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5-HT1A receptor activation reduces fear-related behavior following social defeat in Syrian hamsters

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ABSTRACT

Social defeat leads to selective avoidance of familiar opponents as well as general avoidance of novel, non-threatening intruders. Avoidance of familiar opponents represents a fear-related memory whereas generalized social avoidance indicates anxiety-like behavior. We have previously shown that serotonin signaling alters responses to social defeat in Syrian hamsters, although it is unclear whether serotonin modulates defeat-induced fear, anxiety, or both. In this study we focus on 5-HT1A receptors, in part, because their activation had been linked to the acquisition of conditioned fear. We hypothesized that pharmacological activation of 5-HT1A receptors prior to social defeat would reduce avoidance of familiar opponents and impair Arc expression in the basolateral amygdala (BLA), but not alter anxiety-like behavior. We administered 8-OH-DPAT, a 5-HT1A receptor agonist, prior to 3, 5-minute social defeats and 24 h later exposed hamsters to a social interaction test to measure the conditioned defeat response immediately followed by either a Y-maze test or an open field test. In a separate experiment, we administered 8-OH-DPAT prior to 3, 5-minute social defeats and later removed the brains for Arc immunohistochemistry. Social defeat increased the number of Arc immunopositive cells in the central amygdala (CeA), prelimbic cortex (PL), and BLA, and 8-OH-DPAT treatment reduced Arc immunoreactivity in the PL. These results suggest that 5-HT1A receptor activation impairs the fear memory associated with social defeat, but does not alter defeat-induced anxiety. Overall, 5-HT1A receptor activation may impair Arc expression in select brain regions such as the PL and thereby disrupt the development of a fear memory essential for the conditioned defeat response.

1. Introduction

The type of stress experienced by humans is often psychosocial in nature and stressful events are a contributing factor in the development of affective disorders, such as major depression, generalized anxiety disorder, and post-traumatic stress disorder (Anisman and Zacharko, 1992; Arborelius et al., 1999; Davidson, 2003). Ethologically relevant animal models of social stress are particularly well-suited to investigate the neurobiological mechanisms underlying stress-related psychopathologies (Nestler and Hyman, 2010; Blanchard et al., 1995; Fuchs and Flugge, 2003). Social defeat is a robust stressor that leads to heightened HPA-axis activity (Blanchard et al., 1995) as well as changes in behavior (Ruis et al., 1999; Watt et al., 2009), including increased anxiety-like behavior in the elevated plus maze (Heinrichs et al., 1992; Berton et al., 1998), altered circadian rhythmicity (Tornatzky and Miczek, 1993; Meerlo et al., 1996a), reduced body weight (Bartolomucci et al., 2004; Iio et al., 2012), and reduced locomotor activity (Rygula et al., 2005; Calvo et al., 2011). Following acute social defeat, male Syrian hamsters lose their species-typical territorial aggression and instead show submissive and defensive behavior when a smaller, non-aggressive intruder (NAI) is placed into their home cage (Potegal et al., 1993; Huhman et al., 2003). This change in agonistic behavior is called conditioned defeat and is likely the result of defeat-induced increases in both fear and anxiety.

One advantage of the conditioned defeat model is that because behavioral changes occur following an acute social defeat, neural mechanisms controlling defeat-related memories may be investigated. We know that blockade of NMDA receptors in the basolateral amygdala (BLA) prior to social defeat impairs the acquisition of the conditioned defeat response (Day et al., 2011), whereas overexpression of cAMP response element-binding protein (CREB) in the BLA prior to social defeat increases the acquisition of conditioned defeat (Jasnow et al., 2005). Brain-derived neurotrophic factor (BDNF) mRNA has also been found to increase in the BLA following social defeat, and a TrkB receptor antagonist administered into the BLA prior to social defeat also reduces the acquisition of conditioned defeat (Taylor et al., 2011). Similarly, previous research has shown that NMDA receptors, CREB, and BDNF in the BLA are critical targets controlling the formation of fear-related memories (Rodrigues et al., 2001; Rattiner et al., 2005; Izumi et al., 2011).

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Altogether, these findings suggest that conditioned defeat is controlled by neural circuitry in the BLA known to regulate fear memories. Activity-regulated cytoskeletal-associated protein (Arc/Arg 3.1) is an immediate early gene induced by neural activity and critical for synaptic plasticity in the hippocampus and the consolidation of long-term memory (Steward et al., 1998; Guzowski et al., 2000; Steward and Worley, 2001 — see Ploski et al., 2008 for these references). More recently, Arc expression in the BLA was shown to be necessary for both the consolidation (Ploski et al., 2008), and reconsolidation (Maddox and Schafe, 2011), of Pavlovian fear conditioning. In this study we use Arc expression in the amygdala and medial prefrontal cortex as a cellular marker of synaptic plasticity.

Several lines of evidence suggest that serotonin (5-HT), and particularly neural signaling at 5-HT1A receptors, modulate fear-related and anxiety-like behaviors. In humans, 5-HT1A receptor binding is negatively correlated with anxiety levels (Tauscher et al., 2001). In animal models, 5-HT1A receptor knockout mice show high levels of anxiety-like behavior compared to controls (Gross et al., 2000; Ramboz et al., 1998), and overexpression of 5-HT1A receptors reduces anxiety-like behavior (Kusserow et al., 2004). Consistent with an anxiolytic role for 5-HT1A receptors, Li et al. (2012) found that viral-mediated knockdown of 5-HT1A receptors in the amygdala resulted in increased anxiety in the elevated plus maze in mice. Activation of 5-HT1A receptors also impairs the formation of fear memories. For example, pharmacological activation of 5-HT1A receptors in the hippocampus has been shown to impair the acquisition of both contextual and cued fear conditioning (Stiedl et al., 2000). We have found that pharmacological activation of 5-HT1A receptors in the dorsal raphe nucleus (DRN) (Cooper et al., 2008) and BLA (Morrison and Cooper, 2012) prior to social defeat impairs the acquisition of conditioned defeat in Syrian hamsters. However, it is unknown whether activation of 5-HT1A receptors alters an anxiety component and/or a fear component of the conditioned defeat response.

