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Adolescent traumatic stress experience results in less robust conditioned fear and post-extinction fear cue responses in adult rats☆

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ABSTRACT

Early exposure to a traumatic event may produce lasting effects throughout the lifespan. Traumatic stress during adolescence may deliver a distinct developmental insult compared with more-often studied neonatal or juvenile traumatic stress paradigms. The present study describes the lasting effects of adolescent traumatic stress upon adulthood fear conditioning. Adolescent rats were exposed to a traumatic stressor (underwater trauma, UWT), then underwent fear conditioning during adulthood. Fear extinction was tested over five conditioned suppression extinction sessions three weeks later. The efficacies of two potential extinction-enhancing compounds, endocannabinoid reuptake inhibitor AM404 (10 mg/kg) and M1 muscarinic positive allosteric modulator BQCA (10 mg/kg), were also assessed. Finally, post-extinction fear responses were examined using a fear cue (light) as a prepulse stimulus. Rats traumatically stressed during adolescence showed blunted conditioned suppression on day 1 of extinction training, and AM404 reversed this effect. Post-extinction startle testing showed that fear conditioning eliminates prepulse inhibition to the light cue. Startle potentiation was observed only in rats without adolescent UWT exposure. AM404 and BQCA both ameliorated this startle potentiation, while BQCA increased startle in the UWT group. These results suggest that exposure to a traumatic stressor during adolescence alters developmental outcomes related to stress response and fear extinction compared to rats without adolescent traumatic stress exposure, blunting the adulthood fear response and reducing residual post-extinction fear expression. Efficacy of pharmacological interventions may also vary as a factor of developmental traumatic stress exposure.

1. Introduction

Developmental exposure to a traumatic stress experience may cause lasting changes in behavior. Consequently, the prior stress history of an individual may be a critical factor in determining the severity of responses to stress experiences during adulthood, such as post-traumatic stress disorder (PTSD) (Cabrera et al., 2007; Iversen et al., 2008; McLaughlin et al., 2010). While neonates and juveniles have been the subjects of numerous developmental stress exposure studies (rodent and primate studies are reviewed in Gutman and Nemeroff, 2002; Pryce et al., 2002), fewer studies have specifically addressed adolescence. Adolescent development is distinct from the neonatal and juvenile phases (Spear, 2000), yet many ongoing maturational processes remain susceptible to perturbation during adolescence. Stress during adolescence presents potential for lasting alterations of the developmental course.

Stress insults during adolescent development may cause distinct results on adulthood outcomes compared to insults presented at other timepoints in life, as different ongoing developmental processes may be sensitive to stress-induced change across the course of development (Andersen, 2003). Several studies in rats have shown that juvenile stress can alter adulthood fear-related learning outcomes specifically, including contextual or cued extinction learning (Kosten et al., 2005, 2006; Toledo-Rodriguez and Sandi, 2007; Matsumoto et al., 2008; Koseki et al., 2009). Underlying physiological and signaling changes may also result from a juvenile stress insult in rats (Matsuzaki et al., 2011; Hiraide et al., 2012; Ishikawa et al., 2012). However, the nature of enduring stress effects changes as a factor of age at stress presentation (Wilkin et al., 2012). Little is known about the enduring effects of a mid-adolescence single-event stressor. As fear-related learning phenomena such as intrusive recollection and resistance to extinction learning are contributing factors to mental health conditions such PTSD (APA, 2000), the adolescent traumatic stress history of an individual may be a factor in susceptibility or resilience following a subsequent stress event in adulthood.

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Pharmacological treatment may assist in recovery from anxiety disorders related to intrusive recollection and aversive memory rigidity, possibly by facilitating extinction learning. The endocannabinoid reuptake inhibitor AM404 has been shown to enhance conditioned fear extinction in rats using fear-potentiated startle and contextual fear conditioning approaches (Chhatwal et al., 2005; Bitencourt et al., 2008). However, it has yet to be tested in a conditioned suppression model of fear extinction. Nootropic drugs are also of interest in potentially enhancing extinction learning to aid in recovery from PTSD. To this end, muscarinic cholinergic signaling may play an important role in modulation of extinction learning, shown in studies employing agonist and antagonist modulation of receptors in rats and in brain slices (Boccia et al., 2009; Santini et al., 2012). The M1 muscarinic acetylcholine receptor positive allosteric modulator, BQCA, has previously been used to enhance fear learning and reversal learning, but it has not yet been tested in extinction learning. Further exploration of compounds exploiting these signaling pathways is needed, as efficacy across multiple platforms of behavioral testing would suggest potential therapeutic utility.

