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Brain Research 1043 (2005) 145–154
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Research report

Mapping the neural circuit activated by alarm pheromone perception by c-Fos immunohistochemistry

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Accepted 23 February 2005
Available online 1 April 2005

Abstract

We previously reported that the alarm pheromones released from stressed male rats exaggerated both behavioral and autonomic (stress-induced hyperthermia) responses in recipient rats that were introduced into a novel environment. Subsequent experiments provided evidence that these alarm pheromones could be divided into two functionally different categories based on the site specificity and testosterone dependency of their production. However, the neural mechanisms underlying these behavioral and physiological responses remain unknown. In the present study, we examined Fos expression in 26 brain sites of the recipient rat 60 min after the exposure to the pheromone that aggravated stress-induced hyperthermia. The alarm pheromone-exposed rats showed a concurrent increase in Fos expression, in contrast to control odor-exposed rats in the anterior division lateral and medial group of the bed nucleus of the stria terminalis, paraventricular nucleus, dorsomedial hypothalamic nucleus, anterodorsal medial, lateral and basolateral amygdaloid nucleus, ventrolateral periaqueductal gray, laterodorsal tegmental nucleus, and locus coeruleus. These results provide information about the neural mechanisms in response to a non-sexual pheromone, i.e., an alarm pheromone, and suggest that the perception of the alarm pheromone is related to stress-responsive brains structures, including the hypothalamus and brainstem, as well as to the amygdaloid nuclei.
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Theme: Other systems of the CNS
Topic: Limbic system and hypothalamus

Keywords: Alarm pheromone; Stress-induced hyperthermia; Brain mapping; Rat; Medial amygdala; The bed nucleus of the stria terminalis

1. Introduction

Chemical communication plays important roles in various social interactions among mammals, including sexual [52], territorial [20], and maternal behaviors [33]. Alarm chemosignals, which alert animals to the proximity of conspecific individuals [1,4,53], are considered to be used widely in the animal kingdom. In rodent species, it was reported that rats could distinguish between the odors released from stressed and non-stressed conspecifics [51]. These odors were shown to change the behavior [1,34,35] and immune responses of the recipient [11].

We previously reported that the alarm pheromones released from male rats receiving foot shocks augmented both behavioral (increased sniffing, walking and rearing, and decreased resting behavior) and autonomic (stress-induced hyperthermia, SIH) responses in recipient male rats [26]. We then found that these alarm pheromones could be divided into two functionally different categories based on the androgen-dependent production and the area of the body surface from which they were released, namely, one category of alarm pheromone modifying recipient behavior is released from the whisker pad of the male in a testosterone-dependent manner, whereas the other category of alarm pheromone influencing the autonomic response is released from the perianal region in a testosterone-independent manner [27,28]. Considering that the importance of propagating the notice of a dangerous situation to family or

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0006-8993/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.02.061

group members is not limited to male and that the intensity of SIH reflects the animal's anxiety status [32], it appears reasonable to postulate that the testosterone-independent pheromone that aggravates SIH is biologically more important as compared with the other one. We have therefore decided to focus more attention on this category of alarm pheromone in our subsequent investigation including the present study.

Although behavioral and physiological responses to alarm pheromones have been clearly demonstrated [27–29], the neural mechanism underlying these responses remains largely unknown, with the exception of our preliminary finding that exposure to alarm pheromones increased Fos protein expression in the mitral cell layer of the accessory olfactory bulb [26]. Along with responses elicited due to intra-species chemical communication, a fear or anxiety response has also been shown to be elicited by inter-species chemical communication, e.g., by being exposed to predator odor. It is well known that cat odor can innately elicit a fearful response and risk assessment behaviors in rats [3,37]. C-Fos immunohistochemistry has been most widely used for functional anatomical mapping in laboratory animals, and previous reports have suggested that several brain regions play especially important roles in the stress response in reaction to fear and anxiety [2,31,47]. Recently, a systematical brain mapping study was conducted in rats that had been exposed to the cat odor using c-Fos immunohistochemical analysis [14,38]. It is therefore of interest to compare the neural circuit activated by intra-species chemical communication (e.g., via an alarm pheromone from conspecifics) with that activated by inter-species chemical communication (e.g., by predator odor).

In the present study, we examined Fos expression in 26 brain regions of subjects 60 min after exposure to an alarm pheromone that aggravated the autonomic response [26–28]. To the best of our knowledge, this is the first report to examine Fos expression in response to a non-sexual pheromone in rats. In comparison with the results obtained using control animals, we attempted to map the neural circuit activated by the perception of the alarm pheromone.

2. Experimental procedures

2.1. Animals

Experimentally naïve male Wistar rats were purchased from Clea Japan (Tokyo, Japan) and were used at the age of 10 weeks. Three animals per cage were housed under constant temperature (24 ± 1 °C) and humidity (45 ± 5%) until they were implanted with a telemetry transmitter (see below). Food and water were available ad libitum, and the animals were maintained under a 12:12-h light/dark cycle (lights on at 0800) throughout the experiment. The animals were cared for in accordance with "Policies Governing the Use of Live Vertebrate Animals," set by the University of Tokyo, and based on "The Public Health Service Policy on Humane Care and Use of Laboratory Animals" (revised in 1985) and "The National Institutes of Health's Guide for the Care and Use of Laboratory Animals." The subject animals were implanted with a telemetry transmitter (TA10TA-F40; Data Sciences International, St. Paul, MN) intraperitoneally under anesthesia with ether 10–11 days before the experiment. After surgery, the animals were housed individually on an antenna board (RLA1020 RPC-1; Data Sciences International) in a soundproof chamber (36 cm × 54 cm × 35 cm; Muromachi Kikai, Tokyo, Japan) located in another room that was maintained at a constant temperature (22 ± 1 °C) under a 12:12-h light/dark cycle (lights on at 0800). All animals were handled for 5 min each day beginning 6 days before the experiment, and then each animal was exposed to the test box (see below) for 20 min each day beginning 4 or 5 days before the experiment in order to minimize the effects of novelty stress.

2.2. Pheromone exposure

The general procedures were basically the same as those used in our previous study [27]. Adult male Wistar rats were selected as the pheromone donors and were anesthetized with sodium pentobarbital (50 mg/kg ip, Nembutal; Abbott Laboratories, North Chicago, IL). Anesthetized donor rats bearing two intra-dermal needles (27 G) for electrical stimulation of the neck or perianal region were placed in the acrylic test box (27.5 cm × 20 cm × 27 cm) for 15 min; during this period, the donor rats received 15 electrical stimulations (10 V for 1 s) at 1 min intervals. The electrical stimulation to the perianal region induced the alarm pheromone release that aggravated the SIH response in the other rats [27]. A box in which the neck region of the donor had been electrically stimulated was used as a control box in an attempt to provide the subject with similar amount of the olfactory stimuli derived from the body surface following local stimulation. The neck region was chosen based on our previous finding that the presence of this odor in the test box modify neither the SIH nor behavioral response as compared with the one that were exposed to odors released from the donor receiving no stimulation [27].

After stimulation, the donor rat was removed and the empty box was brought into the room in which the subject animals were maintained after the surgery. Then, the test box was installed on an antenna board in the soundproof chamber and the body temperature (BT) and behavioral responses of each subject rat were recorded. To ensure stable baselines for BT, subject rats were placed in the test box only after showing a BT of less than 37.5 °C for at least 5 min before the experiment in their homecage, and then they were held there for 60 min. The subject rats were randomly assigned to one of two treatments groups, i.e., exposed to either an alarm pheromone (n = 7) or a control odor (n = 8). After being placed in the test box, the BT and

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