Post-Retrieval Extinction in Adolescence Prevents Return of Juvenile Fear
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Supplemental Material

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Supplemental Results

Experiment 1: p17 FC – p45 Intervention

**Adolescent Extinction or Retrieval+Extinction**: During adolescence, freezing during both standard extinction and retrieval+extinction was low (relative to what is usually observed in adults) for all rats fear conditioned at p17. A repeated measures ANOVA with p45 treatment group (Std Ext or Ret+Ext) as the between subjects factor and extinction cue as the within subjects factor revealed a significant within subjects effect (F(17,731)=13.25, p<.001) confirming that rats did significantly reduce their freezing over the course of the extinction session and all rats were freezing at less than 5% by the end of extinction, regardless of treatment (Fig S1a). There was no significant between subjects effect of retrieval (F(1,43)=2.58, p=.115) on freezing during extinction.

**Adult Direct FC (Adult Day 1)**: In experiment 1, p17 fear conditioning did not result in increased freezing when the CS was first presented as adults, but potentiated freezing during re-acquisition in adult rats that received either no intervention or standard extinction during adolescence. All rats showed the expected increase in freezing across conditioning cues when re-conditioned as adults (repeated measures ANOVA F(2,46)=86.13, p<.001). There was a significant between subjects effect of previous fear experience and intervention (F(3,23)=4.839, p=.009). Follow up Tukey HSD mean comparisons revealed that the Naïve-Naïve rats that had never been fear conditioned prior to adulthood froze significantly less during direct fear conditioning than the FC-Ext (p=.017) and FC-No Ext (p=.023) (Fig S1b).

Experiment 2: p25 FC – p45 Intervention

**Adolescent Extinction or Retrieval+Extinction**: Freezing to the first cues of standard extinction or retrieval+extinction during adolescence after p25 fear conditioning was high for both groups (>60%). A repeated measures ANOVA with p45 treatment group (Std Ext or Ret+Ext) as the between subjects factor and extinction cue as the within subjects factor revealed a significant within subjects effect (F(17,918)=20.38, p<.001) confirming that rats did significantly reduce their freezing over the course of the extinction session. The between subjects effect did not quite reach significance (F(1,54)=3.8, p=.056) (Fig S2a).

**Adult FC (Adult Day 1)**: In experiment 2, rats explicitly remembered fear conditioning at p25, and displayed a freezing response to the CS. There was a significant between subjects effect of treatment group (F(3,34)=5.63, p=.003) with post-hoc Tukey mean comparisons revealing that this difference was driven by significant differences in freezing between the Naïve-naïve rats and both the FC-Ext (p=.004) and FC-No Ext (p=.007) during the fear conditioning session of adulthood (Fig S2b).

There was no significant spontaneous recovery of freezing in the ret+ext rats between the end of adolescent extinction performed after a retrieval and freezing to the first CS presentation of adult fear conditioning (t(8)=.872, p=.409). There was spontaneous recovery of freezing in the rats that were fear conditioned at p25 and underwent standard extinction at p45 between the end of extinction and the first cue of fear conditioning (t(9)=-2.43, p=.038) (Fig S2a,b).
However, by the second cue of fear conditioning, all rats that had been fear conditioned as juveniles (regardless of p45 treatment) froze at equivalent levels that were significantly more than the naïve-naïve rats (all ps<.05) as evident by post-hoc Tukey mean comparisons performed after an ANOVA on FC Cue 2 revealed a significant effect of treatment group (F(3,34)=4.27, p=.012). There were no longer any group differences in freezing by the third and final cue of adult fear conditioning (F(3,34)=1.273, p=.299).

Experiment 3: p25 FC – adult intervention – Adult FC

Adult Extinction or Retrieval+Extinction: Rats fear conditioned at p25 and extinguished as adults showed high levels of freezing at the beginning of the extinction sessions and decreased freezing within the session. The between group effect of retrieval approached significance during the extinction session (repeated measures ANOVA F(1,18)=3.95, p=.06), however both groups extinguished to the same final levels (last 3 CS of Ret+ext=13.65%; last 3 CS of Std Ext=15.40%) (Fig S3a).

