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Amnesia for Early Life Stress Does Not Preclude the Adult Development of Posttraumatic Stress Disorder Symptoms in Rats

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Background: Traumatic experience can result in life-long changes in the ability to cope with future stressors and emotionally salient events. These experiences, particularly during early development, are a significant risk factor for later life anxiety disorders such as posttraumatic stress disorder (PTSD). However, because traumatic experience typically results in strong episodic memories, it is not known whether such long-term memories are necessary for particular features of PTSD, such as enhanced fear and anxiety. Here, we used a fear conditioning procedure in juvenile rats before maturation of the neural systems supporting declarative memory to assess the necessity of early memory to the later life development of PTSD-related symptoms.

Methods: Nineteen-day old rats were exposed to unpredictable and inescapable footshocks, and fear memory for the shock context was assessed during adulthood. Thereafter, adult animals were either exposed to single-trial fear conditioning or elevated plus maze or sacrificed for basal diurnal corticosterone and quantification of neuronal glucocorticoid and neuropeptide Y receptors.

Results: Early trauma exposed rats displayed stereotypic footshock reactivity, yet by adulthood, hippocampus-dependent contextual fear-related memory was absent. However, adult rats showed sensitized fear learning, aberrant basal circadian fluctuations of corticosterone, increased amygdalar glucocorticoid receptors, decreased time spent in the open arm of an elevated plus maze, and an odor aversion associated with early-life footshocks.

Conclusions: These results suggest that traumatic experience during developmental periods of hippocampal immaturity can promote lifelong changes in symptoms and neuropathology associated with human PTSD, even if there is no explicit memory of the early trauma.

Key Words: Amnesia, development, early life stress, fear conditioning, hippocampus, rat

Flashback memories of a traumatic experience are a prevalent and distressing component of posttraumatic stress disorder (PTSD), which is further compounded by a persistent avoidance of stimuli associated with the trauma and often comorbid with increased states of anxiety and chronic depression. In the adult human and rodent, memories established within an emotionally charged environment are generally robust and not readily forgotten (1,2) and thus may provide important insights into the link between trauma-related memory and PTSD. This link has been primarily explored in patients with mild traumatic brain injury and/or traumatic asphyxiation injury with posttraumatic amnesia (3–5) as well as in subjects with early life trauma occurring during the period of infantile amnesia (6). Despite this, the link between trauma-related memory and PTSD remains a point of controversy (3,4).

To investigate this potential link, we used a Pavlovian fear conditioning approach in young rats at a developmental age characterized by infantile amnesia (7–9) when declarative hippocampal memory systems are not fully mature (10,11) and then examined its long-term mnemonic and nonmnemonic impact on adult PTSD-related symptomatology. Juvenile rats at postnatal day (P) age 19 and younger have difficulty learning and remembering contextual-spatial features of the environment but generally have little difficulty acquiring and retaining memories of more discrete sensory signals of danger (8,12,13). In fear conditioning, the associative relationships between environmental stimuli and footshocks are established and maintained within the amygdala (14–16), while the hippocampus is crucial for encoding and maintaining a memory of the features of the training context (17,18). Thus, P19 rats are capable of acquiring and retaining cue-based fear memories, while having difficulty with more contextual-spatial fear memories.

Prior animal models focused on efforts to examine early life stress (ELS) during the first 2 weeks of life have mainly used nonpainful stressors, such as mother-pup separation procedures, and have uncovered varying results on adult learning, anxiety, and neuroendocrine function (19–21). Alternatively, a large body of work by Landers and Sullivan (22) using pain-related procedures (odor-footshock/tail pinch pairings and maternal maltreatment) during the same period of development, odor-footshock/tail pinch pairings and maternal maltreatment, attenuated adult fear learning, reducing amygdala neural activity and promoting depressive-like behaviors. These studies have shed important light on the role of attachment and maternal behavior in the maturation of

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cognitive and emotional systems. However, relatively little is known about how painful experiences just before weaning could impact the maturation of neural circuits central to fear and stress regulation. This disparity between physical trauma and the animal mother-pup separation paradigm, which is not amenable to mnemonic analysis, poses a significant hurdle in determining a relationship and/or potential mechanism between early trauma-related memory and adult PTSD.

Here, we describe a rodent model of ELS amenable to mnemonic analysis and the examination of key features of PTSD. Rat pups (19 days old) were exposed to a highly stressful event, repeated footshocks, during a single session. We have previously shown that in adults this procedure causes a sensitized state that models several components of PTSD (23); therefore, it served as our model of trauma. In the present experiments following ELS, adult fear memory of trauma-related memory was assessed 2 months after footshock trauma. At this time, we also determined the acquisition of a novel fear memory. Anxiety in adults was measured by an elevated plus maze procedure, while avoidance behavior related to trauma was assessed by a modified odor choice task. Next, basal levels of corticosterone (CORT) were measured at 4-hour intervals over a 24-hour period to assess homeostatic levels of CORT. Lastly, we measured glucocorticoid receptors (GRs) and neuropeptide Y receptors, which are implicated in stress reactivity and resilience within brain regions critical in fear, memory, and stress regulation.

Methods and Materials

Subjects

Male Long-Evans rats (14 to 16 days old) arrived in litters of eight with surrogate dams (Charles River, Hollister, California). Each litter and dam were housed in plastic cages on a 12:12 light/dark cycle and provided with ad libitum access to food and water. Rats were weaned at 21 days and housed in groups of four, and at 50 to 55 days of age rats were pair-housed. All experimental procedures were approved by the University of California Los Angeles Animal Research Committee.

