Expression of Serotonin Transporter Gene and Startle Response in Rats with Genetically Determined Fear-Induced Aggression

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The study was carried out on Norway rats selected through more than 60 generations for high and low fear-induced aggressiveness towards humans (Institute of Cytology and Genetics). The intensity of aggressive behavior towards man, reflex startle response, and expression of serotonin transporter (5-HTT) gene were studied. Selection for high aggression was associated with more intense startle response. The expression of 5-HTT gene in the frontal cortex was reduced significantly in rats selected for high aggression to humans in comparison with nonaggressive rats. The authors conclude that 5-HTT is involved in the regulation of genetically determined fear-induced aggression.

Key Words: *serotonin; serotonin transporter; gene expression; fear-induced aggression; startle response*

Aggressiveness is the most intensely studied form of behavior, because it underlies asocial and criminal behavior of humans [5]. Numerous studies demonstrated the important role of the serotonin (5-HT) system of the brain in the regulation of animal [13] and human [4] aggressive behavior. Serotonin transporter (5-HTT) plays an extremely important role in the regulation of the function of the brain 5-HT system. The majority of serotonin molecules released in response to a nerve pulse are inactivated by active reuptake from the synaptic cleft into terminal of the serotonin neuron. The reuptake mechanism realized by 5-HTT maintains a certain concentration of the neurotransmitter in the synaptic cleft and at the same time allows repeated use of the same serotonin molecules. In addition, 5-HTT is a molecular target for clinically effective antidepressants [3].

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Despite extremely high significance of 5-HTT, knockout of this gene is not lethal, but leads to significant changes in 5-HT level and metabolism and in behavior. In 5-HTT knockout mice, he level of extracellular serotonin is elevated and hence, the densities of some serotonin receptors and expression of their genes are reduced [6] and the intensity of male-male aggression is lower [8]. Treatment with 5-HTT blockers also leads to desensitization of some serotonin receptor subtypes [11]. All these facts suggest an important role of 5-HTT in the regulation of emotional status and behavioral forms associated with anxiety and fear. One of these forms of behavior is fear-related startle response to an acoustic stimulus and rat aggression towards humans (a form of fear-induced aggressive behavior).

Very little is known about the involvement of 5-HTT in the regulation of fear-induced aggression, but we previously showed that the level of 5-HT, sensitivity and density of 5-HT_{1A} receptors, and expression of the corresponding genes are signi-

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ficantly decreased in the brain of rats selected for high aggression towards humans [14,15].

Here we studied the startle response and expression of 5-HTT gene in the brain of rats selected for high aggression towards humans and nonaggressive rats.

MATERIALS AND METHODS

Experiments were carried out on Norway rats (*Rattus norvegicus*) selected at Institute of Cytology and Genetics over 60 generations for high aggressiveness towards humans and its absence [2,12]. The rats were kept in metal cages (50×33×20 cm) under standard laboratory conditions at natural light with free access to water and food. Three days before the experiment, the animals aged 6 months (300-350 g) were placed into individual cages for elimination of the group effects. All experimental procedures were carried out in accordance with Guidelines for Use of Animals in Neuroscience Research (1992).

Aggressiveness was evaluated by the intensity of aggressive reaction to humans by the glove test using a 5-point scale [12]: 0) the rat allowed being taken without attempts to avoid it; 1) the rats allowed being taken and moved chaotically when taken; 2) the rats ran away from the hand and frantically tried to get free if taken; 3) the rat actively resisted being taken, could squeak loudly, opened its mouth or bit the hand; 4) the rat did not allow being taken and squeaked loudly.

The fear was evaluated by the intensity of startle response caused by an acoustic signal by means of SR-Pilot (San Diego Instruments Inc.). Three minutes after the rat was placed into the device, 4 acoustic signals were presented at 30-sec intervals. The intensity of startle response in each animal was calculated as the mean of 4 measurements.

In order to evaluate the expression of 5-HTT gene, the rats were decapitated and the brain was transferred onto ice, the frontal cortex, hippocampus, and midbrain were isolated, frozen in liquid nitrogen, and stored at -70°C until RNA isolation.

