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Predator odor stress alters corticotropin-releasing factor-1 receptor (CRF1R)-dependent behaviors in rats

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ABSTRACT

Humans with stress-related anxiety disorders exhibit increases in arousal and alcohol drinking, as well as altered pain processing. Our lab has developed a predator odor stress model that produces reliable and lasting increases in alcohol drinking. Here, we utilize this predator odor stress model to examine stress-induced increases in arousal, nociceptive processing, and alcohol self-administration by rats, and also to determine the effects of corticotropin-releasing factor-1 receptors (CRF1Rs) in mediating these behavioral changes. In a series of separate experiments, rats were exposed to predator odor stress, then tested over subsequent days for thermal nociception in the Hargreaves test, acoustic startle reactivity, or operant alcohol self-administration. In each experiment, rats were systemically injected with R121919, a CRF1R antagonist, and/or vehicle. Predator odor stress increased thermal nociception (i.e., hyperalgesia) and acoustic startle reactivity. Systemic administration of R121919 reduced thermal nociception and hyperarousal in stressed rats but not unstressed controls, and reduced operant alcohol responding over days. Stressed rats exhibited increased sensitivity to the behavioral effects of R121919 in all three tests, suggesting up-regulation of brain CRF1Rs number and/or function in stressed rats. These results suggest that post-stress alcohol drinking may be driven by a high-nociception high-arousal state, and that brain CRF1R signaling mediates these stress effects.

1. Introduction

Humans with stress-related anxiety disorders (e.g., post-traumatic stress disorder [PTSD]), exhibit increases in arousal and alcohol drinking, as well as altered pain processing (Engdahl et al., 1998; Norrholm et al., 2011; Roy et al., 2012; Sartor et al., 2010). In animals, a single intense stress or chronic stress produces hyperalgesia (Boccalon et al., 2006; da Silva Torres et al., 2003; Geerse et al., 2006; Quintero et al., 2000; Zhang et al., 2012), increases anxiety-like behavior, and produces hyperarousal in rats (Campos et al., 2013; Kinn Rød et al., 2012; Serova et al., 2013). Alcohol dependence also promotes hyperarousal and hyperalgesia (i.e., increased nociception), each of which may promote compulsive alcohol drinking (Edwards et al., 2012; Egli et al., 2012; Koob, 1999). Our lab utilizes a predator odor stress model that produces lasting increases in alcohol consumption by rats (Edwards et al., 2013), allowing for the hypothesis that hyperarousal and hyperalgesia may be important motivational factors in stress-induced escalation of alcohol drinking.

CRF is important for emotional regulation (Heilig et al., 1994; Pisu et al., 2013; Regev et al., 2011, 2012) and is dysregulated in individuals with anxiety, depression, and drug abuse disorders (Arborelius et al., 1999; Bale and Vale, 2004; Koob, 2010; Risbrough, 2006). Exogenously administered CRF increases anxiety-like behavior in rodents (Arborelius et al., 1999; Swerdlow et al., 1986a). Alcohol- and drug-dependent rats exhibit increases in anxiety-like behavior and escalated alcohol and drug self-administration thought to be driven by CRF hyper-function in the extended amygdala (e.g., George et al., 2007; Gilpin, 2012; Koob, 2008). Brain CRF signaling is also attributed a role in nociceptive processing (Greenwood-Van Meerveld et al., 2006; Lariviere and Melzack, 2000; McNally and Akil, 2002), and CRF-1 receptors (CRF1Rs) mediate increased nociception in rats exposed to stress or made dependent on drugs or alcohol (Edwards et al., 2012; Ji and Neugebauer, 2007, 2008).

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Hyperarousal is a hallmark behavioral effect of stress and also of alcohol dependence (Chester et al., 2008, 2005; Davis et al., 2010; Koob, 1999). The acoustic startle response (ASR) is a reflexive reaction to an auditory stimulus that is mediated by the caudal pontine reticular nucleus (Koch, 1999). Importantly, descending projections from limbic regions (e.g., central amygdala and bed nucleus of stria terminalis) modulate the startle response and CRF signaling in these pathways is particularly important for mediating the startle response under various experimental parameters (Davis et al., 2010; Walker et al., 2009). For example, CRF enhances startle in rats (Liang et al., 1992) and antagonism of CRF1Rs attenuates CRF- and fear-potentiated ASR (Fujiwara et al., 2011; Risbrough et al., 2003; Swerdlow et al., 1986b; Walker et al., 2009).

