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Chronic stress disrupts fear extinction and enhances amygdala and hippocampal Fos expression in an animal model of post-traumatic stress disorder

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ABSTRACT

Chronic stress may impose a vulnerability to develop maladaptive fear-related behaviors after a traumatic event. Whereas previous work found that chronic stress impairs the acquisition and recall of extinguished fear, it is unknown how chronic stress impacts nonassociative fear, such as in the absence of the conditioned stimulus (CS) or in a novel context. Male rats were subjected to chronic stress (STR; wire mesh restraint 6 h/d/21d) or undisturbed (CON), then tested on fear acquisition (3 tone-footshock pairings), and two extinction sessions (15 tones/session) within the same context. Then each group was tested (6 tones) in the same context (SAME) or a novel context (NOVEL), and brains were processed for functional activation using Fos immunohistochemistry. Compared to CON, STR showed facilitated fear acquisition, resistance to CS extinction on the first extinction day, and robust recovery of fear responses on the second extinction day. STR also showed robust freezing to the context alone during the first extinction day compared to CON. When tested in the same or a novel context, STR exhibited higher freezing to context than did CON, suggesting that STR-induced fear was independent of context. In support of this, STR showed increased Fos-like expression in the basolateral amygdala and CA1 region of the hippocampus in both the SAME and NOVEL contexts. Increased Fos-like expression was also observed in the central amygdala in STR-NOVEL vs. CON-NOVEL. These data demonstrate that chronic stress enhances fear learning and impairs extinction, and affects nonassociative processes as demonstrated by enhanced fear in a novel context.

1. Introduction

Post traumatic stress disorder (PTSD) is a debilitating and increasing public health problem, especially in combat-exposed populations. The lifetime prevalence of PTSD in the United States has been reported to be ~6% (Kessler, Petukhova, Sampson, Zaslavsky, & Wittchen, 2012). PTSD develops in a subset of those experiencing a traumatic event (Breslau, Davis, Andreski, & Peterson, 1991), which suggests individual differences in the susceptibility and resilience to the development of the disorder after trauma exposure. One biological risk factor that has been identified for PTSD is reduced hippocampal volume (Gilbertson et al., 2002). Functional imaging studies in PTSD patients corroborate the reduced hippocampal volume findings, but also reveal compromised neural integrity within the hippocampus, reduced volume and responsivity within the medial prefrontal cortex (mPFC), as well as heightened amygdala responsivity (Shin, Rauch, & Pitman, 2006; Shin et al., 2004). Although these observed regional changes provide putative neural substrates for PTSD research, whether these alterations are contributing factors to, or outcomes from the disorder is unknown.

Animal models can help approach questions raised in clinical research in prospective designs under controllable conditions. Chronic stress leads to structural and behavioral alterations in rodents that parallel the changes observed in humans with PTSD. Within the amygdala, chronic stress causes dendritic hypertrophy

Abbreviations: ACG, anterior cingulate gyrus; ANOVA, analysis of variance; BLA, basolateral amygdala; CEA, central amygdala; CON, nonstressed control; CR, conditioned response; CS, conditioned stimulus; DG, dentate gyrus; IL, infralimbic cortex; ITI, inter-trial-interval; MEA, medial amygdala; mPFC, medial prefrontal cortex; OF, open field; PBS, phosphate buffered saline; PL, prelimbic cortex; PTSD, post-traumatic stress disorder; SEM, standard error of the mean; STR, chronic stress; US, unconditioned stimulus.

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(Padival, Blume, & Rosenkranz, 2013; Vyas, Mitra, Shankaranarayana Rao, & Chattarji, 2002; Vyas, Pillai, & Chattarji, 2004) and hyperexcitability (Rosenkranz, Venheim, & Padival, 2010). These stress-induced structural and physiological changes correspond to changes in emotionally-laden behavior including increases in anxiety-like behaviors (Vyas et al., 2002), facilitated acquisition of Pavlovian fear learning (Conrad, LeDoux, Magarinos, & McEwen, 1999; Hoffman, Armstrong, Hanna, & Conrad, 2010; Sandi, Merino, Cordero, Touyarot, & Venero, 2001), and resistance to fear extinction (Izquierdo, Wellman, & Holmes, 2006). In contrast to the amygdala, chronic stress causes dendritic retraction within the hippocampus (McLaughlin, Gomez, Baran, & Conrad, 2007) and mPFC (Brown, Henning, & Wellman, 2005; Cook & Wellman, 2004), changes that correspond to impaired hippocampal-dependent spatial learning and memory (Conrad, 2010; Hoffman et al., 2011) and compromised mPFC-dependent fear extinction retention (Baran, Armstrong, Niren, Hanna, & Conrad, 2009; Miracle, Brace, Huyck, Singler, & Wellman, 2006). Therefore, manipulating chronic stress in animal models allows for the induction of neural and behavioral changes that parallel outcomes that may lead to insights into factors that predispose individuals to develop PTSD symptomatology.

