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Conditioned fear is modulated by D_2 receptor pathway connecting the ventral tegmental area and basolateral amygdala

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ABSTRACT

Excitation of the mesocorticolimbic pathway, originating from dopaminergic neurons in the ventral tegmental area (VTA), may be important for the development of exaggerated fear responding. Among the forebrain regions innervated by this pathway, the amygdala is an essential component of the neural circuitry of conditioned fear. The functional role of the dopaminergic pathway connecting the VTA to the basolateral amygdala (BLA) in fear and anxiety has received little attention. In vivo microdialysis was performed to measure dopamine levels in the BLA of Wistar rats that received the dopamine D_2 agonist quinpirole (1 μg/0.2 μl) into the VTA and were subjected to a fear conditioning test using a light as the conditioned stimulus (CS). The effects of intra-BLA injections of the D_1 antagonist SCH 23390 (1 and 2 μg/0.2 μl) and D_2 antagonist sulpiride (1 and 2 μg/0.2 μl) on fear-potentiated startle (FPS) to a light-CS were also assessed. Locomotor performance was evaluated by use of open-field and rotarod tests. Freezing and increased dopamine levels in the BLA in response to the CS were both inhibited by intra-VTA quinpirole. Whereas intra-BLA SCH 23390 did not affect FPS, intra-BLA sulpiride (2 μg) inhibited FPS. Sulpiride's ability to decrease FPS cannot be attributed to nonspecific effects because this drug did not affect motor performance. These findings indicate that the dopamine D_2 receptor pathway connecting the ventral tegmental area and the basolateral amygdala modulates fear and anxiety and may be a novel pharmacological target for the treatment of anxiety.

1. Introduction

Pavlovian fear conditioning is one of the most widely used paradigms to study the biological basis of emotion, learning, and memory. Although considerable work has been done relating specific circuits of the brain to conditioned fear, less is known about its regulation by neuromodulators such as dopamine (DA), the understanding of which would be therapeutically relevant for fear-related diseases (Brandão, Troncoso, de Souza Silva, & Huston, 2003; Brandão et al., 2005). Dopaminergic systems are also recruited during the formation, retrieval, and expression of affective memory (De la Mora, Gallegos-Cari, Arizmendi-Garcia, Marcellino, & Fuxe, 2010; Millan, 2003). The association between changes in dopaminergic transmission and threatening stimuli has been demonstrated by numerous studies. Acute and chronic stressful stimuli have been found to activate dopaminergic systems (Feenstra, Botterblom, & Van Uum, 1995; Finlay, Zigmond, & Abercrombie, 1995).

The mesocorticolimbic dopaminergic pathway, originating from the ventral tegmental area (VTA), is particularly sensitive to fear-arousing environmental stimuli and has been associated with exaggerated responses to fear situations (Deutch, Tam, & Roth, 1985; Greba, Gifkins, & Kokkinidis, 2001; Guarraci, Frohardt, Falls, & Kapp, 2000; Guarraci & Kapp, 1999). In a recent study, we demonstrated that intra-VTA injection of the dopaminergic D_2 agonist quinpirole attenuated the expression of conditioned fear (Oliveira, Reimer, & Brandão, 2009). Based on quinpirole's high affinity for presynaptic D_2 autoreceptors, we suggested that the reduction in fear was attributable to interference with the ability of the fear-evoking conditioned stimulus to activate dopaminergic neurons in the VTA (i.e., at the origin of the mesocorticolimbic pathway).

At least five dopaminergic receptor subtypes have been characterized (D_1–D_5), and divided into two subfamilies: D_1 and D_2 (Kebabian & Calne, 1979; Millan, 2003; Vallone, Picetti, & Borrelli, 2000). Since 2004, we have been examining the involvement of these receptors in conditioned fear. Using systemic injections of dopaminergic agents, we showed that mainly D_2-mediated

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systems are recruited during conditioned fear (Oliveira, Reimer, & Brandão, 2006). Subsequently, in an attempt to localize these effects in the central nervous system, evidence was obtained supporting the involvement of dopaminergic mechanisms in the VTA and nucleus accumbens in these effects (Martinez, Oliveira, Macedo, Molina, & Brandão, 2008; Oliveira et al., 2009). Other structures of the mesocorticolimbic system have also been implicated in the coordination of conditioned fear responses. Among the forebrain regions innervated by VTA dopaminergic neurons, the amygdala is an essential component of the neural circuitry of conditioned fear (Davis, 1992; Davis, Falls, Campeau, & Kim, 1993; LeDoux, 2000; LeDoux, Iwata, Cicchetti, & Reis, 1988; Oades & Halliday, 1987; Swanson, 1982). Nevertheless, a systematic analysis of the dopaminergic mechanisms in the VTA-amygdala neurocircuit has been lacking.

Thus, in the present study, we pharmacologically assessed the functional role of this pathway in fear- and anxiety-related behaviors using local injections of dopaminergic drugs, microdialysis, and different animal models of anxiety. The present study further assessed the dopaminergic mediation of conditioned fear in the VTA–basolateral amygdala (BLA) neurocircuit using the fear-potentiated startle (FPS) paradigm and a conditioned fear test to measure the freezing response. Although FPS and conditioned freezing are both dependent on the amygdala, different circuits have been shown to be involved in these fear responses (Zhao & Davis, 2004). Moreover, recent findings from this laboratory have shown that FPS and freezing can be pharmacologically dissociated and, as such, should express distinct aspects of fear and anxiety (see Brandão, Zanoveli, Ruiz-Martinez, Oliveira, & Landeira-Fernandez, 2008 for a review).