Stress exposure is an important determinant for alcohol consumption in humans (Keyes et al., 2011). Various stressors increase alcohol consumption in rodent models (Chester et al., 2004; Croft et al., 2005; Logrip and Zorrilla, 2012; Lowery et al., 2008; Meyer et al., 2013), but these effects are transient, inconsistent across stressors and labs, and often highly dependent on experimental parameters (Becker et al., 2011). That said, stress and alcohol dependence each produce increases in extracellular CRF in the central amygdala (CeA), and CRF is a key modulator of behavioral and physiological responses to stress as well as alcohol-related behaviors (for reviews, see Gilpin, 2012; Koob, 2008). In fact, antagonism of CRF1Rs reduces escalated alcohol consumption by alcohol-dependent and stressed rodents (Funk et al., 2007; Lowery et al., 2008; Molander et al., 2012).

Our lab recently described a stress model in which predator odor exposure produces lasting (≥19 days) increases in alcohol self-administration (Edwards et al., 2013). The purpose of this study was to characterize arousal, nociception and alcohol self-administration in rats exposed to predator odor stress and to examine the role of CRF1Rs in those behavioral effects. Because CRF1Rs mediate arousal, nociception, and alcohol consumption, we hypothesized that peripheral administration of R121919, a selective CRF1R antagonist, would attenuate predator odor stress-induced increases in startle reactivity, thermal nociception, and alcohol self-administration.

2. Materials and methods

2.1. General methods

2.1.1. Animals

Male Wistar rats (Charles River) weighing 200–300 g, and aged 6–8 weeks at start of experiment, were housed in groups of two in a humidity- and temperature-controlled (22 °C) vivarium on a reverse 12-h light/dark cycle (lights off at 8 a.m.). Behavioral tests were conducted during the dark period. Animals had *ad libitum* access to food and water.

2.1.2. Drugs

The CRF1R antagonist R121919 (generously supplied by Neurocrine, Inc.) was solubilized first in 1 M HCl (10% final volume), then diluted into 2-hydroxypropyl-β-cyclodextrin (HBC; Sigma-Aldrich, 20% wt/vol final concentration in distilled water) and back-titrated with NaOH to pH 4.5. In Experiments 2 and 3, rats were administered four R121919 doses (0, 5, 10, 20 mg/2 ml/kg s.c.) in a within-subject Latin-square design 60 min prior to behavioral tests. In Experiment 4, rats were repeatedly injected with a single dose of R121919 (10 mg/kg) or equivalent volume of vehicle 60 min prior to drinking sessions, as previously described by our group (Roberto et al., 2010).

2.1.3. Stress exposure

Rats were transferred from the home cage to a clean cage and exposed to predator odor (bobcat urine; stressed) or ambient air (control) for a period of 15 min. Urine was added to a sponge that was placed beside the cage where stressed rats were not able to come in contact with the sponge or urine.

2.1.4. Hargreaves test procedure

Hind paws were individually stimulated from below using a halogen heat source from an IITC model 309 Hargreaves apparatus (IITC Life Sciences, Inc., Woodland Hills, CA). A 20-s cut-off was always employed to prevent tissue damage in non-responsive subjects, although preliminary experiments allowed selection of a light intensity that produced much shorter hind paw withdrawal latencies (~8 s; see Table 1). On test days, rats were placed in the examination room for 5 min to allow for acclimation to the light and testing environment and were then placed in Plexiglas® enclosures with glass floors suspended 30 cm from the table top and allowed to habituate for an additional 5 min prior to testing. On each test day, each hind paw was targeted twice (i.e., left paw, then right paw, 1 min break, then left and right paw again), producing 4 scores that were averaged into one score. The average latency to produce a nocifensive withdrawal response represented an index of thermal nociception (i.e., lower scores indicative hyperalgesia) that was analyzed as described below.