Behavioral Contexts

Early life stress occurred in individual conditioning boxes (32 cm × 25 cm × 25 cm) housed in light- and sound-attenuating chambers (Med Associates, St. Albans, Vermont). Video Freeze software (Med Associates) automatically scored behavior. During each session, the conditioning boxes were configured into one of three distinct contexts based on olfactory, auditory, visual, and tactile stimuli, and the method of transport also varied (Table 1).

Procedures

Figure 1 depicts the overall experimental design for each of the experiments conducted in the present study.

Early Life Stress. In context A, P19 rat pups were given either 15 unsignaled footshocks (1 mA, 1 sec) with a variable intershock interval or no footshocks (23). The duration of each session was 93 minutes.

Adult Memory for Early Life Stress (Context A). Fifty-nine to 61 days later, rats (P78 or P80) were returned for 8 minutes to the ELS context (Figure 1).

Sensitization of One-Trial Fear Conditioning (Context B). Either 1 day before or after ELS testing, rats (P78 or P79) were placed in a novel context (context B) and 3 minutes later received a single footshock (1 mA, 2 sec). One minute later, they were returned to the vivarium. Twenty-four hours later, a test for sensitization of this mild fear conditioning occurred.

Fear Sensitization Test (Context B). Rats (P79 or P80) were returned to the one-shock context (context B) for an 8-minute test to determine if ELS sensitized the development of this mild contextual fear.

Fear Generalization Test (Context C). Half of the rats (P81), 24 hours following the fear sensitization test, were exposed to a novel context (context C) for an 8-minute test of generalized contextual fear.

Odor Choice Test

Sixty days following the 0- or 15-shock conditioning procedure, another set of pair-housed adult rats (P78) were transported to a light- and sound-attenuating room, lit only by a red incandescent light bulb. In the center of the room, an acrylic opaque box was divided into three compartments by two transparent, slotted walls that permitted subjects to move freely between compartments. The lateral compartments were scented with an odor present during P19 context training (Simple Green) or a familiar odor of new cage bedding, while the central compartment was considered neutral and did not contain an odor source. To assess odor choice, each subject was run on four consecutive, 2-minute trials with the location of the odor source counter-balanced. An experimenter blind to the subjects' prior experimental condition used a stopwatch to determine total seconds spent in each of the compartments. Total percent time in each compartment was used to assess odor aversion.

Table 1

Fear Conditioning		Context Configuration			
	Context	Visual/Tactile	Auditory	Odor	Transport
P19-Fear Conditioning (15 Trials) Adult-TrM Test	A	Light Evenly spaced floor bars	Internal ventilation fan	Simple Green	Homecage + Cart + Black plastic cover
Adult-Sensitization Trial Adult-Sensitization Test	B	No light A frame insert Vertically staggered floor bars	None	Acetic acid	Black box + Cart+ Opaque cover
Adult-Context Generalization Test	C	Blue light Curved side and back walls Solid white floor	External fan	Windex	Homecage + Cart + White sheet cover

P, postnatal day; TrM, trauma-related memory.

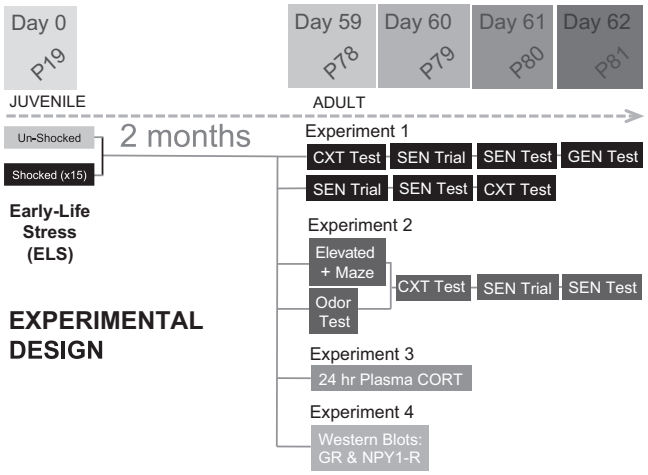

Figure 1. Experimental design: Postnatal day (P) 19 rats were placed in a conditioning context and received no shock (Un-Shocked) or 15 shocks and tested 2 months later. Experiment 1 – At P78 to P81, subjects were tested for context fear memory (CXT), sensitization (SEN), and generalization (GEN). Experiment 2 – At P78 to P81 in a separate group of animals, subjects were tested in elevated plus maze (Elevated + Maze) or odor aversion (Odor Test) and CXT and SEN. Experiment 3 – At P78 to P79 in a separate group of animals, subjects were sacrificed and trunk blood was collected to analyze plasma corticosterone (CORT). Experiment 4 – At P78 to P79 in a portion of animals sacrificed in experiment 3, brains were removed for Western blot analysis for glucocorticoid receptor (GR) and neuropeptide Y type 1 receptor (NPY1-R) proteins.

Elevated Plus Maze

Sixty days following the 0- or 15-shock procedure, a third set of pair-housed adult rats (P78) were transported to a sound-attenuating room, lit by a dim yellow incandescent light. An experimenter blind to the experimental conditions measured the total time each subject spent in the central, open, and closed areas.

