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Glucocorticoids are required for extinction of predator stress-induced hyperarousal

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ABSTRACT

Background: The role of glucocorticoids in extinction of traumatic memories has not been fully characterized despite its potential as a therapeutic target for acquired posttraumatic stress disorder (PTSD). The predator stress paradigm allows us to determine whether glucocorticoids mediate the extinction of both context-dependent and context-independent fear memories.

Methods: Male C57BL/6J mice were exposed to a predator (cat) then repeatedly exposed to the predator stress context in the absence of the cat. Context-dependent (associative) fear memory was assessed as suppression of activity during re-exposure to the predator stress context without the cat (extinction trials). Context-independent fear (non-associative) was assessed seven days after extinction trials using measures of hyperarousal and anxiety-like behaviours in environments unlike the predator stress context. To assess the role of glucocorticoids, mice were injected with metyrapone (50 mg/kg) 90 min prior to extinction trials in predator stressed mice and context-dependent and context-independent fear memories were assessed. Finally, metyrapone-treated predator stressed mice were injected with corticosterone (5 or 10 mg/kg) immediately following extinction trials and context-dependent and context-independent fear memories were assessed.

Results: Repeated re-exposure to the predator stress context without the cat present extinguished context-dependent fear memory, and also reduced hyperarousal, a generalized, chronic PTSD-like symptom. We show that extinction of context-independent predator stress-induced hyperarousal is dependent on endogenous glucocorticoids during the extinction trials. Furthermore, the inhibition of extinction by metyrapone on startle amplitude was reduced by exogenous administration of corticosterone following extinction trials. Overall, these data implicate glucocorticoids in the extinction of hyperarousal, a core symptom of PTSD.

1. Introduction

Exposure to severe stressors such as a traumatic physical or psychological experience may result in the development of affective disorders. One such disorder, posttraumatic stress disorder (PTSD), is characterized by persistent re-experiencing of the trauma, avoidance of trauma-associated stimuli, a general numbing of emotion, and hyperarousal (DSM-IV-TR, 309.81). Between 6.8% and 15% of North Americans develop PTSD following a traumatic event (Kessler et al., 2005). The importance of studying PTSD has increased with the recent terrorist attacks such as September 11, 2001 in New York, which increased prevalence of PTSD (Galea et al., 2008; Kessler & Wang, 2008).

Treatments for acquired anxiety disorders such as PTSD often rely on progressive extinction of fear memories (Bentz, Michael, de Quervain, & Wilhelm, 2010). Unfortunately, the inability to extinguish intense fear memories is an important clinical problem in psychiatric disorders such as PTSD (Blechert, Michael, Grossman, Lajtman, & Wilhelm, 2007; Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007; Yang, Chao, & Lu, 2006). Thus, the purpose of this set of experiments is to use an animal model of PTSD to identify factors that modulate progressive extinction of fear memories produced by severe stress. Knowledge of such factors may suggest more effective extinction treatments.

There is growing evidence from both rodent and human studies that glucocorticoids (corticosterone in animals and cortisol in humans) play a key role in distinct fear memory processes. Glucocorticoids can dose-dependently enhance the consolidation of new fear memoires (Abercrombie, Kalin, Thurow, Rosenkranz, & Davidson, 2003; McGaugh & Roozendaal, 2002; Okuda, Roozendaal, & McGaugh, 2004; Roozendaal, 2002; Sandi & Rose, 1994). On the other hand, pharmacological blockade of corticosterone or cortisol synthesis with metyrapone impairs memory consolidation in both animals and humans (Maheu, Joober, Beaulieu, & Lupien, 2004; Roozendaal, Bohus, & McGaugh, 1996). As in

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consolidation of fear memories, glucocorticoids facilitate the consolidation of extinction (Abrari, Rashidy-Pour, Semnanian, & Fathollahi, 2008; Blundell, Blaiss, Lagace, Eisch, & Powell, 2011; Cai, Blundell, Han, Greene, & Powell, 2006; Yang et al., 2006). Extinction is not only the result of forgetting or of memory erasure, but also involves the formation of new associations that compete with prior fear conditioned associations, hence new extinction memories (Rescorla, 1996). Recently, glucocorticoids have been shown to facilitate the extinction of shock-induced fear memories (Abrari et al., 2008; Cai et al., 2006; Yang et al., 2006), while blocking corticosterone synthesis with metyrapone prevents extinction (Barrett & Gonzalez-Lima, 2004; Blundell et al., 2011; Yang et al., 2006). These effects are mediated in part through the amygdala since intra-amygdalar infusion of a glucocorticoid receptor (GR) agonist facilitates extinction learning, whereas infusion of a GR antagonist blocks extinction learning (Yang et al., 2006). Recent data also suggests that administration of corticosterone blocks retrieval (Cai et al., 2006), while metyrapone enhances retrieval of a shock-induced fear memory. In light of these data, de Quervain and colleagues have suggested that glucocorticoids effects on extinction and retrieval go hand in hand to promote memory extinction processes (de Quervain, Aerni, Schelling, & Roozendaal, 2009).

