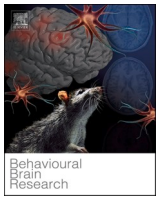
Behavioural Brain Research 438 (2023) 114168

Contents lists available at ScienceDirect

Behavioural Brain Research

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



Research report

On the role of serotonin 5-HT$_{1A}$ receptor in autistic-like behavior: cross talk of 5-HT and BDNF systems

Elena M. Kondaurova, Irina I. Belokopytova, Elisabeth A. Kulikova, Nikita V. Khotskin, Tatiana V. Ilchibaeva, Anton S. Tsybko, Nina K. Popova, Vladimir S. Naumenko *

Federal research center Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Akad. Lavrentyeva Ave. 10, Novosibirsk 630090, Russia

ARTICLE INFO

Keywords:
Serotonin system
5-HT$_{1A}$ receptor
5-HT$_7$ receptor
BDNF system
TrkB
AAV-mediated overexpression
Autism spectrum disorder
Autistic-like behavior
BTBR mice

ABSTRACT

Autism spectrum disorders (ASDs) are some of the most common neurodevelopmental disorders; however, the mechanisms underlying ASDs are still poorly understood. Serotonin (5-HT) and brain-derived neurotrophic factor (BDNF) are known as key players in brain and behavioral plasticity and interact with each other. 5-HT$_{1A}$ receptor is a principal regulator of the brain 5-HT system, which modulates normal and pathological behavior. Here we investigated effects of adeno-associated-virus–based 5-HT$_{1A}$ receptor overexpression in the hippocampus of BTBR mice (which are a model of autism) on various types of behavior and on the expression of 5-HT$_7$ receptor, proBDNF, mature BDNF, and BDNF receptors (TrkB and p75^{NTR}). The 5-HT$_{1A}$ receptor overexpression in BTBR mice reduced stereotyped behavior in the marble-burying test and extended the time spent in the center in the open field test. Meanwhile, this overexpression failed to affect social behavior in the three-chambered test, immobility time in the tail suspension test, locomotor activity in the open field test, and associative learning within the "operant wall" paradigm. The 5-HT$_{1A}$ receptor overexpression in the hippocampus raised hippocampal 5-HT$_7$ receptor mRNA and protein levels. Additionally, the 5-HT$_{1A}$ receptor overexpression lowered both mRNA and protein levels of TrkB receptor but failed to affect proBDNF, mature BDNF, and p75^{NTR} receptor expression in the hippocampus of BTBR mice. Thus, obtained results suggest the involvement of the 5-HT and BDNF systems' interaction mediated by 5-HT$_{1A}$ and TrkB receptors in the mechanisms underlying autistic-like behavior in BTBR mice.

1. Introduction

An autism spectrum disorder (ASD) is a broad term referring to a condition characterized by a lack of social interaction, repetitive behavior of varying severity, and often learning disabilities. ASD diagnosis is still based on observation of atypical behaviors, with such criteria as persistent deficits in social communication and restricted and repetitive patterns of behavior [1]. Autism is one of the most common diseases among children and the most prevalent neurodevelopmental disorder [2]. Current estimates from the Centers for Disease Control and Prevention (CDC) indicate that one in 59 eight-year-olds has autism [3]. Despite intensive studies by numerous research groups, the mechanisms underlying ASDs remain unknown [4,5].

It is well known that brain serotonin (5-HT) plays an important role in the control of normal and pathological behavior [6–9]. Among many 5-HT receptors mediating 5-HT action on neurons, 5-HT$_{1A}$ receptor is one of the most extensively investigated. This receptor is involved in the regulation of the brain 5-HT system's functional activity [10,11] and various physiological functions [9] as well as in mechanisms of different psychopathologies including depression, anxiety, suicide, and schizophrenia [11–18].

It is known that 5-HT$_{1A}$ receptor participates in the mechanisms underlying social and repetitive behaviors [19,20] as well as learning and memory [21], which are known to be impaired in ASDs. Nonetheless, data on 5-HT$_{1A}$ receptor's role in the pathogenesis of autism are scarce and contradictory. One of the few postmortem studies indicates significant reductions in 5-HT$_{1A}$ receptor-binding density in superficial and deep layers of the cingulate cortex and fusiform gyrus: two regions within an extensive limbic-cortical network that contribute to social-emotional behaviors [22]. In a subsequent study, no difference in 5-HT$_{1A}$ receptor concentration and distribution was found between ASD patients and controls subjects [23]. Later, however, deregulation of

* Correspondence to: Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Akad. Lavrentyeva Ave. 10, Novosibirsk 630090, Russia,.
E-mail address: naumenko2002@mail.ru (V.S. Naumenko).

https://doi.org/10.1016/j.bbr.2022.114168
Received 17 August 2022; Received in revised form 11 October 2022; Accepted 17 October 2022
Available online 21 October 2022

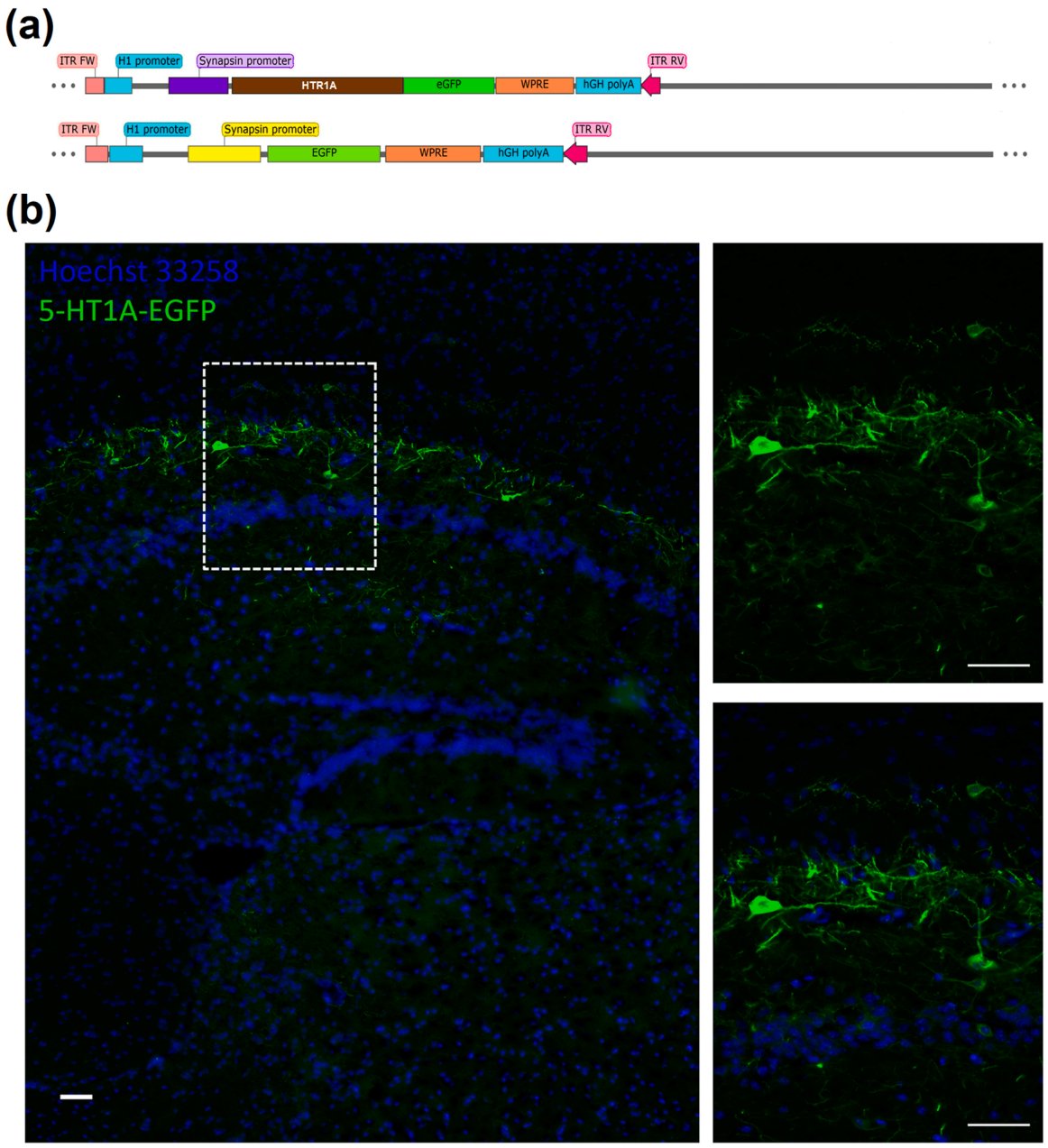


Fig. 1. **(a)** Maps of plasmids AAV-Syn-HTR1A-eGFP and AAV-Syn-eGFP. **(b)** Micrographs of brain slices after AAV-Syn-HTR1A-eGFP injection (scale bar = 100 μm). Cell nuclei were stained with a bis-benzimide solution (Hoechst 33258 dye, 5 μg/ml in PBS, Sigma-Aldrich, Burlington, MA, USA).

