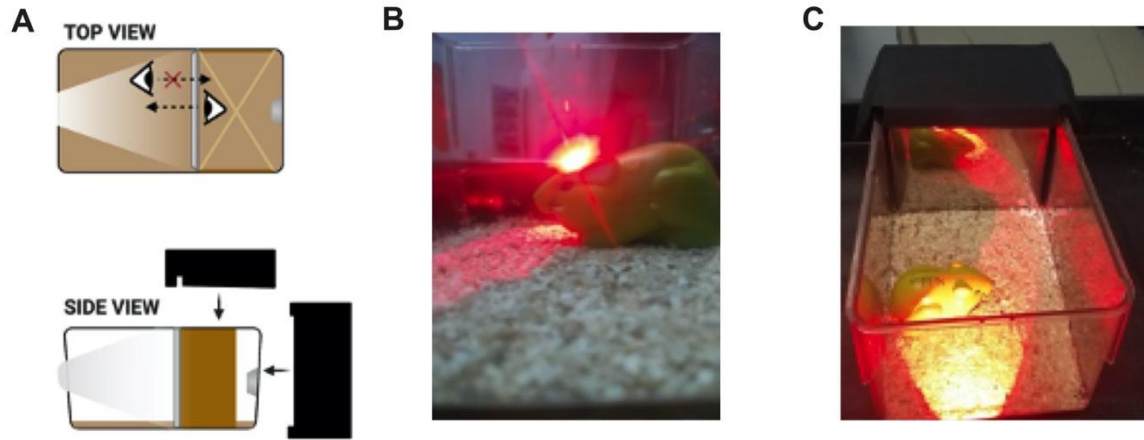
Fig. S1. 1-Way Mirror Prototype, Related to Methods. **A)** Unidirectional visual access allows the recently shocked mouse in the darkened compartment to see the cagemates on the other side of the insert, in the bright compartment. **B** and **C)** The cagemates do not see the shocked cagemate in the dark compartment but rather see their own reflections. Auditory-olfactory stimuli are exchanged over the top and sides of the 7" x 11" x 5" insert.
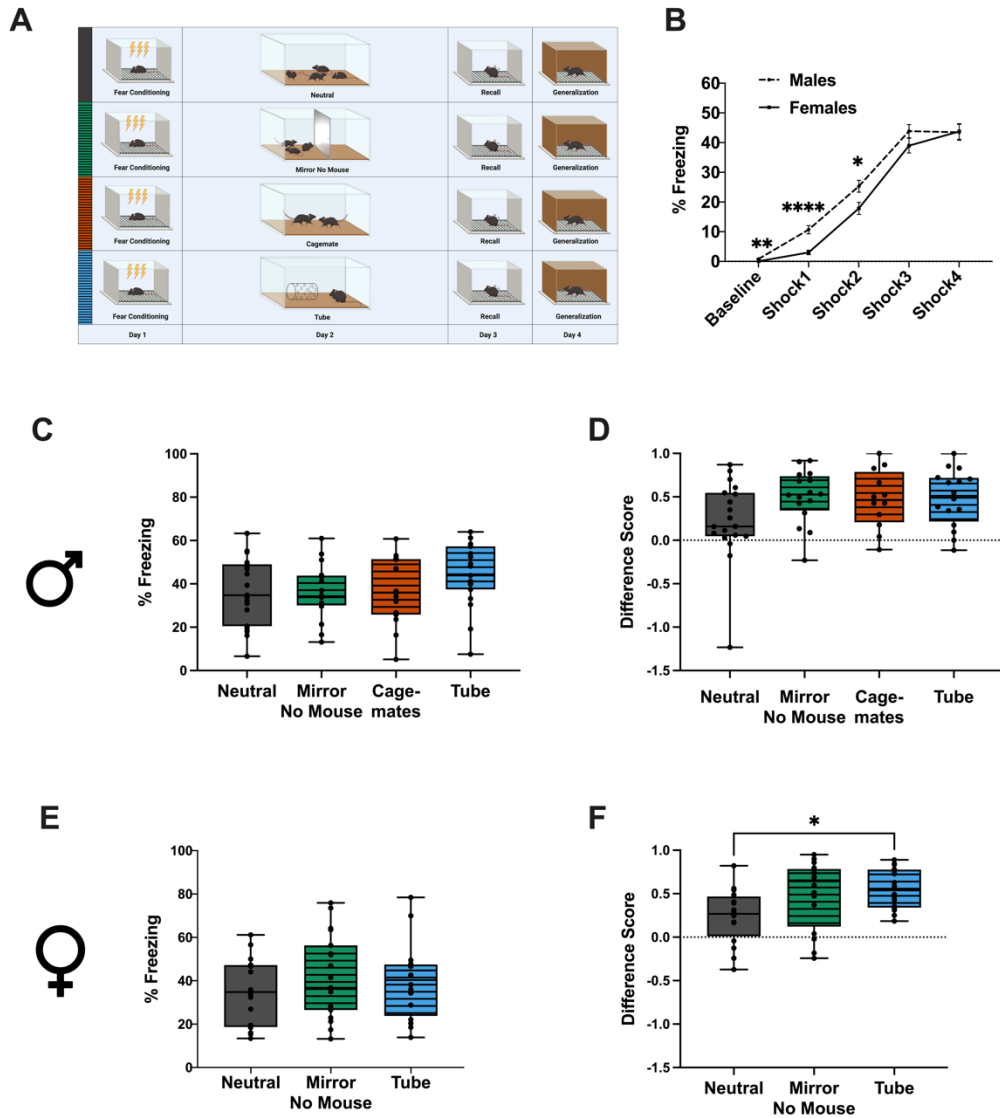
Fig. S2. **Novel objects and neutral social experiences do not impact fear recall. Related to Figure 1.**

