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Alterations in neuronal morphology in infralimbic cortex predict resistance to fear extinction following acute stress

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ABSTRACT

Dysfunction in corticolimbic circuits that mediate the extinction of learned fear responses is thought to underlie the perseveration of fear in stress-related psychopathologies, including post-traumatic stress disorder. Chronic stress produces dendritic hypertrophy in basolateral amygdala (BLA) and dendritic hypotrophy in medial prefrontal cortex, whereas acute stress leads to hypotrophy in both BLA and prelimbic cortex. Additionally, both chronic and acute stress impair extinction retrieval. Here, we examined the effects of a single elevated platform stress on extinction learning and dendritic morphology in infralimbic cortex, a region considered to be critical for extinction. Acute stress produced resistance to extinction, as well as dendritic retraction in infralimbic cortex. Spine density on apical and basilar terminal branches was unaffected by stress. However, animals that underwent conditioning and extinction had decreased spine density on apical terminal branches. Thus, whereas dendritic morphology in infralimbic cortex appears to be particularly sensitive to stress, changes in spines may more sensitively reflect learning. Further, in stressed rats that underwent conditioning and extinction, the level of extinction learning was correlated with spine densities, in that rats with poorer extinction retrieval had more immature spines and fewer thin spines than rats with better extinction retrieval, suggesting that stress may have impaired learning-related spine plasticity. These results may have implications for understanding the role of medial prefrontal cortex in learning deficits associated with stress-related pathologies.

1. Introduction

Deficits in fear extinction are characterized by an inability to suppress a conditioned fear response following the removal of the unconditioned stimulus. This impairment may contribute to the perseveration of fear in stress-sensitive psychopathologies, including post-traumatic stress disorder (PTSD), and may impede any therapeutic techniques targeting the extinction process (Akirav and Maroun, 2007; Myers and Davis, 2007; Herry et al., 2010; Holmes and Quirk, 2010).

Connections between the amygdala and medial prefrontal cortex (mPFC) are critical for extinction learning, and dysfunctional communication between these two structures may lead to extinction deficits (Maroun, 2013). Both acute (Akirav et al., 2009; Maroun et al., 2013) and chronic stress (Izquierdo et al., 2006; Miracle et al., 2006) produce deficits in fear extinction and alter the morphology and function of neurons in basolateral amygdala (BLA; Vyas et al., 2002; Maroun et al., 2013) and mPFC (Cook and Wellman, 2004; Izquierdo et al., 2006). Indeed, BLA is a critical site of plasticity for fear extinction (Orsini and Maren, 2012) and is vulnerable to the effects of stress. For example, acute stress enhances LTP in BLA (Maroun and Richter-Levin, 2003; Maroun, 2006) and leads to dendritic retraction and a rapid increase in spine density here (Maroun et al., 2013). In contrast, chronic stress produces dendritic proliferation in BLA, as well as an increase in spine density (Vyas et al., 2002; Vyas et al., 2004, 2006; Mitra et al., 2005; Roozendaal et al., 2009). In spite of the opposite effects stress has on dendritic morphology in BLA, both acute and chronic stress produce deficits in fear extinction (Maroun et al., 2013) and extinction recall (Miracle et al., 2006; Maroun et al., 2013).

Abbreviations: BLA, basolateral amygdala; IL, infralimbic; PL, prelimbic; mPFC, medial prefrontal cortex.

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Therefore, cortico–amygdalar interactions are likely responsible for the observed behavioral deficits following stress exposure.

Projections from infralimbic (IL) cortex to BLA result in inhibition of BLA projection neurons, and therefore a decrease in fear responding, via the activation of either inhibitory intercalated cells of the amygdala or intra-BLA circuits (Quirk and Mueller, 2008). Indeed, CS-induced firing in IL is correlated with extinction retrieval, and stress-induced decreases in CS-related firing in IL are associated with deficits in extinction retrieval (Wilber et al., 2011). Lesions (Quirk et al., 2000), temporary inactivation (Sierra-Mercado et al., 2011), or inhibition of protein synthesis in IL (Santini et al., 2004) impairs extinction retrieval, which is rescued in chronically stressed animals with IL lesions (Farrell et al., 2010). Potentiation in the mPFC, which is critical for the success of extinction (Herry and Garcia, 2002; Vouimba and Maroun, 2011), is altered following acute stress (Maroun and Richter-Levin, 2003; Rocher et al., 2004; Richter-Levin and Maroun, 2010; Schayek and Maroun, 2015). Additionally, chronic (Cook and Wellman, 2004), mild (Brown et al., 2005), and acute stress (Izquierdo et al., 2006) have all been shown to produce apical dendritic retraction in mPFC. Chronic stress has also been shown to decrease spine density in mPFC (Radley et al., 2006; Liu and Aghajanian, 2008; Radley et al., 2008, 2013), while the effect of acute stress on spine density in mPFC has yet to be examined. Thus, we examined the effect of an acute elevated platform stressor on fear extinction and the morphology of IL neurons.

