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Corticotropin releasing factor type-1 receptor antagonism in the dorsolateral bed nucleus of the stria terminalis disrupts contextually conditioned fear, but not unconditioned fear to a predator odor

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ABSTRACT

The bed nucleus of the stria terminalis (BNST) plays a critical role in fear and anxiety. The BNST is important for contextual fear learning, but the mechanisms regulating this function remain unclear. One candidate mechanism is corticotropin-releasing-factor (CRF) acting at CRF type 1 receptors (CRFr1s). Yet, there has been little progress in elucidating if CRFr1s in the BNST are involved in different types of fear (conditioned and/or unconditioned). Therefore, the present study investigated the effect of antalarmin, a potent CRFr1 receptor antagonist, injected intracerebroventricularly (ICV) and into the dorsolateral BNST ($_{L}$BNST) during single trial contextual fear conditioning or exposure to the predator odor 2,5-dihydro-2,4,5-trimethylthiazoline (TMT). Neither ICV nor $_{L}$BNST antalarmin disrupted unconditioned freezing to TMT. In contrast, ICV and $_{L}$BNST antalarmin disrupted the retention of contextual fear when tested 24 h later. Neither ICV nor $_{L}$BNST antalarmin affected baseline or post-shock freezing—indicating antalarmin does not interfere with the early phases of contextual fear acquisition. Antalarmin did not (1) permanently affect the ability to learn and express contextual fear, (2) change responsivity to footshocks, or (3) affect the ability to freeze. Our findings highlight an important role for CRFr1s within the $_{L}$BNST during contextually conditioned fear, but not unconditioned predator odor fear.

1. Introduction

Corticotropin-releasing factor (CRF) is a 41 amino acid neuropeptide widely studied for its role in the neuroendocrine stress response (Bale and Vale, 2004; Kovács, 2013; Smagin et al., 2001; Vale et al., 1981). In addition to the paraventricular nucleus of the hypothalamus, CRF is also expressed in a number of extrahypothalamic structures including the amygdala and bed nucleus of the stria terminalis (BNST) (Makino et al., 1995; Wong et al., 1994). While these two structures have been investigated for their role in conditioned and unconditioned fear and anxiety-like behaviors (Campeau et al., 1991; Walker and Davis, 1997), our understanding of the function of CRF within these areas is continually expanding.

CRF within the BNST, a part of the extended amygdala, has received substantial attention over the last few decades for its function in fear and anxiety-like behaviors (Walker and Davis, 2008; Walker et al., 2003). Recent work has shed light on how the BNST is involved in associative learning using contextual fear conditioning paradigms (Haufler et al., 2013; Nijsen et al., 2001; Poulos et al., 2010; Resstel et al., 2008; Sullivan et al., 2004). In this paradigm, a neutral context (CS) is paired with a footshock (US) to produce a conditioned response (CR), the most widely studied of which is freezing. Lesions of the BNST disrupt long-term freezing to a context, but not freezing to discrete cues such as tones (LeDoux et al., 1988; Sullivan et al., 2004). Importantly, the BNST may play a significant role in contextual fear learning given that it can compensate for contextual, but not auditory, fear learning when the basolateral amygdala, a structure critical to fear conditioning, is inactivated (Poulos et al., 2010; Zimmerman and Maren, 2011).

The role of the BNST in contextually conditioned fear complements a number of studies showing that the BNST is also essential for modulating other fear and anxiety-like behaviors. For example, lesions of the BNST disrupt the sustained enhancement of startle responses to long-lasting environmental threats (Davis et al., 2010). Both light-enhanced and CRF-enhanced startle, but not fear-potentiated startle to short-duration cues, are blocked by non-selective CRF antagonism in the BNST (Lee and Davis, 1997) and selective CRFr1 antagonism peripherally (Walker et al., 2009b).

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While overexpression of CRF within the BNST has no effect on unconditioned fear-like behavior (in the elevated plus maze), it disrupts sustained fear as measured by enhanced acoustic startle and decreases CRFr1 expression (Sink et al., 2013b). Taken together, these studies suggest that CRF and CRFr1s in the BNST may play an important role in contextually conditioned, but not unconditioned, fear and anxiety-like behaviors.

However, the BNST *is* important for behavioral and endocrine responses to particular types of unconditioned threats—predator odors (Fendt et al., 2003; Rosen et al., 2015; Walker and Davis, 1997). Predator odors are advantageous for investigating unconditioned fear and anxiety-like behaviors for two reasons. First, although laboratory rats have never encountered the odor, they still exhibit robust defensive responses upon the first exposure. Second, predator odors are ethologically relevant to rodents relative to foot-shocks. Inactivation of the BNST, but not key nuclei of the amygdala important for fear conditioning (e.g., the central and basal nuclei), disrupts freezing to the predator odor 2,5-dihydro-2,4,5-trimethylthiazoline (TMT; (Fendt et al., 2003; Rosen, 2004; Wallace and Rosen, 2001)), a synthesized compound derived from the anal secretions of the red fox. TMT exposure also increases numerous immediate-early genes (Day et al., 2004; Kobayakawa et al., 2007) and CRF mRNA across the extended amygdala (Asok et al., 2013a), in addition to elevating corticosterone secretion (Day et al., 2004). These studies suggest that the BNST, and possibly CRF within the BNST, may modulate unconditioned fear-like behavior to predatory threats. However, the role of CRF within the BNST during unconditioned predator odor fear to TMT and contextually conditioned fear has not been contrasted. Therefore, the present study investigated how CRF within the BNST is involved in both conditioned freezing and unconditioned freezing to a predator odor. We evaluated the effects of blocking the anxiogenic CRF type 1 receptor (CRFr1) with a selective CRFr1 antagonist, antalarmin, administered intracerebroventricularly (ICV) or into the dorsolateral BNST ($_{L}$BNST), prior to contextual fear conditioning or exposure to the predator odor TMT.