A) Schematic representation of behavioral schedule. **B)** % freezing throughout fear acquisition in males (dashed) and females (solid). 2 Way RM ANOVA with Sidak's multiple comparison tests of fear acquisition for males vs females, n=69 males, n=63 females; Time x Sex $F_{(4, 520)}$=2.910, p=0.0212; Time $F_{(2.717, 353.3)}$=323.8, p<0.0001; Sex $F_{(1, 130)}$=4.895, p=0.0287; Subject $F_{(130, 520)}$=3.594, p<0.0001; Baseline - t=3.336, p=0.0063; Shock1 - t=4.930, p<0.0001; Shock2 -

t=2.626, p=0.0475; Shock3 - t=1.464, p=0.5452; Shock4 -t=0.07762, p>0.9999. **C** and **E**)
Freezing levels during a 5 minute fear recall test for males (**C**) and females (**E**). Males: 1-Way
ANOVA$F_{(3, 68)}$=1.290, p=0.2848. Females: 1-Way ANOVA$F_{(2, 48)}$=0.5864, p=05603. **D** and **F**)
Differences scores, defined as [(freezing in recall test)-(freezing in generalization test)]/(freezing
in recall test) for males (**D**) and females (**F**). Males: ANOVA$F_{(3, 59)}$=2.172, p=0.1009. Females:
Kruskal-Wallis statistic=8.115, p=0.0173, Dunn's multiple comparisons Tube vs Neutral
z=2.551, p=0.0323. Boxes extend from 25th to 75th percentiles, lines show medians, whiskers
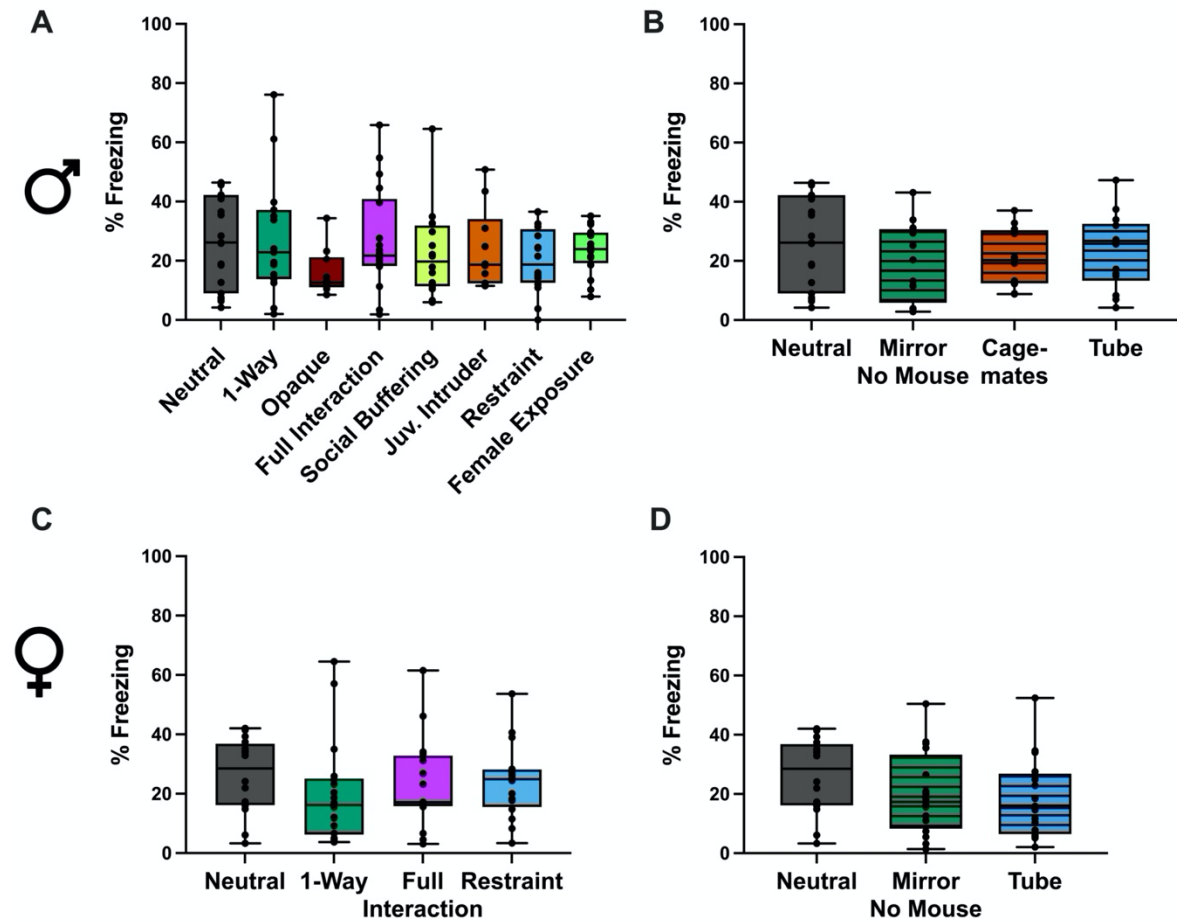extend from minimum to maximum.