5-HT_{1A} receptor density in the striatum of men with an ASD was revealed [24]. Anti–human 5-HT_{1A} antibodies were discovered in the blood of an autistic child, and it was suggested that this finding may have clinical or etiologic significance for this disorder [25]. Other evidence of 5-HT_{1A} receptor's role in the pathogenesis of ASDs comes from pharmacological studies. It has been shown that a 5-HT_{1A} receptor's agonist, 8-OH-DPAT, increases social interaction and improves fear memory extinction in the offspring of a rat model of a valproate-induced ASD; 8-OH-DPAT treatment also reversed the characteristics of miniature excitatory post-synaptic currents as well as paired pulse facilitation observed in lateral amygdala slices [26].

On the other hand, brain-derived neurotrophic factor (BDNF) is now known to take part in the pathogenesis of many disorders of the nervous system [27–33]. Nowadays, this neurotrophic factor is a promising drug target [34–36]. There is some evidence of the involvement of BDNF in the pathogenesis of autism. Several papers indicate high levels of BDNF in the blood and brain in patients with ASDs [37,38]. Genetic research has also linked certain single-nucleotide polymorphism haplotypes of the *BDNF* gene to autism [39]. The interaction between BDNF and the brain 5-HT system as well as the role of this cross talk in different pathologies are well described [40]; however, the interrelations between 5-HT and BDNF systems in autism have not been investigated yet.

The BTBR T^{+} Itpr3tf/J (BTBR) mouse strain is one of the most widely used animal models of autism. These mice have the main behavioral characteristics defining ASDs, including repetitive stereotyped behaviors and impairment of social interactions [41]. The density of 5-HT neurons is reported to be elevated in the midbrain and lowered in the hippocampus of BTBR mice [42]. A year ago, we revealed that a 5-HT_{1A} receptor functional response is significantly reduced in BTBR mice compared to C57BL/6J mice without significant differences in 5-HT_{1A} receptor mRNA and protein levels [43]. It was demonstrated recently that 5-HT_{1A} receptor can form heterodimers with 5-HT_7 receptor that

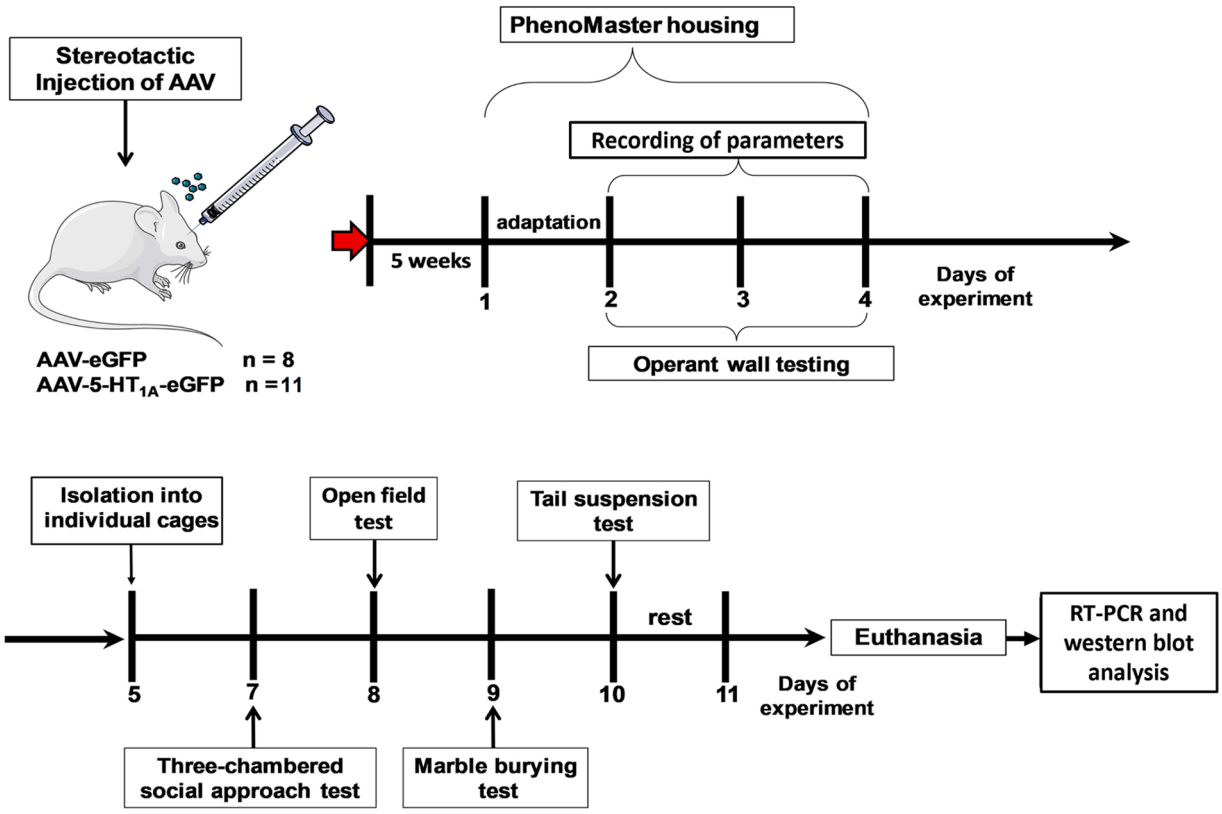


Fig. 2. The experimental design. pAAV-Syn-HTR1A-eGFP (expressing 5-HT_{1A} receptor) was stereotactically administered into the hippocampus of mice of the experimental group. pAAV-Syn-eGFP (expressing only eGFP) was administered into the hippocampus of mice of the control group. Five weeks after recovery (2 days before the behavioral tests), mice were isolated into individual cages. Two days after behavioral testing, the animals were decapitated for subsequent analysis of brain samples.

Table 1
The list of antibodies used and immunodetection conditions.

Antibodies, manufacturer	Dilution	Incubation time, conditions
primary antibodies		
Rabbit polyclonal antibody to 5-HT_{1A} protein, Abcam, Cambridge, United Kingdom, cat. # ab85615	1:1000 in 5% milk powder with TBST	Overnight at 4 °C
Rabbit monoclonal antibody to 5-HT_7 protein, Abcam, Cambridge, United Kingdom, ab128892	1:500 in 5% milk powder with TBST	Overnight at 4 °C
Rabbit antibody to BDNF protein, Abcam, Cambridge, United Kingdom, ab46176	1:1000 in 5% BSA with TBST	Overnight at 4 °C
Mouse antibody to proBDNF protein, Santa-Cruz, Dallas, TX, USA, G-5514	1:250 in 5% milk powder with TBST	Overnight at 4 °C
Rabbit antibody to $p75^{NTR}$ Abcam, Cambridge, United Kingdom, ab38335	1:500 in 5% milk powder with TBST	2 h at room temp.
Rabbit antibody to TrkB Abcam, Cambridge, United Kingdom, ab18987	1:500 in 3% BSA	Overnight at 4 °C
Mouse monoclonal antibody to GAPDH protein, Abcam, Cambridge, United Kingdom, ab8245	1:10000 in 5% BSA with TBST	Overnight at 4 °C
secondary antibodies		
Goat antibody (against rabbit immunoglobulins) conjugated to horseradish peroxidase, Invitrogen, Waltham, MA, USA, G-21234	1:10000 in 5% milk powder in TBST	1 h at room temp.
Goat antibody (against mouse immunoglobulins) conjugated to horseradish peroxidase, Abcam, Cambridge, United Kingdom, ab6728	1:30000 in 5% BSA in TBST	1 h at room temp.

significantly affect 5-HT_{1A} receptor functioning [44] and play an important part in behavioral plasticity [17,45,46]. Consequently, such cross talk of 5-HT_{1A} and 5-HT_7 receptors could also be important for the mechanisms of ASDs, although this notion has not been investigated yet.

According to the above-mentioned data taken together with the crucial function of 5-HT_{1A} receptor in the control of learning and social behavior disrupted in BTBR mice and their stereotyped behavior as well as in the regulation of the brain BDNF system, we decided to investigate the effect of 5-HT_{1A} receptor overexpression in the hippocampus of BTBR mice on their autistic-like behavior and on the expression of 5-HT_7 receptor, proBDNF, mature BDNF, and BDNF receptors (TrkB and $p75^{NTR}$).

