Context fear conditioning inhibits panic-like behavior elicited by electrical stimulation of dorsal periaqueductal gray

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INTRODUCTION

Different patterns of animal defensive behaviors have been employed as useful tools for investigating and understanding the underlying mechanisms for different anxiety disorders [1,2]. Panic disorder is characterized by recurrent panic attacks, which can occur spontaneously or associated with a particular situation. Panic attacks are surges of intense fear or terror accompanied by pounding heart, chest pains, lightheadedness or dizziness, nausea, shortness of breath, shaking or trembling, choking, fear of dying, sweating, feelings of unreality, numbness or tingling, hot flashes or chills, and a feeling of going out of control or going crazy [3]. Electrical stimulation of the DPAG has been proposed as a model of panic attacks [4,5]. According to this model, a stepwise increase in the electrical current intensity to stimulate the DPAG produces alertness, then freezing and finally the panic-like behavior characterized by running and jumping responses [4]. Pharmacological results support the isomorphism between the escape responses induced by the stimulation of the DPAG and human panic attacks. Panicolytic drugs, such as clomipramine and fluoxetine increased the electrical current threshold to elicit the running and jumping behaviors whereas the panicogenic drug pentylentetrazole decreased this threshold [4,5]. In humans, DPAG electrical stimulation produced closely related panic attack symptoms such as heart-pounding terror and feelings of imminent death accompanied by diffuse face and chest pain [6].

Considerable evidence also indicates that freezing response to contextual cues previously associated with electrical footshocks is an animal model of anxiety [7–10]. According to this model, an animal exposed to unsignaled footshocks starts to freeze shortly after the shock as well as when the animal is returned to the same chamber some time after the presentation of the electrical footshocks [7]. Bidirectional modulation of anxiety at the benzodiazepine receptor has been employed to validate the context fear conditioning paradigm as an experimental model of anxiety in rodents. Benzodiazepine receptor agonist, such as diazepam or midazolam, reduced the amount of freezing elicited by contextual cues previously associated with footshock whereas the benzodiazepine inverse agonist dimethoxy-beta-carboline produces freezing behaviour similar to the one elicited by the context fear conditioning [8]. Finally, anxiolytic-like substances such as 5-HT1A receptor agonists, selective serotonin reuptake inhibitors, and mono-
amine oxidase inhibitors with verified clinical efficacy in the treatment of anxiety symptoms attenuate conditioned behavior in rats indicating a considerable construct and face validity of this paradigm to human anxiety [9,10]. Although there are natural differences between animal and human behavior, context fear conditioning and electrical stimulation of the DPAG might be two useful animal models to clarify the relationship between anxiety and panic attack. This is an important issue especially because experiments with human patients have lead to conflicting results. There are evidences supporting the view that anxiety might either facilitate [11] or inhibit [12] the occurrence of panic attack. Therefore, the present study employed these two animal models in the same experimental design in order to investigate whether context fear conditioning might increase or decrease the occurrence of active defensive behavior evoked by electrical stimulation of the DPAG.

MATERIALS AND METHODS
Male albino Wistar rats weighing about 250 g were employed as subjects. Animals were housed in individual Plexiglas-walled cages with free access to food and water in a 12:12 h light:dark cycle (lights on 07:00 h). The experiment was conducted during the light phase of the cycle. Room temperature was maintained at 23 ± 1°C. The experiments were performed in compliance with the recommendations of SBNeC (Brazilian Society of Neuroscience and Behavior), which are based on the US National Institutes of Health Guide for Care and Use of Laboratory Animals.

Rats were anaesthetized with tribromoethanol (250 mg/kg, i.p.) and fixed on a stereotaxic instrument (David Kopf, USA). With the skull horizontal between bregma and lambda, an electrode was inserted into the DPAG (angle 16°, 1.9 mm lateral to lambda at a depth of 5.2 mm below the bony surface). The electrode was made of stainless steel wire 250 μm diameter, insulated except at the cross-section of the tip. The electrode was attached to the skull by means of acrylic resin and two stainless steel screws. The electrode could be connected to a male pin so that it could plugged into a amphenol socket at the end of a flexible electrical cable and used for brain stimulation.

Five days after the surgery, each animal was placed inside the experimental chamber for 6 min habituation period. After that, the DPAG aversive baseline threshold was determined through an electrical stimuli (AC, 60 Hz, 15 s) presented through the implanted electrode. The electrical interstimulus interval was 15 s. The current intensity started at 20 μA and was increased by steps of 8 μA until the rat presented a stereotyped escape response defined as running or jumping reactions. The aversive baseline threshold was defined as the lowest current intensity that produced the escape behavior in three successive trials of electrical stimulation. Animals with aversive baseline thresholds > 200 μA were discarded from the experiment. Following the baseline aversive threshold procedure, one group of animals (DPAG/SHOCK) was submitted to the context fear conditioning procedure. Conditioning consisted in the presentation of five unsignaled 2 s 1 mA electrical foot-shocks with a 1 min intershock interval. A no-shock control group (DPAG/NO SHOCK) had exactly the same procedure as the first group with the exception that no footshock was delivered. Testing session took place two hours later. During this phase, all animals were reexposed to the experimental chamber and freezing behavior was recorded for 5 min. Freezing was scored through a time-sample procedure. Every 2 s an experimenter rated the animal’s behavior as freezing or activity. Freezing was defined as absence of visible movements, except those due to respiration. At the end of the fifth minute a new DPAG aversive threshold to induce an escape response was determined.

At the end of the experiment, animals were sacrificed with an overdose of sodium thiopental and perfused intracardially with saline followed by 10% formalin containing 1% ferrocyanide. A DC current from a 9 V battery (20 s) was applied to the brain electrode to stain the brain tissue around its tip. The brains were then removed and further fixed for a minimum of 3 days with 10% formalin. Serial 60 μm brain slices were sectioned using the cryostatic method. The stimulation sites were identified and plotted on diagrams according to the Paxinos and Watson [13] rat brain atlas.

RESULTS
A representative histological section showing the location of the electrode tip is presented in Fig. 1. Histological examination of the brain slices indicated that all electrode tips were located inside or at the borders of DPAG. The final group samples were DPAG/NO SHOCK, n = 8; DPAG/SHOCK n = 14. All the animals presented aversive baseline thresholds < 200 μA. As reported previously [14], freezing and escape behaviors occurred in a stepwise fashion as the intensity of electrical current applied to the DPAG increased. Escape behavior stopped as soon as the DPAG electrical stimulation was switched off. No differences in the basal aversive threshold between shock (DPAG/SHOCK) and no-shock control (DPAG/NO SHOCK) groups were found (p > 0.5).

Fig. 1. Photomicrograph showing a typical example of a stimulation electrode site (arrow). Aq, aqueduct of Sylvius; DPAG, dorsal periaqueductal gray; DIPAG, dorsolateral periaqueductal gray; VIPAG, ventro-lateral periaqueductal gray; VPAG, ventral periaqueductal gray. The histological section was located 76 mm posterior to bregma.
Our results also have an immediate consequence on the comprehension about the relationship between anxiety and panic attack in humans. According to evolutionary ap-
acutely treated with the 5-HT releaser and uptake blocker attacks [24]. On the other hand, panic disorder patients 

Contextual cues previously associated with footshock 

CONCLUSION

Contextual cues previously associated with footshock induced defensive freezing behavior and increased DPAG 

electrical current threshold to elicit flight reactions. Since context fear conditioning is a model of anxiety whereas electrical stimulation of the DPAG is related to panic attack, it is concluded that an increase in anxiety might cause a decrease in panic attack.

REFERENCES


