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Differences in Stress-Induced Changes in Extinction and Prefrontal Plasticity in Postweanling and Adult Animals

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ABSTRACT

BACKGROUND: Postweaning is a critical developmental stage during which the medial prefrontal cortex (mPFC) undergoes major changes and the brain is vulnerable to the effects of stress. Surprisingly, the engagement of the mPFC in extinction of fear was reported to be identical in postweanling (PW) and adult animals. Here, we examined whether the effect of stress on extinction and mPFC plasticity would be similar in PW and adult animals.

METHODS: PW and adult animals were fear conditioned and exposed to the elevated platform stress paradigm, and extinction and long-term potentiation were examined. The dependency of stress-induced modulation of extinction and plasticity on *N*-methyl-D-aspartate receptors was examined as well.

RESULTS: We show that exposure to stress is associated with reduction of fear and enhanced induction of long-term potentiation (LTP) in PW pups, in contrast to its effects in adult animals. Furthermore, we report opposite effects in the occlusion of LTP following the enhanced or impaired extinction in the two age groups and that the reversal of the effects of stress is independent of *N*-methyl-D-aspartate receptor activation in PW animals.

CONCLUSIONS: Our results show that qualitatively different mechanisms control the modulatory effects of stress on extinction and plasticity in postweanling pups compared with adult rats. Our results point to significant differences between young and adult brains, which may have potential implications for the treatment of anxiety and stress disorders across development.

Keywords: Amygdala, Extinction, LTP, Metaplasticity, Postweanling, Prefrontal cortex, Stress

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The interaction between the basolateral amygdala (BLA) and the medial prefrontal cortex (mPFC) is crucial for extinction of fear (1–5).

Fear associations can be learned by animals throughout development (6). However, developmental differences among different brain regions determine the age at which different subtypes of learned fear associations can be acquired. For example, whereas amygdala-dependent auditory fear conditioning emerges by postnatal day (PND) 16 to 18, hippocampal-dependent contextual fear conditioning was reported around PND 23 and has been attributed to the ongoing maturation of the hippocampus (7–12).

However, the mPFC and the BLA are late maturing structures and undergo major changes during postweaning (childhood in humans) in both rats and humans (13–18). In the mPFC, both interneurons (19,20) and *N*-methyl-D-aspartate receptors (NMDARs) (21,22) undergo dramatic changes during cortical development at postweaning (23). This may suggest that the inhibitory function of the mPFC on the BLA in early life may be dissimilar across development (24–27). Similarly, differences were reported between adults and postweanlings (PWs) in the hypothalamic-pituitary-adrenal axis response (28,29). Furthermore, the regulation of the hypothalamic-pituitary-adrenal axis undergoes extensive morphologic and functional remodeling during this period (23,30–32).

Together, these observations raise the question of whether stress would similarly affect mPFC-dependent functions in the PW and adult animals.

In the adult animal, we and others have reported that exposure to behavioral stressors is associated with impairments in high-frequency stimulation (HFS)-induced potentiation (long-term potentiation [LTP]) in the BLA-mPFC pathway (33–35) and with impairments in extinction of fear (36–38). We have also shown that stress induces an NMDAR-dependent type of metaplasticity in the adult mPFC (34). In this study, we sought to examine 1) the effects of exposure to behavioral stressors on extinction and HFS-induced LTP in the BLA-mPFC pathway in the PW pup compared with the adult animal; 2) whether prior exposure to stress and extinction training would differentially affect the ability for further induction of HFS-induced LTP in both age groups; and 3) the role of NMDA receptors in occlusion of LTP in both age groups.

Our results clearly establish qualitative differences between the two age groups and show that stress differentially modulates extinction and plasticity in PW and adult animals.

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METHODS AND MATERIALS

Adult (~60 days old) and postweanling (24–27 days old) male Sprague Dawley rats from the local animal colony at the Haifa University were used (for details, see Supplement 1).

Surgery and electrophysiological recordings were previously reported in our studies (39,40) (Supplement 1).

Stress procedure was detailed elsewhere (39,40) (Supplement 1). Corticosterone assessment is detailed in Supplement 1.

Detailed description of the procedure of fear conditioning and extinction was previously detailed in our work [e.g., (41)] (Supplement 1).

Details of the drugs that were used appears in our previous work (40) (Supplement 1).

The locations of the stimulating electrodes in the BLA were verified histologically (Figure S1 in Supplement 1; see Supplement 1 for details).

Data were analyzed using SPSS 19 Statistics software (IBM, Chicago, Illinois) (Supplement 1).

RESULTS

Effects of Exposure to Stress on Extinction in Adult and Postweanling Rats

Adult animals (adults, 60 days old) and postweanling pups (PW, 24–27 days old) were trained to associate a tone with an electrical footshock on the conditioning day. Twenty-four hours after conditioning, the animals were exposed to three tones for fear memory retrieval and immediately thereafter were either exposed to the elevated platform stressor (EP; adults-EP; $n = 8$; PW-EP; $n = 7$) or placed back in their home cage (adults-control; $n = 8$; PW-control; $n = 8$).

