Selective enhancement of fear learning and resistance to extinction in a mouse model of acute early life trauma

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Early life stress (ELS) experiences can cause changes in cognitive and affective functioning. This study examined the persistent effects of a single traumatic event in infancy on several adult behavioral outcomes in male and female C57BL/6j mice. Mice received 15 footshocks in infancy and were tested for stress-enhanced fear learning, extinction learning, discrimination and reversal learning, and novel object recognition. Infant trauma potentiated fear learning in adulthood and produced resistance to extinction but did not influence other behaviors, suggesting restricted effects of infant trauma on behaviors reliant on cortico-amygdala circuitry.

Exposure to traumatic events early in childhood is associated with the development of psychiatric disorders (Copeland et al. 2018) and deficits in cognitive and affective functioning (Pechtel and Pizzagalli 2011) in adulthood. In humans, early life stress (ELS) is defined as experiencing traumatic events in childhood (Pechtel and Pizzagalli 2011). Rodent models of ELS suggest that acute versus chronic stress may differentially alter systems that regulate the stress response, producing different behavioral outcomes in adulthood (Pryce et al. 2002; Musazzi et al. 2017).

Stress-enhanced fear learning (SEFL), a powerful preclinical model of PTSD- and addiction-like behaviors, captures the enduring, maladaptive effects of a single traumatic event on behavior (Rau et al. 2009; Meyer et al. 2013; Radke et al. 2019). In these studies, exposure to 15 footshocks enhances contextual fear conditioning later in life. For infant SEFL, enhanced contextual fear conditioning occurs months after the initial stressful experience and in the absence of memory for the context in which ELS was experienced (Poulos et al. 2014; Quinn et al. 2014). SEFL protocols have been used to model adult or infant trauma in rats (e.g., Rau et al. 2009; Poulos et al. 2014; Quinn et al. 2014) and have been extended to adult mice (Sillivan et al. 2017; Hassien et al. 2020; Pennington et al. 2020). However, to our knowledge, no studies have established the use of acute, infant footshock as a model of ELS in mice. We sought to characterize the effects of acute, infant trauma exposure across several types of learning in adult mice. We tested mice exposed to 15 footshocks on postnatal day (PND) 17 for contextual fear learning, extinction of fear, discrimination and reversal learning, and novel object recognition. Our results suggest that exposure to acute infant trauma enhances fear learning and resistance to extinction in adulthood, but does not alter other types of learning.

Male and female C57BL/6j mice were generated from breeding pairs from The Jackson Laboratory. Mice were group-housed (two to four mice/cage) post-weaning and were provided food and water ad libitum, unless otherwise specified. Mice were on a 12:12 light/dark cycle. Other than trauma exposure, all behavioral tests were conducted during adulthood (PND 60+). Animals were cared for in accordance with the guidelines set by the National Institutes of Health and all procedures were approved by the Institutional Animal Care and Use Committee at Miami University.

We first established that exposure to infant footshock produced enhanced contextual fear conditioning in adulthood. Mice were placed in a MED-Associates conditioning chamber (context A) on PND 17 (Fig. 1A; after Quinn et al. 2014). Context A was brightly lit, contained a uniform grid floor, was scented with vanilla (50%), and was cleaned with odorless 5% sodium hydroxide. Mice received either 0 or 15 footshocks (1 mA, 1 sec) during a 60-min session beginning 180 sec following placement in the chamber. Progressive scan video cameras containing visible light filters monitored mice throughout the session. Video Freeze software (Med Associates, Inc.) analyzed the video and data were expressed as percent of time spent freezing during the session. Fear conditioning experiments were powered to detect sex differences with an n of eight per sex. Because differences were not observed when sex was included as a factor in analyses, all reported results represent data from both sexes. Due to computer malfunction, fear conditioning sessions from 14 mice were hand scored according to standard time-sampling procedures (Chowdhury et al. 2005) and data from three mice had to be excluded on extinction session 2.