2. Materials and methods

2.1. Animals

Experiments were carried out on specific-pathogen-free adult (P60) male mice of the BTBR inbred strain. The mice were housed at the Center for Genetic Resources of Laboratory Animals (unique identifier RFME-FI62119X0023) at the Institute of Cytology and Genetics, the Siberian Branch of the Russian Academy of Sciences (ICG SB RAS), under standard laboratory conditions on a 14/10 h light/dark cycle with water and feed available ad libitum. The primary source of mice was Charles River Laboratories (Wilmington, NC, USA). Two days before a behavioral experiment, the mice weighed 25 ± 1 g and were isolated into individual cages to prevent the group effect. The number of animals per group was 7–10. All experimental procedures were in compliance with the Guide for the Care and Use of Laboratory Animals, Eighth Edition, Committee for the Update of the Guide for the Care and Use of Laboratory Animals; National Research Council (© 2011 National Academy of Sciences;

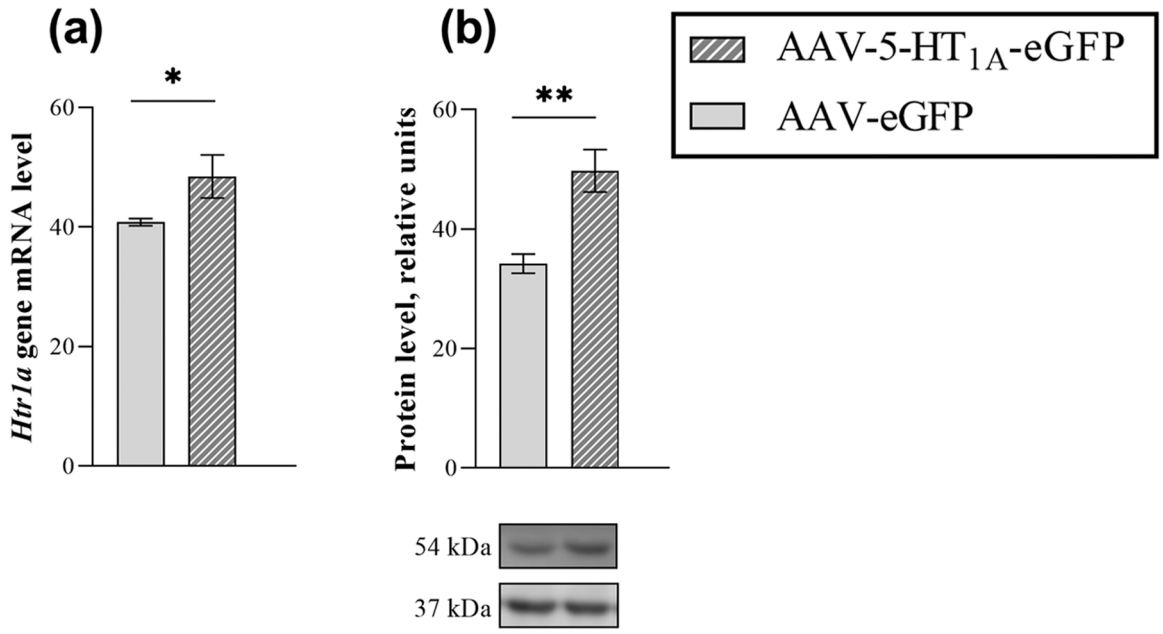


Fig. 3. The influence of AAV-Syn-HTR1A-eGFP administration into the hippocampus on 5-HT_{1A} receptor mRNA (a) and protein (b) levels in the hippocampus of BTBR mice. Gene expression is presented as the number of the gene's cDNA copies per 100 cDNA copies of *rPol2*. The protein was quantitated in chemiluminescence relative units and normalized to GAPDH chemiluminescence relative units. All the values are means ± SEM (n ≥ 7).

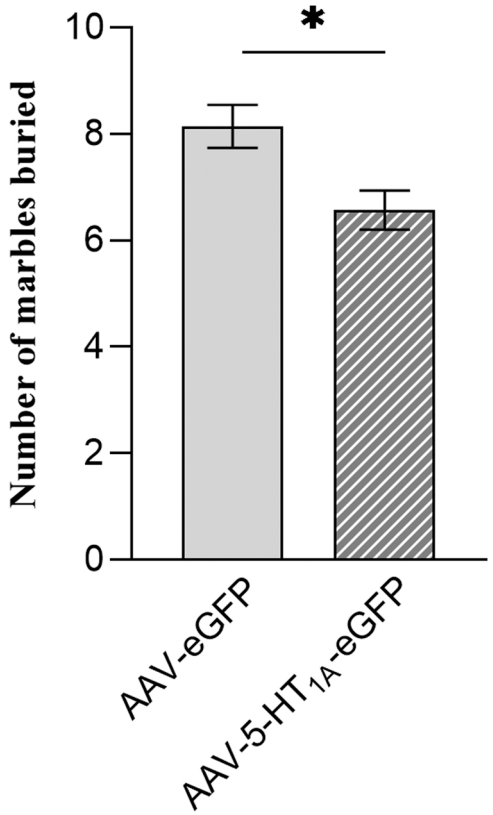


Fig. 4. The impact of the 5-HT_{1A} receptor overexpression in the hippocampus of BTBR mice on behavior in the marble-burying test. All the values are presented as mean ± SEM (n = 7).

Washington, DC, USA) and were approved by the ethical committee of the ICG SB RAS (Protocol No. 106 of 23 November 2021). All surgical procedures were performed under anesthesia, and every effort was made to minimize the suffering of the animals.

2.2. The cell line

HEK 293FT cells (ATCC cat. # PTA-5077, Manassas, VA, USA) were used to produce recombinant adeno-associated virus (rAAV) vectors. The cell line was maintained in DMEM containing 10% (v/v) of FBS (F2442, Sigma-Aldrich, Burlington, MA, USA) and 100 U/ml penicillin/streptomycin (P4333, Sigma-Aldrich, Burlington, MA, USA) at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. The cells were split at 70% confluence, and the culture medium was refreshed every 2 or 3 days.

2.3. Production of rAAV vectors

To estimate the influence of 5-HT_{1A} receptor overexpression on the autistic-like behavior and brain 5-HT and BDNF systems, we employed two types of genetic constructs: pAAV-Syn-HTR1A-eGFP expressing 5-HT_{1A} receptor (for the experimental group) and pAAV-Syn-eGFP expressing only eGFP (for the control group). pAAV-Syn-HTR1A-eGFP was obtained by cloning of the cDNA encoding mouse *Htr1a* into the pAAV-Syn-eGFP vector. Maps of the plasmids AAV-Syn-HTR1A-eGFP and AAV-Syn-eGFP utilized in the current study are displayed in Fig. 1a. The packaging of pAAV-Syn-HTR1A-eGFP or control pAAV-Syn-eGFP plasmids DNA into AAV capsids was performed by cotransfection with plasmids AAV-DJ and pHelper (Cell Biolabs, Inc., San Diego, CA, USA). Viral particles were harvested after 48 h, according to the protocol described by Grimm and coauthors [47]. The number of the obtained viral particles was determined by real-time PCR with the following primer pair: F 5′-CCTGGTTGCTGTCTCTTTATGAGG-3′; R 5′-TGACAGGTGGTGGCAATGC-3′. Serial dilutions of an original plasmid of known concentration served as standards for quantifying the viral particles. The AAV vectors used in this study had similar genomic titers (10^9 viral genomes per microliter).

