Behavioural Brain Research 343 (2018) 102–110

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Genetically defined fear-induced aggression: Focus on BDNF and its receptors

Tatiana V. Ilchibaeva*,[1], Anton S. Tsybko[1], Rimma V. Kozhemyakina, Elena M. Kondaurova, Nina K. Popova, Vladimir S. Naumenko

The Federal Research Center Institute of Cytology and Genetics, The Siberian Branch of Russian Academy of Sciences, Lavrentyeva av.10, Novosibirsk, 630090, Russia

ARTICLE INFO

Keywords:
BDNF
proBDNF
BDNF pro-peptide
Full-length TrkB receptor
Truncated TrkB receptor
$p75^{NTR}$ Receptor
Expression
Fear-induced aggression

ABSTRACT

Brain-derived neurotrophic factor (BDNF), its precursor proBDNF, BDNF pro-peptide, BDNF mRNA levels, as well as TrkB and $p75^{NTR}$ receptors mRNA and protein levels, were studied in the brain of rats, selectively bred for more than 85 generations for either the high level or the lack of fear-induced aggressive behavior. Furthermore, we have found that rats of aggressive strain demonstrated both high level of aggression toward humans and increased amplitude of acoustic startle response compared to rats selectively bred for the lack of fear-induced aggression. Significant increase in the BDNF mRNA, mature BDNF and proBDNF protein levels in the raphe nuclei (RN), hippocampus (Hc), nucleus accumbens (NAcc), amygdala, striatum and hypothalamus (Ht) of aggressive rats was revealed. The BDNF/proBDNF ratio was significantly reduced in the Hc and NAcc of highly aggressive rats suggesting prevalence of the proBDNF in these structures. In the Hc and frontal cortex (FC) of aggressive rats, the level of the full-length TrkB (TrkB-FL) receptor form was decreased, whereas the truncated TrkB (TrkB-T) protein level was increased in the RN, FC, substantia nigra and Ht. The TrkB-FL/TrkB-T ratio was significantly decreased in highly aggressive rats suggesting TrkB-T is predominant in highly aggressive rats. The $p75^{NTR}$ expression was slightly changed in majority of studied brain structures of aggressive rats. The data indicate the BDNF system in the brain of aggressive and nonaggressive animals is extremely different at all levels, from transcription to reception, suggesting significant role of BDNF system in the development of highly aggressive phenotype.

1. Introduction

Brain-derived neurotrophic factor (BDNF) is one the best-studied and well-characterized neurotrophins, realizing many crucial functions within the central nervous system (CNS). The BDNF plays a key role both in physiological processes, such as neuronal maturation and synaptic plasticity, and in mechanisms underlying a number of psychiatric disorders [1]. The polyfunctionality of the BDNF is based on its structural and functional complexity that is displayed in the synthesis of diverse precursor isoforms (pre-pro-BDNF, proBDNF) and in the ability to act via two different receptors (TrkB and $p75^{NTR}$) regulating the opposite effects. The pre-pro-BDNF is cleaved into the pro-BDNF [2] that preferentially binds with the low-affinity neurotrophin $p75^{NTR}$ receptor. The proteolytic cleavage of the pro-BDNF produces functionally active N-terminal pro-domain (BDNF pro-peptide) realizing its action via the $p75^{NTR}$ receptor [3] and the mature BDNF that binds to the receptor tyrosine kinase TrkB [4]. While the BDNF–TrkB pathway has been implicated in neuronal growth and survival, the proBDNF via $p75^{NTR}$ receptors promotes apoptosis [5]. It should be taken into account that, in addition to the full-length TrkB (TrkB-FL), there exists a truncated isoform (TrkB-T) [6]. By heterodimerization with TrkB-FL, the truncated form of TrkB receptor acts as a dominant-negative inhibitor of BDNF signaling [7]. TrkB-T has other BDNF-dependent and BDNF-independent functions, such as BDNF sequestration and translocation, induction of neurite outgrowth and regulation of cytoskeletal changes in non-neuronal cells [6]. Hence, the pro-peptide, proBDNF, and mature BDNF and their receptors play bidirectional role in synaptic plasticity and cell survival, and the balance between BDNF isoforms and TrkB-FL/TrkB-T/$p75^{NTR}$ receptors activation is crucial for determining BDNF activity [4,8].