Diurnal Corticosterone Levels

Sixty days following a 0- or 15-shock fear-conditioning procedure, pair-housed rats (P78) were moved into a quiet room and maintained on a 12:12 light/dark cycle 24 hours before blood collection. Every 4 hours, separate groups of rats were moved to an adjacent room and rapidly decapitated. Trunk blood was collected in a heparinized 500 μL tube and then centrifuged (1400 rpm, 3 min) for plasma extraction. Plasma was stored at −80°C and thawed to room temperature before CORT quantification by immunoassay.

Enzyme-Linked Immunosorbent Assay

Corticosterone enzyme-linked immunoassay kits were purchased from Assay Designs (Ann Arbor, Michigan). Plasma samples were diluted into assay buffer solution at a 1:40 ratio. Thereafter, we followed the procedures provided by the manufacturer.

Western Blotting

Fifty-nine days following a 0- or 15-footshock fear-conditioning procedure, subjects (P78) were sacrificed for immunoblotting of basal GR and neuropeptide Y type 1 receptors (NPY1-Rs). Glucocorticoid receptors bind the stress-related and regulatory steroid, CORT, while neuropeptide Y receptors bind the anxiety and feeding regulatory peptide, neuropeptide Y. We quantified each of these proteins in the amygdala, hippocampus, and medial prefrontal cortex (mPFC). Whole brains were immediately flash frozen and subsequently stored at −80°C. Brains were thawed by immersion in Allprotect tissue reagent (Qiagen, Germantown, Maryland) at −20°C for 7 days. Bilateral amygdala (lateral, basolateral, and central), dorsal hippocampus, and mPFC (anterior cingulate, prelimbic, and infralimbic cortices) tissues were dissected on a cold plate and stored in Allprotect before homogenization in a buffer. Immunoblots were performed from crude homogenates using an antibody concentration of 1:1000 GR (H-300) and NPY1-R (H-91) (Santa Cruz Biotechnologies, Dallas, Texas). Total protein controlled for receptor values from each individual sample, which were then normalized to the mean of the unshocked control group, such that the average unshocked control value equaled 100%. Receptor levels are represented as the percent change from the unshocked group.

Results

Experiment 1: Long-Term Memory and Sensitization of Early-Life Trauma

In this experiment, adult rats (ELS $n = 9$; no ELS $n = 7$) previously exposed to strong early-life fear conditioning exhibited little or no fear of the ELS context (Figure 2A). Similarly, subsequent exposure to a novel context (context B) failed to elicit

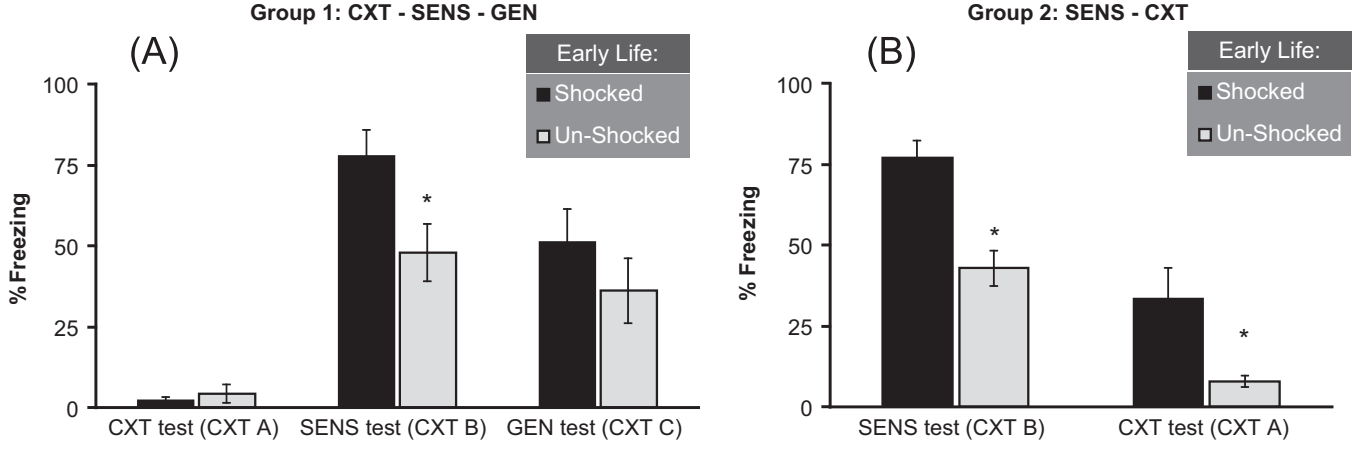

Figure 2. Percent freezing was used to assess trauma-related context fear memory (CXT), sensitization (SENS), and generalization (GEN) of fear. **(A)** Group 1: Initially tested for CXT, followed by SENS and then GEN. **(B)** Group 2: Initially tested for SENS, followed by CXT. *$p < .05$.