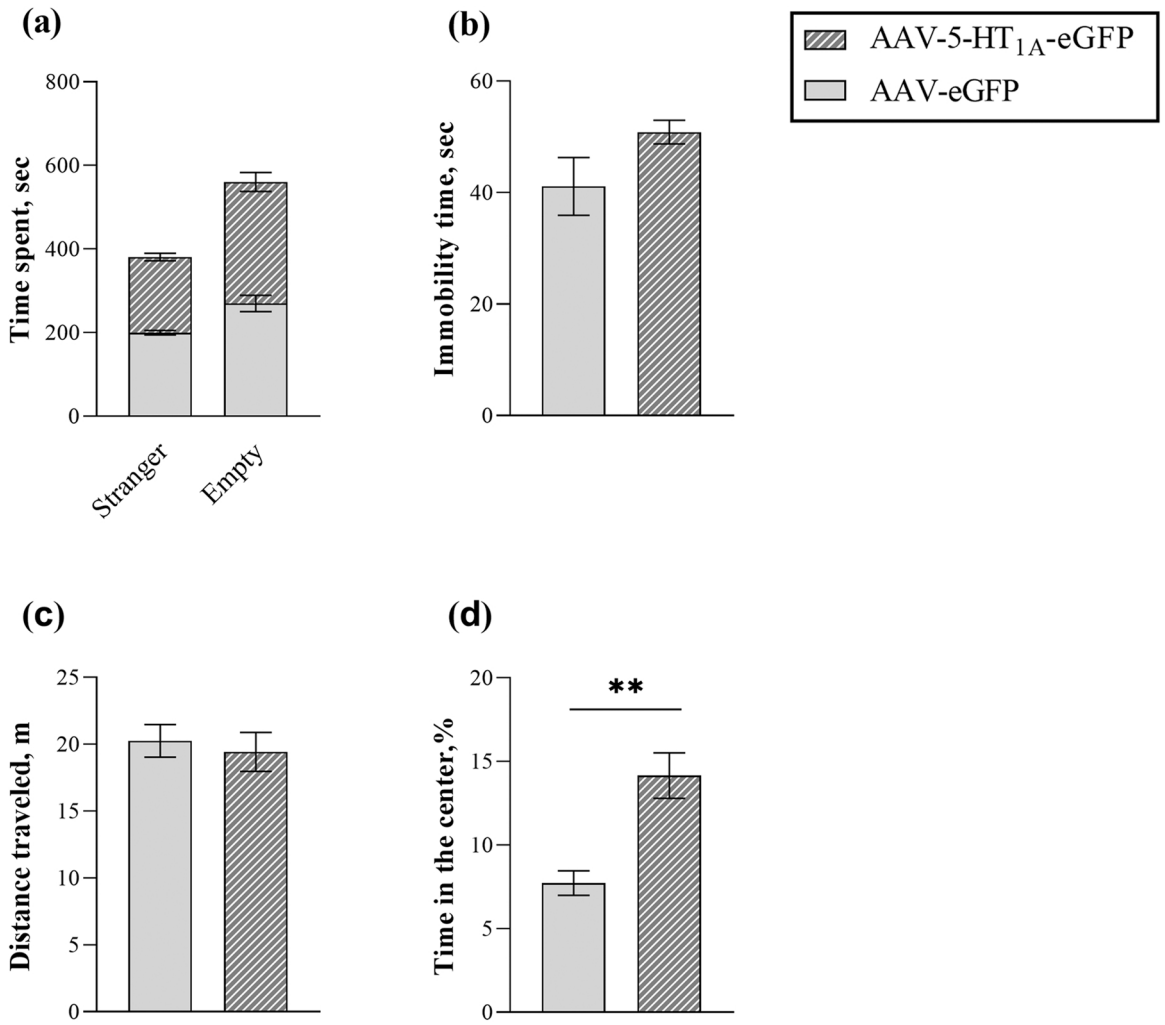


Fig. 5. Effects of the 5-HT_{1A} receptor overexpression in the hippocampus of BTBR mice on behavior in (a) the three-chambered social test, (b) the tail suspension test, on the distance traveled (c) and time spent in the center of the arena (d) in the open field test. All the data are presented as mean ± SEM (n = 8).

2.4. Stereotaxic microinjections

Mice were anesthetized by an intraperitoneally (i.p.) administered solution (1 ml/kg) of 2,2,2-tribromoethanol (T48402–25 G, Sigma-Aldrich, Burlington, MA, USA) in 2-methyl-2-butanol (240486, Sigma-Aldrich, Burlington, MA, USA). The AAV vectors (carrying plasmid pAAV-Syn-HTR1A-eGFP or pAAV-Syn-eGFP) were bilaterally injected into the hippocampus at the following coordinates: AP-1, L± 1, DV 2.5 and AP-2, L± 2, DV 1.5 (according to preliminary experiments and the mouse brain atlas) [48]. A viral vector (0.5 μl, 10^9 ng/μl) was microinjected into the site at the rate of 0.2 μl/min via a Hamilton syringe. The syringe was left in place for 3 min and then removed slowly.

2.5. Behavioral tests

After 5 weeks of postoperative recovery, the mice underwent a behavioral test battery according the experimental design showed in Fig. 2. Daily dynamics of *locomotor activity, sleep,* and *water and feed consumption* were investigated in the PhenoMaster system (TSE, Bad Homburg, Hessen, Germany) according to the manufacturer's instructions. The device consists of seven individual cages equipped with infrared sensors that trace an animal's movements. Drinking bowls and feeders were also equipped with sensors, allowing for accurate measurement of water and feed consumption. The data from the sensors were recorded each minute and processed by the software from the manufacturer. The animals learned to use the drinking bowls and feeders for 2 successive days, and then they were isolated in PhenoMaster cages, and their locomotion, sleep duration, water and feed consumption were registered for 48 h. The first 24 h (1–24 h) were considered an adaptive period and were disregarded. The dynamics of locomotor (m) and exploratory activities (counts) as well as water (ml) and feed (g) consumption were assessed as described elsewhere [49].

The "operant wall" was used to estimate the impact of 5-HT_{1A} receptor overexpression on associative learning in BTBR mice. The "operant wall" unit is a metal wall mounted in each home cage of the PhenoMaster system. There are three recesses and eight light indicators on the wall. One of them lit up when the device was turned on and went out when the animal obtained the maximum number of pellets, thereby signaling the beginning and end of a trial. Other light indicators served as markers that prompted the animal the correct the order of interactions with the "operant wall." Every recess contains a laser sensor that detects the immersion of a mouse nose into it ("nose poke"). To receive the "reward," the animal must perform nose pokes in the correct order, after which the "reward" indicator in the middle recess lights up, and a sweet pellet is dispensed. The number of available rewards varied from five on the training day to 10 on each experimental day.

After a mouse habituated to the home cage (a day after being placed in the device), at the beginning of the dark phase of the day, the "operant wall" was turned on, and the animal was given 2 h to get acquainted with the device and the learning task. Because mice were not subjected

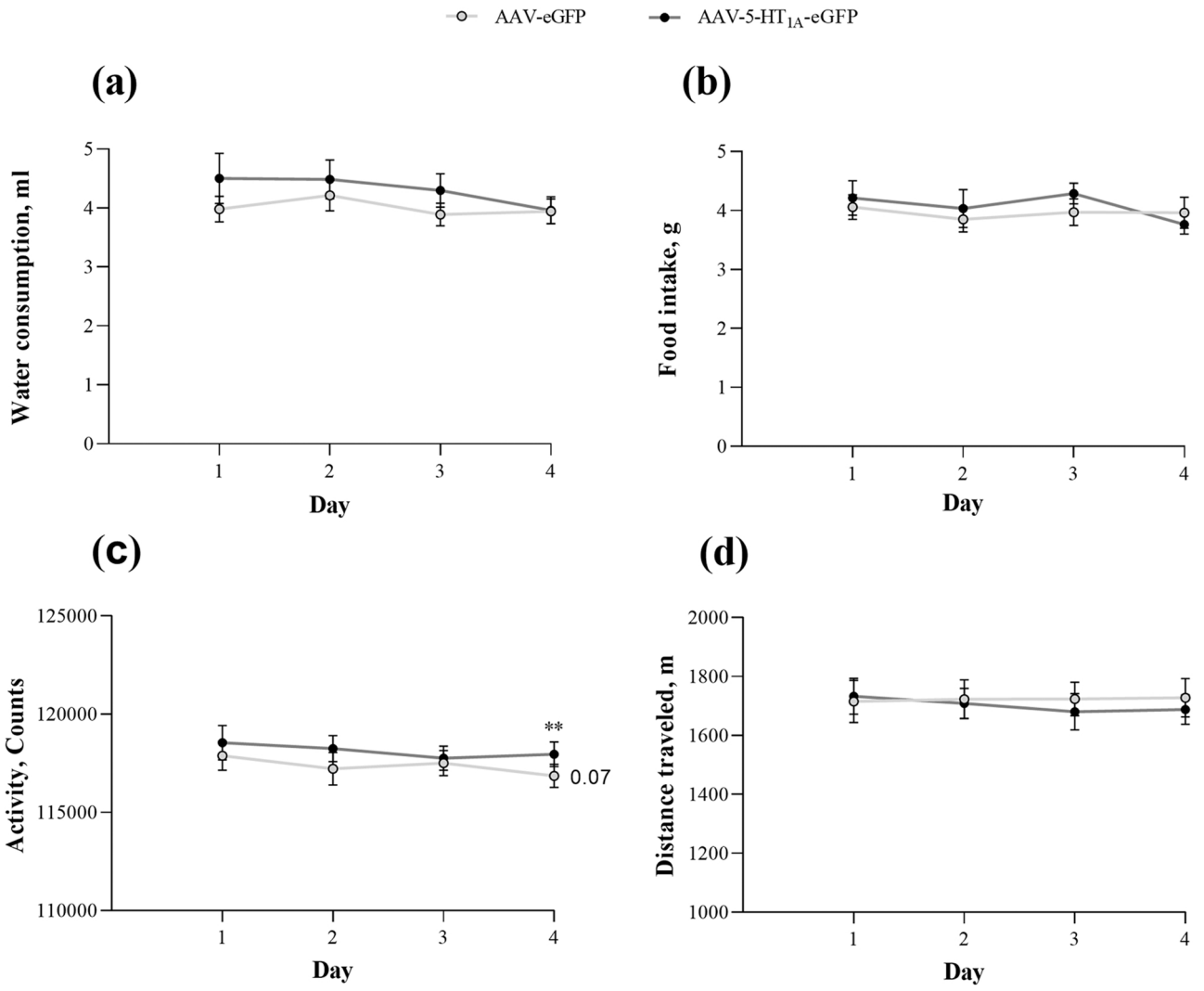


Fig. 6. Effects of the 5-HT_{1A} receptor overexpression in the hippocampus of BTBR mice on behavior in a home cage: (a) water consumption, (b) feed intake, (c) activity, and (d) distance traveled. All the values are presented as mean ± SEM (n ≥ 7).

to food deprivation, on the "training" day, one pellet was placed in the central recess for familiarization with the food reward and to arouse the animal's interest. On the first, "training" day, the task was simple: to get a reward, it was enough to stick one's nose into the hole marked with a lit up light bulb, but the task became more difficult with each subsequent day, reaching a maximum of complexity by the third day. On the second day, to get the reward, it was necessary to perform two nose pokes in the two marked holes. On the third day, the task did not differ from the previous day, but there was no light indication: the animal had to recall the necessary sequence of actions on its own. Cumulative numbers of pellets and nose pokes on the third day were recorded.