The expression of 5-HTT gene was evaluated as described previously [1,9,10] using DNA-dependent RNA polymerase (rPol II) mRNA as the internal standard and mouse genome DNA of a known concentration as the external standard. The primers for amplification of 5-HTT cDNA (F 5'-gttacgccatc tgcatcatcgc and R 5'-cagcgtccaggtgatgttgtcc) were developed on the base of published sequence [7] using the Nucleotide EMBL database and synthesized at the Biosan Company. The PCR was carried out according to the following protocol: 3 min at 94°C (1 cycle); 10 sec at 94°C, 30 sec at 64°C (for rPol II) or 63°C (for 5-HTT), and 15 sec at 72°C: 25 cycles for 5-HTT and rPol II in the midbrain, 26 cycles for rPol II in the frontal cortex and hippocampus, or 32 cycles for 5-HTT in the frontal cortex and hippocampus; 2 min at 72°C (1 cycle).

The expression of 5-HTT gene was shown as the number of cDNA transporter copies per 100 copies of rPol II cDNA.

The data were expressed as *m±SEM* and compared using unifactorial analysis of dispersions.

RESULTS

The rats selected for high aggressiveness exhibited more pronounced aggressive reaction in the glove test in comparison with nonaggressive rats ($F_{1.113}$= 2035.8; p<0.001). The mean score was 3.9±0.04 for highly aggressive and 0.50±0.07 for nonaggressive animals.

Highly aggressive and nonaggressive rats differed significantly by the intensity of the startle response to the acoustic signal (Fig. 1). The rats selected for high fear-induced aggression demonstrated 2-fold higher startle amplitude in comparison with the nonaggressive animals ($F_{1.62}$=10.26; p<0.01). This, no doubt, indicated that selection for high level of fear-induced aggression not only led to more pronounced aggressive behavior, but also stimulated the fear reaction.

A significant reduction of 5-HTT mRNA level in the frontal cortex was detected in rats selected for high fear-induced aggression in comparison with nonaggressive rats ($F_{1.15}$=22.13; p<0.001). No appreciable differences between these groups of

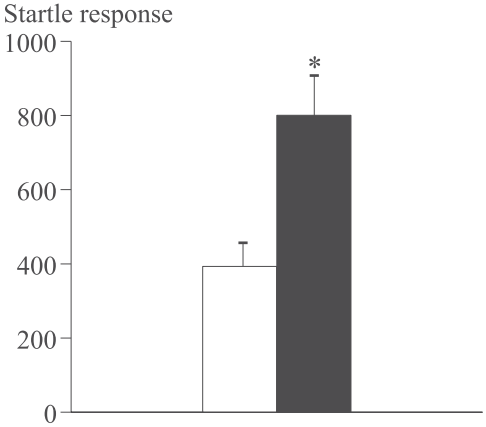

Fig. 1. Intensity of startle response to an acoustic signal in aggressive and nonaggressive rats. Here and in Fig. 2: light bars: nonaggressive rats (n=8); dark bars: aggressive rats (n=8). Startle amplitude (ordinate) is presented in arbitrary units and calculated as the means of 4 measurements. *p<0.01 compared to nonaggressive rats.

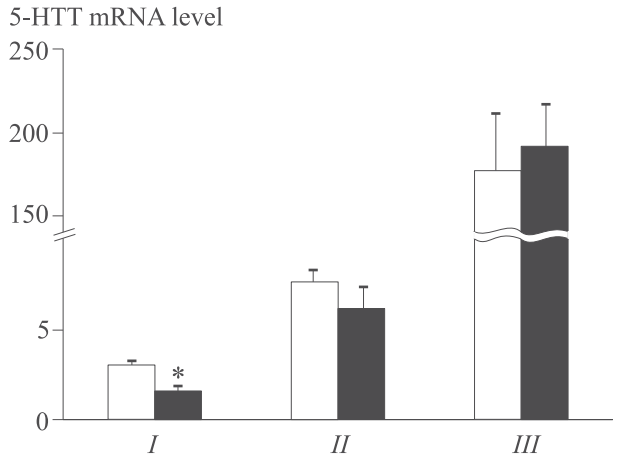

Fig. 2. Level of 5-HTT mRNA in brain structures of highly aggressive and nonaggressive rats. I) frontal cortex; II) hippocampus; III) midbrain. The level of 5-HTT mRNA is expressed as the number of transporter cDNA copies per 100 copies of rPol II cDNA. *$p<0.001$ compared to nonaggressive rats.