We previously characterized the acute and subacute effects of a traumatic stress experience (underwater trauma, UWT) upon behavior, serum corticosterone, peripheral blood oxygenation, and lung tissue during adolescence. This previous study demonstrated that UWT caused lasting behavioral change and altered neuroendocrine signaling without hypoxic insult (Moore et al., 2012). The present study works to characterize the lasting effects of a traumatic adolescent stress experience upon adulthood fear conditioning and extinction of conditioned fear. We examined baseline adulthood behavior and acute fear conditioning response in rats with or without adolescent traumatic stress exposure. We then tested fear extinction with or without pharmacological treatment, and finally measured residual effects after fear extinction was complete.

2. Methods

2.1. Subjects

102 male Sprague–Dawley rats (postnatal day, P22–24) were received from Charles River Laboratories (Wilmington, MA) and housed under a 12:12 light–dark cycle. Rats were pair housed, and acclimated to handling for four days before experimental manipulations took place.

2.2. Experimental design

Fig. 1 shows the experimental timeline of the study. Rats received either traumatic stress (UWT) or control swim procedures during adolescence (P37). Rats in each group were counterbalanced to receive either fear conditioning or control (no footshock) in adulthood (P115). Before and after adolescent trauma, adulthood fear conditioning, and fear extinction, a battery of dependent measures including behavioral and endocrine status was collected from each rat. Rats were assigned to UWT or Swim condition during adolescence based upon open arm exploration in the adolescent pre-test. UWT and Swim treated adult rats were assigned to Fear Conditioning or Sham groups based upon open arm exploration in the adulthood pre-test. Drug treatment assignments were randomized within groups. Four stress history groups were defined: *C–C* (no adolescent stress, no fear conditioning, n = 30), *U–C* (adolescent UWT only, no fear conditioning, n = 12), *C–F* (no adolescent stress, adulthood fear conditioning only, n = 29), and *U–F* (adolescent UWT and adulthood fear conditioning, n = 31) (Table 1). Only twelve rats were used in the UWT only (U–C) group as no drug treatments were administered to this control group. All rats underwent five days of extinction training using conditioned suppression of operant behavior three weeks after fear conditioning or control (no footshock). At the end of extinction, a prepulse startle test using a light prepulse stimulus was also run.

2.3. Underwater trauma

Procedures were performed as previously described (Moore et al., 2012). As the prior study showed no decrease in peripheral blood oxygen saturation, this measure was not collected in the present study. Briefly, UWT rats swam for 40 s in a 12 L tank of normal saline at room temperature, then were gently submerged for 20 s. Rats were removed from the tank and briefly dried before returning to the home cage. To provide a control for effects of the procedure in absence of the traumatic submersion exposure, control rats swam for the entire 60-sec time window, then were removed from the tank and briefly dried before returning to the home cage. Saline was used to minimize the effects of submersion on mucous membranes of the rats.

2.4. Operant conditioning

Adult (P70) rats were singly housed and began food restriction to maintain weight at approximately 330 g beginning one week prior to operant conditioning. Sessions were conducted using MED Associates (St Albans, VT) rodent operant conditioning chambers containing two stimulus lights and a sonalert tone generator. Food reinforcement pellets were from Bio-Serv (Frenchtown, NJ). Rats were first trained to press an active lever for a food reward, under a continuous schedule of reinforcement. After five training sessions, rats were transitioned to a variable interval, 32-sec reinforcement schedule (VI32). A food pellet was presented on the first active lever press following a pseudo-randomized interval with an average duration of 32 s and a range of 0.8–127.9 s. VI32-schedule operant behavioral sessions ran for 30 min, five days per week, for six weeks prior to fear conditioning. The number of active lever presses per minute (response rate) was monitored throughout the training period. Response rates stabilized across days within this six-week period.

2.5. Test battery

Before and after fear conditioning and extinction sessions, a test battery consisting of corticosterone (CORT) measurement, elevated

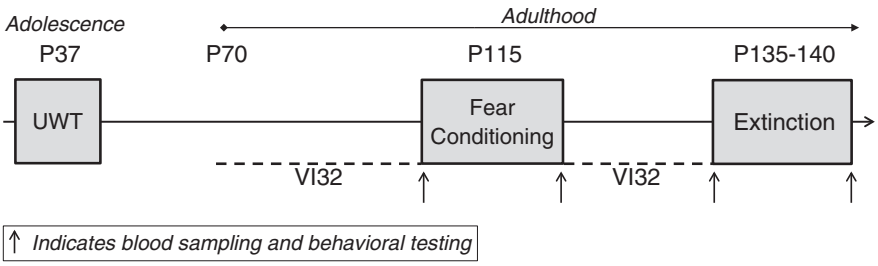

Fig. 1. Experimental design. Following an acclimation period, adolescent traumatic stress (underwater trauma, UWT) was delivered at P37. Daily operant behavioral sessions (VI32) began at P70. At P115, fear conditioning took place. A test battery (arrows; blood sample, Acoustic Startle Reflex, and Elevated Plus Maze) was run before and afterward. Daily operant behavioral sessions resumed for three weeks until the five-day extinction protocol began at P135. The same test battery (arrows) was run before the first extinction session and after the last session.