For assessment of locomotor activity, *the open field test* was carried out. A circular arena (40 cm in diameter) bordered by a white plastic wall and illuminated through a mat and semitransparent floor was used. A mouse was placed near the wall and tested for 5 min. The total distance traveled was measured in meters automatically by means of the EthoStudio software [50].

The tail suspension test was performed to assess depressive-like behavior. Briefly, mice were suspended by the tail using adhesive tape on a horizontal bar positioned at 50 cm height. The behavior of mice was registered for 6 min by the EthoStudio software. Total time of immobility was determined by an experienced rater blinded to group assignment of the mice.

Assessment of stereotyped behavior was performed by the *marble-burying test* [51,52]. Marble burying is considered a test for repetitive and anxiety-related behavior in rodents [52]. Mice were taken from their home cages and placed individually in polypropylene cages (42 × 24 × 12 cm), containing 20 clean glass marbles 1.5 cm in diameter, evenly spaced on 5-cm-deep sawdust without feed or water. The ceiling was composed of a metal grid. The marbles buried at least two-thirds deep were counted 30 min later.

The three-chambered social approach test was performed to investigate mice's sociability, as described previously [53]. Each mouse was placed separately in a rectangular socialization device (60 × 40 × 22 cm) made of clear polycarbonate. The test consisted of two stages: the habituation phase and socialization phase. Tested mice were placed in the central chamber during the habituation phase to freely explore the three chambers for 10 min. In the socialization phase, a new mouse (S) and a new object (O) were placed on each side of the chamber, and each experimental mouse was allowed to explore all three chambers for 10 min. The time spent in each chamber and the sniffing time (nose toward the cage at a distance of less than 2 cm) for each mouse during each 10-min phase were detected and recorded using the EthoStudio software [50]. The preference index was calculated as the difference in time between the new mouse and the new object divided by the total time spent in the two side chambers or sniffing targets (S-O/total time). The equipment was cleaned after each test with 70% alcohol and water.

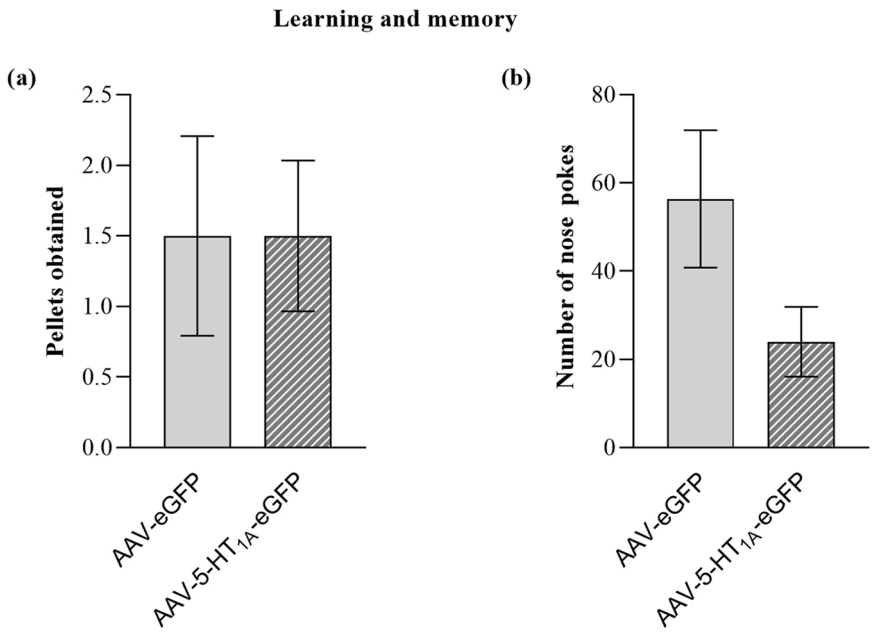


Fig. 7. The influence of the 5-HT_{1A} receptor overexpression in the hippocampus of BTBR mice on (a) the number of obtained pellets and (b) the number of nose pokes within the "operant wall" paradigm. All the values are means ± SEM (n = 8).

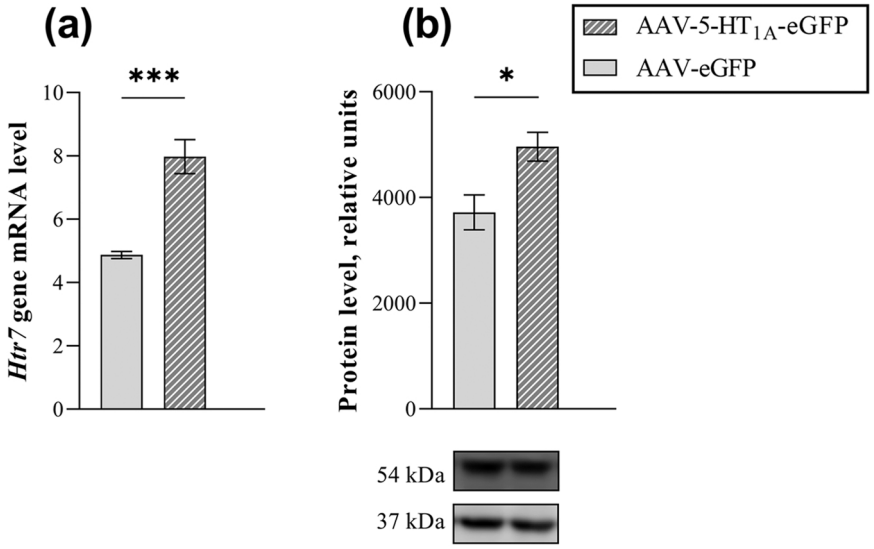


Fig. 8. Effects of the 5-HT_{1A} receptor overexpression in the hippocampus on 5-HT_7 receptor mRNA (a) and protein (b) levels in the hippocampus of BTBR mice. Gene expression is presented as the number of the gene's cDNA copies per 100 cDNA copies of *rPol2*. All the data are indicated as mean ± SEM (n ≥ 7).

2.6. Isolation of brain structures

Two days after behavioral testing, the animals were decapitated, and the hippocampus was excised on ice, frozen in liquid nitrogen, and stored at − 80 °C until subsequent procedures.

2.6.1. RT-PCR

Total RNA was isolated with the TRIzol reagent (15596026, Invitrogen, Waltham, MA, USA) and then treated with RNA-free DNase (Promega, Madison, WI, USA); 1 μg of the mRNA was used for cDNA synthesis with a random hexanucleotide primer. PCR was conducted as in our previous works [54–56]. Quantitative real-time PCR was carried out on a LightCycler 480 (Roche Applied Science, Switzerland) using the following primers: *Htr1a* F 5′-GACTGCCACCCTCTGCCCTATATC-3′ and R 5′-TCAGCAAGGCAAACAATTCCAG-3′; *Htr7* F 5′-GGCTACACGATCTACTCCACCG-3′ and R 5′-CGCACACTCTTCCACCTCCTTC-3′; *Bdnf* F 5′-TAGCAAAAAGAGAATTGGCTG-3′ and R 5′-TTTCAGGTCATGGATATGTCC-3′; *Ntrk2* F 5′-CATTCACTGTGAGAGGCAACC-3′ and F 5′-ATCAGGGTGTAGTCTCCGTTATT-3′; *Ngfr* F and R 5′-CACAACCACAGCAGCCAAGA-3′; *rPol2* F 5′-GTTGTCGGGCAGCAGAATGTAG-3′ and R 5′-TCAATGAGACCTTCTCGTCCCC-3′. A calibration curve in a plot of C_t (threshold cycle) versus minus lg P (decimal logarithm of the amount of a DNA standard) was constructed automatically by the LightCycler 480 System software. Gene expression is presented as the number of cDNA copies per 100 copies of *rPol2* cDNA [57–59]. A melting-curve analysis was performed at the end of each run for each primer pair, allowing us to control amplification specificity.

