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Roles of the anterior basolateral amygdalar nucleus during exposure to a live predator and to a predator-associated context

Ricardo Passoni Bindi[a], Marcus Vinicius C. Baldo[b], Newton Sabino Canteras[a,*]

[a] Dept. Anatomy, Institute of Biomedical Sciences, University of São Paulo, São Paulo, SP 05508-000, Brazil
[b] Dept. Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo, SP 05508-000, Brazil

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ABSTRACT

The basolateral amygdala complex, which includes the lateral, basolateral and basomedial nuclei, has been implicated in innate and contextual fear responses to predator threats. In the basolateral complex, the lateral and posterior basomedial nuclei are able to process predator odor information, and they project to the predator-responsive hypothalamic circuit; lesions in these amygdalar sites reduce innate responses and practically abolish contextual fear responses to predatory threats. In contrast to the lateral and posterior basomedial nuclei, the basolateral nucleus does not receive direct information from predator olfactory cues and has no direct link to the predator-responsive hypothalamic circuit. No attempt has previously been made to determine the specific role of the basolateral nucleus in fear responses to predatory threats, and we currently addressed this question by making bilateral N-methyl-D-aspartate lesions in the anterior basolateral nucleus of the amygdala (BLAa), which is often regarded as being contiguous with the lateral amygdalar nucleus, and tested both innate and contextual fear in response to cat exposure. Accordingly, BLAa lesions decreased both innate and contextual fear responses to predator exposure. Considering the targets of the BLAa, the nucleus accumbens appears to be a potential candidate to influence innate defensive responses to predator threats. The present findings also suggest that the BLAa has a role in fear memory of predator threat. The BLAa is likely involved in memory consolidation, which could potentially engage BLAa projection targets, opening interesting possibilities in the investigation of how these targets could be involved in the consolidation of predator-related fear memory.

1. Introduction

During recent years, a great deal has been learned about the neural systems involved in processing innate and conditioned contextual fear response to predatory threats (see [1]). Of particular relevance for the present account, lesions emcompassing large part of the basolateral amygdalar complex, which includes the lateral, basolateral and basomedial nuclei, have been shown to disrupt unconditioned fear responses elicited by cat fur [2] or cat odor [3]; and pharmacological inactivation studies have shown that the basolateral amygdalar complex is involved in contextual fear memory consolidation to a cat odor-related environment [3].

In the basolateral complex, the lateral and posterior basomedial nuclei are known to receive predator-related kairomone information through projections from the medial amygdalar nucleus [4,5] and up-regulate FOS expression during exposure to a live cat [6]. Notably, the lateral nucleus projects massively to the posterior basomedial nucleus [7], and together are likely to work as a distinct functional unit within the amygdala, providing direct inputs to the hypothalamic predator-responsive circuit [8], which is known to critically influence the expression of anti-predatory defensive responses [1]. Bilateral NMDA cytotoxic lesion confined either to the lateral or the posterior basomedial nuclei reduced innate responses and practically abolished contextual fear responses to predator stimuli [6].

In contrast to the lateral and posterior basomedial nuclei, the basolateral nucleus does not receive direct information from predator olfactory cues and has no direct link to the hypothalamic predator-responsive circuit. Nevertheless, previous findings from our laboratory showed that the anterior basolateral nucleus (BLAa) presented an increase in Fos expression during exposure to a live cat [6] likely related to the fact that major sources of inputs to the BLAa, such as the prelimbic and anterior cingulate areas [9], the dorsal raphe nucleus and the locus coeruleus [10], present significant activation during predator exposure [11–13] and are presumably involved in providing the predator-related attentional and motivational statuses.

However, no attempt was made to determine the specific role of the basolateral nucleus in fear responses to predatory threats. In the present investigation, we addressed this question by making bilateral N-methyl-

* Corresponding author at: Department of Anatomy, Institute of Biomedical Sciences, University of São Paulo, Av. Lineu Prestes, 2415, CEP 05508-900 São Paulo, SP, Brazil.
E-mail address: newton@icb.usp.br (N.S. Canteras).

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D-aspartate (NMDA) lesions in the anterior basolateral nucleus of the amygdala (BLAa), which is anatomically contiguous with the lateral amygdalar nucleus, and then testing innate and contextual fear in response to cat exposure. The results will be discussed in the context of the connective pattern of the BLAa, revealing alternative paths involved in mediating innate and contextual fear of predatory threats.

