European Neuropsychopharmacology (2013) **23**, 379–389

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Conditioned fear response is modulated by a combined action of the hypothalamic-pituitary-adrenal axis and dopamine activity in the basolateral amygdala

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Received 9 February 2012; received in revised form 17 May 2012; accepted 19 May 2012

KEYWORDS
Fear conditioning;
HPA axis;
Corticosterone;
Metyrapone;
Basolateral amygdala;
Dopamine D_2 receptors

Abstract
The present study sought to determine the extent to which the combined activity of the hypothalamic-pituitary-adrenal (HPA) axis and dopaminergic systems is important for the expression of conditioned fear responses. The first experiment examined changes in plasma corticosterone concentration and the conditioned freezing response in rats treated with the dopamine D_2 receptor agonist quinpirole (0.25 mg/kg), the dopamine D_2 receptor antagonist sulpiride (40 mg/kg), corticosterone (3 or 6 mg/kg), or the corticosterone synthesis blocker metyrapone (30 mg/kg) and subjected to a conditioned fear test. A second experiment assessed the effects of corticosterone (3 or 6 mg/kg) and metyrapone (30 or 60 mg/kg) on fear-potentiated startle. A third experiment assessed the HPA axis modulation of conditioned fear using *in vivo* microdialysis targeted at dopaminergic neurotransmission in the basolateral amygdala (BLA). Quinpirole and sulpiride decreased conditioned freezing but did not affect plasma corticosterone concentration. Corticosterone and metyrapone did not affect fear-potentiated startle, but metyrapone attenuated conditioned freezing, suggesting that the expression of conditioned freezing requires HPA axis activation. Metyrapone inhibited the increase in dopamine levels in the BLA in response to the conditioned stimulus, whereas corticosterone had no significant effect. These results suggest that HPA axis activation is an initial step in an integrated neuroendocrine-neurochemical-behavioral response when the organism evaluates a threat associated with an environmental stimulus and triggers defense reactions to cope with this situation.

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http://dx.doi.org/10.1016/j.euroneuro.2012.05.007

1. Introduction

Pavlovian aversive conditioning is one of the most commonly used paradigms to investigate the neurobiological basis of emotion, learning, and memory. During the acquisition phase of the experiment, an emotionally neutral stimulus, such as a light, is paired with an aversive unconditioned stimulus (US), such as a footshock. The light then acquires conditioned aversive properties (i.e., becomes the conditioned stimulus [CS]) and during the expression phase of the experiment elicits conditioned fear responses. Freezing behavior is an important response to cues associated with footshock and is a widely used measure of conditioned fear in rodents (Bolles and Collier, 1976; de Oliveira et al., 2006, 2011; Fendt and Fanselow, 1999; Reimer et al., 2008). Another often-used measure of conditioned fear is fear-potentiated startle (FPS), in which the acoustic startle reflex (i.e., a skeletal muscle contraction in response to a sudden and intense burst of noise) is enhanced in the presence of a stimulus that has been previously paired with footshock (Brown et al., 1951; Davis et al., 1993; de Oliveira et al., 2006, 2009, 2011; Reimer et al., 2008; Santos et al., 2005).

Studies on aversive conditioning have proposed that the amygdala is a key component of the neural circuitry involved in the acquisition and expression of conditioned fear (Davis, 1992; Fanselow and LeDoux, 1999; LeDoux, 2003; Martinez et al., 2007; Pare et al., 2004). The amygdala integrates stimulus inputs from the environment and signals to other structures the degree of threat that these stimuli represent (Davis, 1992; LeDoux, 2003). Although FPS and conditioned freezing are both dependent on the amygdala, different neural circuits may be associated with each of these responses (Zhao and Davis, 2004). Moreover, FPS and conditioned freezing can be pharmacologically dissociated and therefore could reflect different aspects of fear and anxiety (Brandão et al., 2008).