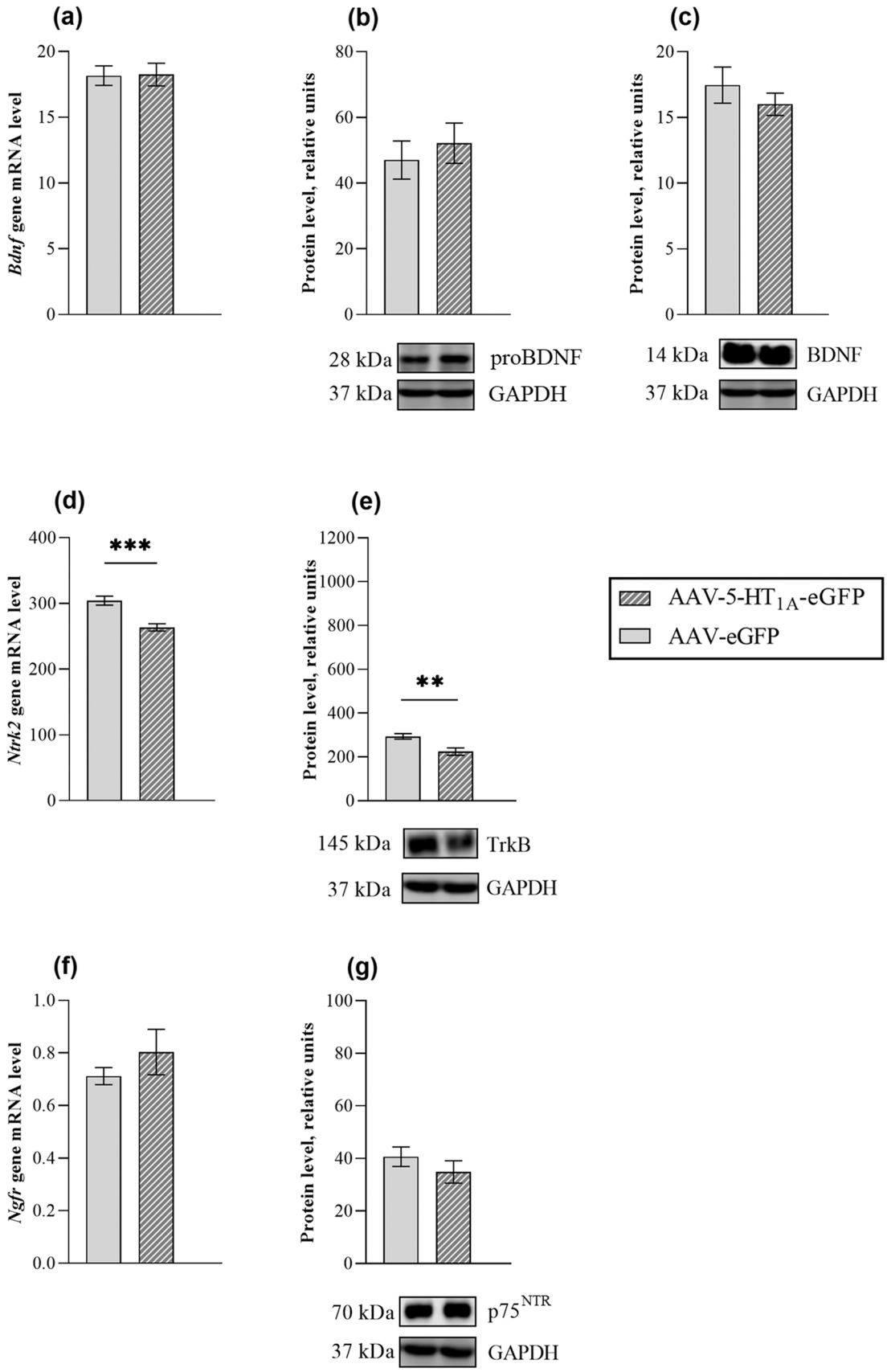


Fig. 9. Effects of the 5-HT_{1A} receptor overexpression in the hippocampus on (a) BDNF mRNA and (c) protein levels, (b) on the proBDNF protein level, (d) on TrkB mRNA and (e) protein levels, and (f) on $p75^{NTR}$ mRNA and (g) protein levels in the hippocampus of BTBR mice. Gene expression is presented as the number of a gene's cDNA copies per 100 cDNA copies of *rPol2*. Protein levels were determined in chemiluminescence relative units and normalized to GAPDH chemiluminescence relative units. All the values are means ± SEM ($n \geq 7$).

2.7. Western blotting

For assessment of total protein levels, a tissue sample was homogenized in LB buffer (300 mM NaCl, 100 mM Tris-HCl pH 8, 4 mM EDTA, 0.2% Triton X-100, 1 mM $NaVO_4$, 2 mM PMSF, and a protease inhibitor cocktail), incubated 60 min on ice, and centrifuged (12,000 × *g*, 15 min). Supernatant protein was transferred to a clean tube and kept at − 80 °C. Protein concentration was estimated spectrophotometrically with the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) on a NanoDrop 2000 C spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA), followed by adjustment of the samples to the same concentration with 2 × Laemmli sample buffer. Proteins in the samples were denatured by boiling for 10 min at 95 °C. Proteins in the resultant extracts (30 μg of total protein per lane for BDNF and pro-BDNF, and 15 μg of total protein per lane for other proteins: 5-HT_{1A}, 5-HT_7, $p75^{NTR}$, and TrkB) were resolved by SDS-PAGE and blotted onto a nitrocellulose membrane (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using a Trans-Blot Turbo Transfer System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The membranes were blocked in TBS-T containing 5% of nonfat dry milk (NFDM-TBST) for 1 h, rinsed, and next incubated with primary antibodies (Table 1). After protein detection (as described below), all blots were stripped and then reprobed with anti-GAPDH as a loading control. For protein detection, the membranes were washed in TBST (5 × 5 min), followed by incubation with a secondary antibody conjugated with horseradish peroxidase. After washing, the blots were incubated with the Clarity Western ECL Substrate (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's instructions. Protein bands were detected using a C-DiGit Blot Scanner (LI-COR, Lincoln, NE, USA). Quantification of protein bands was performed in the ImageStudio software (LI-COR Image Studio Software, Lincoln, NE, USA). Target protein levels were normalized to GAPDH levels.

2.8. Fluorescent microscopy of mouse brain sections

Mice were transcardially perfused with phosphate-buffered saline (PBS) and a 4% paraformaldehyde solution under anesthesia at 5 weeks after the AAV injection. The brain was removed and postfixed with 4% paraformaldehyde for 6 h and immersed in 30% sucrose in PBS for 2-day incubation. Sequential 14-μm slices were prepared on a cryostat (Thermo Scientific, Inc., Waltham, MA, USA). Cell nuclei were stained with a bis-benzimide solution (Hoechst 33258 dye, 5 μg/ml in PBS, Sigma-Aldrich, Burlington, MA, USA). Finally, the sections were mounted in an antiquenching medium (Fluoromount G; Southern Biotechnology Associates) followed by examination under a Zeiss AxioImager2 microscope with 10 × and 40 × air-immersion objectives. Cryosections from three animals were analyzed to obtain representative images. These analyzed mice received an injection of AAV-5-HT1A-eGFP but were not subjected to the behavioral testing.

2.9. Statistics

Statistical analysis was performed in the STATISTICA software, version 8.0. Data distribution was evaluated for normality by the Shapiro–Wilk test. The Dixon test was used to find and exclude outliers from the analysis. For nonparametrically distributed data, the Mann–Whitney *U* test was carried out. For parametric data, two-tailed Student's *t* test was applied to determine statistical significance of a difference between two experimental groups. Feed and water consumption as well as locomotor and exploratory activities in a home cage were assessed by repeated-measures two-way ANOVA. The statistical significance was set to $p < 0.05$. The results are presented as mean ± SEM.

3. Results

The site of the in vivo injection of the viral construct was confirmed by fluorescent microscopy (Fig. 1b). In micrographs of brain slices, the fluorescence of the 5-HT_{1A} receptor fused with eGFP could be observed in the hippocampus region of mice injected with AAV-Syn-HTR1A-eGFP.

Administration of the AAVs caused an expected increase in 5-HT_{1A} receptor mRNA ($t = 2.2$, $df = 13$, $p < 0.05$) and protein levels ($t = 3.99$, $df = 14$, $p < 0.01$) in the hippocampus of BTBR mice from the experimental group (Fig. 3).

