Supplemental Methods

Subjects

Of the 176 Sprague-Dawley rats used in this study, 144 (used in Experiments 1, 1b, 2, and 4) were derived from the breeding colony maintained by the School of Psychology at UNSW and 32 (used in Experiment 3) were obtained from the Animal Resources Centre (Perth, Australia; which was the supplier of the breeders used to produce the other animals used in this study). Animals were maintained on a 12 h light/ dark cycle (lights on at 0700) in a humidity- and temperature-controlled colony room with food and water available ad libitum.

Apparatus

MED Associates chambers [24cm (length) x 30 cm (width) x 21cm (height)] were used, and each was enclosed in a sound- and light-attenuating cabinet where ventilation fans provided constant low level (approx. 58dB) background noise. Two chambers, referred to as Context A, were constructed primarily of Perspex with stainless steel sidewalls. The floor was made of stainless steel rods (4 mm wide), spaced 16 mm apart, just above a stainless steel tray filled with corncob bedding. A clear Perspex dividing wall diagonally bisected the chamber, creating a triangle-shaped space. Infrared lighting was the only light source in Context A. The two chambers used as Context B differed in size, flooring, lighting, and visual features to those in Context A. The Context B chambers did not have a dividing wall but did have a clear Perspex insert covering the grid floor. Sheets of paper with vertical black and white stripes (2.5 cm width) were attached to the ceiling and front wall of the chamber. In addition to infrared lighting, a white light above the chamber provided low-level illumination inside the chamber (~ 4 lux). All chambers were cleaned with tap water after each experimental session.
Procedure

Rats in Experiment 1 and 1b were handled for 3-4 min on P24 but did not receive any context pre-exposure. In Experiments 2-4, rats were handled for 3-4 min each day and then placed in Context A for 10 min (two days in Experiments 2 and 4, and 3 days in Experiment 3). Conditioning occurred in the Context A chambers while extinction and test occurred in the Context B chambers.

Each session began with a 2 min adaptation period. During conditioning, there were 3 pairings of a white noise conditioned stimulus (CS; 8dB above background, 10 sec duration) and a scrambled footshock unconditioned stimulus (US; 1 sec duration) delivered through the grid-floor. In Experiments 1, 1b, 3, and 4 a 0.4mA footshock was used while in Experiment 2 the intensity of the footshock was 0.5mA. The CS co-terminated with the US in all cases. The intertrial intervals were 135 and 85 sec. Extinction training consisted of 30 non-reinforced presentations of the CS (10 sec each; 10 sec inter-trial interval). Presentations of the CS and US were controlled by a computer running Med-PC IV software (Med Associates). The animals’ behavior was recorded via a camera mounted on the rear wall of each cabinet.

Analysis

Pre-CS freezing and test data were assessed using two-way between-subjects analysis of variance (ANOVA) for Experiments 1-3 and independent-samples t-tests in Experiments 1b and 4. Conditioning and extinction training data were analyzed using a mixed-design ANOVA or mixed-design Analysis of Covariance (ANCOVA), where appropriate. Simple main effects analyses or independent samples t-tests were used to explore significant interactions. Whenever a mixed-design ANOVA was used violations of the assumption of sphericity (as determined by Mauchly’s test) led to the use of Greenhouse-Geisser corrections. The assumption of homogeneity of variances was tested using Levene’s test, and
in those cases where this assumption was violated the correct \( t \) and \( p \) values are reported. Planned contrasts were used to analyze test data in Experiment 2 as both comparisons were replications of previous findings. The decision-wise error rate was controlled at the 0.05 level for each contrast tested.

Two rats were excluded from the analyses in this study because they were statistical outliers (\( \geq 2.7 \) standard deviations above the mean) based on their test scores. One of these rats came from group Saline AdolesCond-Ext in Experiment 2 and one rat came from group Saline in Experiment 4.

Results

Baseline freezing

**Experiment 1**: There were no group differences in pre-CS freezing levels prior to fear conditioning, extinction training, or extinction recall (largest \( F_{1,42} = 2.91, p > .05 \); see Table S1).

**Experiment 1b**: There were no differences in pre-CS freezing levels between groups prior to fear conditioning (\( t_{7} = 1.00, p = .35 \)), extinction training (\( t_{8.45} = 1.55, p = .16 \)), or test (\( t_{14} = .00, p = 1.00 \)).

**Experiment 2**: As shown in Table S1, levels of pre-CS freezing were similar across groups during all stages of the experiment (conditioning: \( F_{5} < 1 \); extinction day 1: \( t_{21} = .29, p = .77 \); extinction day 2: largest \( F_{1,51} = 2.34, p = .13 \); test: largest \( F_{1,51} = 1.10, p = .29 \)).