There are certain data indicating involvement of the BDNF in the mechanisms of aggressive behavior. Studies involving rodent models of

* Corresponding author at: Laboratory of Behavioral Neurogenomics, The Federal Research Center Institute of Cytology and Genetics, The Siberian Branch of Russian Academy of Sciences, Lavrentyeva av.10, Novosibirsk, 630090, Russia.
E-mail address: rbicehok@mail.ru (T.V. Ilchibaeva).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbr.2018.01.034
Received 8 September 2017; Received in revised form 30 January 2018; Accepted 31 January 2018
Available online 06 February 2018
0166-4328/

BDNF deficiency, including knockout ($BDNF^{+/-}$) and conditional knockout ($BDNF^{2L/2LCk-Cre}$ and $BDNF^{2L/1LNes-cre}$), showed increased intermale aggression [9–11]. In addition, BDNF $^{-/-}$ knockout in hippocampal CA3 area of mice caused elevation of aggression, compared to wild-type animals [12], suggesting an important role of the hippocampal BDNF in the control of aggressive behavior.

In contrast, a significantly higher BDNF protein level was demonstrated in the hippocampus, frontal cortex and striatum of AB-Halle (ABH) mice, characterized by a high level of isolation-induced aggression, compared to closely related but nonaggressive AB-Gatersleben (ABG) mice [13]. Moreover, central BDNF administration produced asocial behavior (aggressive attacks towards juvenile mice) in AKR mice [14].

There are a number of works devoted to the study of expression and/or the BDNF levels in the context of repeated aggression, as well as in the situation of victory or defeat in agonistic interactions. Aged dominant CD-1 mice showed increased BDNF and TrkB mRNA levels in the subventricular zone and hippocampus [15,16]. The BDNF mRNA level is increased in the raphe nuclei area of mice, 20-times winners in daily agonistic interactions [17]. Also, syrian hamsters, who won in battles, had significantly higher BDNF mRNA levels in the dentate gyrus of the dorsal hippocampus than the losers [18]. The increase of the BDNF level in the hypothalamus of dominants was also promoted by social isolation and depleted environment (although in the enriched environment, the baseline level of the BDNF also increases) [19].

Some studies on humans indicate a cross-relation between the BDNF and aggressive behavior, although the extant data are contradictory. An association between the aggressive behavior and the BDNF Met allele in Val66Met polymorphism in a population of schizophrenic patients was revealed [20]. However, the other studies did not confirm the data [21,22]. At the same time, an association between adolescent aggression and Val66Met polymorphism was found [23].

Therefore, the data on the implication of the BDNF in the regulation of aggressive behavior are scarce and contradictory. Moreover, there is a lack of data on the cross-relation between genetically determined aggression and BDNF expression.

Long-lasting selective breeding of wild rats for the high level or for the lack of aggressive response to humans led to development of two strains absolutely different in aggressive behavior – completely non-aggressive, “tame” rats and demonstrating maximal scores in the handling test of highly aggressive rats. It should be noted that extremely aggressive behavior towards humans is accompanied by enhanced aggressiveness in zoosocial interactions [24]. Recently we have revealed increased predatory and pathological (infanticide) aggression, as well as decreased social interaction in highly aggressive rats [25]. Additionally, highly aggressive rats demonstrated impaired learning in the Morris water maze [26] and increased amplitude of the acoustic startle response, reflecting elevation of fear and anxiety [27–29].

