Neurobiology of Learning and Memory 147 (2018) 26–34

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Altering D1 receptor activity in the basolateral amygdala impairs fear suppression during a safety cue

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ARTICLE INFO

Keywords:
Amygdala
Fear
Safety
Dopamine

ABSTRACT

Accurate discrimination among cues signifying reward, danger or safety initiates the proper emotional response in order to guide behavior. Appropriate conditioned inhibition of fear in the presence of a safety cue would allow an organism to engage in reward seeking behaviors. There is currently little known about the mechanisms of reward, fear and safety cue discrimination and how a safety cue can inhibit fear and release reward seeking from inhibition. Here we assess reward, fear and safety cue learning together using a behavioral paradigm that has identified neurons that discriminate among these cues in the basolateral amygdala (BLA) (Sangha, Chadick, & Janak, 2013). Dopamine signaling in the BLA has been implicated in discriminatory reward learning, learned fear responses and fear extinction. We tested the hypothesis that D1 receptor activity will influence reward-fear-safety cue discrimination by using the D1 receptor agonist, SKF-3839, and antagonist, SCH-23390, either systemically or within the BLA during discrimination learning in male Long Evans rats. We show that both the agonist and antagonist interfered with fear suppression in the presence of the safety cue, when administered systemically or when infused directly into the BLA. This indicates that altering D1 receptor activity in the basolateral amygdala impairs fear suppression during a safety cue. Neither the agonist or antagonist had a consistent negative impact on discriminatory reward seeking when infused into the BLA. However, systemic administration of the D1 receptor agonist did reduce reward seeking behavior during a task that included fear and safety cues. We did not observe a negative impact on reward seeking during systemic administration of a D1 receptor agonist in a task that only included reward cue + sucrose and nonreward cue + no sucrose pairings. This indicates the impairments we saw with the systemically applied agonist in the safety-fear-reward cue discrimination task were more likely due to effects on fear and/or motivation rather than on cue discrimination. Together, our data indicate that altered dopamine D1 receptor activity in the BLA may be a potential mechanism that leads to the impairment in fear suppression to the safety signal seen with PTSD patients.

1. Introduction

Post-traumatic Stress Disorder (PTSD) affects approximately 8.7% of the general population within their lifetime (Kessler et al., 2005), and these patients are impaired in learning to suppress their fear response in the presence of a safety cue (Jovanovic et al., 2009), and to extinguish fear (Morriss, Christakou, & Van Reekum, 2015). PTSD is also comorbid with substance abuse disorders such as the use of alcohol, opioid, cocaine (Ouimette, Read, Wade, & Tirone, 2010), and smoking (Forbes et al., 2015), indicating there is an additional reward dysregulation in PTSD comorbid with substance abuse disorders.

Accurate discrimination among cues signifying danger, safety or reward initiates the proper emotional response in order to guide behavior. Since potentially rewarding and dangerous stimuli often occur simultaneously leading to opposing behaviors, reward- and fear-related circuits must interact in order to mediate these antagonistic behaviors. The amygdala is critical for both fear and reward learning (Wassum & Izquierdo, 2015). In order to investigate how the fear, safety and reward circuits integrate, we have been training Long Evans rats to discriminate among (a) a fear cue paired with footshock, (b) a safety cue in the presence of the fear cue resulting in no footshock, and (c) a reward cue paired with sucrose delivery. A selective increase in freezing to the fear cue and reward seeking to the reward cue indicate good fear and reward discrimination, respectively. This procedure also produces significant suppression of freezing to the fear cue if in the presence of a safety cue (Sangha, 2015; Sangha, Chadick, & Janak, 2013; Sangha, Greba, Robinson, Ballendine, & Howland, 2014; Sangha, Robinson, Greba, Davies, & Howland, 2014). Using this task, we have previously

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https://doi.org/10.1016/j.nlm.2017.11.011
Received 10 March 2017; Received in revised form 9 November 2017; Accepted 19 November 2017
Available online 22 November 2017

identified neurons in the basolateral amygdala (BLA) that discriminate among these cues (Sangha et al., 2013) and have shown differential roles for the infralimbic and prelimbic regions of the prefrontal cortex in this task (Sangha et al., 2014).

Dopaminergic signaling within the BLA is important for both fear and reward learning. Within the BLA, dopamine levels increase during learned fear responses (de Oliveira et al., 2011) and D1 receptors are required for fear extinction (Hikind & Maroun, 2008). Dopamine signaling is also implicated in discriminatory reward learning (Eagle, Olumolade, & Otani, 2014). Both D1 and D2 receptor activity modulate risk decisions during a reward uncertainty task (Larkin, Jenni, & Floresco, 2016). Dopamine D1 and D2 receptors are differentially expressed throughout the amygdala: D2 receptors are more selectively expressed in the central amygdala whereas D1 receptors are more selectively expressed in the BLA (Abraham, Neve, & Lattal, 2014; Weiner et al., 1991).

Taken together, these findings suggest that dopamine signaling should modulate fear suppression and reward discrimination in our safety-fear-reward cue discrimination task. We tested this hypothesis by administering a D1 dopamine receptor agonist or antagonist, systemically or infused into the BLA, before training sessions in which rats were learning to discriminate among fear, safety and reward cues.