2. Materials and methods

2.1. Subjects

Male Sprague-Dawley rats (8–11 weeks of age) obtained from Harlan breeders (Indianapolis, IN) and weighing between 280 and 330 g were used for all experiments. Rats were maintained on a 12 h light/dark cycle (lights on at 7:00 AM.) at constant temperature with free access to food and water. Following arrival in the animal colony, rats were left undisturbed for seven days prior to the start experimental procedures. Rats were pair-housed in opaque polycarbonate cages with wood shavings for the duration of the study. All animals were handled by the experimenter for 2 consecutive days (~5 min/day) prior to the start of behavioral experiments. All procedures were approved by the University of Delaware's Institutional Animal Care and Use Committee.

2.2. Surgery

Rats were anesthetized with a ketamine/xylazine cocktail (85/15 mg/kg) prior to stereotaxic surgery. For rats that received ICV surgery, a single 26-gauge 5 mm guide cannula (Plastics One, Roanoke, VA) was implanted 1 mm above the rat's right lateral ventricle using the following coordinates: AP = −0.1, ML = −1.8, DV = −3.2. For rats that received cannula implanted into the dorsolateral division of the bed nucleus of the stria terminalis ($_{L}$BNST), two 26-gauge guide cannula were angularly implanted using the following coordinates: AP = −0.1 mm, ML = ±3.8 mm, DV = −5.4 mm, at a 19° angle. Following surgeries, a dummy cannula that extended 1 mm beyond the tip of the guide cannula was inserted to prevent blockage.

2.3. Drug preparation and delivery

The selective CRFr1 receptor antagonist antalarmin hydrochloride (Sigma, St. Louis, MO) was used for all experiments (Zorrilla et al., 2002b). Antalarmin was dissolved in dimethyl sulfoxide (DMSO) as a vehicle for all experiments. For ICV experiments, rats received either 3 μL of DMSO vehicle or 3 μL of DMSO vehicle containing 20 μg antalarmin. This dose was selected because it was in range with previous ICV and peripheral studies (Deak et al., 1999; Zorrilla et al., 2002a,b). For BNST infusions, rats received either 0.2 μL of DMSO vehicle or 0.2 μL of DMSO containing antalarmin. Three doses were tested. Antalarmin was dissolved in DMSO to a final concentration of either 10 μg/μL (for 2 μg dose), 1 μg/μL (for 0.2 μg dose), or 0.01 μg/μL (for 0.02 μg dose). These doses were selected because the BNST is part of the extended amygdala and other studies have used a similar dose range for antalarmin infused into the amygdala (Vicentini et al., 2014; Wellman et al., 2013).

Antalarmin or vehicle was administered 30 min prior to fear conditioning, TMT exposure, or shock responsivity testing using an electronic infusion pump (Harvard Apparatus, Holliston, MA). One μL Hamilton syringes were connected to polyethylene tubing, and capped with a cannula injector that extended 1 mm below the end of the guide cannula. Solutions were infused at a rate of 1 μL/min for ICV and 0.2 μL/min for BNST. The vehicle and administration time point were chosen based off of previous studies evaluating the pharmacokinetic profile of antalarmin (Sanghvi et al., 2009).

2.4. Contextual fear conditioning

Contextual fear conditioning was conducted in four identical Plexiglas/metal chambers (25 cm × 31 cm × 32 cm) containing metal grid floors (19 stainless steel bars, 0.5 cm in diameter, and 1.25 cm apart). All groups were counterbalanced within and across days. For conditioning, each animal was placed in the chamber for 180 s (baseline freezing measurement), followed by a single 1 s 1.5 mA shock, followed by a 300 s shock-free period (post-shock freezing measurement). Twenty-four hours later, animals were returned to the same chamber and tested for freezing to the context for 300 s (retention freezing measurement). All chambers were cleaned with a 5% ammonium hydroxide solution between sessions. A camera positioned at the top of each chamber recorded behavior for each animal and transmitted the signal to a computer running FreezeFrame software (Actimetrics, Wilmette, IL). Freezeframe was configured to score freezing as 0.75 s bouts without changes in pixel luminance and then verified offline by an experimenter (see Asok et al., 2014).

2.5. Contextual fear re-training in an alternate context

Rats were re-trained in a different environment without any drug and under identical contextual fear conditioning parameters (e.g., 180 s baseline, a single 1 s 1.5 mA shock, 300 s measure of post-shock freezing, and 300s retention test) in an alternate context consisting of four rectangular Plexiglas chambers (16.5 cm × 12.1 cm × 21.6 cm) with metal grid floors (9 stainless steel bars, 4 mm in diameter, and 1 cm apart) inside a fume hood. All chambers were cleaned with a 70% ethanol solution between sessions.