Previously we have found some differences in BDNF gene expression, as well as in the protein level in the midbrain, hippocampus and frontal cortex between highly aggressive and nonaggressive rats [30]. However, taking into account complexity of the BDNF system it is need to investigate both BDNF and its isoforms more detailed in majority of brain structures directly involved in the regulation of aggressive behavior. Extremely interesting to understood of how isoforms of the TrkB receptor, as well as the $p75^{NTR}$ receptor, are involved in the regulation of genetically defined fear-induced aggressive behavior, since the balance between the BDNF isoforms and the corresponding receptors is crucial for BDNF activity. These findings can provide a more complete picture of the state of the BDNF system in highly aggressive rats.

Thus, the aim of our study was to investigate the expression of the BDNF and its receptors in the brain structures of Norway rats selectively bred for either a high level or the lack of fear-induced aggression. For this purpose, we examined the BDNF, TrkB and $p75^{NTR}$ mRNA levels, as well as proBDNF, BDNF pro-peptide, BDNF, TrkB-FL/TrkB-T and p75 ^{NTR} protein levels in the frontal cortex, hippocampus, amygdala, nucleus accumbens, hypothalamus, striatum, substantia nigra and raphe nuclei area of the midbrain in genetically defined highly aggressive and nonaggressive rats.

2. Materials and methods

2.1. Animals

The experiments were carried out on adult male Norway rats (*Rattus norvegicus*), selectively bred for 85 generations for either a high level or the lack of fear-induced aggression at the Institute of Cytology and Genetics, Novosibirsk (Naumenko et al., Plyusnina and Oskina) [31,32]. The animals were housed in steel cages (50 × 33 × 20 cm) under standard laboratory conditions in a natural light-dark cycle (12 h light and 12 h dark) with free access to water and food in groups of four individuals. Two days before the experiments, 6 month-old rats weighted 300–350 g (20 animals of each strain) were isolated into individual cages to rule out the group effect. All the experimental procedures were in compliance with the Guidelines for the Use of Animals in Neuroscience Research, 1992. All efforts were made to minimize the number of animals used and their suffering. The study was carried out on the base of the ICG SD RAS vivarium (RFMEFI61914 × 0005 and RFMEFI61914 × 0010).

2.2. Handling (glove) test

The response to handling by gloved hand was used for estimation of aggressiveness. Two days before the presentation of the glove test, 20 tame and 20 aggressive males rats (experimental group 1) were housed singly in a standard home cage with the front wall opening as a door. A gloved hand was placed into the cage in which a rat was kept, and behavior towards human was estimated by a variations of point scores ranging from −4 to +4 [32]. According to the scores emotional positive/negative response or aggressiveness is estimated, for example, as: +4, the rat freely explores the hand, without displaying negative responses when handled, tolerates handling; −4, the rat remains at the door, emits threatening vocalization, attacks the hand promptly as it starts to approach.

2.3. Startle response

Fear responses were assessed in terms of the intensity of the startle response evoked by an acoustic signal using an SR-Pilot apparatus (San Diego Instruments Inc.) on animals of experimental group 2 (8 tame and 8 aggressive males rats). The apparatus consisted of a chamber of size 15 × 19 × 25 cm. A Plexiglas platform was placed on the chamber floor and was attached to a piezo probe; a loudspeaker was attached to the ceiling and was used to deliver sound signals into the chamber. White noise was initially delivered (65 dB). The rat was placed in the chamber for 3 min for adaptation, after which the main signal (115 dB, 40 msec) was presented. The piezo probe was automatically switched on at the same time, and data from the probe were processed by the built-in computer and the intensity of the startle response was shown on the display of the apparatus, in relative units. Three minutes after the animal was placed in the apparatus, four acoustic signals were delivered with 30-s intervals. The extent of the startle reflex of each animal was calculated as the mean of the four measurements.

2.4. RT-PCR

To prepare the brain samples, the rats of experimental group 1 were decapitated three days after behavioral testing, brains were removed on ice and the hypothalamus, frontal cortex, nucleus accumbens, hippocampus, striatum, amygdala, substantia nigra and raphe nuclei area of the midbrain were dissected according to the coordinates from the online rat brain atlas (https://scalablebrainatlas.incf.org/rat/