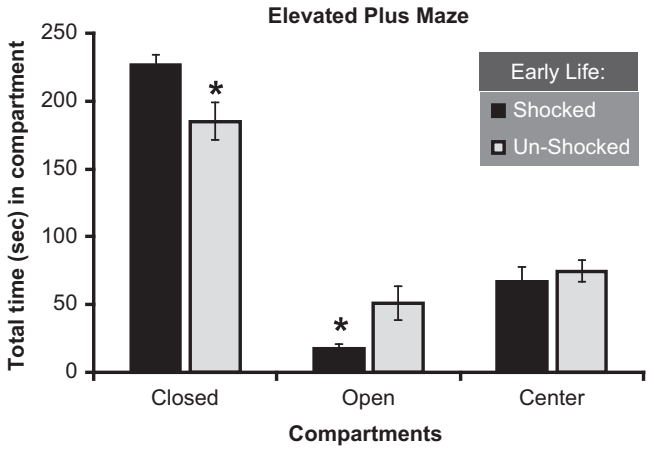

Figure 3. Total time (sec) spent in the open arms of an elevated plus maze was used to assess anxiety. Also depicted are total times in closed arm and central portion of maze. *$p < .05$.

freezing (data not shown). However, one-trial fear conditioning in this novel context resulted in significantly greater freezing in P19 shocked versus unshocked control rats (Figure 2A; analysis of variance [ANOVA], $F_{1,14} = 6.804$, $p = .021$). The following day both P19 shocked and unshocked control rats displayed similar levels of generalization to novel context C (Figure 2A) as confirmed by ANOVA, $F_{1,14} = 1.338$, $p = .67$.

In a second group of rats (ELS $n = 6$, no ELS $n = 6$), the order of the trauma-related memory test and single-shock trials were reversed. Again, adult fear conditioning in P19 shocked animals resulted in a significantly greater freezing than in unshocked control animals, replicating the first set of results ($F_{1,10} = 19.962$, $p = .001$; Figure 2B). Upon return to the environment associated with early-life P19 shock (context A), animals froze significantly more then unshocked control animals (ANOVA; $F_{1,10} = 6.98$, $p = .025$), but this freezing was similar to the first set of rats tested in a novel context (context C). This result suggests that such freezing was not due to fear directly conditioned by ELS but rather was indicative of context fear generalized from being shocked in context B. In other words, ELS did not result in an enduring associative context fear memory but left rats in a sensitized state that enhanced subsequent adult fear conditioning. This greater level of adult fear conditioning resulted in more fear to generalize to a novel context.

Experiment 2: Long-Term Anxiety and Avoidance Following ELS

Adult rats exposed to P19 shocks displayed a long-lasting anxiety-like phenotype as measured by the elevated plus maze task. Shocked rats ($n = 6$) spent significantly less time in the open arms of an elevated plus maze than unshocked rats ($n = 6$; ANOVA; $F_{1,10} = 5.468$, $p = .041$). As shown in Figure 3, this corresponded with ELS animals spending a significantly greater percentage of time in the closed arms, as compared with