Contextual fear conditioning occurred on PND 60 in a novel context (context B). Context B was dark with a staggered grid floor and cleaned and scented with acetic acid (5%). Baseline freezing during the first 180 sec in this novel context was assessed and used to measure generalization between the stress exposure context (context A) and the novel fear conditioning context (context B). Mice received either 0 or 1 footshocks (1 mA, 1 sec) 180 sec into a 3.5-min session. Thus, there were four groups (infant trauma/adult fear conditioning): no/no shock, n = 14; 15/no shock, n = 15; no/one shock, n = 15; and 15/one shock, n = 18. To test extinction of fear memory, mice were reintroduced to context B for two 8-min sessions, separated by 24 h. Finally, mice were reintroduced to context A for an 8-min retention test of the original context.

Mice exposed to 15 footshocks in infancy and conditioned with one footshock as adults exhibited robust SEFL. There were...
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Since PTSD is associated with deficits in fear extinction (Zuij et al., 2016), we next examined extinction learning using 30-min sessions (Fig. 2A). Mice were exposed to acute infant trauma (0 or 15 footshocks) on PND 17. On PND 60, mice were reintroduced to context A for an 8-min test of memory for the original ELS context prior to fear conditioning (no/one shock = 2.41 ± 0.58; 15/one shock = 3.50 ± 0.42; no/three shocks = 1.86 ± 0.43). On PND 61 fear conditioning occurred in context B. Since we observed that trauma-naive mice displayed very little fear conditioning following one footshock (Fig. 1B,C), mice in this experiment received either one or three footshocks during adult fear conditioning. There were three groups (infant trauma/adult fear conditioning): no/one shock; n = 17; 15/one shock; n = 17; no/three shocks; n = 18. Retention of fear memory was tested for five subsequent days (PND 62–66) in context B during 30-min sessions.

On the first extinction session, there were significant main effects of time (F(29,1421) = 2.23, P < 0.001) and group (F(2,49) = 5.54, P = 0.007) and a significant interaction (F(58,1421) = 1.93, P < 0.001). Holm–Sidak follow-up comparisons revealed that trauma-exposed mice (15/one shock) and mice conditioned with three footshocks during adulthood (no/three shocks) froze more than the no/one shock group during the first 3 min (P < 0.05 for minute 1 and P < 0.01 for minutes 2 and 3) of the first extinction session (Fig. 2B). Trauma-exposed mice continued to freeze more than the no/one shock group during minutes 4–7 (P < 0.01) and minute 11 (P < 0.05). Freezing in the no/3 shocks group was similar to trauma-exposed mice for the first 4 min but diminished sooner, evidenced by a significant difference between these groups during minutes 5, 6, and 9 (P < 0.05). These results suggest that conditioning with three footshocks produces similar levels of fear in trauma-naive mice as conditioning with one footshock in trauma-exposed mice, but that within-session extinction is delayed in the trauma-exposed group.

To examine extinction across the five sessions, we averaged freezing during the first 8 min of each session. We found significant main effects of session (F(4,196) = 22.92, P < 0.001) and group (F(2,49) = 6.38, P = 0.003) and a significant interaction (F(8,196) = 2.75, P = 0.007). Holm–Sidak follow-up comparisons revealed that mice exposed to infant trauma (15/one shock) and mice in the no/three shock group froze more than those in the no/one shock group on extinction session 1 (P < 0.01 and P < 0.05, respectively) (Fig. 2C). On session 2, freezing was greater in trauma-exposed mice versus both other groups (P < 0.01). Percent freezing in infancy did not correlate with freezing behavior in adulthood. Activity bursts in infancy correlated with freezing behavior in adulthood on extinction test 1 (r = −0.558, P = 0.020). These results further suggest that infant trauma produces resistance to extinction.