Fig. S3. **Non-normalized freezing generalization context does not differ between groups. Related to Figure 1.** Each graph shows the % freezing during the generalization test, with the Neutral group included as reference. **A)** Experimental Males: Brown-Forsythe ANOVA $F_{(7, 87.07)}$=1.273, p=0.2730; **B)** Control Males: 1-Way ANOVA $F_{(3, 53)}$=0.8275, p=0.4846; **C)** Experimental Females: ANOVA $F_{(3, 62)}$=0.5153, p=0.6733; **D)** Control Females: ANOVA $F_{(2, 45)}$=1.452, p=0.2448. Boxes extend from 25th to 75th percentiles, lines show medians, whiskers extend from minimum to maximum.
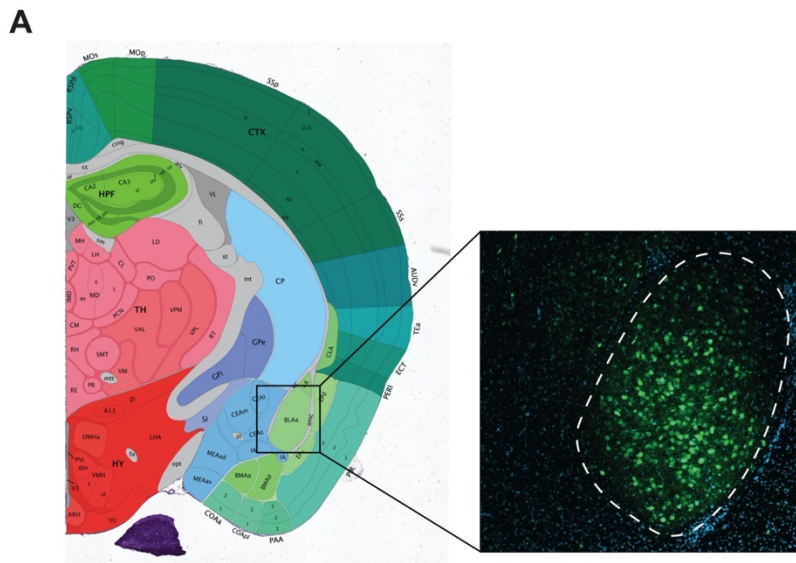
Fig. S4. BLA histology. Related to Figure 2. A) Representative 10x confocal image demonstrating the bounds used to define the BLA.
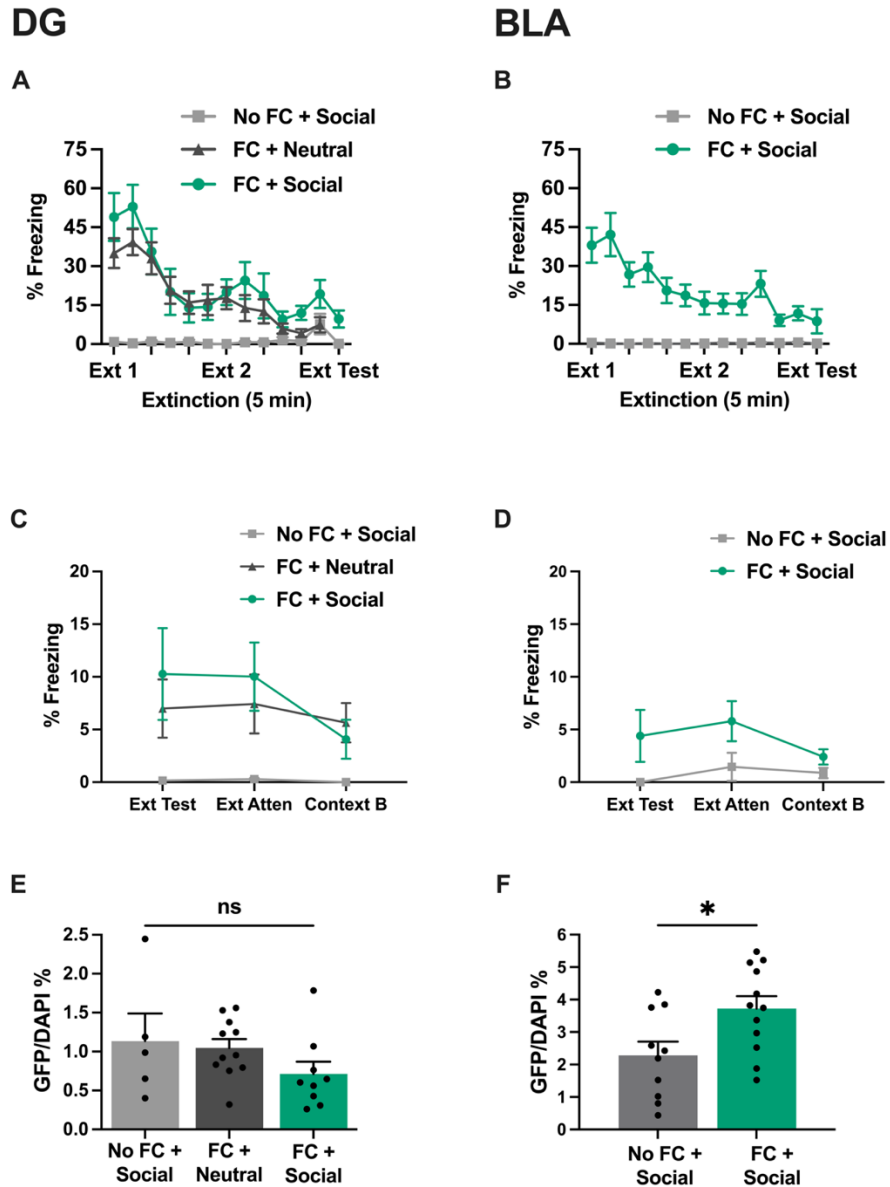
DG