Neurochemical experiments from our laboratory revealed an increase in the extracellular concentration of dopamine in the basolateral amygdala (BLA) during the expression of conditioned fear (de Oliveira et al., 2011). We also showed that this increase is attributable to the activity of the ventral tegmental area (VTA). Furthermore, microinjection of sulpiride (a dopamine D_2 receptor antagonist) into the BLA decreased FPS. These findings reinforce the importance of D_2 receptors in the BLA in the expression of conditioned fear and suggest that interference with the ability of the conditioned stimulus to activate dopaminergic neurons in the BLA reduces fear.

Dopamine is one of the most active neuromodulators that underlie states of fear and anxiety (Carvalho et al., 2009; de Oliveira et al., 2006, 2009, 2011; Finlay et al., 1995; Martinez et al., 2008; Reis et al., 2004; Zweifel et al., 2011) and plays an important role in the pathogenesis of several psychiatry disorders (Calzavara et al., 2009; Eilam and Szechtman, 2005; Elsworth and Roth, 1997; Goldstein and Deutch, 1992). The elucidation of the mode by which dopamine participates in the generation of a particular emotional behavior would be therapeutically relevant for fear-related diseases. Dopaminergic systems were more commonly associated with the reinforcing effects of various stimuli (Volkow et al., 2002; Wise, 2009), and the involvement of dopamine in Pavlovian aversive conditioning raises the issue of how distinct stimuli are interpreted as aversive or appetitive. An interaction between dopaminergic activity and other neurotransmitters or neurohormones may underlie the behavioral response exhibited by animals, depending on the environmental context, whether aversive or reinforcing. Thus, dopaminergic neurotransmission associated with activation of the hypothalamic–pituitary–adrenal (HPA) axis, for example, may determine aversive states (Barr et al., 2009; Sapolsky, 2009).

The activation of the HPA axis, reflected by an increase in plasma corticosterone in rodents and cortisol in primates, has been considered an important part of the stress reaction and can be triggered either by innate or conditioned fear stimuli (Albrechet-Souza et al., 2007; Coco et al., 1992; Cordero et al., 1998; Mason, 1968; Reis et al., 2012). Once in the circulation, corticosterone modulates emotional behavior by acting on two types of receptors: mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) (Joë]ls and Baram, 2009; Lu et al., 2006; Tasker et al., 2006; Ulrich-Lai and Herman, 2009). Previous studies confirmed that both corticosteroid receptors are present in the amygdala (Conrad et al., 2004; Furay et al., 2008; Harfstrand et al., 1986; Lu et al., 2006; Roozendaal et al., 1996; Yang et al., 2006).

Despite the recognized involvement of corticosteroids in the modulation of emotional behavior, the specific role of corticosterone in the expression of conditioned fear responses is still open to investigation. Understanding how distinct neuromodulators are called into action during the expression of conditioned fear may contribute to better knowledge of the neurobiology of fear and anxiety. The aim of the present study was to determine the extent to which the combined action of the HPA axis and dopaminergic neurotransmission is important for the expression of conditioned fear responses. The first experiment examined changes in plasma corticosterone concentration and the conditioned freezing response in rats systemically treated with the dopamine D_2 receptor agonist quinpirole, the dopamine D_2 antagonist sulpiride, corticosterone (i.e., the final product of HPA axis activation), and the corticosterone synthesis blocker metyrapone and subjected to a conditioned fear test. To further explore the importance of the hormonal response in the expression of conditioned fear, a second experiment assessed the effects of systemic corticosterone and metyrapone administration on FPS. A third experiment combined pharmacology and neurochemistry using *in vivo* microdialysis to examine the role of HPA axis activation in dopaminergic neurotransmission in the BLA during the expression of conditioned fear.

2. Experimental procedures

2.1. Animals

One-hundred ninety-eight naive male Wistar rats from the animal facility of the University of São Paulo at Ribeirão Preto were used. The rats, weighing 270–290 g, were housed in groups of five in plastic boxes and maintained under controlled conditions (23 ± 1 °C; 12 h/12 h light/dark cycle, lights on at 07:00 AM) with food and water freely available. The procedures were approved by the Committee for Animal Care and Use of University of São Paulo at Ribeirão Preto (No. 10.1.595.53.7).