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Short communication

c-Fos activation and intermale aggression in rats selected for behavior toward humans

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HIGHLIGHTS

- The neuronal activation pattern in tame and aggressive rats after exposure to R–I test was studied.
- Social encounter caused similar brain activation pattern in rats of selected lines.
- Activation was shown in bed nucleus, hypothalamic attack area and medial amygdala.
- Activation of the hypothalamic attack area was higher in aggressive males then in tame rats.

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ABSTRACT

Tame and aggressive rat lines were created during the long-term selection of Norway rats for elimination and enhancement of aggressiveness toward humans, respectively. Our previous experiments have demonstrated that selection for the elimination of defensive aggression toward humans is associated with attenuated intraspecific intermale aggression. However, the neuronal mechanisms underlying low and high intermale aggression in the tame and aggressive rats remain unclear. Here, we used c-Fos immunoreactivity to evaluate neuronal activation patterns in the main aggression-related areas in selected lines under basal conditions and after the resident–intruder (R–I) test. Although agonistic behavior of the tame and the aggressive rats differed significantly, social encounter caused similar brain activation patterns in both groups; we observed increased neuronal activation in the bed nucleus of stria terminalis, the hypothalamic attack area, and the medial amygdala 1 h after the R–I test. However, neuronal activation in the hypothalamic attack area was significantly higher in the aggressive males compared to their tame counterparts. We propose that lower activation of the hypothalamic area is associated with the attenuation of intraspecific intermale aggression during selection for the elimination of aggressiveness toward humans.

Immediate early gene expression (e.g., c-Fos) is a widely used functional marker of neuronal activation and is often employed to identify cells and brain circuits that respond to various stimuli [4,2,5]. Assessment of immediate early gene expression has been used to provide an accurate account of brain structures involved in the regulation of aggressive behavior. The study of c-Fos activation in rat and mice lines selected for behavior is of particular interest, and these studies have revealed genetically determined neuronal mechanisms underlying different types of stress-induced behavior. Intraspecific confrontation has been used as a biologically and ecologically relevant model of social stress in laboratory conditions [12]. c-Fos protein immunohistochemistry studies have demonstrated that agonistic confrontation causes differential brain activation in rats selected for high and low anxiety that differed in aggressive behavior [23], and in mice selected for long and short attack latency [8]. Another valuable model that can show either intense or low aggression is the Norway rat, which has been selected for elimination (tame) and enhancement (aggressive) of aggressiveness toward humans using the glove test [13,17,18]. This selection regime also affected physiological and neurochemical characteristics—notably those closely associated with specific behavioral responses and stress hormones [13,17,14]. Moreover, tame rats differ from aggressive rats in that they have increased serotoninergic activity [13,19].

Abbreviations: BNST, bed nucleus of stria terminalis; CeA, central nucleus of the amygdala; CoA, cortical amygdala; DR, dorsal raphe nucleus; HAA, hypothalamic attack area; HAB, high-anxiety behavior; LAB, low-anxiety behavior; LS, lateral septum; MeA, medial nucleus of the amygdala; MR, medial raphe nucleus; PAG, periaqueductal gray matter; PVN, paraventricular nucleus; R–I, resident–intruder.

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Our previous experiments have demonstrated that selection for elimination of aggressiveness toward humans is associated with the attenuation of intraspecific intermale aggression. Tame rats display lower intermale aggression than both aggressive and unselected rats; there is no significant difference in intermale aggression between aggressive and unselected rats [18]. However, the neuronal mechanisms underlying intermale aggression in tame and aggressive rats remain unknown. The current work sought to investigate the neural background of agonistic behavior in tame and aggressive rats by using c-Fos immunohistochemistry to assess neural activation.

Experiments were carried out with adult male gray rats (*Rattus norvegicus*) selected for 78 generations for elimination (tame) and enhancement (aggressive) of aggressiveness toward humans. The neutral opponents in the resident–intruder (R–I) test were naïve adult male Wistar rats from the IC&G animal facility. Rats from different lines were housed in separate rooms. After weaning on the 28th day, rats were housed in metal cages (50 cm × 33 cm × 20 cm) in groups of four. Animals were kept under standard laboratory conditions in a natural light–dark cycle of 11:13 h with free access to food and water. Experiments were carried out in the light phase of the day between 14:00 and 18:00. All experiments were performed according to the Guide for the Care and Use of Laboratory Animals approved by the Ministry of Public Health of Russia (Supplement to order No. 267 of June 19, 2003).

In order to investigate whether line differences in aggression are accompanied by differences in neuronal activation, aggressive ($n = 8$) and tame ($n = 8$) males from different litters were housed in observational cages. After 1 week of single housing, males from each line were divided into two equal groups – control and experimental. Males in the experimental group were exposed to a standard 10-min R–I test [9]. One hour later, they were deeply anesthetized with pentobarbital (40 mg/kg) and perfused intracardially with 0.1 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Males from control groups were anesthetized and perfused without agonistic confrontation. Their brains were removed, post-fixed overnight in 4% paraformaldehyde and cryoprotected by soaking in 20% sucrose in PBS at 4 °C for 5 days. Coronal sections were cut at 40 μm using a cryostat. Immunohistochemistry was carried out on free-floating sections with a validated protocol [8,6]. The c-Fos protein was labeled with a rabbit polyclonal antibody (ab53036; Abcam, Cambridge, UK). The primary antibodies (1:200) were detected with biotinylated anti-rabbit goat IgG from a rabbit specific horseradish-peroxidase/diaminobenzidine detection kit (ab64261, Abcam). Section planes were standardized according to the atlas of Ref. [16].