The upregulation of the hippocampal 5-HT_{1A} receptor in BTBR mice led to a significant reduction of stereotyped behavior as evidenced by a lower number ($U = 6.5$, $p < 0.05$) of buried marbles in the marble-burying test (Fig. 4). Overexpression of the 5-HT_{1A} receptor in the hippocampus failed to affect social behavior in the three-chambered test ($t = 0.8$, $df = 14$, $p > 0.05$), immobility time in the tail suspension test ($t = 0.6$, $df = 14$, $p > 0.05$), and locomotor activity in the open field test ($t = 0.4$, $df = 14$, $p > 0.05$) (Fig. 5a, b, c). At the same time, 5-HT_{1A} receptor overexpression in the hippocampus of BTBR mice extended the time spent in the center in the open field test ($t = 4.2$, $df = 14$, $p < 0.001$) (Fig. 5d). A slight decline of mouse activity on the fourth day in the home cage was noted for mice from the experimental group ($p < 0.01$), whereas in control mice, this difference was only marginally significant ($p = 0.07$). Feed ($F_{3,39} = 1.2$, $p > 0.05$) and water ($F_{3,39} = 0.2$, $p > 0.05$) consumption as well as distance traveled ($F_{3,42} = 1.0$, $p > 0.05$) did not change in BTBR mice of the experimental group (Fig. 6). Within the "operant wall" paradigm, the upregulation of the hippocampal 5-HT_{1A} receptor in BTBR mice failed to improve associative learning (Fig. 7). No effect was detectable for both the number of obtained pellets ($U = 30$, $p > 0.05$) and the number of nose pokes ($t = 1.9$, $df = 14$, $p > 0.05$).

Of note, the 5-HT_{1A} receptor overexpression in the hippocampus was accompanied by a significant increase in hippocampal 5-HT_7 receptor mRNA ($t = 6.1$, $df = 13$, $p < 0.001$) and protein levels ($t = 2.9$, $df = 14$, $p < 0.05$) (Fig. 8).

The 5-HT_{1A} receptor overexpression resulted in significant changes in the BDNF system functioning. A considerable decline of mRNA ($t = 4.5$, $df = 13$, $p < 0.001$) and protein levels ($t = 3.3$, $df = 14$, $p < 0.01$) of TrkB receptor (which mediates the action of mature BDNF) was demonstrated in the hippocampus of the experimental group of BTBR mice (Fig. 9d, e). Meanwhile, the upregulation of the 5-HT_{1A} receptor in the hippocampus was not accompanied by significant expression changes for BDNF ($p > 0.05$ for mRNA and protein level), its precursor proBDNF ($p > 0.05$), and $p75^{NTR}$ receptor ($p > 0.05$) (Fig. 9a–c, f, g).

4. Discussion

5-HT_{1A} receptor is implicated in an amazingly wide range of behaviors. There is evidence of involvement of 5-HT_{1A} receptors in locomotor activity and a novelty response [60], in alcohol consumption [61, 62], feeding behavior [63], water intake [64], drug addiction [65], and mechanisms of cocaine [66–68] and opioid action [69]. There are data indicating a role of 5-HT_{1A} receptor in the mechanisms of social behavior [24,70,71], stereotyped behavior [72], learning and memory [73,74], and active stress avoidance [75,76], which are known to be disrupted in ASDs [77–79]. Nevertheless, the data on 5-HT_{1A} receptor in ASDs in humans and on its relevance to the regulation of autistic-like behavior in animal models of autism are extremely limited.

In the present paper, we demonstrated that administration of AAV-Syn-HTR1A-eGFP into the hippocampus of BTBR mice leads to an expected increase of 5-HT_{1A} receptor mRNA and protein levels in the hippocampus.

Mice of the BTBR strain, a widely used model of autism, manifest the main behavioral characteristics defining ASD, including impairment of social interactions and repetitive stereotyped behaviors [41]. Here we showed that AAV-based 5-HT_{1A} receptor overexpression in the hippocampus of BTBR mice significantly alleviates stereotyped behavior. Overall, our results on the suppressive effect of the hippocampal 5-HT_{1A}

receptor overexpression on stereotyped behavior are in agreement with other reports, which indicate that 5-HT_{1A} receptor overexpression reduces repetitive and restricted behaviors [19,20]. At the same time, our data are suggestive of the importance of hippocampal 5-HT_{1A} receptor for the regulation of this type of autistic-like behavior in BTBR mice.

The overexpression of 5-HT_{1A} receptor in the hippocampus of BTBR mice failed to significantly influence mouse behavior in a home cage. Feed and water consumption as well as distance traveled did not change in mice of the experimental group. Nonetheless, we noticed a slight decline of murine activity on the fourth day in the home cage for mice from the experimental group, whereas in control mice, this difference was only marginally significant. Such a decrease could be considered a normal reaction to a familiar environment, and the same direction of behavioral alterations in the comparison of the experimental and control groups does not allow to make any firm conclusions about 5-HT_{1A} receptor's function in the regulation of this behavioral parameter in BTBR mice.

In line with this notion, the 5-HT_{1A} overexpression in the hippocampus of BTBR mice failed to produce any changes in locomotor activity of the experimental animals on the novel territory in the open field test. Nevertheless, this overexpression significantly extended the time spent in the center of the arena. This finding is suggestive of enhancement of exploratory behavior. On the other hand, we should take into account the exaggerated stress avoidance of BTBR mice [80] together with the fact that the center of the arena is open and illuminated space that should provoke physiological anxiety-related fear in mice. Therefore, our results may also indicate some anxiolytic-like response to the 5-HT_{1A} receptor overexpression in the hippocampus. This idea is actually in good agreement with ample data on the anxiolytic effect of 5-HT_{1A} receptor agonists [81,82] and with reports that clinically used anxiolytic drugs mostly possess 5-HT_{1A} agonistic activity [83].

Unfortunately, the 5-HT_{1A} receptor overexpression in the hippocampus failed to restore another kind of autistic-like behavioral trait of BTBR mice: the social behavior deficit. It has been demonstrated earlier that stimulation of 5-HT_{1A} receptor with its agonist 8-OH-DPAT enhances social interaction in a rat model of a valproate-induced ASD [26, 84]. Nevertheless, taking into account low specificity of systemic administration of a 5-HT_{1A} receptor agonist to brain structures, our data suggest that at least hippocampal 5-HT_{1A} receptors are not significantly involved in the social behavior deficit in an ASD. At the same time, differences in the pathogenesis of autistic-like behavior between genetic (BTBR mice) and pharmacological (valproate-induced) models must be considered as well.

A positive influence of 5-HT_{1A} receptor stimulation on fear memory extinction has been demonstrated in the same pharmacological model (rat model of a valproate-induced ASD) [26]. In our study, we did not reveal an effect of the hippocampal 5-HT_{1A} receptor overexpression on associative learning within the "operant wall" paradigm. On the other hand, this is a completely different type of learning. Taken together with the well-known role of 5-HT_{1A} receptor in learning and memory [21], our results suggest that hippocampal 5-HT_{1A} receptors likely cannot be used as a target to restore associative learning in an ASD.

Usually, immobility in the tail suspension test is thought to reflect depressive-like behavior [85,86]. By contrast, in BTBR mice, reduced immobility time is more related to active stress avoidance rather than antidepressive-like behavior [80]. In our work, we did not demonstrate any impact of hippocampal 5-HT_{1A} receptors on this type of autistic-like behavior.

Very interesting results were obtained after our measurement of the effect of the 5-HT_{1A} receptor overexpression on the expression of 5-HT_7 receptor. It is well known that 5-HT_{1A} can form heterodimers with 5-HT_7, which significantly affect 5-HT_{1A} receptor activity [44]. This heterodimerization plays crucial role in the regulation of depressive-like behavior and in the response to antidepressant drugs [17,45,46]. In the current paper, we revealed that the 5-HT_{1A} receptor overexpression in the hippocampus significantly raises 5-HT_7 receptor mRNA and protein levels in this brain structure. Given that 5-HT_{1A}/5-HT_7 receptor heterodimerization promotes internalization of 5-HT_{1A} receptor, thereby suppressing its function [44], it is possible that the 5-HT_7 receptor overexpression may be a response of the brain aimed at attenuating the excessively enhanced 5-HT_{1A} receptor expression. It is noteworthy that earlier, we have shown that chronic 5-HT_7 receptor stimulation lowers both 5-HT_7 and 5-HT_{1A} receptors expression in the frontal cortex and midbrain but not in the hippocampus [87]. It has been proposed that this phenomenon is linked with a low quantity of 5-HT_{1A}/5-HT_7 heterodimers in the hippocampus [87], in agreement with evidence of moderate expression of 5-HT_7 in this brain structure [44,46,88,89]. Nonetheless, this receptor may play an independent part in the regulation of autistic-like behavior, and the behavioral response to hippocampal 5-HT_{1A} overexpression may be at least partially mediated by 5-HT_7 receptor. This idea suggests that it would be worthwhile to further focus on the involvement of this receptor in ASD mechanisms. Additionally, the observed alterations of 5-HT_7 receptor expression in the hippocampus possibly indicate that 5-HT_{1A}/5-HT_7 receptor heterodimers are a possible target for ASD treatment.

