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Propranolol produces short-term facilitation of extinction in a rabbit model of post-traumatic stress disorder

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ABSTRACT

Post-traumatic stress disorder (PTSD) is a learning-based anxiety disorder with significant public health challenges due to difficulties in treating the complex, multiple symptomology. We have developed an animal model of PTSD, based on Pavlovian eyeblink conditioning in rabbits, that addresses two key features: conditioned responses (CRs) to cues associated with an aversive event and a form of conditioned hyperarousal referred to as conditioning-specific reflex modification (CRM). We have found previously that unpaired extinction is ideal for reducing both CRs and CRM simultaneously and shows sensitivity to systemic serotonergic and glutamatergic manipulations. The following study aimed to extend our work to examine the role of the noradrenergic system, dysregulation of which is strongly implicated as part of the neurobiology of PTSD and which may also play a role in the balance shift from fear reconsolidation to extinction during treatment. The goal of the following two studies was to examine whether the β-adrenergic receptor antagonist propranolol combined with either a full or brief course of unpaired extinction treatment could enhance extinction of CRs and/or CRM. Results showed a within-session facilitation of propranolol on extinction of CRs, particularly during the first extinction session, and a short-term enhancement of extinction of CRM when extinction treatment was brief. However, neither benefit translated to long-term extinction retention for the majority of subjects. Findings suggest that propranolol may provide the most therapeutic benefit in situations of high arousal early in treatment, which may be more important for future patient compliance rather than long-term treatment outcomes.

1. Introduction

Post-traumatic stress disorder (PTSD) is a multifaceted anxiety disorder that develops in response to trauma exposure in approximately 7% of individuals in the United States (Kessler et al., 2005), with estimates often higher in those exposed to military or combat trauma (Donoho et al., 2017; Ramchand et al., 2010; but see also Wisco et al., 2016). Treatment of PTSD remains a significant public health challenge due to difficulty in finding treatments that can address the complex nature of PTSD symptomology, which includes persistent re-experiencing of the event, avoidance of stimuli associated with trauma, and generalized hyperarousal such as increased startle reflexes and hypervigilance (American Psychiatric Association, 2013). Not surprisingly, research into the neurobiology of PTSD has suggested the involvement of multiple neural/neurochemical systems that may each play a role in one or more aspects of PTSD symptomology (Kelmendi et al., 2016; Pitman et al., 2012). In addition, there is a learning, or more specifically, a dysfunctional fear conditioning component underlying PTSD (Lissek and van Meurs, 2014; VanElzakker et al., 2014) that cannot easily be resolved by pharmacological treatment alone. The use of cognitive behavioral therapy aimed at extinguishing abnormally conditioned fear is another factor to consider in the development of PTSD treatments, further increasing the pool of pharmacological targets to include those that may be used in conjunction with therapy to improve efficacy, by enhancing fear extinction for example (Fitzgerald et al., 2014; Singewald et al., 2015). Because of the complexity of PTSD and the fact that it is often co-morbid with other disorders such as depression, animal models are crucial to further our understanding of why certain treatments work and how they may be combined to provide better treatment outcomes in the clinical population.

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We have developed an animal model of PTSD that addresses two key features: conditioned responses (CRs) to trauma-associated cues and hyperarousal (Burhans et al., 2008; Schreurs and Burhans, 2015). This model is based on conditioning of the rabbit's nictitating membrane response (NMR), also known as eyeblink conditioning. One of the unique aspects of the eyeblink conditioning paradigm is that it has historically been used in *both* humans and animals as a means of assessing associative learning (Solomon, 2002), allowing strong translatability from the bench to the clinic and vice versa. While human studies have used it to document learning phenotypes for disorders like schizophrenia (Kent et al., 2015; Marenco et al., 2003) and anxiety disorders including PTSD (Burriss et al., 2007; Handy et al., 2018), parallel work in animals has delineated the behavioral laws of acquisition and extinction and the critical neural circuitry (Christian and Thompson, 2003; Freeman and Steinmetz, 2011), giving a neurobiological perspective to those phenotypes. In a typical experiment in our laboratory, rabbits are evaluated for reflexive eyeblink responding to varying intensities (from 0.1 to 2.0 mA) of a periorbital shock unconditioned stimulus (US) prior to and following eyeblink conditioning during which they learn to associate an auditory tone conditioned stimulus (CS) with a 2.0-mA US. We have established that this paradigm results in a form of hyperarousal called conditioning-specific reflex modification (CRM), manifesting as increased and exaggerated responding to shock intensities, particularly lower intensity shocks that elicited little or no responding prior to conditioning. CRM is "conditioning-specific" in that it does not develop in the same way in rabbits that receive explicitly unpaired presentations of the CS and US. We have validated this model by demonstrating it shares many commonalities with PTSD development; for example, CRM is stronger when more aversive (i.e. more traumatic) stimuli are used as the US during conditioning (Buck et al., 2001; Seager et al., 2003) and, like PTSD in the clinic, may worsen after an incubation period (Schreurs et al., 2011a). Importantly, strong CRM only develops in a limited number of subjects (Smith-Bell et al., 2012), just as not all those exposed to trauma develop PTSD. We have also found that the amygdala, dysfunction of which is strongly implicated in PTSD (Hughes and Shin, 2011), can modulate acquisition of CRs to the tone CS and expression of CRM (Burhans and Schreurs, 2008). CRM-like changes have also been documented by others in rabbits (Gruart and Yeo, 1995; Wikgren et al., 2002) and rats (Servatius et al., 2001).