Adult FC: Both adult standard extinction and retrieval+extinction of p25 fear memories resulted in spontaneous recovery of freezing when presented with the CS 55 days after intervention. Paired samples t-tests were performed for each treatment group to compare freezing during the last 3 cues of extinction to the first cue of fear re-conditioning in order to test for spontaneous recovery of freezing. There was a significant increase in freezing between the last 3 cues of extinction and the first cue of fear re-conditioning, indicating that spontaneous recovery occurred, after both FC-Ret+Ext (t(9)=5.22, p=.001) and FC-Ext (t(9)=3.12, p=.013) (Fig S3a,b). Repeated measures ANOVA with fear conditioning cue as the within subjects factor and treatment group as the between subjects factor revealed a significant between subjects effect of treatment group (F(3,40)=5.05, p=.005) on freezing across all 3 of the CS-US pairings.

Experiment 4: adult FC – adult intervention – adult FC

Adult Extinction or Retrieval+Extinction: Rats fear conditioned as adults and extinguished as adults 20 days later showed high levels of freezing at the beginning of the extinction sessions and decreased freezing within the session (repeated measures ANOVA: F(17,204)=13.861, p<.001). There was not a significant between subjects effect of treatment group (F(1,12)=1.974, p=.185) (Fig S4a).

Adult Direct FC: Both adult standard extinction and retrieval+extinction of p25 fear memories resulted in spontaneous recovery of freezing when presented with the CS 55 days after intervention. There was a significant increase in freezing between the last 3 cues of extinction and the first cue of fear re-conditioning, indicating that spontaneous recovery occurred, for both FC-Ret+Ext-FC (paired samples: t(7)=4.685, p=.002) and FC-Ext-FC (paired samples: t(5)=6.167, p=.002) (Fig S4b).

Repeated measures ANOVA with fear conditioning cue as the within subjects factor and treatment group as the between subjects factor revealed no between subjects effect of treatment group (F(3,22)=.385, p=.765) on freezing across the 3 CS-US pairings.
Figure S1: Experiment 1 – freezing after p45 intervention of p17 fear memories

A) p45 extinction following either a retrieval (FC-Ret+Ext) no retrieval (FC-Ext) in rats that were fear conditioned to the same cue at p17 showed very little freezing that decreased to <5% in all rats. B) Freezing during direct FC on 1 rat of each triad as adults. Std Ext or No Ext at p45 resulted in higher freezing at the end of the adult fear conditioning session compared to the Naïve-Naïve rats that were fear conditioned as adults (p<.05) and a trend towards an increase compared to rats that received the Ret+Ext at p45 (p=.08).
Figure S2: Experiment 2 – freezing after intervention of p25 fear memories
Freezing was measured during cue presentation during adolescence (A) and adulthood (B) after p25 fear conditioning. A) Freezing during standard extinction or retrieval+extinction at p45. B) Retrieval+extinction at p45 targeting p25 fear memories prevented the spontaneous recovery of freezing during the first cue of adult fear conditioning. Error bars +/- SEM. * indicates p<.05.
Figure S3: Experiment 3 – Freezing after adult intervention of p25 fear memories

A-B) Rats displayed expected within session extinction but both retrieval+extinction and standard extinction resulted in spontaneous recovery of freezing when the first CS was presented 55 days later. B) Freezing during direct fear re-conditioning and C) long term memory tests. Adult retrieval+extinction of p25 fear resulted in a more rapid decrease in freezing over 3 CS only presentations after re-conditioning. Error bars represent +/- SEM. * indicates p<.05
Figure S4: Experiment 4 – freezing after adult intervention on remote adult fear memories

A) Freezing during retrieval+extinction and standard extinction on 20 day old memories.
B) Long-term memory tests 24 hours after extinction indicate that both ret+ext and ext reduce freezing in the short term, however, 45 days later all groups exhibited spontaneous recovery of freezing. C) freezing during fear re-conditioning approximately 45 days after extinction. D) Freezing during long-term memory tests 24 hours after fear re-conditioning indicate less retention of the fear memory after ret+ext. Error bars +/- SEM. * indicates p<.05