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Serotonin 2C receptor antagonist improves fear discrimination and subsequent safety signal recall

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ABSTRACT

The capacity to discriminate between safety and danger is fundamental for survival, but is disrupted in individuals with posttraumatic stress disorder (PTSD). Acute stressors cause a release of serotonin (5-HT) in the forebrain, which is one mechanism for enhanced fear and anxiety; these effects are mediated by the 5-HT$_{2C}$ receptor. Using a fear discrimination paradigm where a danger signal conditioned stimulus (CS+) co-terminates with a mild footshock and a safety signal (CS−) indicates the absence of shock, we demonstrate that danger/safety discrimination and fear inhibition develop over the course of 4 daily conditioning sessions. Systemic administration of the 5-HT$_{2C}$ receptor antagonist SB 242084 (0.25 or 1.0 mg/kg) prior to conditioning reduced behavioral freezing during conditioning, and improved learning and subsequent inhibition of fear by the safety signal. Discrimination was apparent in the first recall test, and discrimination during training was evident after 3 days of conditioning versus 5 days in the vehicle treated controls. These results suggest a novel therapeutic use for 5-HT$_{2C}$ receptor antagonists to improve learning under stressful circumstances. Potential anatomical loci for 5-HT$_{2C}$ receptor modulation of fear discrimination learning and cognitive performance enhancement are discussed.

Ethical Statement: John P. Christianson and Allison R. Foilb, the authors, verify that animal research was carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and all procedures involving animals were reviewed and approved by the Boston College Animal Care and Use Committee. All efforts were made to limit the number of animals used and their suffering.

1. Introduction

The ability to differentiate between danger and safety is necessary for survival. Exposure to traumatic stress can alter this fundamental process and individuals with post-traumatic stress disorder (PTSD) display an inability to utilize environmental safety signals (Jovanovic et al., 2009), overgeneralize fear (Rauch, et al., 2006a), and fail to extinguish trauma-induced fear responses (Orr et al., 2000; Milad et al., 2009). A major effort in translational neuroscience has revealed much of the neural circuitry underlying fear learning and recall (LeDoux, 2000; Johansen et al., 2011; Beyeler et al., 2014) and we are beginning to understand how stressors modulate these systems (Baratta et al., 2007; Rodrigues et al., 2009; Martijena and Molina, 2012). Yet, little is known regarding the neural mechanisms underlying the discrimination learning that is critical to recognizing and utilizing environmental safety signals (Christianson et al., 2012).

In preclinical models of PTSD, exposure to uncontrollable traumatic stress leads to enhanced fear conditioning, expression, and interference with extinction (Rau et al., 2005; Baratta et al., 2007, 2008, 2015). Uncontrollable stress triggers a release of serotonin (5-HT) in the brain, specifically in regions known to modulate fear learning and recall including the medial prefrontal cortex (Kawahara et al., 1993; Bland et al., 2003), basolateral amygdala (Kawahara et al., 1993; Amat et al., 1998b) and hippocampus (Amat et al., 1998a). Acute increases in extracellular 5-HT are sufficient to induce anxiety-like states and enhance the expression of fear by action at 5-HT$_{2C}$ receptors (Martin et al., 2002; Campbell and Merchant, 2003; Burghardt et al., 2007; Greenwood et al., 2008). With regard to the consequences of uncontrollable stress, 5-HT$_{2C}$ receptor antagonists prevent the social anxiety (Christianson et al., 2010; Christianson et al., 2013), fear enhancement (Baratta et al., 2015) and instrumental learning deficits (Strong et al., 2009) that typically follow uncontrollable stress (for review see Christianson and Greenwood, 2014). Furthermore, selective activation of the 5HT$_{2C}$ receptor is sufficient to induce stress-like anxiety (Christianson et al., 2013) and fear expression (Campbell and Merchant, 2003; Greenwood et al., 2008).

The expression of fear and anxiety can be modulated by learned safety signals (Christianson et al., 2008; Pollak et al., 2008; Christianson et al., 2011; Christianson et al., 2013). A safety signal is a stimulus that is a good predictor of the non-occurrence of danger or aversive stimuli

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and is a specific type of a conditioned inhibitor (Christianson et al., 2013). Unlike conditioned exciters, which come to trigger the response that normally follows exposure to an unconditioned stimulus, i.e. freezing to a tone after pairing with footshock, conditioned inhibitors counteract the expression of conditioned responses even in the presence of conditioned exciters (Rescorla, 1969). Myers and Davis (2004) established conditioned inhibition of fear using a fear discrimination paradigm in which one conditioned stimulus (CS+) was repeatedly paired with a footshock, while another stimulus (CS−), the safety signal, was never paired with footshock. This approach leads to fear discrimination within one or two training sessions (Chen, Foilb & Christianson, under review); yet conditioned inhibition is only apparent after many training sessions (see Experiment 1). Thus, this paradigm allows for translational research into ways to improve or accelerate the acquisition of safety signals that might be useful in the treatment or prevention of PTSD.