2. Materials and methods

2.1. Subjects

Seventy Long Evans male rats (Harlan/Envigo) weighing 300–350 g were single housed (12 h light/dark cycle, lights on 09:00) and handled for 1 week before commencing experiments. All procedures were performed during the light cycle and approved by the Purdue Animal Care and Use Committee. Rats had *ad libitum* access to food and water up until the first training session, when they were restricted to 20 g of food per day for the remainder of the experiment.

2.2. Apparatus

Operant chambers were Plexiglas boxes (32 cm length × 25 cm width × 30 cm height) encased in sound-attenuating chambers (Med Associates, ST Albans, VT). 10% liquid sucrose (100 μL) was delivered through a recessed port 2 cm above the floor in the center of one wall. Port entries and exits were monitored through an infrared beam. Two lights (28 V, 100 mA) located 10.5 cm from floor on either side of the port served as the 20-s continuous light cue. A light (28 V, 100 mA) 27 cm above the floor on the wall opposite the port provided constant illumination. Auditory cues were delivered via a “tweeter” speaker (ENV-224BM) located 24 cm from the floor on the same wall as the port. Footshocks were delivered through a grid floor via a constant current aversive stimulator (ENV-414S). A side video camera located on the door of the sound-attenuating cubicle recorded the rat’s behavior for offline video analyses.

2.3. DC behavioral training procedure

The three cues signifying reward, fear or safety were a 20 s continuous 3 kHz tone (70 dB), a 20 s pulsing 11 kHz tone (200 ms on, 200 ms off; 70 dB), or a 20 s continuous light (28 V, 100 mA), respectively. The stimuli were not counterbalanced for this study since our previous study did not find significant differences in conditioned freezing or reward seeking to any of these stimuli (Sangha et al., 2013).

Rats first received 5 reward-only sessions on 5 separate days, which consisted of 25 pairings (ITI, 90–130 s) of the reward cue with a 3 s delivery of 10% sucrose solution (100 μL; pseudorandom delivery 10–20 s after cue onset) into a port. Rats then received a single habituation session consisting of 25 trials of reward cue-sucrose pairings, as well as 5 additional trials each of the fear cue presented alone and safety cue presented alone (ITI, 90–130 s). This procedure allows the animals to habituate and reduce their baseline freezing to the novel cues but does not contain enough presentations to produce latent inhibition (Sangha et al., 2013). Rats then received 4 sessions of discriminative conditioning (DC) on 4 separate days, which consisted of 15 reward cue-sucrose pairings, 4 fear cue-footshock pairings (0.5 s, 0.45 mA footshock at cue offset), 15 trials of the fear cue and safety cue presented simultaneously without footshock, and 10 trials of the safety cue presented alone without footshock (total 44 trials, ITI 100–140 s). Inclusion of trials where the safety cue was presented alone was to provide the animal with additional trials with a safety cue-no shock contingency and to assess if freezing developed to the safety cue.

2.4. Reward-nonreward behavioral training procedure

Rats first received 5 reward-only sessions on 5 separate days, which consisted of the same 25 pairings of the reward cue with 3 s delivery of 10% sucrose solution as mentioned above. Rats then received a single habituation session consisting of 25 trials of reward cue-sucrose pairings, as well as 5 additional trials of a non-reward cue presented alone (ITI, 90–130 s). Rats then received 4 sessions of reward vs non-reward discrimination training on 4 separate days, which consisted of 15 reward cue-sucrose pairings and 15 non-reward cue presented alone without sucrose (total 30 trials, ITI 100–140 s).

2.5. Systemic injections

Systemic s.c. injections of a D1 receptor agonist (10 mg/kg SKF-38393) (Doty et al., 1998; Inoue, Izumi, Maki, Muraki, & Koyama, 2000), antagonist (3. 33 μg/kg SCH-23390) (Sciascia, Mendoza, & Chaudhri, 2014) or saline were administered 20 min prior to each DC session. Similarly, the same dose of D1 receptor agonist or saline were administered 20 min prior to each Reward vs nonReward training session. To acclimate the animals to the injection procedure, all rats also received saline injections 20 min prior to the last reward session and habituation session.

2.6. Surgery

Rats were anesthetized with isoflurane and stereotaxically implanted bilaterally with stainless steel 27-gauge guide cannula dorsal to the BLA (AP −2.2 mm; ML ± 4.9 mm; DV −7.5 mm). During infusions, 32 gauge needles extended 1 mm beyond the guide cannulas into the BLA. Rats were allowed 7–10 days to recover in which they had *ad libitum* access to food and water. Stainless steel 32-gauge dummy cannulas were inserted into the guide cannulas between infusions.

2.7. BLA infusions

D1 dopamine receptor agonist SKF-38393 and antagonist SCH-23390 were each dissolved in 0.9% sodium chloride with concentrations of 1 μg/0.5 μL (Zarrindast, Rezayof, Sahraei, Haeri-Rohani, & Rassouli, 2003) and 0.25 μg/0.5 μL (Hikind & Maroun, 2008), respectively. Twenty minutes prior to each DC session, 0.5 μL of the mixture was infused (0.25 μL/s) into the BLA bilaterally. The injectors were left in place for 2 min post-infusion to allow for drug diffusion. A separate group of animals received saline infusions instead. In order to habituate animals to the infusion procedure, all animals received sham infusions 20 min prior to the last reward session and habituation session.

2.8. Histology

Rats were deeply anesthetized with sodium pentobarbital, and then perfused with PBS followed by 10% formalin. Tissues were then post-fixed in 30% sucrose formalin and sectioned at 50 μm with a cryostat. Sections were then plated on glass slides, stained with cresyl violet and