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Research report

Neural mechanisms of predatory aggression in rats—Implications for abnormal intraspecific aggression

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HIGHLIGHTS

- We studied the neural background of predatory aggression in rats.
- We quantified c-Fos expression in 15 aggression-related brain regions.
- The neural substrates of predatory aggression are similar in rats and cats.
- Predatory and abnormal social aggression are controlled by similar mechanisms.

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ABSTRACT

Our recent studies showed that brain areas that are activated in a model of escalated aggression overlap with those that promote predatory aggression in cats. This finding raised the interesting possibility that the brain mechanisms that control certain types of abnormal aggression include those involved in predation. However, the mechanisms of predatory aggression are poorly known in rats, a species that is in many respects different from cats. To get more insights into such mechanisms, here we studied the brain activation patterns associated with spontaneous muricide in rats. Subjects not exposed to mice, and those which did not show muricide were used as controls. We found that muricide increased the activation of the central and basolateral amygdala, and lateral hypothalamus as compared to both controls; in addition, a ventral shift in periaqueductal gray activation was observed. Interestingly, these are the brain regions from where predatory aggression can be elicited, or enhanced by electrical stimulation in cats. The analysis of more than 10 other brain regions showed that brain areas that inhibited (or were neutral to) cat predatory aggression were not affected by muricide. Brain activation patterns partly overlapped with those seen earlier in the cockroach hunting model of rat predatory aggression, and were highly similar with those observed in the glucocorticoid dysfunction model of escalated aggression. These findings show that the brain mechanisms underlying predation are evolutionarily conservative, and indirectly support our earlier assumption regarding the involvement of predation-related brain mechanisms in certain forms of escalated social aggression in rats.

1. Introduction

The general functions of aggression fall into two main categories, particularly social competition and predation [1–3]. The first entails conspecifics, which fight for access to resources in a broad meaning (e.g. food, territory, social rank). This form of aggression is associated with high physiological arousal, and covers social communication. For example, threat signals convey information on 'attack intentions'; moreover, threats may replace actual fights by the process of ritualization. In contrast, predation aims at killing an individual that belongs to a different species. This behavior is associated with minimal arousal, and does not involve social communication. These two forms of aggression are controlled by distinct neural circuits as shown by feline stimulation studies [4–6]. Based on phenomenological and physiological similarities, these types of aggressive behavior were proposed to be analogous with particular forms of psychopathological human aggression [7–10]. For example, exacerbated affective aggression is seen in intermittent explosive disorder, which is a violent response to a perceived threat. Other forms of pathological aggression, e.g. those seen in antisocial personality disorder, have different characteristics: such aggression is often gain-oriented, and is associated with limited

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emotional arousal and low intention signaling [7–9]. The phrase 'predatory aggression' is frequently used to emphasize these characteristics [11–13].

In recent years, the differentiation of the two types of aggression and the idea that they are governed by distinct neural mechanisms gained attention in both human and animal research. In humans, a psychiatric inventory was developed to differentiate reactive and proactive aggression [14], and current theories deal with their neural underpinnings differentially [15,16]. We recently developed two laboratory models that mimic important characteristics of affective/reactive and instrumental/proactive forms of aggression, and proposed behavioral methods to differentiate species-typical and abnormal forms of aggression [17–20]. Importantly for the present study, we found that rats submitted to one of these models – the glucocorticoid dysfunction model of abnormal aggression – deliver bites to vulnerable body parts of conspecifics (head, throat and belly), which is accompanied by low intention signaling by threats, disturbed social behavior, and reduced autonomic arousal, features that are in many respects to similar to the symptoms of aggressive antisocial personality disordered subjects [17–20]. We found that in this model, aggressive encounters increase the activation of the lateral hypothalamus, central amygdala and ventral periaqueductal gray (PAG) above the levels seen in controls submitted to fights (i.e. these regions were overactivated) [4,18,19,21,22]. Moreover, the activation of the central amygdala and lateral hypothalamus correlated significantly with the share of abnormal, predatory-like attacks in this model [22]. As the very same brain regions were shown to control predatory attacks in cats [1,4–6], we proposed that antisocial-like aggressiveness in rats has a 'predatory dimension' as it regards both behavior and brain function. Unfortunately, however, the brain mechanisms of predatory aggression are less well known in rats than in cats. Early electrophysiological studies identified the hypothalamic locus of control of frog and mouse killing in rats but in contrast to cats, such studies provided limited information on other modulatory brain regions [23–26]. More recently, neural mechanisms were evaluated in rats by using c-Fos immunohistochemistry to investigate brain activation patterns of cockroach hunting as a model of predatory aggression [27,28]. In these studies, food intake inherently associated with cockroach hunting was carefully controlled. While the activated brain areas overlapped in many respects with the circuitry that controls predation in cats, important differences were also observed. For example, the lateral hypothalamus showed similar levels of activation in insect hunting rats and their feeding controls, despite the fact that this brain area is considered the most important control region of rat killing in cats and frog and mouse killing in rats [1,4,29]. According to our own observation, muricide and insect hunting are behaviorally different, which may explain these discrepancies. As such, studies using muricide as a model seem necessary to fully understand the brain mechanisms of predatory aggression in rats, but such studies are unavailable at present.