Fear discrimination conditioning involves repeated sessions of unavoidable footshocks, which are sufficient to trigger acute increases in extracellular 5-HT (Shanks et al., 1991; Inoue, 1993; Kawahara et al., 1993; Kirby et al., 1997; Hajós-Korcsok et al., 2003). Given the fear-enhancing effects of 5-HT and the $5HT_{2C}$ receptor, we hypothesized that fear discrimination and conditioned inhibition could be facilitated by $5HT_{2C}$ receptor antagonist administrations prior to conditioning. Using a fear discrimination paradigm, in which discrete auditory or visual cues served as the conditioned stimuli, we established danger/safety discrimination. A recall test comprised of presentations of the CS+ cue, the CS+ and CS− cues in compound (CS+/− cue), and the CS− cue alone provided a means to assess fear recall, conditioned inhibition, and discrimination, respectively. In Experiment 1 we determined the number of training sessions necessary for CS+/CS− discrimination during training, and discrimination and conditioned inhibition measured in later recall tests. In Experiment 2 we tested the hypothesis that systemic $5HT_{2C}$ receptor antagonist SB 242084 administration would improve fear discrimination learning, recall and conditioned inhibition.

2. Materials & methods

2.1. Animals

A total of 48 adult (250–300 g) male Sprague–Dawley rats from Charles River Laboratories (Wilmington, MA) were used. Rats were housed in groups of 2 and had free access to food and water at all times. Rats were given 7–10 days to acclimate to colony housing and were kept on a 12-h light/dark cycle with lights on at 0700. All procedures were reviewed and approved by the Boston College Institutional Animal Care and Use Committee.

2.2. Apparatus

Rats were conditioned in 10 × 11 × 6 in (L × W × H) cages made of black plastic with wire mesh lids and a floor of stainless bars attached to a shocking grid (Model H10-11R-TC-SF, Coulbourn Intruments, Whitehall, PA). Each cage was housed within a 15 × 12 × 27 in (L × W × H) light and sound-attenuated chamber. The chamber was illuminated from above by 2 infrared LEDs arrays (CMVision Model IR30) and behavior was recorded by overhead cameras (Model VX-5000, Microsoft, Redmond, VA) with the infrared blocking filters replaced with infrared passing filters. ANY-Maze software (version 4.99, Stoelting, Wood Dale, IL) was used for freezing detection using the manufacturer's recommended settings as previously (Christianson et al., 2011). A white LED array (Model LPL620WTHD, Hampton Bay) and a speaker mounted at the top of the chamber were used for conditioned stimuli. A fan provided ventilation and masking noise of ~55 dB.

2.3. CS+/CS− conditioning and discrimination tests

As in Chen et al. (Chen, Foilb and Christianson, under review) and adapted from Myers and Davis (2004) to quantify fear using behavioral freezing, conditioning sessions consisted of 15 CS+ and 15 CS− trials. A flickering LED light (264.0 Lux, 20 ms on/off) and a white noise pip (pip duration = 10 ms, interval = 3 Hz, 75 dB) were used as the stimuli. Assignment of light or pip as CS+ or CS− was counterbalanced in each experiment. Each conditioning trial began with a 5 s, 1 kHz tone (75 dB), followed by a presentation of either the CS+ or CS− cue for 15 s. The cues were presented in a quasi-random order so that no cue was presented more than twice is succession. CS+ trials co-terminated with a 500 ms, 1.2 mA shock (Model H13-15, Coulbourn Instruments); CS− cues were not accompanied with shock. Each training session consisted of 15 presentations of each cue, with a 70 s inter-trial-interval, so that each training session was a total of 45 min. Training was conducted from 1200 to 1400 each day for 4 or 5 consecutive days.

In a pilot experiment we found that fear discrimination and conditioned inhibition recall manifest equally when tested in the familiar conditioning context or in a novel context (A. R. Foilb and J. P. Christianson, unpublished data). Therefore, recall tests were conducted in the conditioning apparatus. Discrimination recall tests were conducted at 0900 each day after conditioning. Rats were transferred to the conditioning apparatus and after 2 min of context exposure they were presented with the CS+, the CS+ in compound with the CS− (CS+/−) and finally the CS− alone.

2.4. Drugs

The highly selective $5HT_{2C}$ receptor antagonist SB 242084 was purchased from Tocris and dissolved in 50% dimethyl sulfoxide (DMSO) in saline. The doses of 0.25 and 1 mg/kg were chosen to capture the range of effective doses (0.2 mg/kg to 1 mg/kg) found in several recent reports (Burghardt et al., 2007; Strong et al., 2009; Christianson et al., 2010, 2013). Importantly, these doses do not alter locomotor activity (Martin et al., 2002). Intraperitoneal (i.p.) injections were made at a volume of 1 ml/kg.

2.5. Experimental procedures

2.5.1. Experiment 1

The purpose of Experiment 1 was to establish the time course of fear discrimination learning. To this end, 16 rats were given CS+/CS− conditioning on four consecutive days. Recall tests were given in the morning following the most recent conditioning session to gauge fear recall, CS+/CS− discrimination and conditioned inhibition.

2.5.2. Experiment 2

To determine the effect of $5HT_{2C}$ receptor antagonist administration on the acquisition of conditioned fear discrimination 32 rats were assigned to one of three treatment groups: vehicle ($n = 10$), 0.25 ($n = 10$) or 1.0 mg/kg ($n = 12$) SB 242084. Systemic SB242084 has been effective when given 45 min to 1 h before testing (Burghardt et al., 2007; Christianson et al., 2010), therefore injections were made in the vivarium 15 min before the 45 min conditioning sessions. Training and testing were performed as in Experiment 1.

2.6. Data analysis

Time spent freezing to the relevant cues was converted to a percentage of time based on the length of each cue. For example, the total time spent freezing to the CS+ during training was divided by the number of cues (15) multiplied by the number of seconds per cue (15 s) and then multiplied by 100 to provide a percentage. To examine discrimination and inhibition ratios were computed of freezing to the CS− relative to the CS+ (discrimination ratio) or the CS+/− compound to the CS+