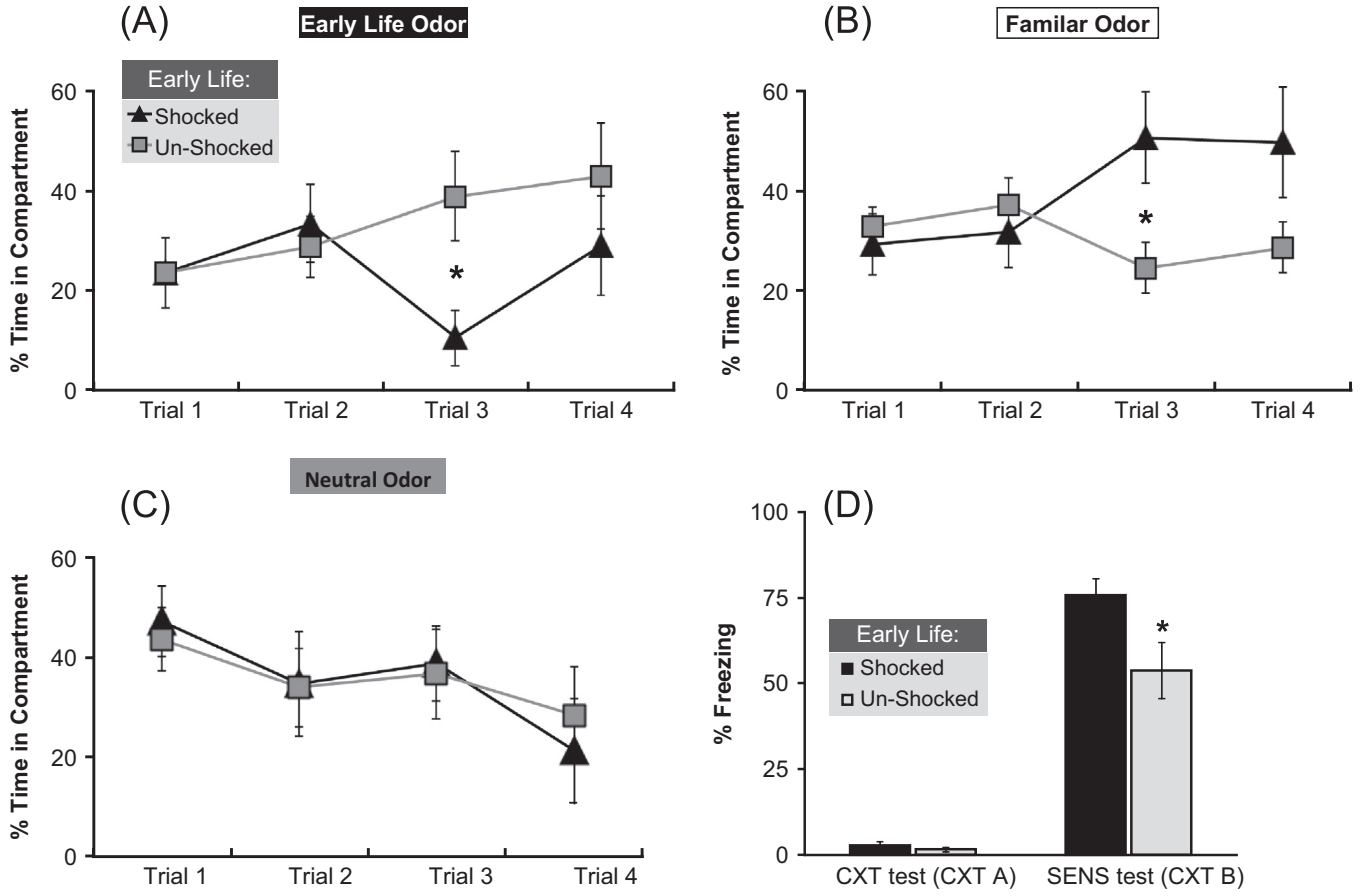

Figure 4. Percent time in compartment was used to assess odor aversion across four trials. Plotted are the mean percent time each group explored one of three compartments baited with **(A)** early life odor (Simple Green), **(B)** familiar odor (new cage bedding), and **(C)** neutral odor (no odor). *$p < .05$. **(D)** Percent freezing was used to assess fear during context fear memory (CXT) and sensitization (SENS).

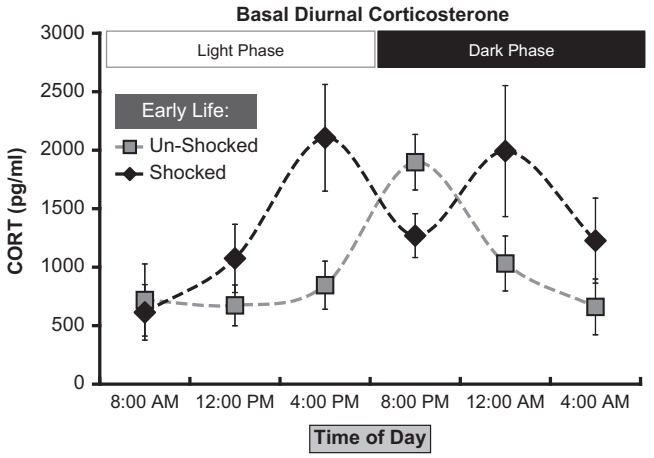

Figure 5. Total plasma concentration of corticosterone (CORT) across 24 hours: light phase (7:00 AM–7:00 PM) and dark phase (7:00 PM–7:00 AM). Each data point represents mean CORT collected from trunk blood at one of four time points.

unshocked rats (ANOVA; $F_{1,10} = 5.370$, $p = .043$). No significant differences were detected in time spent in the central portion of the maze (ANOVA; $F_{1,10} = .268$, $p = .617$).

Next, to examine any potential associative learning that persisted from P19 shocks, adult animals were tested in an odor choice task. Unshocked control animals ($n = 7$) gradually increased time spent in the compartment baited with the early-life odor (Simple Green), whereas P19 shocked subjects ($n = 7$) decreased time in the same compartment over the final two trials (Figure 4). Analysis of variance revealed a significant decrease in percent time P19 shocked animals spent in the early-life odor compartment on the third trial ($F_{1,12} = 7.378$, $p = .019$) but not at other trials (trial 1: $F_{1,12} = .00$, $p = 1.00$, trial 2: $F_{1,12} = .221$, $p = .647$, trial 4: $F_{1,12} = .970$, $p = .360$) as compared with unshocked control animals. This change corresponded with P19 unshocked animals showing a gradual decline in percent time in the compartment with the familiar odor (new cage bedding), while P19 shocked animals gradually increased their time in this compartment. This increase in the familiar odor compartment was statistically significant at trial 3 ($F_{1,12} = 6.153$, $p = .029$). No group differences in the percent time spent in the neutral, nonodor baited compartment were detected at any trials between groups (trial 1: $F_{1,12} = .145$, $p = .710$; trial 2: $F_{1,12} = .004$, $p = .950$; trial 3: $F_{1,12} = .037$, $p = .851$; trial 4: $F_{1,12} = .250$, $p = .626$). At best, the odor aversion to the original early life contextual odor was weak, yet these rats failed to display freezing to the P19 shocked context that had been scented with this odor. However, as demonstrated in experiment 1, P19 shocked animals showed increased adult contextual fear conditioning (ANOVA; $F_{1,14} = 5.560$, $p = .033$; Figure 4B).

Experiment 3: Long-Term Neuroendocrine Effects of ELS

We examined the impact of P19 fear conditioning on adult basal plasma levels of CORT over a 24-hour period. Plasma CORT data were analyzed using ANOVA. Figure 5 represents the diurnal cycle for basal measurements of CORT in rats that either received ELS ($n = 41$) or not ($n = 40$). Fifty-nine days later, control rats displayed basal CORT levels that peaked just before lights-off and troughed before the lights-on period during a 12:12 light-dark cycle. In contrast, P19 shocked rats displayed basal CORT levels that peaked at 4:00 PM and 12:00 AM. Overall, a heightened level of basal CORT was observed in P19 shocked versus unshocked rats. A 2 × 6 factorial ANOVA detected main effects of an early-life experience ($F_{1,80} = 4.462$, $p = .038$) and time of day ($F_{5,80} = 2.854$, $p = .021$) and a significant interaction ($F_{5,80} = 2.954$, $p = .002$). When the data from the two groups were analyzed separately using a trend analysis, a significant quartic function for P19 shocked rats ($F_{1,40} = 5.0042$, $p = .03$) confirmed a double peak diurnal profile, while a significant quadratic function for unshocked control rats ($F_{1,39} = 10.8100$, $p < .01$) indicated a single peak diurnal profile.

