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.