2. Materials and methods

2.1. Animals

Adult male Wistar rats ($n = 23$), weighing approximately 250 g and obtained from the local breeding facilities, were used in the present study. The animals were maintained under controlled temperature (23 ± 2 °C) and illumination (12 h cycle; lights on – 10 AM/lights off – 10 PM) in the animal quarters, and, before the animals were placed in the experimental apparatus, they 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 The Care and Use of Laboratory Animals of the Institute of Biomedical Sciences–University of São Paulo, Brazil (Protocol number 085/2012). In the present study, the experiments were planned to minimize the number of animals used and their suffering. In addition, all surgical procedures were performed under deep anesthesia, and analgesic and antibiotic medication were given postoperatively.

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 bilaterally centered in BLAa ($n = 15$; coordinates: anteroposterior, −2.2 mm from bregma; lateral, 4.7 mm from the midline of the sagittal sinus; dorsoventral, 6.8 mm from the surface of the brain). In addition, in 8 other animals, control saline injections (sham group) were performed bilaterally at the same coordinates used for the BLAa. NMDA deposits were performed over a 15 min period through a glass micropipette (30 μm tip diameter) using a constant-current device (model CS3, Midgard Electronics, Canton, MA, USA) set to deliver −15 μA, with 7 sec pulse and interpulse durations. Animals received postoperative analgesics (Ibuprofen; Medley; Campinas, SP, Brazil; 30 mg/kg in drinking water) and antibiotics (Pentabiótico©; Zoetis; Campinas, SP, Brazil; 0.1 ml/100 g, i.p.). After a 1-week post-surgical period, the animals were placed in the experimental apparatus and the behaviors were scored during the three phases of the behavioral testing.

2.4. Experimental apparatus and procedure

The experimental protocol currently used to investigate innate and contextual fear related to predator exposure followed Ribeiro-Barbosa et al. [13]. The experimental apparatuses were made of clear Plexiglas. Each consisted of a 25 × 25 × 25 cm^3 home cage connected to another 25 × 25 × 25 cm^3 chamber (the food compartment) by a hallway 12.5 cm wide and 100 cm long, with walls 25 cm high. Between the home cage and the hallway, there was a sliding door (12.5 cm wide and 26 cm high), which was opened when the animals were allowed to explore the rest of the apparatus. During 9 days, each animal was isolated in the home cage, and, at the beginning of the dark phase, the animals were allowed to explore the rest of the apparatus and obtain food pellets stored in the food compartment. The testing procedure consisted of three phases of a 10 min observation period, during the beginning of the dark phase of the light/dark cycle.

Phase 1. After the habituation period, on the 10th day, animals were allowed to explore the familiar environment, providing a low-defense baseline.

Phase 2. On the 11th day, a neutered 2-year-old male cat was placed and held in the food compartment by an experimenter, and, as the rat's home cage door was opened, they were exposed to a live cat during a 10 min period. During this period, the cat was held by an experimenter and remained relatively calm and quiet without attempting to attack the rat. 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.

Phase 3. On the day after cat exposure, the sliding door was opened, and the animals were exposed to the environment where the predator had been previously encountered, providing high levels of contextual conditioned fear responses.

For all testing periods, the pellets in the home cage were removed 3 h before the beginning of the dark phase and, after the testing periods, placed back into the home cage. During the tests, the animals were recorded using a horizontally mounted video camera, under 50 W red light illumination.

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, The Netherlands). As for the experimental protocol, the behavioral analysis currently used to investigate innate and contextual fear related to predator exposure followed Ribeiro-Barbosa et al. [13]. The analysis comprised of spatiotemporal and behavioral measurements. The spatiotemporal measurements were the amounts of time spent in the home cage, the 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.
- Risk-assessment behaviors: comprising crouch-sniff (animal immobile with the back arched, but actively sniffing and scanning the environment) and 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).
- Fearless exploration: including nondefensive locomotion (locomotion with arched back) and exploratory up-right position (i.e., animals actively exploring the environment, standing over the rear paws and leaning on the walls with the forepaws).

All behavioral scoring was conducted by an observer who was blind to the rat's condition.

2.6. Histology

Upon completion of behavioral testing, all rats were injected with sodium pentobarbital (Cristália; 40 mg/kg, i.p.) and perfused transcardially with a solution of 4.0% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4; the brains were removed and placed overnight in a solution of 20% sucrose in 0.1 M phosphate buffer at 4 °C. The brains were then frozen, and four series of 30 μm sections were cut with a sliding microtome in the frontal plane. One series of sections was mounted on gelatin-coated slides and stained with thionin to serve as the reference series for cytoarchitectonic purposes.