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The differential mice response to cat and snake odor

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HIGHLIGHTS

- We analyzed the differences between mice exposed to two different predator odor.
- Cat odor increased the Fos expression in predator-responsive hypothalamic circuit.
- Snake odor did not change Fos expression in predator-responsive hypothalamic circuit.
- Mice showed defensive behavior in front of cat odor, but not snake odor.
- Snake odor seemed not to be considered a threat to Swiss mouse.

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ABSTRACT

Studies from the last two decades have pointed to multiple mechanisms of fear. For responding to predators, there is a group of highly interconnected hypothalamic nuclei formed by the anterior hypothalamic nucleus, the ventromedial hypothalamic nucleus and the dorsal premammillary nucleus — the predator-responsive hypothalamic circuit. This circuit expresses Fos in response to predator presence or its odor. Lesion of any component of this system blocks or reduces the expression of fear and consequently defensive behavior when faced with a predator or its cue. However, most of the knowledge about that circuit has been obtained using the rat as a model of prey and the cat as a source of predator cues. In the present study, we exposed mice to strong cat or snake odors, two known mice predators, and then we used the rat exposure test (RET) to study their behavior when confronted with the same predator's odor. Our data point to a differential response of mice exposed to these odors. When Swiss mice were exposed to the cat odor, they show defensive behavior and the predator-responsive hypothalamic circuit expressed Fos. The opposite was seen when they faced snake's odor. The acute odor exposure was not sufficient to activate the mouse predator-responsive hypothalamic circuit and the mice acted like they were not in a stressful situation, showing almost no sign of fear or defensive posture. This leads us to the conclusion that not all the predator cues are sufficient to activate the predator-responsive hypothalamic circuit of mice and that their response depends on the danger that these predators represent in the natural history of the prey.

1. Introduction

Fear and anxiety are adaptative responses of animals against a real or ambiguous threat. When this threat becomes a matter of life and death, the physiological and behavioral responses must be fast and precise.

Independent of the source, fear-triggered behaviors, such as freezing, fleeing and flight, are very similar among the animal species, and because of this, early theories of fear processing pointed to a single response mechanism [1,2,3]. However, evidence accumulated over the past two decades suggests that there are at least 3 parallel circuits that process distinct types of fear: fear of pain, fear of aggressive conspecifics and fear of a predator [4].

Fear of pain is directed against physically harmful or aversive stimuli; fear of aggressive conspecifics (social fear) is a reaction to an

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aggressive social interaction; and fear of a predator is directed against predators or their cues [4]. An important distinction is made between innate fear responses that are activated by intrinsically threatening stimuli, and learned fear that is elicited by neutral stimuli that have been associated with innate threat.

Since an encounter with a predator can be the last moment of an animal, this powerful selective pressure has generated in some species a neural system whose objective is not only to react appropriately faced with predators, but also to avoid them. The now-called predator-responsive circuit is composed of a predator-sensitive amygdala, a predator-responsive hypothalamic circuit and a column of the periaqueductal gray matter. The posteroventral division of the medial nucleus of the amygdala (processing predator odor), the lateral amygdala, and the posterior division of the basolateral nucleus of the amygdala (processing predator polimodal sensory cues) project to a highly interconnected predator-responsive hypothalamic circuit. The anterior hypothalamic nucleus (AHN), dorsomedial division of the ventromedial nucleus of the hypothalamus (VMHdm) and dorsal premammillary nucleus (PMd) of this circuit process not only the sensorial but also the contextual cues before sending them to the dorsolateral column of the periaqueductal gray matter, responsible for the execution of the defensive reactions [5,6,7]. Studies using Fos as a marker of neuronal activity show that all this circuitry is activated when the animals are in the presence of a predator, its cue, or in a context associated with predator exposure [8,9,10,11].

Neuroanatomical and ethological studies of the past two decades seem to have established a predator-responsive circuit. The question is if it works in a similar way in different species? And with different predators? Since most knowledge about neurobiology of predator fear has been acquired using rats as prey and a cat or its smell as the predator cue [8,12,11,13,14] we chose to study this circuit in a different species of rodent exposed to different sources of predator cues.

