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

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Context fear conditioning has been widely used as an animal model of anxiety whereas electrical stimulation of the dorsal portion of the periaqueductal gray (DPAG) as a model of panic attack. The present study employed these two animal models in order to investigate the influence of anxiety in the occurrence of panic attack. Results indicated that animals exposed to contextual cues that were previously associated with electrical footshocks engaged in robust defensive freezing behavior and were less likely to display flight evoked by electrical stimulation of the DPAG when compared with control animals that were not exposed to the context fear conditioning procedure. These results indicate that activation of the brain mechanisms that underlie anxiety produces an inhibitory effect on panic attack. *NeuroReport* 14:1641–1644 © 2003 Lippincott Williams & Wilkins.

Keywords: Animal defensive behavior; Context fear conditioning; Electrical brain stimulation; Generalized anxiety disorder; Panic attack; Periaqueductal gray

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 pentylenetetrazole 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-β-carboline produces freezing behaviour similar to the one elicited by the context fear conditioning [8]. Finally, anxiolytic-like substances such as 5-HT_{1A} 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 behaviour 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 $23 \pm 1^{\circ}C$. 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 footshocks 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 fixated 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 ($p > 0.5$).

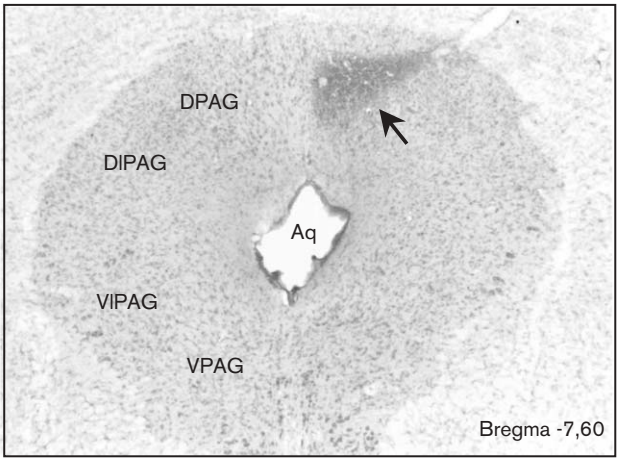

Fig. 1. Photomicrograph showing a typical example of a stimulation electrode site (arrow). Aq, aqueduct of Sylvius; DPAG, dorsal periaqueductal gray; DlPAG, dorsolateral periaqueductal gray; VlPAG, ventrolateral periaqueductal gray; VPAG, ventral periaqueductal gray. The histological section was located 7.6 mm posterior to bregma.

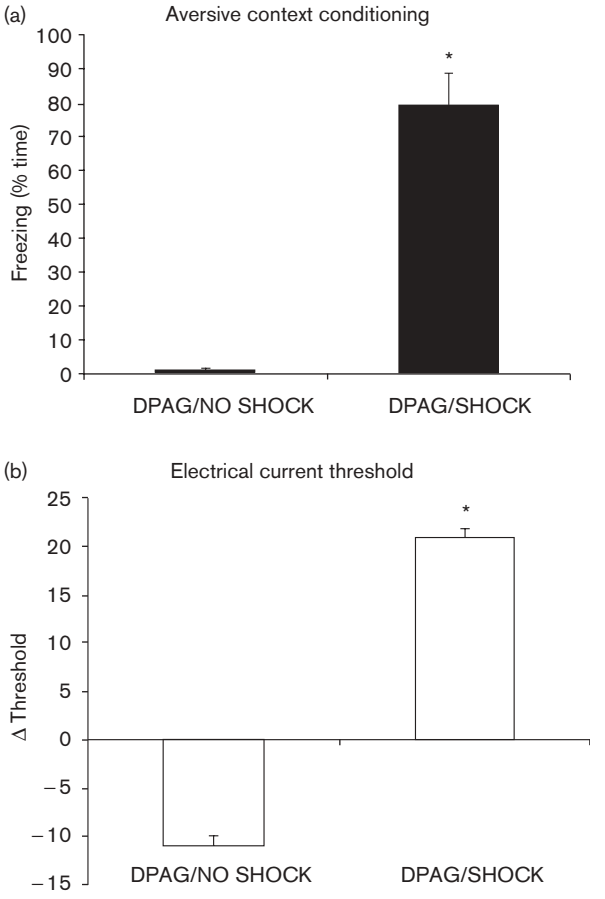

Fig. 2. (**a**) Mean ± s.e.m. percentage time spent engaged in freezing behavior during testing session 2 h after rats were exposed (DPAG/SHOCK) or not exposed (DPAG/NO SHOCK) to five unsignaled footshocks. (**b**) Mean ± s.e.m. difference (Δ) between testing and baseline DPAG electrical current threshold to induce an escape behavior. $*p < 0.05$.

