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Evidence for the thalamic targets of the medial hypothalamic defensive system mediating emotional memory to predatory threats

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ABSTRACT

Previous studies from our laboratory have documented that the medial hypothalamic defensive system is critically involved in processing actual and contextual predatory threats, and that the dorsal premammillary nucleus (PMd) represents the hypothalamic site most responsive to predatory threats. Anatomical findings suggest that the PMd is in a position to modulate memory processing through a projecting branch to specific thalamic nuclei, i.e., the nucleus reuniens (RE) and the ventral part of the anteromedial nucleus (AMv). In the present study, we investigated the role of these thalamic targets in both unconditioned (i.e., fear responses to predatory threat) and conditioned (i.e., contextual responses to predator-related cues) defensive behaviors. During cat exposure, all experimental groups exhibited intense defensive responses with the animals spending most of the time in the home cage displaying freezing behavior. However, during exposure to the environment previously associated with a cat, the animals with combined RE + AMv lesions, and to a lesser degree, animals with single AMv unilateral lesions, but not animals with single RE lesions, presented a reduction of contextual conditioned defensive responses. Overall, the present results provide clear evidence suggesting that the PMd's main thalamic targets (i.e., the nucleus reuniens and the AMv) seem to be critically involved in the emotional memory processing related to predator cues.

1. Introduction

Motivated responses such as feeding, reproductive and defensive behaviors are critically important in assuring the survival of the animal, as well as the species. Specifically, defensive reactions are displayed in situations of threat to the physical integrity or survival of the organism, such as predator confrontation and agonistic interactions with animals of the same species (Motta et al., 2009). Predator exposure and predator-derived odors can be highly effective stimuli for eliciting defensive reactions in laboratory rodents, and these have been largely used in neurobiological and behavioral studies of unconditioned and conditioned fear (Apfelbach, Blanchard, Blanchard, Hayes, & McGregor, 2005; Blanchard & Blanchard, 1988).

In this context, a number of studies have provided evidence to elucidate the hypothalamic circuits putatively involved in the integration and expression of innate and learned fear responses to predatory threat (Canteras, Chiavegatto, Valle, & Swanson, 1997; Cezario, Ribeiro-Barbosa, Baldo, & Canteras, 2008; Dielenberg, Hunt, & McGregor, 2001; Staples, Hunt, Cornish, & McGregor, 2005). These studies indicate that predator threats are processed by a distinct medial hypothalamic circuit formed by the anterior hypothalamic nucleus, the dorsomedial part of the ventromedial nucleus, and the dorsal premammillary nucleus – the so-called medial hypothalamic defensive system (Canteras, 2002). These studies further suggest that the dorsal premammillary nucleus (PMd) is one of the most responsive hypothalamic sites during exposure to a predator or its odor, and to a context previously associated with a predatory threat. In line with this view, lesions centered in the PMd severely reduce the defensive responses to both a live cat and its odor (Blanchard et al., 2003; Canteras et al., 1997; Cezario et al., 2008). In addition, pharmacological inactivation of the PMd, immediately before the exposure to a context previously associated with a predator, was able to practically abolish the contextual conditioned responses (Canteras, Kroon, Do-Monte, Pavesi, & Carobrez, 2008; Cezario et al., 2008; Do Monte, Canteras, Fernandes, Assreuy, & Carobrez, 2008).

Anatomical and functional findings have proposed that descending projections from the PMd to the dorsolateral periaqueductal gray are critical for the expression of unconditioned defensive reactions during exposure to a predator or its odor, as well as

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contextual conditioned responses to an environment previously associated with a predator threat (Cezario et al., 2008). Moreover, recent studies have indicated that, besides mediating unconditioned and contextual conditioned responses, PMd projections may influence the mnemonic processes related to the contextual defensive responses to predator cues (Canteras et al., 2008; Do Monte et al., 2008). In such studies, it has been demonstrated that beta-adrenoceptor (Do Monte et al., 2008) or NMDA receptor (Canteras et al., 2008) blockade in the PMd, prior to cat odor exposure, interferes with contextual conditioned responses to cat odor. It has been postulated that the PMd's role in modulating memory processing would involve its projecting branch to two specific thalamic nuclei: the rostral half of the nucleus reuniens (RE) and the ventral part of the anteromedial nucleus (AMv) (Canteras & Swanson, 1992; Risold, Thompson, & Swanson, 1997). In addition, the rostral nucleus reuniens is also targeted by other elements of the medial hypothalamic defensive system, such as the anterior hypothalamic nucleus (Risold, Canteras, & Swanson, 1994; Risold et al., 1997). Therefore, in the present study, we investigated the role of these thalamic targets in both unconditioned (i.e., fear responses to predatory threat) and conditioned (i.e., contextual fear responses to predator-related cues) defensive behaviors.