In Syrian hamsters acute social defeat results in a specific memory of the aggressive opponent. For example, social defeat results in increased social avoidance of familiar opponents compared to unfamiliar opponents (Petrulis et al., 2004; McCann and Huhman, 2012). Similarly, using a Y-maze test for individual recognition after social defeat, hamsters show increased avoidance of familiar winners compared to unfamiliar winners and familiar neutral animals (Lai and Johnston, 2002; Lai et al., 2005). Also, systemic administration of the protein synthesis inhibitor anisomycin blocks defeat-induced social avoidance in the Y-maze (Huang et al., 2011). Anxiety-like behavior following social defeat has been measured in an open field test in several rodent species. Defeated animals typically show reduced locomotion and reduced time spent in the center of the arena (Raab et al., 1986; Meerlo et al., 1996b, 1996c; Kinsey et al., 2007).

The purpose of this study was to investigate the mechanisms by which 5-HT1A receptors modulate the development of conditioned defeat. We hypothesized that activation of 5-HT1A receptors prior to social defeat would impair the acquisition of conditioned defeat by disrupting memory for the defeat experience and decreasing defeat-induced Arc expression in the BLA.

2. Methods

2.1. Animals

We used adult male Syrian hamsters (*Mesocricetus auratus*) that weighed 130–170 g (3–4 months of age) at the start of the study, and were individually housed for 10–14 days prior to testing. Older hamsters that weighed 180–200 g (>6 months) were individually housed and used as resident aggressors for social defeat training. Younger hamsters that weighed 90–120 g (2 months) were group-housed (4 per cage) and used as nonaggressive intruders for conditioned defeat testing. All animals were housed in polycarbonate cages (12 cm × 27 cm × 16 cm) with corncob bedding, cotton nesting materials, and wire-mesh tops. Animal cages were not changed for at least 1 week prior to testing to allow individuals to scent mark their territory. Animals were housed in a temperature-controlled colony room (20 ± 2 °C) and maintained on a 14:10 h light:dark cycle with food and water available *ad libitum*. Subjects were handled for 7–10 days prior to social defeat training and all behavioral testing occurred in the first 3 h of the dark cycle. All procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee and follow the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Behavioral testing

2.2.1. Social defeat training

Social defeat training consisted of three, 5-minute aggressive encounters in the home cage of a larger resident aggressor (RA). To facilitate similar amounts of aggression, the start of social defeat began at the first attack, which usually occurred within the first 60 s of the encounter. When drug treatments were administered prior to social defeat training, defeats were digitally recorded to identify any nonspecific effects of drug treatments. The number of attacks and total duration of aggressive behavior received by subjects were later quantified. In experiments where drug treatments altered the duration of conditioned defeat behavior, no defeat controls were used to assess whether the treatments altered agonistic behavior in the absence of social defeat stress. No defeat control animals were exposed to three different empty RA cages for 5 min each.

2.2.2. Conditioned defeat testing

Conditioned defeat testing occurred 24 h following social defeat training and consisted of one, 5-minute encounter with a novel, non-aggressive intruder (NAI) in the home cage of the subject. Testing was recorded and later scored by a researcher blind to the experimental conditions using Noldus Observer. A second researcher scored a subset of testing sessions, and inter-observer reliability was greater than 90% agreement. We quantified the duration of four categories of behavior: submissive/defensive (flee, avoid, upright and side defensive postures, tail-up, stretch-attend, head flag); aggressive (chase, attack, upright and side offensive postures); non-agonistic social (nose touching, sniff, approach); and nonsocial (locomotion, grooming, nesting, feeding) (Albers et al., 2002). We also quantified the frequency of flees, attacks, and stretch-attend postures displayed by the subject.

2.2.3. Y-maze testing

In order to evaluate avoidance of the RA, a Y-shaped acrylic maze was used. The Y-maze is divided into eight rectangular regions (10 cm wide × 10 cm high). The base of the Y (89 cm long) is divided into start box (20 cm) and stem (69 cm), and the two arms of the maze are 70 cm long and are divided into 3 sections. The sections closest to the stem are the basal parts (25 cm) of the arm while the compartments farther from the base are the distal parts (25 cm) of the arm. Subjects have access to all compartments of the Y-maze except for the most distal parts of the Y, which are the stimulus boxes (20 cm) where the RA is located during testing. The RA and subject are separated through a perforated Plexiglas wall (0.8 cm thick) to allow for the movement of air throughout the maze. The screen in front of the stimulus box is permanent whereas the screen that separates the start box from the stem is removable to allow the subject to explore the maze. Air is drawn from the stimulus boxes to the start box by a fan mounted on the outside of the start box.

Y-maze testing consisted of two, 3-minute trials as described by Lai et al. (2005). In the first trial, the stimulus box was empty and the subject's preferred arm was determined. In the second trial, one of the former RAs that defeated the subject was placed in the stimulus box of the subject's preferred arm to avoid confounding side preferences with avoidance of the RA. The Y-maze was cleaned with 70% ethanol