**Experiment 3**: Although levels of pre-CS freezing were very low for all groups in all phases of this experiment (i.e., all < 5%; see Table S1) there was a significant effect of interval in the conditioning phase (\( F_{1,28} = 7.93, p = .009 \)). That is, those animals to be extinguished 1 day after conditioning had higher levels of pre-CS freezing during the adaptation period in the
conditioning phase relative to those animals to be extinguished 10 days after conditioning. There was also a significant effect of interval for the extinction phase ($F_{1, 28} = 43.95, p = .04$), such that animals extinguished 1 day after conditioning had higher levels of pre-CS freezing than animals extinguished 10 days after conditioning.

**Experiment 4:** There were no significant group differences in pre-CS freezing in either the conditioning or test phase ($F_{s} < 1$). There was a significant group difference in pre-CS freezing levels in the extinction phase of this experiment ($t_{23} = 2.09, p = .05$), with those animals injected with saline having higher levels of pre-CS freezing than those given MK801.
CS-elicited freezing during conditioning

**Experiment 1**: Across CS-US pairings, levels of CS-elicited freezing increased ($F_{1.60, 67.06} = 31.47, p < .001$), and there were no effects of group or drug ($F$s < 1). However, there was a block $\times$ group $\times$ drug interaction ($F_{1.60, 67.06} = 3.91, p = .03$; Figure S1, panel A).

**Experiment 1b**: Across conditioning trials, levels of CS-elicited freezing increased ($F_{1.35, 18.89} = 31.50, p < .001$). There was no effect of the drug or block $\times$ drug interaction ($F$s < 1; Figure S1, panel B).

**Experiment 2**: CS-elicited freezing increased across conditioning trials ($F_{1.53, 78.01} = 75.53, p < .001$; Figure S1, panel C). There was no significant effect of condition ($F_{1.51} = 3.45, p = .069$) or drug ($F < 1$), or interaction between these factors ($F < 1$), indicating that all groups had similar overall levels of CS-elicited freezing during conditioning, and comparable rates of acquisition (largest $F_{1.53, 78.01} = 1.95, p = .15$).

**Experiment 3**: Due to the significant effect of interval on baseline freezing levels at the time of conditioning (see above), CS-elicited freezing during conditioning was analyzed with ANCOVA using pre-CS freezing as a covariate. Groups showed equivalent rates of acquisition across trials ($F_{2.54} = 40.67, p < .001$), with a trial $\times$ drug interaction ($F_{2.54} = 3.35, p = .04$; Figure S1, panel D).

**Experiment 4**: Both groups exhibited an equivalent increase in levels of freezing across training trials ($F_{1.59, 36.58} = 19.02, p < .001$), and neither the effect of drug, nor the block $\times$ drug interaction was significant ($F$s < 1; Figure S1, panel E).
Experiment 1b

Experiment 1 demonstrated that rats conditioned as juveniles and extinguished as adolescents do not use NMDARs in extinction when a delay of ~10 d exists between these procedures. However, if NMDAR-independent extinction in these animals is indeed determined by the transition to adolescence, rather than the presence of an interval of ~10 d, a shorter interval between conditioning and extinction should not render extinction in these animals to be NMDAR-dependent. Therefore, we tested whether MK801 impaired extinction retention in rats with a shorter delay of 6 days between conditioning at P26 and extinction at P32. We also tested the boundary conditions of the extinction retention profile of rats conditioned within the juvenile period and extinguished in adolescence by conditioning animals at an older juvenile age of P26, rather than P24 as in Experiment 1, and administering extinction training at a younger age of P32 within adolescence, rather than P34-36 as in Experiment 1. Half the animals were injected with MK801 prior to extinction training, while the other half were injected with saline (see Figure S2A for a schematic of the procedure).

Baseline data for conditioning, extinction, and test are presented in Table S1. The statistical analyses for baseline freezing and conditioning were reported above, with the other experiments. During extinction training, there was a main effect of block ($F_{2.56, 35.72} = 19.80$, $p < .001$), drug ($F_{1, 14} = 5.16, p = .03$), and a block × drug interaction ($F_{2.56, 35.72} = 4.03, p = .02$; see Figure S2B). That is, CS-elicted freezing was higher in saline-treated animals than MK801-treated animals but decreased across extinction blocks. At the end of extinction, CS-elicted freezing was comparable in saline- and MK801-treated rats. At test, there were no differences in CS-elicted freezing between groups ($t_{14} = 1.16, p = .27$; see Figure S2C). Therefore, MK801-treated rats did not show a higher return of fear (i.e., impaired extinction retention) relative to saline-treated rats at test.