Pavlovian fear conditioning is a widely used model to study the neurobiology of fear and PTSD. In this paradigm, a neutral stimulus (such as a tone) serves as the conditioned stimulus (CS) and is paired with an aversive stimulus (such as a footshock) – the unconditioned stimulus (US). The animal learns the association between CS and US, and exhibits a conditioned response (CR, such as freezing) in the presence of the CS. Analogous to exposure therapy in humans, a common PTSD treatment approach, fear extinction occurs with repeated unreinforced CS presentations that result in a new, inhibitory memory trace, or a CS-no US association. One challenge with PTSD populations is the relapse of symptoms between extinction sessions, i.e., fear responding recovers between exposure therapy sessions and outside the therapy context (discussed in Hamner, Robert, & Frueh, 2004). Previous work has shown that chronic stress facilitates the spontaneous recovery of extinguished cue-elicited fear (Baran et al., 2009; Miracle et al., 2006), which is consistent with the fear responding recovery in PTSD cases. However, it is unknown how a history of chronic stress impacts nonassociative fear, such as in the absence of the CS or in a novel context (Kamprath & Wotjak, 2004), which is pertinent to the hyperarousal symptom cluster in PTSD patients (Yehuda & LeDoux, 2007). Furthermore, how the chronically stressed brain becomes engaged during the retrieval of a fear memory has been virtually unexplored. The current study aimed to investigate (1) how a history of chronic stress impacts both cued and context extinction following cued fear conditioning, (2) how chronic stress affects fear responding in a novel context following extinction, and (3) how chronic stress impacts functional activation of limbic structures involved in fear learning and extinction during retrieval of a cued fear memory.

2. Method

2.1. Subjects

Twenty male Sprague–Dawley rats weighing approximately 250–275 g upon arrival (approx. 2 months old; Charles River Laboratories) were pair-housed in light and sound attenuating chambers (21–22 °C) on a 12:12 reverse light cycle (lights off at 6 am) according to conditions specified by the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Science, National Research Council, 1996). Food and water were available *ad libitum* except during restraint procedures (described below). All procedures occurred during the dark phase of the light cycle.

Prior to group assignments, all animals were tested in a single open field (OF) for anxiety-like behavior and locomotion profiles. OF testing was consistent with our previously published procedures (Huynh, Krigbaum, Hanna, & Conrad, 2011) and helped to distribute similar profiles across groups (Bellani, Luecken, & Conrad, 2006). Briefly, animals were placed at pseudorandom locations in an open square arena (110 cm × 110 cm, 30 cm height) under low light intensity (200 lx) and given 10 min to explore then returned to their home cage. The OF arena was cleaned after each trial with pet deodorizer. OF behavior was recorded using an overhead video camera for offline scoring. Behavior was scored using (1) grid crossings, defined as the front two paws traversing a center or peripheral grid line, and (2) center grid time, recorded from the time the front two paws crossed the center grid until the front two paws exited the center grid.

Following OF testing, animals were divided into non-stressed control (CON) or chronically stressed groups (STR), $n = 10$/group, and further subdivided into subgroups for the same and novel context testing condition (described below). All groups had similar locomotor and anxiety-like behavior profiles in OF (data not shown).

2.2. Stress manipulation

Rats were chronically stressed via repeated wire mesh restraint (STR) or not (CON), and were weighed weekly. During the designated restraint period, STR rats were restrained in their home cages in wire mesh restrainers for 6 h/d/21d. Wire mesh restrainers were 18 cm circumference × 24 cm long (wire mesh from Flynn and Enslow Inc., San Francisco, CA) with wire ends sealed with grip guard sealer (ACE Hardware). CON rats were handled briefly each day, with their food and water restricted while the STR rats were restrained to keep food and water access similar across treatment conditions.

2.3. Fear conditioning: apparatus

Rodent fear conditioning chambers (25 cm depth × 29 cm height × 26 cm width: Coulbourn Instruments, E10-18TC) were contained in sound-attenuating cubicles (Coulbourn, E10-23, white). A PC interface card (Coulbourn, PCI-3-KIT) adapted to a PC, a universal link (Coulbourn, L91-04S), and Graphic State software (v 3.03 GS3.03) controlled the stimulus presentation. A frequency generator (Coulbourn, E12-01) produced a tone (75 dB, ~3.0 kHz) through a speaker located in the side panel of the conditioning chamber. The shock (500 ms, 0.35 mA, Coulbourn Animal Shock Generator, H13-15) was administered as a current, equally distributed through a metal grid floor (Coulbourn, E10-18RF). Behavior was videotaped for off-line analysis using a camera (Coulbourn, E27-91) mounted on the ceiling and a videocassette recorder. Infrared lights were located on the side panels of the chamber to denote the onset and offset of the tone, because no audio was recorded. A house light (Coulbourn, E11-01) was mounted in the side panel to illuminate the chamber at all times.

Two distinct chamber contexts (contexts A and B) were utilized for different fear conditioning testing phases. Context A consisted of white and silver paneled walls, a wire bar shock floor with a white catch pan, and was cleaned with 70% ethanol. Context B consisted of striped paneled walls, a smooth Plexiglas® floor insert and a dark catch pan, and was cleaned with an orange scented cleaner (method® clementine all purpose natural surface cleaner, methodhome.com).

2.4. Fear conditioning: procedure

During the last two days of restraint stress, all testing groups were transported by cart in their home cage into the fear conditioning testing room and left on the cart for 30 min to acclimate to the

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