Experiment 4: Glucocorticoid and Neuropeptide Y Receptors

In the final experiment, we examined GR and NPY1-R receptor protein levels within context fear-related brain regions: amygdala, hippocampus, and mPFC. As shown in Figure 6A, adult amygdala levels of GR were altered by early-life experience (shocked $n = 10$, unshocked $n = 12$). An ANOVA revealed ELS significantly increased amygdala levels of GR ($F_{1,19} = 5.520$, $p = .007$) and marginally decreased NPY1-R ($F_{1,20} = 1.832$, $p = .053$; Figure 6B). Even though hippocampal receptor protein levels tended to be greater in P19 shocked subjects, these changes were not statistically significant (GR: $F_{1,20} = 1.604$, $p = .220$; NPY1-R: $F_{1,20} = 3.227$, $p = .088$). Lastly, both GR and NPY1-R levels within the mPFC

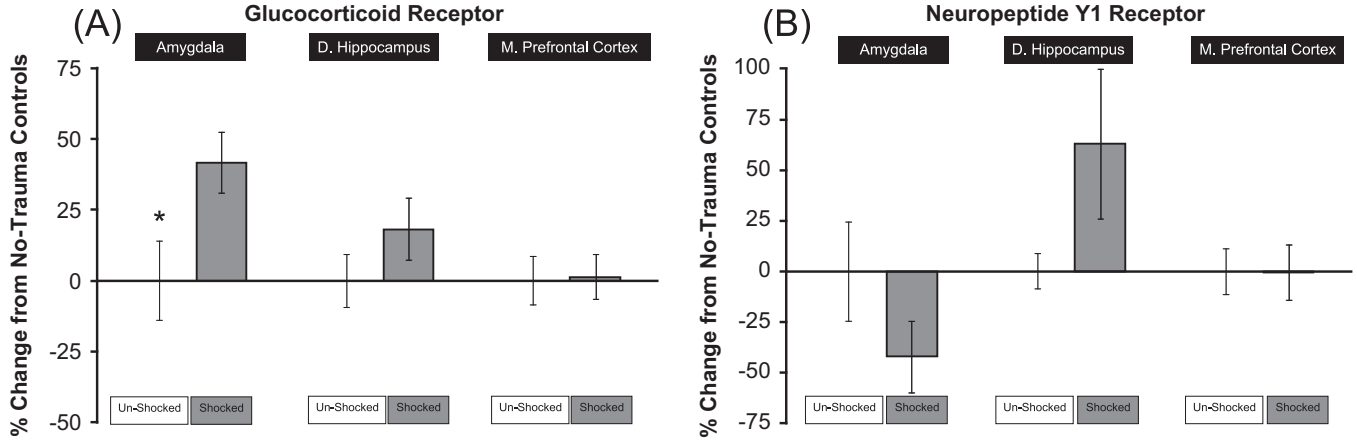

Figure 6. Percent change in protein levels in shocked from unshocked within the amygdala, dorsal (D.) hippocampus, and medial (M.) prefrontal cortex. **(A)** The glucocorticoid receptor. (B) The neuropeptide Y1 receptor. *$p < .05$.

were not altered by P19 footshocks (GR: $F_{1,20} = .014$, $p = .908$, NPY1-R: $F_{1,20} = .001$, $p = .976$).

A Pearson product-moment correlation coefficient was computed to assess the relationship between GRs and NPY1-Rs within each brain region (Figure 7). No significant correlations were found between receptors within the amygdala (shocked $r = -.0366$, $n = 10$, $p = .6021$; unshocked $r = -.064$, $n = 11$, $p = .425$) or hippocampus (shocked: $r = .0407$, $n = 10$, $p = .455$, unshocked $r = .1382$, $n = 11$, $p = .342$). However, within the mPFC, there was a strong negative correlation between GR and NPY1-R in the P19 shocked ($r = -.8077$, $n = 10$, $p = .002$) but not in the unshocked group ($r = -.0508$, $n = 11$, $p = .441$).