2. Material and methods

2.1. Animals

Healthy adult Swiss mice, male, weighing about 36 g, were housed four per cage until the time of the experiments in the animal care facility of our institution. The animals were maintained on a 12-h light/dark cycle (lights on at 6 a.m.) in a temperature-controlled environment (22 ± 2 °C) and were given free access to food and water. All experiments were carried out in accordance with the guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996) and by the Federal University of Rio Grande do Norte Committee for Research and Animal Care (CEUA-UFRN; protocol 018/2009). We attempted to minimize the number of mice used and every effort was made to ensure that no mouse suffered unnecessarily.

2.2. Analysis of Fos expression

2.2.1. Acute odor exposure

For 4 days, 21 mice, housed individually, had the bedding in their cages changed for a cleaning once daily, at 4 p.m. (2 h before the dark period). On day 5, the animals were randomly separated into 3 groups of 7: CT mice had their bedding changed for a clean bedding again; CO mice had their bedding changed for bedding with cat odor (acquired by rubbing a towel on cat fur for 1 min); SO mice had their bedding changed for bedding with snake odor (acquired from a bedding where a snake (*Boa constrictor*) had been for five days and including little pieces of snake skin). All the groups were made in different months in order for one do not to affect the others.

2.2.2. Perfusion and imunohistochemistry

Ninety minutes after the odor exposure, the mice were deeply anesthetized with an intraperitoneal injection of Ketamine 10% (0,2 mL/10 g,

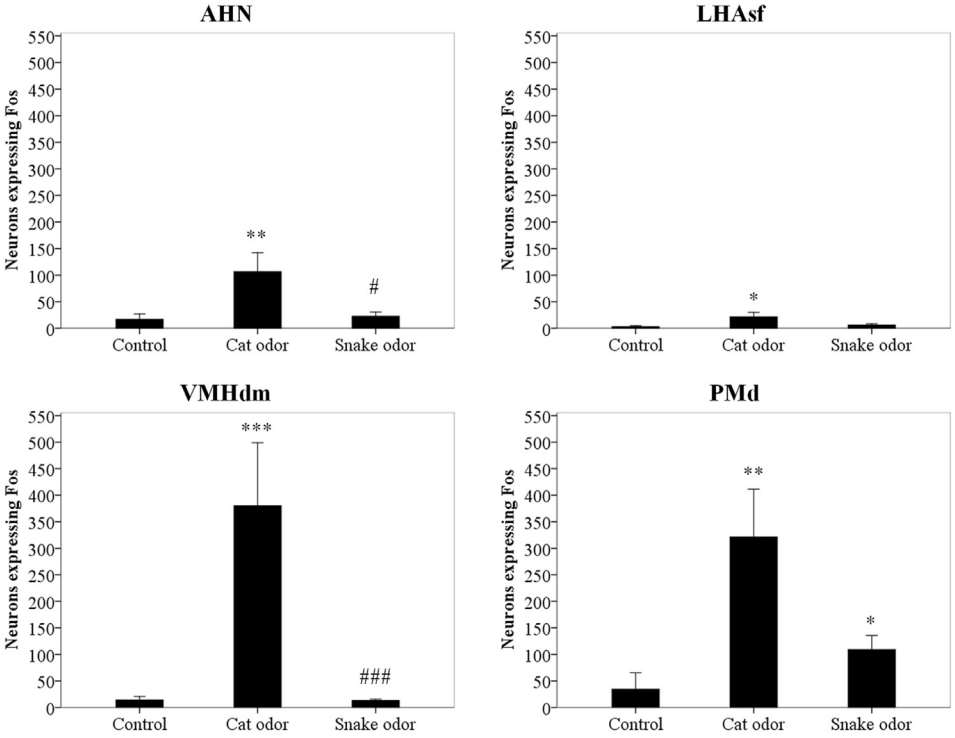

Fig. 1. Bar graphs showing the number of Fos-expressing neurons in the anterior hypothalamic nucleus (AHN), subfornical region of the lateral hypothalamic area (LHAsf), dorsomedial division of the ventromedial nucleus of the hypothalamus (VMHdm) and the dorsal pre-mammillary nucleus (PMd) exposed to different sources of predator odor. Values expressed as mean ± S.E.M. (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001 compared to the control group. #p ≤ 0.05; ##p ≤ 0.01; ###p ≤ 0.001 compared to cat odor group).