Two-way analysis of variance (ANOVA) (2 × 2: group [stress (EP), control]; age [adult, PW]) of freezing levels during the retrieval test showed no significant main effect for any of the variables or for the interaction (group: $F_{1,28} = .3$, not significant (ns); age: $F_{1,28} = .09$, ns; age × group: $F_{1,28} = .11$, ns; Figure 1), which suggests comparable freezing levels during the retrieval test.

Twenty-four hours after the retrieval session, the four groups of animals underwent extinction training during which 10 tones were presented in the absence of footshock. Repeated measures on the five extinction blocks showed no significant effect of group ($F_{1,28} = .15$, ns) or of age ($F_{1,28} = .3$, ns). However, there was a significant effect for the interaction between age and group ($F_{1,28} = 24.9$; $p < .0001$), suggesting that the two age groups behaved differently. Furthermore, there was a significant effect of block ($F_{1,28} = 24.3$, $p < .001$) in the absence of any significant interaction with the other variables ($F_{1,28} = .8$, ns), which suggests that the different age groups extinguished fear memory similarly over the extinction blocks.

Follow-up analysis of the observed interactions of age × group using one-way ANOVA with repeated measures on the extinction trials showed differences between the adults-control and adults-stress groups ($F_{1,15} = 11.08$, $p < .005$, adults-control: 52.29 ± 5.05%; adults-EP: 76.78 ± 5.3%). The results show that stress impaired extinction of fear in adults.

In the postweanling animals, one-way ANOVA also revealed significant differences between the groups, with the PW-stress group showing lower freezing levels than the control group ($F_{1,13} = 52.2$, $p < .0001$, PW-control: 81.3 ± 2.6%; PW-EP: 55.4 ± 2.44%). This result suggests that stress facilitated extinction of fear in postweanling pups.

Similarly, two-way ANOVA on the retrieval of extinction 24 hours after extinction training showed a significant interaction effect (group × age: $F_{1,28} = 41.3$; $p < .0001$) without any significant effect of group ($F_{1,28} = .6$, ns) or of age ($F_{1,28} = .85$, ns). A follow-up *t* test on the observed interactions showed that exposure to stress was associated with impaired extinction in adult rats ($t_{15} = 5.6$, $p < .001$, adults-control: 37.7 ± 4.9%; adults-EP: 75.3 ± 4.15%). In postweanling pups, the differences between the PW-control and PW-EP were also maintained during extinction retrieval, with the PW-EP group showing better fear extinction than control pups ($t_{13} = 4.5$, $p < .005$; PW-control: 68.6 ± 4.1%; PW-EP: 42.9 ± 3.8%).

Thus, stress exerts opposite effects on fear extinction in postweanling pups compared with adult rats.

These results show that the similar kinetics of extinction in all groups suggest a reduction in expression of fear rather than facilitated extinction. To better dissociate between the two, we

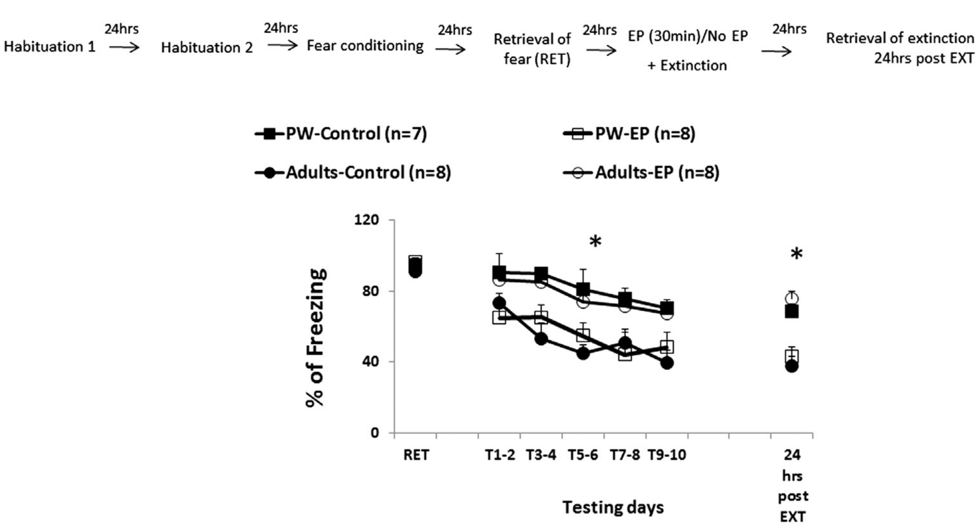

Figure 1. Schematic representation of the experimental protocol. A total of four groups were tested: postweanling (PW) pups and adult rats were conditioned on the first day. The next day they underwent retrieval testing (RET) and 24 hours later, the four groups of animals underwent extinction training (EXT). The results indicate that stress increased the expression of fear in adults and reduced it in PW pups (*$p < .001$ for significant effect between adults-control and adults-elevated platform stressor [EP] and PW-control and PW-EP). These differences were maintained 24 hours later (24 hrs post-EXT; *$p < .005$).