Figure 2a presents the mean ± s.e.m. freezing behavior during the 5 min testing session 2 h after rats were exposed (DPAG/SHOCK) or not (DPAG/NO SHOCK) to five unsignaled footshocks. The results are clear cut and indicate that animals exposed to contextual cues that were previously associated with electrical footshocks displayed more freezing than control non-shocked animals ($t(20) = 5{,}8$; $p < 0.001$). The high levels of freezing among the animals in the DPAG/SHOCK group is in accordance with previous findings which report that unsignaled footshock reliably leads to context fear conditioning [7]. Figure 2b shows the mean change in DPAG electrical current aversive threshold between test and baseline phases of the experiment. As can be observed, current threshold difference (Δ) between testing and baseline phases was greater among animals previously exposed contextual cues previously associated with electrical footshocks when compared with the no-shock control group ($t(20) = 2{,}3$; $p < 0.05$). The fact that DPAG/NO SHOCK group presented a negative Δ threshold indicates that some animals in this group presented a lower current aversive threshold during the test phase compared with their baseline aversive threshold. The opposite pattern was observed among the animals in the DPAG/SHOCK group. Therefore, these results indicate that context fear conditioning was able to inhibit active flight reactions evoked by electrical stimulation of the DPAG.

DISCUSSION

Results from the present experiment clearly indicate that contextual cues previously associated with footshock induced defensive freezing behavior and inhibited active defensive reaction evoked by electrical stimulation of the DPAG. These results are in conformity with the view that animal defensive responses are organized hierarchically. According to this view, freezing is the main response to innate or learned aversive stimuli and can inhibit active forms of nociceptive behavior to a variety of threatening situations [8]. For example, context fear conditioning can trigger an analgesic reaction that can inhibit withdrawal reflex to painful stimuli, such as tailflick to radiant heat, or more complex and elaborated nociceptive behaviours elicited in the formaline-induced nociception [8]. Moreover, it has been shown that context fear conditioning can inhibit vigorous running and jumping response trigger by footshock [15] as well as shock-induced defensive fight [16].

It is noteworthy that our aversive threshold procedure involved a stepwise increase in the electrical stimulation of the DPAG. According to this procedure, as the electrical intensity increased, the animals suddenly stopped, became immobile and often urinated and defecated. With higher intensities, freezing behavior was followed by vigorous running and jumps. Therefore, freezing behavior always preceded the occurrence of escape defensive reactions during the DPAG electrical stimulation procedure. Since animals exposed to the contextual cues previously associated with footshock were already in a freezing posture, one would expect that aversive threshold among these animals would be lower than in control non-shocked animals. However, our results indicated that although context fear conditioning in fact induced a defensive freezing response, the DPAG electrical stimulation to induce a flight response suffered an increase instead of a decrease. Therefore, defensive freezing triggered either by contextual cues associated with footshock or by electrical stimulation of the DPAG might have different natures. This suggestion is in agreement with the notion that there are at least two different patterns of inhibitory defensive behavior with distinct pathways [2,17]: one related to the ventral portion of the periaqueductal gray (VPAG) and the other linked to the DPAG [3]. It has been reported that VPAG electrolytic lesions disrupt freezing to contextual cues associated with footshock but do not affect freezing induced by DPAG electrical or chemical stimulation [18]. Moreover, experiments employing the fear-potentiated startle response paradigm indicates that a cue signaling intense but not moderate footshocks involve activation of the DPAG [19]. Finally, freezing induced by electrical stimulation of the VPAG decreases with termination of the stimulation whereas electrical stimulation of the DPAG induced a long-lasting freezing behavior that remains at high levels after the stimulation [20].

Our results also have an immediate consequence on the comprehension about the relationship between anxiety and panic attack in humans. According to evolutionary ap-

proach, anxiety disorders are interpreted as pathologies related to the malfunctioning of distinct defensive systems that were developed through a phylogenetic mechanism [1]. Well-controlled laboratory experiments indicate that there are at least two related but different neural circuitries responsible for the occurrence of opposite patterns of animal defensive behavior. One, related to a defensive freezing posture, involves the activation of the amygdaloid complex and the VPAG. It is possible that the malfunctioning of this defensive system seems to be related to pathological forms of anxiety present in generalized anxiety disorder [1,2]. The other system is responsible for a completely opposite pattern of defensive behavior. It involves extremely vigorous forms of active behavior, such as flight-or-fight reactions. The DPAG is closely associated with this stereotyped active defensive responses and its overactivation appears to be related to panic attack [4–6], the core phenomenon of panic disorder [3]. Since our results indicated that context fear conditioning induced defensive freezing behavior and inhibited active defensive reaction evoked by electrical stimulation of the DPAG it can be implied that generalized anxiety and panic disorder have an inverse relationship. To our knowledge, the present findings represent the first experimental demonstration of inhibitory relationship between two different anxiety-related disorders. In accordance with this view, it has been reported that relaxation therapy employed to reduce anxiety symptoms may precipitate panic attacks [21]. The frequency of panic attacks is higher at the beginning of agoraphobia, when there is little anticipatory anxiety, than in the late phase, when anxiety has fully developed [22]. Pharmacological results also support the suggestion that enhancement of anxiety inhibits the occurrence of panic attacks. For example, clinical studies indicated that 5-$HT_{2A/2C}$ receptor antagonist ritanserin alleviate generalized anxiety symptoms [23], but if anything exacerbate the incidence of panic attacks [24]. On the other hand, panic disorder patients acutely treated with the 5-HT releaser and uptake blocker D-fenfluramine presented a decrease in panic attacks but an increase in anxiety [25]. Therefore, it appears that activation of neural circuitry involved in anxiety might in fact inhibit the incidence of panic attacks.

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.

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