2.1.5. Acoustic startle response testing

Acoustic startle response (ASR) testing was conducted with a commercial startle reflex system (S-R Lab; San Diego Instruments, San Diego, CA). The sound-attenuated test chamber includes an exhaust fan, a sound source, and an internal light that is off during testing. Inside the test chamber, a single Plexiglas rodent cylinder (8.7 cm internal diameter) sits on a 12.5 × 25.5 cm Plexiglas stand. The acoustic startle response was transduced by a piezoelectric accelerometer mounted below the Plexiglas stand and then converted into arbitrary units by a personal computer program. Prior to testing, an S-R calibrator tube was used to calibrate the chambers. Each test session was preceded by a 5-min habituation period during which 70 dB of background white noise is present. This background white noise was present throughout the test session. The test session consists of 31 trials with startle stimuli of three different decibel levels. During each of the 31 trials, a 750-ms burst of 95 dB, 105 dB, or 115 dB white noise was presented. The startle response of the rat was recorded for each of the first 100 ms of each trial. The main dependent variable, average ASR, is an average of these 100 (one per ms) response outputs. The 95 dB and 115 dB stimuli were each presented 8 times and the 105 dB stimulus presented 9 times, each separated by a 30-s fixed intertrial interval.

2.1.6. Operant alcohol oral self-administration

Rats were trained to orally self-administer ethanol or water in a concurrent, two-lever, free-choice contingency that did not incorporate a sweet fading procedure. Prior to placement in operant boxes, rats were given a single 24-hr period of access to 10% w/v ethanol vs. water in the home cage. Intakes were not measured during this 24-hr home cage ethanol access, the purpose of which was to prevent neophobia upon presentation of ethanol in operant boxes. Rats were then given a single 15-hr operant session to learn to press a single lever for water (right lever; FR1) in the presence of *ad libitum* food on floor of operant chamber. Rats were then allowed one 3-hr two-lever operant session for 10% w/v ethanol (right lever; FR1) vs. water (left lever; FR-1), one 2-hr session, then one 1-hr session, followed by daily 30-min sessions on the two-lever contingency until reaching stable intake rates. All operant sessions after the initial 15-hr session occurred in the absence of food. Rats underwent daily 30-min sessions for ~15 days, at which point ethanol response rates were stable for individual rats across days.

2.2. Experimental procedures

2.2.1. Experiment 1

One week after arrival into colony rooms, rats ($n = 21$) were subjected to an intensity response curve to determine a light intensity that produces moderate hind paw withdrawal latencies. The selected intensity (75 A.I.) produced an average baseline threshold of approximately 8 s (see Table 1). Five days following predator odor stress, control ($n = 5$) and stressed ($n = 16$) rats were tested for stress-induced changes in hind paw withdrawal latency (i.e., thermal nociception) at light intensity = 75 A.I.

2.2.2. Experiment 2

One week after arrival into colony rooms, rats ($n = 31$) were tested for baseline nociception. On days 1 and 2, animals were tested for hind paw withdrawal latency at light intensity = 75 A.I. On days 3 and 4, animals were injected with vehicle 60 min prior to testing (pre-treatment time based on (Funk et al., 2007)) and again tested for hind paw withdrawal latency at light intensity = 75 A.I. Injections did not affect hind paw withdrawal latency (see Table 1). Rats were divided into two groups counterbalanced for baseline withdrawal latency and five days later, approximately

Table 1
Hargreaves intensity response and baseline. Table depicts the mean paw withdrawal latency of rats ($n = 21$; top panel) during light intensity-response tests to determine appropriate light intensity for pharmacology tests using the Hargreaves test of thermal nociception. A separate group of rats ($n = 31$; bottom panel) were tested for baseline nociception with and without vehicle injection 60 min prior to testing over four days. There was no effect of vehicle injection on paw withdrawal latencies in unstressed rats.

Intensity response			
25 AI	50 AI	75 AI	99 AI
18.77 ± 0.38	15.08 ± 0.43	8.79 ± 0.25	5.95 ± 0.21
Baseline (75 AI)			
No injection	No injection	Vehicle injection	Vehicle injection
7.81 ± 0.30	8.18 ± 0.34	8.04 ± 0.24	8.20 ± 0.14