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Research report

Dopamine D_2 receptors regulate unconditioned fear in deep layers of the superior colliculus and dorsal periaqueductal gray

Sangu Muthuraju[a,b], Teddy Talbot[b,c], Marcus Lira Brandão[a,b,*]

[a] Laboratory of Neuropsychopharmacology, FFCLRP, Universidade de São Paulo, Campus USP, Ribeirão Preto, SP 14049-901, Brazil
[b] Instituto de Neurociencias e Comportamento, Avenida do Café, 2450, Ribeirão Preto, São Paulo, 14050-000, Brazil
[c] Department of Neurosciences and Behavior, Ribeirão Preto School of Medicine, University of São Paulo, Ribeirao Preto, São Paulo, Brazil

HIGHLIGHTS

- D_2 receptor inhibition in the dlSC increased the number of switch-off responses to light.
- D_2 receptor inhibition in the dlSC and dPAG caused proaversive effects in the elevated plus maze.
- Dopamine D_2 receptors in the dlSC and dPAG regulate unconditioned fear.

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ABSTRACT

Rationale: Electrical and chemical stimulation of the dorsal periaqueductal gray (dPAG), deep layers of the superior colliculus (dlSC), and inferior colliculus (IC) causes freezing and escape behavior in rodents. Systemic injections of the selective dopamine D_2 receptor antagonist sulpiride increased the number of switch-off responses (SORs) to light and auditory evoked potentials in response to loud sounds. Dopamine D_2 receptor inhibition in the IC was shown to enhance unconditioned fear. Nevertheless, the role of dopamine receptors in the dlSC and dPAG in the mediation of unconditioned fear has not yet been demonstrated.
Objectives: The purpose of the present study was to characterize the effects of sulpiride injections (4 and 8 μg/0.2 μl) in the dlSC and dPAG in rats that were subjected to unconditioned fear paradigms.
Methods: Switch-off responses to light and exploratory behavior in the elevated plus maze were used to evaluate unconditioned fear in rats.
Results: Intra-dlSC microinjections of sulpiride increased the number of SORs to light. Intra-dlSC and intra-dPAG injections of sulpiride reduced the number of entries into and time spent on the open arms and decreased end-arm exploration and head dipping in the elevated plus maze.
Conclusion: These findings suggest that dopamine, through D_2 receptors in the dlSC and dPAG, is involved in defense reactions that are organized in the midbrain tectum.

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1. Introduction

Electrical and chemical stimulation of the dorsal periaqueductal gray (dPAG), deep layers of the superior colliculus (dlSC), and inferior colliculus (IC) causes a characteristic pattern of active unconditioned defense reactions, including alertness, freezing, escape responses, and autonomic changes that resemble anxiety disorder [1–6]. The behavioral repertoire of defense reactions that are displayed in response to stimulation of the dPAG in animals has been effectively used as a model of panic attacks in humans [1,7–10]. Comparable responses can be elicited by stimulation of the dlSC [11,12] and IC [13], and the midbrain tectum has been considered to be part of an encephalic aversion system (EAS) [14]. We recently showed that fear-evoking stimuli increased the magnitude of auditory evoked potentials (AEPs) that were directly recorded from the IC in response to loud sounds [5,6,15]. γ-Aminobutyric acid (GABA), serotonin, opioids, excitatory amino acids, and nitric oxide have been reported to modulate the activity of the EAS, but

Abbreviations: DA, dopamine; EAS, encephalic aversion system; dPAG, dorsal periaqueductal gray; IC, inferior colliculus; SOR, switch-off response; AEP, auditory evoked potential; dlPAG, dorsolateral periaqueductal gray; EPM, elevated plus maze; AP, anterior/posterior; ML, medial/lateral; DV, dorsal/ventral; ANOVA, analysis of variance; GABA, γ-aminobutyric acid.

* Corresponding author at: Laboratory of Neuropsychopharmacology, FFCLRP, Universidade de São Paulo, Campus USP, Ribeirão Preto, SP 14049-901, Brazil. Fax: +55 16 33154830.
E-mail address: mbrandao@usp.br (M.L. Brandão).

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little is known about the role of dopaminergic neurotransmission in the dlSC and dPAG in unconditioned fear.

The association between changes in dopaminergic transmission and conditioned fear has been previously demonstrated in numerous studies. Recent studies reported that systemic and intra-amygdala injections of the dopamine D_2 receptor antagonist sulpiride attenuated the expression of conditioned fear [16–20]. These studies and others from several laboratories [21,22] indicate a key role for dopamine in the neurobiology of anxiety [for review,see 3]. Unknown is the extent to which dopamine also mediates unconditioned fear. Two studies showed that systemic sulpiride injections caused anxiogenic-like effects in the elevated plus maze and switch-off procedure, in which rats can interrupt light presentations simply by crossing to the other side of a compartment of a switch-off [23,24]. Recent evidence also supports a role for dopamine receptors in defensive behavior that is associated with unconditioned fear [3,6]. Additionally, systemic administration of the dopamine receptor antagonist haloperidol increased AEPs in response to loud tones (i.e., unconditioned stimuli) [6]. However, the role of D_2 receptors in other structures of the brain aversion system, such as the dPAG and dlSC, has not yet been clearly studied. The present study investigated dopamine's mediation of unconditioned fear that is organized at the level of these two structures of the midbrain tectum. We used switch-off responses (SORs) to light and the elevated plus maze (EPM) to evaluate the effects of sulpiride injections [25,26] in the midbrain tectum in rats.