In searching for behavioral treatments that can reduce PTSD-like symptoms in our model, we have established an extinction treatment that can reduce both CRs and CRM simultaneously (Burhans et al., 2015; Schreurs et al., 2011b). While CRs to the tone CS can be extinguished by CS-alone presentations and CRM by US-alone presentations (but not vice versa), extinction sessions including unpaired presentations of the CS and US can extinguish both. Importantly, this can be achieved even when the US is reduced sixfold from the training intensity (Schreurs et al., 2011b). In translating this to the clinic, our findings suggest that adding random presentations of innately but mildly stressful stimuli like skin stimulation to traditional therapies such as exposure therapy may help address the hyperarousal symptoms of PTSD (Haesen and Vervliet, 2015). We have also been able to use the CRM model to investigate pharmacological treatments, both alone and in conjugation with behavioral extinction treatment. Previous work has delineated that different aspects of our model are sensitive to serotonergic and glutamatergic manipulations (Burhans et al., 2013, 2017), reinforcing the notion that a multi-factor approach to treatment is needed to address multiple PTSD symptoms.

The following study aimed to further extend our work in the CRM model to examine the role of the noradrenergic system. Dysregulation of norepinephrine (NE), normally released during stressful or fearful situations as part of the fight or flight response, has been strongly implicated as part of the neurobiology of PTSD (Hendrickson and Raskind, 2016; Southwick et al., 1999; Strawn and Geracioti, 2008). In support of this, for example, it has been found that NE is elevated in PTSD patients both at baseline levels and in response to trauma-associated stimuli (Blanchard et al., 1991; Geracioti et al., 2001; Liberzon et al., 1999). The crucial role NE plays in emotional arousal and modulation of emotional memory formation is believed to occur specifically through actions at β-adrenergic receptors (McIntyre et al., 2012), making these receptors a prime target for pharmaceutical intervention. Propranolol is a β-adrenergic receptor antagonist historically prescribed as a treatment for hypertension that has gained renewed interest as a treatment for PTSD, although with some mixed results (Giustino et al., 2016; Southwick et al., 1999). Systemic propranolol has also been previously examined in eyeblink conditioning paradigms and was found to impair acquisition in both rabbits (Gould, 1998) and rats (Cartford et al., 2002), although effects on extinction are less clear with some evidence for enhancement (Gould, 1998). However, there is a vast literature supporting a role for propranolol in enhancing fear extinction and blocking fear memory reconsolidation in animal models as well as healthy humans (as reviewed in Giustino et al., 2016). In the following series of two experiments, we tested the hypothesis that propranolol could enhance extinction of CRs and CRM in our rabbit model of PTSD. In the first experiment, we assessed the effects of propranolol combined with a full, six-session course of unpaired extinction treatment with weak shock. In Experiment 2, we assessed the effects of propranolol combined with a brief course of unpaired extinction, which we have previously shown to be less successful at extinguishing CRs and CRM, with evidence it may even worsen CRM (Schreurs et al., 2011b).

2. Materials and methods

2.1. Subjects

The subjects were 43 male, New Zealand White rabbits (*Oryctolagus cuniculus*), 2–3 months of age weighing approximately 1.8–2.3 kg upon delivery from the supplier (Harlan, Indianapolis, IN). Prior to behavioral training, one rabbit was removed due to a failure to adapt to restraint. Rabbits were housed in individual cages on a 12 h light-dark cycle and given *ad libitum* access to food and water. They were maintained in accordance with the guide for the care and use of laboratory animals issued by the National Institutes of Health, and the research was approved by the West Virginia University Animal Care and Use Committee.

2.2. Apparatus

The apparatus and recording procedures for NMR conditioning have been detailed elsewhere by Schreurs and Alkon (1990) who modeled their apparatus based on those described by Gormezano (Coleman and Gormezano, 1971; Gormezano, 1966). Briefly, rabbits were restrained in a Plexiglas box placed inside a sound-attenuating, ventilated chamber (Coulborn Instruments, Allentown, PA; Model E10-20). Inside the chamber, a stimulus panel containing a speaker and houselight (10-W, 120 V) was mounted at a 45° angle 15 cm anterior and dorsal to the rabbit's head. An exhaust fan created a constant ambient noise level of 75 dB inside the chamber. Periorbital electrical stimulation was delivered by a programmable two-pole stimulator (Colbourn Instruments, Model E13-35) via stainless steel Autoclip wound clips (Stoelting, Wood Dale, IL) that were positioned 10 mm ventral and 10 mm posterior to the dorsal canthus of the right eye. Stimulus delivery, data collection, and analysis were all accomplished using the LabVIEW