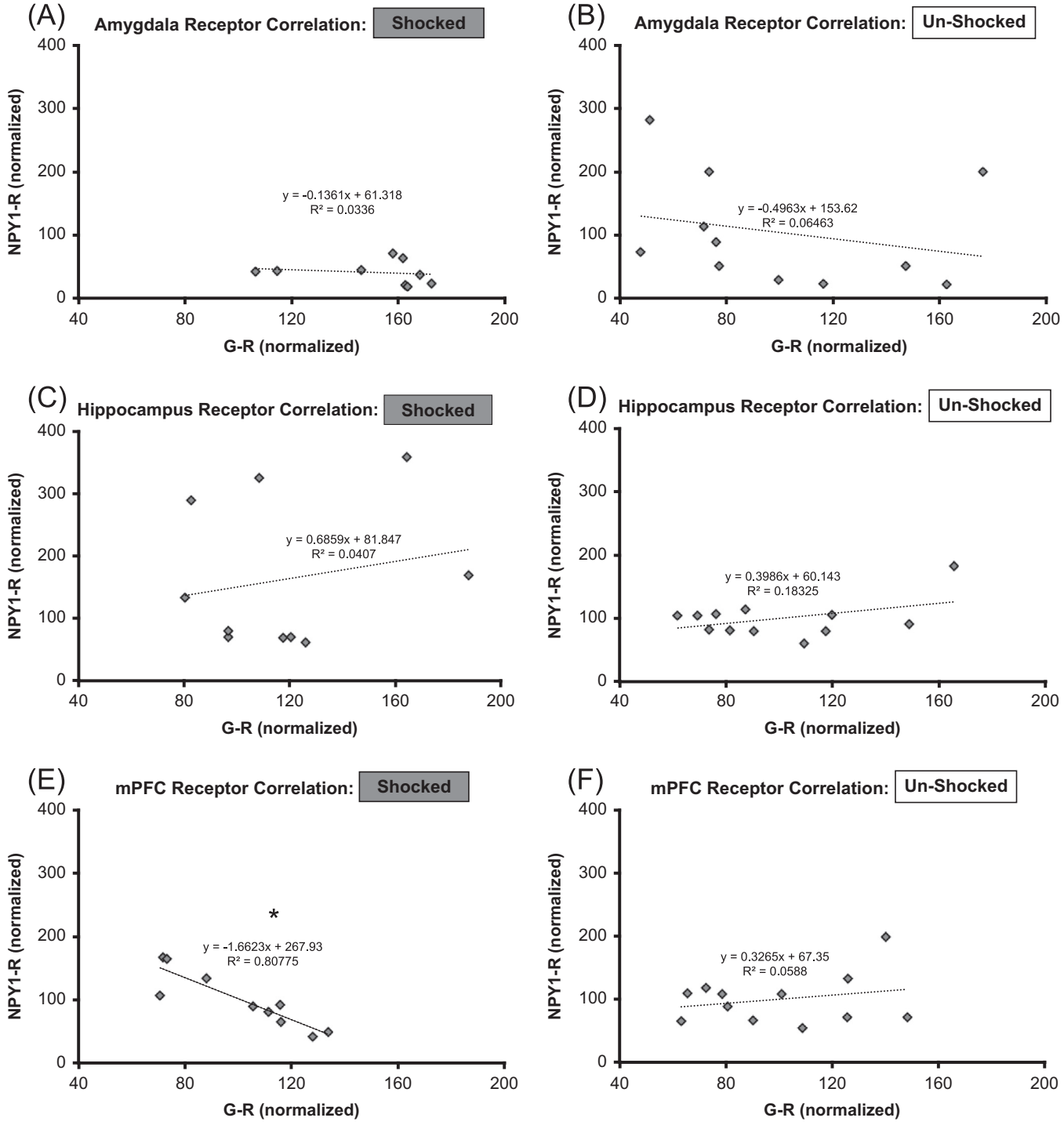

Figure 7. Scatter plot of normalized (to unshocked control animals) level of total glucocorticoid receptor (GR) as compared with neuropeptide Y type 1 receptor (NPY1-R) for individual subjects. **(A)** Amygdala: shocked; **(B)** amygdala: unshocked; **(C)** hippocampus: shocked; **(D)** hippocampus: unshocked; **(E)** medial prefrontal cortex (mPFC): shocked; **(F)** mPFC: unshocked. *$p < .05$.

Discussion

It has been argued that the lack of memories about a trauma may be protective against the development of PTSD, as observed in traumas resulting from brain injury (3). The present results suggest that in a developmental animal model of early-life trauma amenable to mnemonic analysis, trauma-related memory is not essential to produce an adult PTSD-like phenotype. Adult animals that failed to retrieve a hippocampal-dependent memory of early-life trauma displayed increased anxiety, a mild trauma-related odor avoidance, dysregulation of diurnal CORT, and sensitization to novel fear experiences. This sensitization of fear and altered CORT corresponded to an amygdala specific increase in GRs and small decrease in NPY1-Rs.

Juvenile, 19-day-old rats exposed to a 15-shock fear-conditioning procedure, which in adults normally results in a robust lifelong fear memory (2,24), failed to produce a lasting contextual fear memory of early-life experience in adulthood (P78–P80). The absence of contextual fear conditioning at this age is consistent with prior studies indicating that hippocampal-dependent memory systems are not functionally mature until 21 to 24 days of age (8,9). This transitional phase in rodent memory development has been described to be analogous to a period of infant/childhood amnesia for episodic hippocampal-dependent memories in humans (8).

This normally potent conditioning procedure in rats 21 days and older failed in P19 pups to result in a context fear memory, yet still resulted in an increased vulnerability or predisposition to future fear experiences. This was evident, as pups exposed to repeated footshocks showed greater adult fear conditioning without an increase in pain sensitivity (Figure S1 in Supplement 1) to a single shock in a novel context than previously unshocked animals. In contrast, Landers and Sullivan (22) have demonstrated rats exposed to repeated footshocks at an earlier age (P8–P12) fail to acquire contextual fear and as adults display suppressed amygdala activity and attenuated fear conditioning. We have previously shown that in adults, pharmacologic blockade of trauma-related memory by the amnestic *N*-methyl-D-aspartate antagonist, AP-5, failed to disrupt subsequent fear sensitization (23). In both of these cases, a specific memory for the trauma context is not necessary for sensitization of subsequent fear learning. This augmented fear conditioning is consistent with reports from PTSD patients exhibiting a greater propensity to develop new phobias and a greater reactivity to emotionally salient stimuli, even under conditions in which patients may fail to remember the precipitating trauma (4).