To determine whether the behavioral alterations observed in mice exposed to infant trauma extend to other types of learning, we tested the effects of infant footshock on operant discrimination and reversal learning for food reward and novel object recognition. For discrimination and reversal learning, we used a subset of mice
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from the first experiment (no shock = 20, 15 shocks = 17) restricted to 85% of free-feeding weight. Mice underwent one habituation day with ten 14-mg grain pellets (Bio Serv) in their home cages. The following day, mice began 15-min training sessions in a standard mouse operant chamber (Med Associates). There were two nose-poke holes and a reward receptacle on one wall of the chamber and a house light and speaker on the opposite wall. The chamber was housed in a sound and light-attenuating box and patterned walls (Panlab) for a 10-min habituation session to the chamber. Twenty-four hours later, mice were returned to the apparatus for a 3-min session (1-h test) where one of the sample objects was replaced with a novel object (a 2.5 × 1.2 × 1.5-cm Lego tower). The object replaced was alternated for each mouse. Mice were returned to the same box 24 h later for another 3-min session (24-h test) with the opposite cap replaced with a second novel object (a 3 × 1.5-cm black binder clip). ANY-maze software recorded each session. Time spent interacting with each object was measured by two independent raters and averaged (after Bevins and Besheer 2006).

A three-way ANOVA revealed a significant main effect of object (familiar vs. novel; \( F_{1,60} = 59.84, P < 0.001 \)). There was also a main effect of testing session (1-h vs. 24-h test; \( F_{1,60} = 7.89, P = 0.007 \)) and an interaction of object × testing session (\( F_{1,60} = 11.48, P = 0.001 \)) (Fig. 3C,D). Interrater reliability was confirmed using Pearson’s correlation (\( r = 0.942 \)). These results indicate that acute infant trauma does not influence hippocampal-dependent object recognition memory.

Our results demonstrate that an acute traumatic experience during infancy affects some learned behaviors during adulthood. As previously reported in rats (Quinn et al. 2014; Poulos et al. 2014), 15 footshocks on PND 17 increased adult contextual fear was met (≥30 rewards with 85% reinforced responses over two consecutive sessions).

Neither sex nor adult fear conditioning affected any measure of discrimination and reversal learning, so all results are reported collapsed across these two factors. Two trauma-exposed females did not acquire the discrimination in 25 sessions and were not advanced to reversal. Data were analyzed using mixed-effects analyses with phase (i.e., acquisition and reversal) as the within-subjects factor. There were no differences between groups in the total number of sessions required to complete discrimination (no shock = 5.70 ± 0.69; 15 shock = 6.82 ± 1.50) or reversal (no shock = 9.40 ± 0.98; 15 shock = 7.73 ± 1.21). Mice made more total reinforced (main effect of phase: \( F_{1,33} = 11.62, P = 0.002 \)) and total nonreinforced (main effect of phase: \( F_{1,33} = 42.54, P < 0.001 \)) responses during reversal but there were no effects of infant trauma on behavior (Fig. 3A,B). These results indicate that acute infant trauma does not influence acquisition of operant discrimination learning or behavioral flexibility in adulthood.
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Acute infant trauma does not affect discrimination and reversal learning or novel object recognition. (A,B) Following infant trauma on PND 17 (no footshock, blue, or 15 footshocks, black), adult mice were trained to respond for a food pellet in an operant, spatial discrimination and reversal learning task. Following acquisition of the discrimination (left or right nose-poke hole reinforced 100% of the time) the contingencies of the responses were reversed. Mice made more total reinforced (A) and unreinforced (B) responses during reversal versus acquisition across sessions (**P < 0.01, main effect of training phase) but there were no effects of infant trauma exposure. (C) A separate cohort of mice exposed to infant trauma (no or 15 footshocks on PND 17) was tested for novel object recognition by assessing exploration of a novel object 1 and 24 h after exposure to the familiar object. Mice explored the novel object more (**P < 0.01, main effect of object, main effect of testing session, and interaction of object × testing session) but there were no effects of infant trauma exposure. Data are means ± SEM.

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