Here we investigated c-Fos activation in 15 aggression-related brain regions in adult male Wistar rats that spontaneously killed a mouse in their home-cage. Rats which did not attack the mice and rats without mouse exposure were used as controls. This study was motivated by multiple goals. Firstly, we aimed at describing brain activation patterns associated with muricide, a work that has not been performed so far. Secondly, we aimed at comparing these findings with those obtained earlier in cat stimulation studies to establish the cross-species stability of predation-related brain mechanisms. We also aimed at comparing findings with those obtained in the cockroach-hunting model, to investigate the impact of the pray on brain mechanisms. Finally, we aimed at providing a more proper comparison for the recently described "predatory-like aggression network" activated in the aforementioned model of violent social aggression [22].

2. Material and methods

2.1. Animals

Subjects were adult male Wistar rats raised in the breeding facility of our Institute. Parents were obtained from Charles River Laboratories (Germany). Rats were housed in macrolon cages in groups of 4–6. Food and water were available ad libitum throughout, temperature and relative humidity was kept at $22 \pm 2\,^{\circ}C$ and $60 \pm 10\%$, respectively. Rats were maintained in a light cycle of 12:12 h with lights off at 1000 h. The weight of subjects was 350–450 g when behaviorally tested. Behavioral tests were conducted in the early phase of the dark period, under dim red illumination. 50–70 days-old male CD1 mice from the same source were used as stimulus animals. Mice were housed in a different room, but otherwise were maintained under similar conditions. The experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were reviewed and approved by the Animal Welfare Committee of the Institute of Experimental Medicine.

2.2. Experimental procedure

Subjects were housed individually for one week before behavioral testing but otherwise were maintained under the same conditions as earlier. The experiment was started by placing a mouse in the home-cage of the rat. Subjects have never encountered a mouse before. If the rat killed the mouse, the latency to kill was recorded, the killed mouse was removed immediately, and at the same time, another, uninjured mouse was removed from the home cage of a randomly chosen rat to assure that the time of stimulation/interaction was equal between groups ("muricide" vs. "no muricide" control). The cut-off time for mouse-killing was 20 min. On each experimental day, control rats unexposed to mice ("no mouse" control) were also randomly chosen from rats that were not exposed to mice to assess baseline c-Fos activity. The experiment was continued until sample sizes reached 7 per group.

2.3. Brain processing and immunohistochemistry

Rats were left undisturbed for 120 min after the encounters to allow c-Fos signal to develop. Afterwards, they were deeply anesthetized by an i.p. injection of a mixture of ketamine, xylazine and pipolphen (5, 10 and 5 mg/kg, respectively) and perfused through the ascending aorta with 100 ml ice-cold 0.1 M phosphate-buffered saline followed by approximately 200 ml 4% paraformaldehyde dissolved in 0.1 M phosphate-buffered saline. The brains were removed, post-fixed in the same solution for 3 h and cryoprotected overnight by 20% sucrose in phosphate-buffered saline at $4\,^{\circ}C$. 30 μm frozen sections were cut in the frontal plane on a sliding microtome. The c-Fos protein was labeled with a rabbit polyclonal antibody raised against the amino terminus of c-Fos p62 (Santa Cruz Biotechnology, USA, sc-52) as described earlier [20,22]. The primary antibody (1:5000) was detected by biotinylated anti-rabbit goat serum (1:1000; Jackson Laboratories, USA) and avidin–biotin complex (ABC, 1:1000; Vector Laboratories, Burlingame, CA, USA). The peroxidase reaction was developed in the presence of diaminobenzidine tetrahydrochloride (0.2 mg/ml), nickel–ammonium sulphate (0.1%) and hydrogen peroxide (0.003%) dissolved in Tris buffer.

Table 1 shows the brain structures investigated in the present study; anteroposterior levels and frame sizes are also shown. The number of investigated levels depended on the length of the particular brain region. At each level, the c-Fos signal was counted bilaterally, and the average of counts was considered. Section planes were standardized according to the atlas of