BLA

A

B

C

D

E

F

Fig. S5. **Ensemble size and extinction learning curves for optogenetically manipulated groups**. **Related to Figure 4. A** and **B**) % freezing throughout extinction training across groups. Mixed-effects model (REML), **A**) time p<0.0001, group=0.0025. **B**) time p=0.0016, group <0.0001. **D** and **D**) % freezing during ext test, extinction attenuation post stimulation, and context b. **E** and **F**) % Chr2-GFP positive cells over total DAPI positive cells. **E**) 1-Way ANOVA $F_{2, 22}$=1.497, p=0.2458. **F**) unpaired 2-tailed t-test t=2.528, df=20, *p=0.02.

Movie S1.

Representative video of 1-way mirror paradigm for fear conditioned cagemates.

Movie S2.

Representative video of juvenile intruder paradigm for fear conditioned cagemates. The experimental resident mouse has two marks at the base of his tail, the intruder has a long solid line from the base to the middle of his tail.

Dataset S1: Data for Figure 1

Dataset S2: Data for Figure 2

Dataset S3: Data for Figure 3

Dataset S4: Data for Figure 4

Dataset S5: Data for Supplemental Figure 2

Dataset S6: Data for Supplemental Figure 3

Dataset S7: Data for Supplemental Figure 5