animals were detected for the 5-HTT mRNA levels in the hippocampus and midbrain (Fig. 2). As 5-HTT gene is expressed only in the midbrain (which is confirmed by reduction of its level in the structures distant from the midbrain), presumably, rat selection for fear-induced aggression leads to changes in the genetic system, reducing the 5-HTT mRNA life span. The detected reduction of 5-HTT gene expression is in good agreement with the data on reduced level of 5-HT, desensitization and reduced density of 5-HT_{1A} receptors, and expression of their gene in the brain of highly aggressive rats [14,15], but does not agree with the data obtained on mice with 5-HTT genetic knockout [6]. However, different changes in 5-HTT status in rats and mice eventuated in similar consequences. An increase of serotonin level, induced by 5-HTT gene knockout in mice, led to a reduction of the level of male-male aggression [8]. On the other hand, increase of serotonin level, caused by long selection for reduced level of fear-induced aggression, led to an increase of 5-HTT gene expression. This can be explained by differences in the regulation of aggressive behavior in mice and rats or by differences in the two forms of aggressive behavior (aggression towards humans in rats and male-male aggression in mice). However, the effect of serotonin on aggressive behavior is inhibitory in both cases.

Hence, genetically determined fear-induced aggression is linked with intensification of the startle response and reduced expression of 5-HTT gene. Selection for high level of fear-induced aggression leads to a reduction of 5-HTT mRNA level in the frontal cortex, which presumably leads to changes in the transporter content. In addition, it is obvious that genetically determined high defense aggression is associated with inhibition of the entire serotonin system. This is confirmed not only by reduced expression of 5-HTT gene, but also by reduced level of 5-HT [14], low sensitivity and density of 5-HT_{1A} receptors [15] in the brain of rats selected for high level of aggression towards humans.

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REFERENCES

1. V. S. Naumenko and A. V. Kulikov, *Mol. Biol.*, **40**, 37-44 (2006).
2. E. M. Nikulina, P. M. Borodin, and N. K. Popova, *Zh. Vyssh. Nervn. Deyat.*, **35**, 703-709 (1985).
3. E. L. Barker and R. D. Blakey, *Psychopharmacology. The Fourth Generation of Progress*, Eds. F. E. Bloom and D. J. Kupfer, New York (1995), pp. 321-333.
4. M. E. Berman, J. I. Tracy, and E. F. Coccaro, *Clin. Psychol. Rev.*, **17**, No. 6, 651-665 (1997).
5. D. C. Blanchard, M. Hebert, and R. J. Blanchard, *Animal Models of Human Emotion and Cognition*, Eds. M. Haug and R. E. Whalen, Washington D. C. (1999), Vol. 3, pp. 297-316.
6. R. R. Gainetdinov and M. G. Caron, *Annu. Rev. Pharmacol. Toxicol.*, **43**, 261-284 (2003).
7. B. J. Hoffman, E. Mezey, and M. J. Brownstein, *Science*, **254**, 579-580 (1991).
8. A. Holmes, D. L. Murphy, and J. N. Crawley, *Psychopharmacology* (Berl.), **161**, No. 2, 160-167 (2002).
9. A. V. Kulikov and V. S. Naumenko, *New Messenger RNA Research Communications*, Ed. L. B. Kwang, N. Y. (2007), pp. 53-68.
10. V. S. Naumenko, D. V. Osipova, E. V. Kostina, and A. V. Kulikov, *J. Neurosci. Methods*, **170**, No. 2, 197-203 (2008).
11. T. Pejchal, M. A. Foley, B. E. Kosofsky, and C. Waeber, *Br. J. Pharmacol.*, **135**, No. 5, 1115-1122 (2002).
12. I. Plyusnina and I. Oskina, *Physiol. Behav.*, **61**, No. 3, 381-385 (1997).
13. N. K. Popova, *Complex Brain Functions: Conceptual Advances in Russian Neuroscience*, Eds. R. Millar *et al.* (1999), pp. 317-329.
14. N. K. Popova, A. V. Kulikov, E. M. Nikulina, *et al.*, *Aggress. Behav.*, **17**, 207-213 (1991).
15. N. K. Popova, V. S. Naumenko, I. Z. Plyusnina, and A. V. Kulikov, *J. Neurosci. Res.*, **80**, No. 2, 286-292 (2005).