Odor aversions related to traumatic events have been long noted by clinicians as potential triggers of fear-related memories and anxiety (25). In the present experiments, even though ELS rats failed to display a long-term conditional fear response to an odor that composed part of the trauma environmental context, these same rats displayed a mild avoidance of this odor that persisted into adulthood. Importantly, both groups were exposed to the environmental odor (Simple Green), but it was only animals paired with shock that resulted in a mild odor avoidance as an adult. This avoidance corresponded with shocked-exposed animals spending more time in the vicinity of a familiar odor (new cage bedding), as compared with unshocked rats. A possible explanation for the decreased time in the Simple Green scented compartment is that early-life footshocks may have facilitated neophobia (26). However, this was not supported here, as P19 shocked adults did not differ in time spent in the Simple Green scented compartment during the initial two trials as compared with unshocked control rats. Alternatively, it has been hypothesized that the mature dorsal hippocampus encodes a unified multimodal sensory representation of the fear context that includes odor (27,28). Because of the immature status of the dorsal hippocampus at P19 (9), the fear context may not have been encoded as a unified representation by the hippocampus. Rather, a specific element of the context (i.e., odor) may have become associated with the shock. Such an elemental association would not require hippocampal processing [e.g., (29,30)] but would still depend on the amygdala (29,31). Consistent with this, it has been demonstrated that the emergence of amygdala-dependent odor-shock aversions are the earliest forms of associative learning to develop (32).

Amygdalar CORT is important for the acquisition of context fear memories (32,33) and altered peripherally in patients with PTSD (34). As shown in the present studies, adult rats exposed to early-life footshocks displayed an overall basal increase in circulating CORT across the diurnal cycle. This is particularly evident in shocked animals at 4:00 PM (3 hours before lights off in the vivarium) and 12:00 AM (5 hours after lights off). However, though not statistically significant, a small dip in CORT was detected at 8:00 PM (1 hour after lights off), which coincides with the beginning of the active phase in the nocturnal rat. This time period may be analogous to the waking period in humans and is normally when CORT peaks. Interestingly, Yehuda *et al.* (34) and Lanius *et al.* (35) have reported persons with PTSD, rather than having increased levels of cortisol, have decreased morning levels of basal cortisol. Here, we provide evidence of large diurnal fluctuations in CORT that are consistent with decreased morning cortisol in PTSD patients and increased CORT, particularly evident at transitional phases between nadir and peak. These collective changes in basal diurnal CORT could represent deficiencies in hypothalamic-pituitary-adrenal activity, feedback inhibition, and/or changes in glucocorticoid metabolism (36) that are reflected in region-specific changes in neuronal receptors.

Here, we identified amygdala-specific increases in GRs of subjects exposed to early-life footshocks. Recently, Gueze *et al.* (37) showed that increased GRs are not only a vulnerability factor for the development of PTSD but may also lead to increased amygdala activity following a severe stress. In rodents, peripheral and amygdala targeted injections of CORT increase basolateral amygdala neural activity (38) and fear conditioning (39). This increase in amygdala GRs, but not within the hippocampus or mPFC, suggests amygdala neurons containing GRs are particularly sensitive and persistently affected by a single ELS. Additionally, ELS rats displayed a trend toward reduced NPY1-Rs in the amygdala. In humans, decreased levels of peripheral neuropeptide Y are correlated with increased vulnerability to develop PTSD (40,41). In rats, basolateral amygdala targeted antagonism or genetic deletion of NPY1-R enhanced fear conditioning (42), while neuropeptide Y agonists decreased fear conditioning (43). Even though amygdala NPY1-Rs in early-life shocked rats were not statistically lower ($p = .053$) than in unshocked control rats, this subtle decrease in NPY1-Rs and increased GRs may act either individually or synergistically to enhance vulnerability and/or conditionability to emotionally salient events. Another result of interest, even though no changes in GRs and NPY1-Rs were found in either the hippocampus or PFC as a result of ELS, a robust negative correlation in GRs to NPY1-Rs was found in the PFC of ELS rats. This correlation at the exclusion of an overall change in receptor number could reflect differential changes in the ratio of NPY1-Rs to GRs within subregions such as the prelimbic and infralimbic cortices that play heterogeneous roles in stress and

emotion regulation. McKlveen *et al.* (44) recently demonstrated that viral knockdown of GRs within infralimbic, but not prelimbic, cortices caused stress sensitization and depressive-like behaviors. Studies dissecting regional changes of both GRs and NPY1-Rs to early-life footshock could shed further light into the source of the present PFC correlations.

Collectively, these results suggest that in preadolescent animals, normally painful, stressful, and aversive stimuli fail to result in a long-lasting contextual fear memory, yet they behaviorally potentiate new adult fear conditioning, induce anxiety, and promote trauma-related odor avoidance. Physiologically, this corresponds to a dysregulation of circadian entrained levels of circulating CORT and a selective increase in amygdala GRs. Importantly, this behavioral and physiological characterization of an ELS model may in the near future promote a greater mechanistic understanding of the neuroendocrine changes resulting from juvenile aversive experiences, as well as refining future pharmacologic treatments for PTSD.

Conclusion

A potent ELS did not result in a specific context memory of the trauma. However, it left rats in a sensitized state that persisted into adulthood. This state consisted of increased susceptibility to acquiring new fears, anxiety, a disturbed circadian rhythm for basal CORT, and increased GRs in the amygdala.

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