FPS has been one of the most widely used tests for evaluating amygdala-dependent fear behavior (Davis, 1992; Davis et al., 1993). Basically, the acoustic startle reflex is a skeletal muscle contraction in response to a sudden and unexpected burst of noise (Fleshler, 1965; Koch, 1999; Yeomans & Frankland, 1996). When the startle-inducing noise occurs in the presence of a conditioned stimulus (CS) previously paired with an aversive unconditioned stimulus (US; e.g., footshock), the startle response is enhanced. This increase in the startle reflex – termed FPS – is considered an index of conditioned fear (Brown, Kalish, & Farber, 1951; Davis et al., 1993). FPS is sensitive to the anxiolytic action of benzodiazepines and has been considered a model for generalized anxiety disorder (Berg & Davis, 1984; Brodkin, Busse, Sukoff, & Varney, 2002; Davis, 1979; Reimer, Oliveira, & Brandão, 2008). Complementing FPS, the conditioned freezing response was also analyzed in this study.

The drugs used for the pharmacological evaluation were SCH 23390, a D_1 antagonist (Andersen, 1988; Fletcher & Starr, 1988; Greba & Kokkinidis, 2000; Hyttel, 1983), quinpirole, a D_2 agonist (Levant, Grigoriadis, & DeSouza, 1993), and sulpiride, a D_2 antagonist (Standish-Barry, Bouras, Bridges, & Watson, 1983; White & Wang, 1984). Based on the results of the behavioral studies, the role of dopaminergic systems in the VTA–BLA pathway in the expression of conditioned fear was also examined in a combined pharmacological/neurochemical study using in vivo microdialysis.

2. Methods

2.1. Subjects

Subjects were male Wistar rats ($n = 97$; 260–280 g) from the animal facility of the University of São Paulo at Ribeirão Preto. Animals were housed in groups of five at 23 ± 1 °C under a 12 h/12 h light/dark cycle (lights on at 07:00 am). Food and water were available *ad libitum*. Procedures were approved by the Committee for Animal Care and Use of University of São Paulo at Ribeirão Preto (No. 06.1.123.8.53.9). All behavioral tests in this study are routinely used in this laboratory (Oliveira et al., 2006, 2009).

2.2. Surgery

A detailed description of surgical procedure has been provided previously (Oliveira et al., 2009). Briefly, rats were anesthetized with ketamine/xylazine (100/7.5 mg/kg, intraperitoneal) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, USA). For FPS, bilateral BLA cannulation for drug injections was performed. For microdialysis, two guide-cannulae were implanted, one over BLA for dialysis probe (CMA/12; CMA/Microdialysis AB, Stockholm, Sweden) and one over VTA for drug injection, both in the right hemisphere. Previous studies from this laboratory have shown that microdialysis from cannula unilaterally implanted into the amygdala accurately measures changes in extracellular concentrations of DA in this structure (Macedo, Martinez, de Souza Silva, & Brandão, 2005). With bregma as the reference point, the coordinates used were (Paxinos & Watson, 2007): BLA (anterior/posterior, −2.3 mm; medial/lateral, ±5.5 mm; dorsal/ventral, −7.0 mm) and VTA (anterior/posterior, −5.8 mm; medial/lateral, ±0.8 mm; dorsal/ventral, −7.6 mm). Afterward, the rats were allowed 5 days to recover from the surgical procedure. The experimental design used in this study is described below and presented schematically in Fig. 1.

2.3. Fear-potentiated startle

2.3.1. Matching

The rats' startle reaction put pressure on a response platform generating analog signals that were analyzed by Startle Reflex software (Med Associates, St. Albans, USA). The testing cage was a wire-grid cage (16.5 × 7.5 × 7.5 cm) which was fixed to the response platform by four thumb screws. The testing cage and the response platform were located inside a ventilated sound-attenuating plywood-chamber (64 × 60 × 40 cm). A loudspeaker located 10 cm behind the testing cage delivered both the startle stimulus (100 dB; 50 ms burst of white noise) and continuous background noise (55 dB). The startle reaction was recorded within a time window of 100 ms after the onset of the startle stimulus. For the first 2 days, the animals were placed in the testing cage for a 5 min habituation period and afterward received a total of 30 startle stimuli with an interstimulus interval of 30 s. Each matching session was 20 min in duration, including the habituation period. In order to match the animals of the control and drug groups according to scores achieved in the matching phase, animals were assigned to the different groups in a way that each group had the same average startle amplitude based on the last matching day.

2.3.2. Training

Animals were conditioned to light-CS in a cage (20 × 20 × 25 cm) with stainless steel side and back walls, a transparent Plexiglas ceiling and front door and grid floor consisted of stainless-steel rods. This training cage was located within a ventilated and sound-attenuated chamber (45 × 45 × 45 cm) and was different from the testing cage to avoid conditioning of the context. After recovery from surgery, animals were placed in the training cage, and each rat received 10 CS–US pairings after a habituation-phase of 5 min using a 4 s, 6 W light-CS, coterminating with a 1 s, 0.6 mA footshock-US. The inter-trial interval varied randomly between 60 and 180 s. The duration of each training session was about 25 min, including habituation time.

2.3.3. Testing

Test sessions were conducted without footshock presentation in the same cages used for matching. Twenty-four hours after train-