Microscopic analyses were conducted using the "Axioskop" 2 Plus software (Zeiss, Oberkochen, Germany). Images were digitized with a high-resolution color CCD-camera AxioCam HRc (Zeiss, full resolution 3600 × 3030 pixels), and the number of positive cells was counted by means of the AxioVision software (Zeiss). Specific brain regions were selected based on evidence from previously published studies regarding encounter-related brain activation [6,15,24]. A standard, area-specific frame was used to outline regions of interest [6,2]. All areas were analyzed bilaterally in two parallel sections except the dorsal and medial raphe (where the analysis was performed in the midline). The lateral septum (LS) was a rectangular region ($10 \times 10^4\ \mu m^2$) at the level where the anterior commissure lies below the lateral ventricle (interaural 9.20). The bed nucleus of stria terminalis (BNST) was a circular area ($9.7 \times 10^4\ \mu m^2$) dorsolateral to the anterior commissure in the same level. The paraventricular nucleus (PVN) was divided into two circular parts – lateral (magnocellular; $4 \times 10^4\ \mu m^2$) and dorsal (parvocellular; $4 \times 10^4\ \mu m^2$) – and one triangular part (medial, parvicellular $3.3 \times 10^4\ \mu m^2$). The cortical amygdala (CoA) was an oval region ($12 \times 10^4\ \mu m^2$) medial to the cortex–amygdala transition zone at the same level as the PVN (interaural 9.20). The central nucleus of the amygdala (CeA) was counted where the optic tract was medially close (oval frame, $3.37 \times 10^4\ \mu m^2$). This plane (interaural 6.20) was also used to analyze the hypothalamic attack area (HAA, oval frame $6.5 \times 10^4\ \mu m^2$), and the medial nucleus of the amygdala (MeA, oval frame $0.65 \times 10^4\ \mu m^2$). The periaqueductal gray matter (PAG, interaural $13.6 \times 10^4\ \mu m^2$) was counted directly lateral to the aqueductus (rectangular frame $5.49 \times 10^4\ \mu m^2$; interaural 1.36), and the dorsal raphe nucleus (DR, oval frame $4.04 \times 10^4\ \mu m^2$) and medial raphe nucleus (MR, oval frame $7.9 \times 10^4\ \mu m^2$) were analyzed in the same level [6,2]. For semiquantitative evaluation, the following rating scale was used: 3 – intense; 2 – moderate; 1 – weak; and 0 – no reactivity. Quantitative analysis was performed in regions where semiquantitative evaluation revealed significant differences between groups [6]. The attack latency in the R–I test was analyzed using the nonparametric Kruskal–Wallis one-way analysis of variance (ANOVA) with the rat line as a factor. Interline differences were analyzed using Mann–Whitney *U* test. c-Fos activation data were analyzed using a two-way ANOVA; factor 1 was rat line, and factor 2 was fighting experience. Fisher post hoc comparisons were made if significant differences were observed.

In the present study, tame males were characterized by the attenuation of intermale aggression in comparison with their aggressive counterparts. Attack latency in the R–I test was significantly higher in the tame rats compared with the aggressive ($H(1, n = 8) = 9.54$, $p = 0.002$). Semiquantitative evaluation of c-Fos activation (Table 1) showed that aggressive interactions induced activation in the BNST, HAA, MeA, and CoA in both lines. Aggressive interactions induced 'moderate' c-Fos activation in the BNST and MeA and 'intense' activation in the CoA in the tame and aggressive males. However, there were differences in HAA c-Fos activation after the R–I test between rats from selected lines. HAA activation after aggressive interactions was 'very intense' in the aggressive and 'intense' in the tame rats. No changes in c-Fos activation were observed in other regions following confrontation in either rat line.

Quantitative analysis was performed for areas where large differences between fight-exposed and control animals were observed. It confirmed the qualitative evaluation data (Fig. 1). In the BNST, HAA, MeA, and CoA, c-Fos intensity was dependent on the agonistic encounter ($F(1,12) = 23.35$, $p < 0.001$; $F(1,12) = 61.72$, $p < 0.001$; $F(1,12) = 16.47$, $p = 0.001$; $F(1,12) = 4.61$, $p = 0.05$, respectively). The BNST did not show line differences, but it was strongly activated by agonistic encounters in both lines. Agonistic

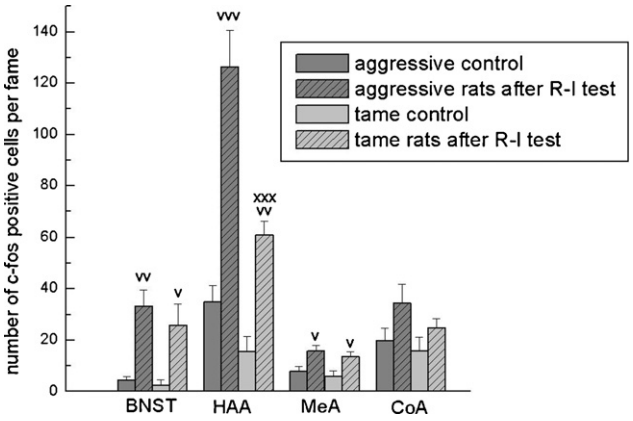

Fig. 1. The number of c-Fos positive cells in the bed nucleus of stria terminalis (BNST), hypothalamic attack area (HAA), medial (MeA) and cortical (CoA) amygdala in the tame and the aggressive rats after agonistic confrontation in the resident–intruder test and without it. $^{xxx}p < 0.001$ in comparison with aggressive rats (Fisher post hoc), $^{v}p < 0.05$, $^{vv}p < 0.01$, $^{vvv}p < 0.001$ in comparison with rats not exposed to resident–intruder test (Fisher post hoc).