Cai et al. (2006) and others (Abrari, Rashidy-Pour, Semnanian, Fathollahi, & Jadid, 2009; Blundell et al., 2011; Yang et al., 2006) proposed that the glucocorticoid surge during reactivation of a shock-induced fear memory is necessary for the extinction of the fear memory. In the absence of the glucocorticoid surge, fear memories persist which may ultimately lead to at least one of the core symptoms of PTSD, the persistent associative fear memories. This is consistent with the human data showing decreased cortisol levels in patients with PTSD (Heim, Ehlert, Hanker, & Hellhammer, 1998; Kellner et al., 2000; Mason, Giller, Kosten, Ostroff, & Podd, 1986; Yehuda, 2002, 2009; Yehuda, McFarlane, & Shalev, 1998; Yehuda et al., 1990), suggesting that low cortisol levels may contribute to a hyper-retrieval of fear memories (de Quervain et al., 2009), or an inability to extinguish the fear memory. Indeed, cortisol administration is helpful in the treatment of established disorders of emotional memories such as PTSD and phobias (Aerni et al., 2004; Schelling, Roozendaal, & de Quervain, 2004; Schelling et al., 2006; Soravia et al., 2006). Furthermore, de Quervain and colleagues have recently shown that glucocorticoids can promote extinction-based psychotherapy (de Quervain et al., 2011). Taken together, these preclinical and clinical studies suggest that glucocorticoids may play a critical role in the development and subsequent treatment of acquired anxiety disorders such as PTSD.

Predator stress is an ecologically relevant model of PTSD in that it presents animals with a traumatic event (exposure to a predator or predator cues) that they may encounter in nature (Adamec & Shallow, 1993; Cohen & Zohar, 2004; Munoz-Abellan, Andero, Nadal, & Armario, 2008). Predator stress paradigms reliably induce hyperarousal (enhanced acoustic startle response) which closely parallels symptoms seen in human PTSD patients (Adamec, Head, Soreq, & Blundell, 2008; Adamec, Strasser, Blundell, Burton, & McKay, 2006; Adamec, Blundell, & Burton, 2003; Blundell & Adamec, 2007; Cohen & Zohar, 2004). Furthermore, predator stress often causes a long-lasting increase in anxiety-like behaviour as measured in the elevated plus maze, light/dark box, and hole board (Adamec & Shallow, 1993; Adamec, Walling, & Burton, 2004; Blundell & Adamec, 2007; Cohen & Zohar, 2004). Increased generalized anxiety is co-morbid with PTSD (Pitman, Orr, & Shalev, 1993). Thus, the predator stress paradigm allows us to determine if pharmacologically targeting extinction not only affects subsequent context-specific symptoms (*i.e.* persistent trauma-associative fear memories), but also more generalized context-independent symptoms of hyperarousal and anxiety.

Despite the merits of predator stress as a model of PTSD, extinction of a predator stress-induced fear memory has not been assessed. Thus, the first goal of these experiments was to determine if the memory of the predator stress encounter (predator stress-induced contextual fear memory) can be extinguished. Repeated exposure to the predator stress room (in the absence of the predator) extinguished the predator stress-induced contextual fear memory (extinction of an associative fear memory). Even more interesting was the novel finding that repeated re-exposure to the predator stress context extinguished predator stress-induced hyperarousal, a context-independent fear memory (or non-associative fear memory). Hyperarousal is context-independent because it is measured in an environment very different from the cat exposure room (stress context). As described above, extinction of a contextual shock-induced fear memory is glucocorticoid-dependent (Abrari et al., 2008; Blundell et al., 2011; Cai et al., 2006; Yang et al., 2006). However, it is unknown if extinction of hyperarousal, another core symptom of PTSD is also glucocorticoid-dependent. Thus, the second goal of these experiments was to determine if extinction of predator stress-induced fear memories is also glucocorticoid-dependent. Our findings suggest that the endogenous glucocorticoid surge is necessary during predator stress-induced fear memory reactivation for normal extinction of hyperarousal, a non-contextual fear memory. Overall, our data suggest that decreased glucocorticoids during extinction contribute to hyperarousal, a core symptom of PTSD.

2 Methods

2.1. Study 1

2.1.1. Subjects

A total of 45 male C57BL/J6 (Charles River, Canada) mice were used in Study 1. Mice arrived at 6 weeks of age and were housed individually in clear plastic cages with wire covers (42 cm × 25 cm × 20 cm) and provided food and water *ad libitum*. Mice were adapted to a 12 h light/dark cycle (lights off at 7 AM) for one week. Prior to treatment, mice were handled daily for 5 days. Following treatment, predator stressed mice were housed in a separate colony room from mice which were not predator stressed to reduce olfactory stress contagion from stressed mice to unstressed controls.

2.1.2. Groups

There were three groups ($n = 15$): handled control (HC), predator stressed (PS), and predator stressed extinction (PSE). Mice in the handled control (HC) group were not exposed to a cat. Instead, they were handled on predator exposure day, and then remained undisturbed in their home cage until behavioural testing. Predator stressed (PS) mice underwent a 10 min unprotected exposure to a cat, and were then returned to their home cage where they were left undisturbed until behavioural testing. A detailed description of the predator stress exposure can be found in the section entitled "Testing". Predator stressed extinction (PSE) mice were exposed to the cat in the same manner as were the PS mice. Over 5 days after cat exposure, PSE mice were re-exposed to the cat exposure context (predator stress context) for 10 min without the cat present (extinction trials).

Seven days after the final extinction trial (a total of 12 days after predator exposure), all mice underwent several tests of anxiety and hyperarousal including elevated plus maze (EPM), hole board test (HB), light/dark box (LD), and response to acoustic startle. Behavioural tests were run over three days with HB and EPM on the first testing day, LD on the second day and acoustic startle response on the third. A detailed description of the behavioural tests can be found below in the section entitled "Testing".