2. Methods and materials

2.1. Animals

A total of 115 male Wistar rats, weighing 250–300 g, were obtained from the animal facility of the University of Sao Paulo at Ribeirao Preto. The animals were housed in groups of three in plastic boxes and maintained under a 12 h/12 h light/dark cycle. The rats were allowed free access to food and water throughout the experiment. The experiments were performed during the light phase of the cycle. All of the experiments received formal approval from the Committee on Animal Research and Ethics of the University of Sao Paulo, Brazil (process no. 10.595.53.7).

2.2. Surgery

The animals were anesthetized with a ketamine/xylazine mixture (100/7.5 mg/kg, intraperitoneal) and fixed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The upper incisor bar was set 3.3 mm below the interaural line, such that the skull was horizontal between bregma and lambda. Guide cannulae (0.6 mm outer diameter) for drug injections were implanted in the midbrain, aimed at the dPAG and dlSC. The cannula was introduced unilaterally using the following coordinates, with lambda as the reference for each plane: dPAG (anterior/posterior [AP], 0.0 mm; medial/lateral [ML], ±1.9 mm; dorsal/ventral [DV], 5.1 mm; 16° angle) and dlSC (AP, 0.0 mm, ML, ±1.4 mm; DV, 4.5 mm; 0° angle) [27]. The cannulae were fixed to the skull with acrylic resin and two stainless-steel screws. Each guide cannula was sealed with a stainless steel wire to protect it from blockage. Afterward, the rats were allowed 5 days to recover from surgery.

2.3. Light switch-off response

The experimental chamber consisted of a two-compartment shuttle box (30 cm × 25 cm × 25 cm; Insight, Ribeirão Preto, Brazil). The ceiling, side walls, and back wall of the chamber were made of black Plexiglas while the front door was made of transparent Plexiglas, which was covered with opaque paper. The experimental chamber was equipped with a compartmentalized flip-flop grid floor with 15 stainless-steel rods (2.0 mm diameter) spaced 1.2 mm apart. The shuttle behavior of the animals was measured during the session by counting the number of times the floor moved over the fulcrum in the shuttle box. This arrangement allowed the detection of shuttle locomotion from one compartment to the other. Two 28-V light bulbs one in the center of each side of the rear wall of the chamber, 12 cm from the floor, went off after each switch-off response and remained lit in the absence of this response. The lights produced no noise when turned on or off. The experimental chamber was located within a small, ventilated attenuating box (50 cm × 40 cm × 35 cm). The behavior of the animals during the test sessions was recorded by a video camera (Everfocus, Duarte, California, USA) that was positioned in the lateral wall of the observation chamber, thus allowing the detection of all behaviors. The video signal was relayed via a closed circuit to a monitor that was located in an adjacent room.

Each animal was placed inside the shuttle box and allowed 5 min for acclimatization to the experimental context before the beginning of the session. Sessions consisted of 40 associations with an unconditioned stimulus (US: light). Each animal was subjected to 20 s of US exposure. The light stimulus was approximately 120 lux, measured at the floor level of the cage with a luxmeter (LX103; Lutron, Coopersburg, PA, USA). The software and an appropriate interface connected to a computer was provided by the manufacturer of the equipment (insight). This equipment allowed the recording and analysis of the frequencies of the escape responses. The presentation and sequencing of the light stimuli were also controlled by the same software, which also collected data in blocks of 10 trials during the entire session (Reis et al. [24]).

Whenever a rat passed from one compartment to the other while the light was on, this response switched the light off (SOR, i.e., responses within 20 s). Two successive trials were separated by a random variable interval of 10–50 s. Transitions between compartments were scored. The software controlled the presentation and termination of the stimuli and collected all data. In the test conditions, each animal was submitted to only one session. The aforementioned study from our laboratory [24], compared the two-way avoidance (TWA) test with the results of the light switch-off (SOR) test, showing that learning, if any, in the SOR test was very weak compared with the TWA test. This lack of learning during the 40-trial session suggests that behavior in this test reflects an escape response to the light rather than a conditioned learning response.

2.4. Elevated plus maze

We used a wooden EPM which consisted of two open arms (50 cm × 10 cm) and two enclosed arms of equal size, surrounded by 50 cm high walls. The maze was set in such a way that arms of the same type were opposite to each other, and the whole apparatus was elevated 50 cm from the floor. A raised transparent Plexiglas edge (1.0 cm) surrounded the open arms providing additional grip for the rats.

Testing was carried out during the light phase of the light/dark cycle between 9:00 AM and 11:00 AM. The apparatus was located inside a room with constant background noise (50 dB). Behavior was recorded by a video camera (Everfocus, Duarte, California, USA) that was positioned 1.5 m above the maze. The video signal was relayed via a closed circuit to a monitor in an adjacent room. Luminosity at the floor level of the open arms was 30 lux. The rats were individually placed in the center of the maze, with the nose facing one of the closed arms and allowed 5 min of free exploration. Videotapes were subsequently scored by an observer using the Observer 3.0 ethological analysis software (Noldus, Wageningen, The Netherlands). This software allows measurements of the num-

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