2. Materials and methods

2.1. Subjects and stressor

All procedures were conducted in accordance with NIH Guidelines and International Guiding Principles for Biomedical Research Involving Animals, and were approved by the Bloomington Animal Care and Use Committee and the University of Haifa Ethics and Animal Care Committee. Young adult male Sprague Dawley rats (age ~60 days; 250–300 g; Harlan Laboratories, Jerusalem, Israel) weighing 200–280 g were group housed (two rats per cage) in Plexiglas cages and maintained on a 12:12 light/dark cycle with free access to food and water. Stress was evoked as previously described (Xu et al., 1997; Maroun et al., 2013). Briefly, each rat was placed on an elevated platform (12 × 12 cm) in a brightly lit room for 30 min. This was previously demonstrated to effectively impair fear extinction (Akirav and Maroun, 2007; Maroun et al., 2013), as well as LTP in mPFC (Maroun and Richter-Levin, 2003; Rocher et al., 2004; Richter-Levin and Maroun, 2010).

2.2. Fear conditioning and extinction

Fear conditioning was conducted in 'context A,' a chamber with a grid floor and transparent Plexiglas walls. The conditioning procedure was performed as previously described (Hikind and Maroun, 2008; Maroun et al., 2013), and was comprised of three pairings of a conditioned stimulus and unconditioned stimulus (120-s inter-pairing interval) after a 120-s no-stimulus baseline. The CS was a 4-kHz, 80-dB, 30-s tone that co-terminated with delivery of the 0.8-mA, 1-s footshock unconditioned stimulus. On the day after conditioning, rats were placed in 'context B,' a chamber with transparent Plexiglas walls and a black Plexiglas floor, and were subjected to fear retention testing via three CS presentations (note that rats had been habituated to context B for 20 min on each of 3 days prior to conditioning).

Immediately after retention testing, stressed rats (n = 11) were exposed to the elevated platform, whereas unstressed rats (n = 8) were returned to their home cages. One day later, rats were given an extinction session composed of 10 CSs in context B. Extinction retrieval was carried out in context B on the following day, by presentation of three CSs. Additional groups of rats (n = 8, unstressed; n = 7, stressed) underwent stress/no-stress, but were not subjected to fear conditioning or extinction testing.

Freezing, i.e. the absence of all movement except for respiration (Blanchard and Blanchard, 1972; Kim et al., 1992), was quantified from video with image-based software (P. Schmid, Behavioral Neurobiology Laboratory, Swiss Federal Institute of Technology, Zurich), and expressed as the percentage of time spent freezing during tone presentation. The behavioral data reported here were used in previous analyses (Maroun et al., 2013).

2.3. Golgi histology and dendritic analysis

Following extinction retrieval testing, tissue was processed using Glaser and van der Loos' modified Golgi stain, as described previously (Glaser and Van der Loos, 1981; Martin and Wellman, 2011). Rats were deeply anesthetized with equithesin and perfused with 0.9% saline. Brains were then removed and immersed in Golgi-Cox solution for 12 day and then moved to 30% sucrose in saline. Brains were then dehydrated, embedded in 8% celloidin, and sectioned at 180 μm on a sliding microtome (AO860; American Optical Company, Buffalo, NY, USA). Free-floating sections were alkalinized, developed in Dektol (Kodak), fixed in Ilford rapid fixer, dehydrated in a graded series of ethanols, cleared in xylenes, mounted, and coverslipped.

Pyramidal neurons in IL of mPFC were drawn. IL is readily identifiable by its position on the medial wall of rostral cortex, and its location just ventral to prelimbic cortex, which has more well-defined layers (Zilles and Wree, 1995). Pyramidal neurons were identified by a distinct, single apical branch extending from the apex of the soma towards the pial surface of the cortex, two or more basilar dendrites, and dendritic spines. Neurons selected for reconstruction did not have truncated branches and were not obscured by neighboring neurons, with dendrites that were easily discernable by focusing through the depth of the tissue. In 3 evenly spaced sections through the rostral-caudal extent of IL, all pyramidal neurons meeting these criteria were identified. A random number generator was used to select four of these neurons per section, two from each hemisphere (one superficial, one deep, for a total of 12 neurons per animal). All neurons were drawn at 600× and morphology of apical and basilar arbors was quantified in three dimensions using a computer-based neuron tracing system (Neurolucida; MBF, Bioscience, Williston, VT) with the experimenter blind to the condition.

Spines were also counted on 12 neurons per animal. For each neuron, a segment averaging 40 μm in length was drawn from one terminal apical and one terminal basilar dendrite. We examined distal branches because corticosterone has been shown to induce dendritic remodeling in these branches (Wellman, 2001; Cook and Wellman, 2004; Liu and Aghajanian, 2008). Spines were classified as stubby, thin, or mushroom, based on standard morphological criteria (Peters and Kaiserman-Abramof, 1970).

2.4. Statistical analyses

For statistical analyses, extinction trials were collapsed into blocks of two trials. Percentage freezing during each testing phase was compared between unstressed and stressed groups using two-way repeated measures ANOVAS (for conditioning, retention, and extinction retrieval, stress × trial; for initial extinction, stress × block). When appropriate, subsequent planned comparisons were conducted, consisting of two-group F-tests performed within the overall ANOVA (Hayes, 1994; Maxwell and Delaney,