2. Materials and methods

2.1. Animals

Adult male Wistar rats ($n = 67$), weighing about 250 g and obtained from the local São Paulo breeding facilities, were used in the present study. The animals were kept under controlled temperature (23 °C) and illumination (12-h cycle) in the animal quarters, and had free access to water and standard laboratory diet.

2.2. Ethics

Experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, 1996). All experimental procedures had been previously approved by the Committee on Care and Use of Laboratory Animals of the Institute of Biomedical Sciences – University of São Paulo, Brazil (Protocol No. 084/2005). In the present study, we attempted to minimize the number of animals used and their suffering.

2.3. Surgery

For the lesion procedure, rats were deeply anesthetized with sodium pentobarbital (Cristália; Itapira, SP, Brazil; 40 mg/kg, i.p.) and were placed in a stereotaxic apparatus. Bilateral iontophoretic deposits of a 0.15 M solution of N-methyl-D-aspartate (NMDA, Sigma, St. Louis, MO, USA) were placed in the rostral half of the nucleus reuniens ($n = 10$; coordinates: anteroposterior, 1.50 mm from bregma; laterolateral, 0.0 mm from midline of the sagital sinus; dorsoventral, 6.40 mm from the surface of the brain), in the ventral part of the anteromedial thalamic nucleus uni- ($n = 30$) or bilaterally ($n = 10$; coordinates: anteriorposterior, 1.50 mm from bregma; laterolateral, ±1.0 mm from midline of the sagital sinus; dorsoventral, 6.35 mm from the surface of the brain), in addition to control saline injections that were made in other 17 rats. NMDA deposits were produced over 15 min through a glass micropipette (30 μm tip diameter), using a constant-current device (model CS3, Midgard Electronics, Canton, MA) set to deliver −10 μA, with 7-s pulse and interpulse durations. After a 1-week post-surgical period, the animals were placed in the experimental apparatus.

2.4. Experimental apparatus and procedure

The experimental apparatus was made of clear Plexiglas, and consisted of a 25 × 25 × 25 cm home cage connected to another 25 × 25 × 25 cm chamber (the food compartment) by a hallway 12.5 cm wide and 100 cm long, with 25-cm high walls. Between the home cage and the hallway, there was a sliding door (12.5 cm wide and 26 cm high) that remained closed most of the time—except when the animals were allowed to explore the rest of the apparatus. During 10 days before the testing procedures (habituation period), each animal was isolated and left living in the home cage and, at the beginning of the dark phase, the home cage door was opened, and the animals were allowed to explore the rest of the apparatus and obtain food pellets stored in the food compartment. During all habituation and testing periods, to increase the search for food, all the pellets in the home cage were removed 3 h before the beginning of the dark phase. After the testing procedures, the food pellets were placed back into the home cage. The testing procedure consisted of three phases. All tests, in phases 1–3, consisted of a 10-min observation period during the beginning of the dark phase of the light/dark cycle. During the tests, the animals were recorded using a horizontally mounted video camera, under 50-W red light illumination.

2.4.1. Phase 1 – exposure to the familiar environment

On day ten of habituation, we observed the animals' behavior in a familiar environment. In order to keep the animals active, but not eating, no food pellet was offered.

2.4.2. Phase 2 – cat exposure test

On the 11th day, an adult male cat was placed and held in the food compartment by an experimenter, as the rat's home cage door was opened. Thus, the second phase of the testing procedure involved observation of the animals' behavior during a 10-min cat exposure to a live cat. After the cat was removed at the end of the 10-min period, the hallway and food compartment were cleaned with 5% alcohol and dried with paper towels.

2.4.3. Phase 3 – context exposure test

On the day after cat exposure, the 10-min test period permitted observation of animals in the environment where the predator had been previously encountered (i.e., the predatory context). Note that the animals were living in the home cage, and were exposed to the predatory context only after the home cage door had been opened, when the animals were exposed to the part of the apparatus where the predator had been previously encountered. As for the other phases, no food pellet was offered during the test period.

2.5. Behavior analysis

Behaviors were scored by a trained observer using the ethological analysis software 'The Observer' (version 5.0, Noldus Information Technology, Wageningen, Netherlands). The analysis comprised of spatiotemporal and behavioral measurements. The spatiotemporal measurements were the time spent in the home cage, hallway, or the food compartment. The behavioral data were processed in terms of duration (total duration per session). The following behavioral items were encoded: freezing (cessation of all movements, except for those associated with breathing), crouch-sniff (animal immobile with the back arched, but actively sniffing and scanning the environment), stretch postures (consisting of both *stretch attend posture*, during which the body is stretched forward and the animal is motionless, and *stretch approach*, consisting of movement directed toward the food compartment with the animal's body in a stretched position), locomotion, grooming, exploratory up-right position (i.e., animals actively exploring the environment, standing over the rear