BDNF is now known to participate in the pathogenesis of many disorders of the nervous system [27–33], and the interaction between BDNF and the brain 5-HT system is well documented [40]. We showed that the 5-HT_{1A} receptor overexpression in the hippocampus of BTBR mice results in a significant decrease of TrkB receptor mRNA and protein levels in the hippocampus. It must be noted that in the majority of existing studies, different interventions result in unidirectional changes in the expression of 5-HT_{1A} and TrkB receptors [90–93]. A knockout of 5-HT_{1A} receptor induces a reduction in TrkB receptor expression [94]. Taking into account the above-mentioned observation, our data on the significant decline of TrkB receptor mRNA and protein levels in the hippocampus of BTBR mice overexpressing hippocampal 5-HT_{1A} receptor probably indicate an impairment of the 5-HT_{1A}–TrkB receptors interaction in autism. On the other hand, 5-HT_{1A} receptor overexpression in the hippocampus of BTBR mice failed to alter the expression of BDNF, of its precursor proBDNF, and of proBDNF receptor $p75^{NTR}$, implying that this cross talk between 5-HT_{1A} receptor and the BDNF system is mediated by TrkB receptor.

Our notion of the impaired 5-HT_{1A}–TrkB interaction in autism is consistent with the results on reduced anxiety and ameliorated stereotyped behavior accompanied by TrkB receptor downregulation in BTBR mice; the latter findings contradict available literature data. In multiple articles, an association between a lowered TrkB receptor level and enhanced stereotyped behavior [95–97] and anxiety [98–101] has been documented.

Thus, our results point to the importance of hippocampal 5-HT_{1A} receptor for the regulation of stereotyped behavior and anxiety in BTBR mice: a widely used model of autism. Our data indicate that cross talk between brain 5-HT and BDNF systems—and between serotonin 5-HT_{1A} and 5-HT_7 receptors—participates in mechanisms of autistic-like behavior.

Author contributions

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Conceptualization*, Vladimir S. Naumenko; *Methodology*, Elena M. Kondaurova, Irina I. Belokopytova, Tatiana V. Ilchibaeva; *Investigation*, Elena M. Kondaurova, Irina I. Belokopytova, Elizabeth A. Kulikova, Tatiana V. Ilchibaeva, Nikita V. Khotskin; *Formal Analysis*, Elena M. Kondaurova, Irina I. Belokopytova, Tatiana V. Ilchibaeva; *Writing—Original Draft*; *Writing—Review & Editing*, Vladimir S. Naumenko, Elena M. Kondaurova, Tatiana V. Ilchibaeva, Anton S. Tsybko; *Visualization*, Tatiana V. Ilchibaeva, Alexander Ya. Rodnyy; *Supervision*, Vladimir S. Naumenko, Nina K. Popova; *Funding Acquisition*, Nina K. Popova.

Data Availability

Data will be made available on request.

Acknowledgements

The cost of animal housing was compensated by basic-research project No. FWNR-2022–0023. The study was supported by the by Russian Science Foundation, project No. 22–15-00028. The English language was corrected by shevchuk-editing.com.

References

[1] A. Masi, M.M. DeMayo, N. Glozier, A.J. Guastella, An overview of autism spectrum disorder, heterogeneity and treatment options, Neurosci. Bull. 33 (2) (2017) 183–193.
[2] L. Rylaarsdam, A. Guemez-Gamboa, Genetic causes and modifiers of autism spectrum disorder, Front. Cell. Neurosci. 13 (2019) 385.
[3] D.L. Christensen, J. Baio, K. Van Naarden Braun, D. Bilder, J. Charles, J. N. Constantino, J. Daniels, M.S. Durkin, R.T. Fitzgerald, M. Kurzius-Spencer, L. C. Lee, S. Pettygrove, C. Robinson, E. Schulz, C. Wells, M.S. Wingate, W. Zahorodny, M. Yeargin-Allsopp, C. Centers, C. centers for disease, prevention, prevalence and characteristics of autism spectrum disorder among children aged 8 years–autism and developmental disabilities monitoring network, 11 sites, united states, 2012, morbidity and mortality weekly report, Surveill. Summ. 65 (3) (2016) 1–23.
[4] D.G. Amaral, G.M. Anderson, A. Bailey, R. Bernier, S. Bishop, G. Blatt, R. Canal-Bedia, T. Charman, G. Dawson, P.J. de Vries, E. Dicicco-Bloom, C. Dissanayake, Y. Kamio, R. Kana, N.Z. Khan, A. Knoll, F. Kooy, J. Lainhart, P. Levitt, K. Loveland, N. Minshew, R.A. Mueller, D. Murphy, P. Mundy, S. Palencia, J. Pinto-Martin, A. Rattazzi, S. Rogers, W.L. Stone, S.J. Webb, A. Whitehouse, Gaps in current autism research: the thoughts of the autism research editorial board and associate editors, Autism Res.: Off. J. Int. Soc. Autism Res. 12 (5) (2019) 700–714.
[5] K. Yenkoyan, A. Grigoryan, K. Fereshetyan, D. Yepremyan, Advances in understanding the pathophysiology of autism spectrum disorders, Behav. brain Res. 331 (2017) 92–101.
[6] J. Harro, L. Oreland, Depression as a spreading neuronal adjustment disorder, Eur. Neuropsychopharmacol.: J. Eur. Coll. Neuropsychopharmacol. 6 (3) (1996) 207–223.
[7] R.S. Duman, G.R. Heninger, E.J. Nestler, A molecular and cellular theory of depression, Arch. Gen. Psychiatry 54 (7) (1997) 597–606.
[8] L.A. Jans, W.J. Riedel, C.R. Markus, A. Blokland, Serotonergic vulnerability and depression: assumptions, experimental evidence and implications, Mol. Psychiatry 12 (6) (2007) 522–543.
[9] N.K. Popova, V.S. Naumenko, 5-HT1A receptor as a key player in the brain 5-HT system, Rev. Neurosci. 24 (2) (2013) 191–204.
[10] N.M. Barnes, T. Sharp, A review of central 5-HT receptors and their function, Neuropharmacology 38 (8) (1999) 1083–1152.
[11] T. Sharp, N.M. Barnes, Central 5-HT receptors and their function; present and future, Neuropharmacology 177 (2020), 108155.
[12] P.R. Albert, S. Lemonde, 5-HT1A receptors, gene repression, and depression: guilt by association, Neuroscientist 10 (6) (2004) 575–593.
[13] P. Blier, N.M. Ward, Is there a role for 5-HT1A agonists in the treatment of depression? Biol. Psychiatry 53 (3) (2003) 193–203.
[14] C.J. Hong, T.J. Chen, Y.W. Yu, S.J. Tsai, Response to fluoxetine and serotonin 1A receptor (C-1019G) polymorphism in Taiwan Chinese major depressive disorder, Pharm. J. 6 (1) (2006) 27–33.
[15] S. Lemonde, G. Turecki, D. Bakish, L. Du, P.D. Hrdina, C.D. Bown, A. Sequeira, N. Kushwaha, S.J. Morris, A. Basak, X.M. Ou, P.R. Albert, Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide, J. Neurosci.: Off. J. Soc. Neurosci. 23 (25) (2003) 8788–8799.
[16] H.Y. Meltzer, T. Sumiyoshi, Does stimulation of 5-HT(1A) receptors improve cognition in schizophrenia? Behav. brain Res. 195 (1) (2008) 98–102.
[17] A.V. Kulikov, R.R. Gainetdinov, E. Ponimaskin, A.V. Kalueff, V.S. Naumenko, N. K. Popova, Interplay between the key proteins of serotonin system in SSRI antidepressants efficacy, Expert Opin. Ther. Targets 22 (4) (2018) 319–330.
[18] N.K. Popova, A.S. Tsybko, V.S. Naumenko, The implication of 5-HT receptor family members in aggression, depression and suicide: similarity and difference, Int. J. Mol. Sci. 23 (2022) 8814.
[19] E. Lacivita, M. Niso, M. Mastromarino, A. Garcia Silva, C. Resch, A. Zeug, M. I. Loza, M. Castro, E. Ponimaskin, M. Leopoldo, Knowledge-based design of long-chain arylpiperazine derivatives targeting multiple serotonin receptors as potential candidates for treatment of autism spectrum disorder, ACS Chem. Neurosci. 12 (8) (2021) 1313–1327.
[20] J.T. Dunn, J. Mroczek, H.R. Patel, M.E. Ragozzino, Tandospirone, a Partial 5-HT1A Receptor Agonist, Administered Systemically or Into Anterior Cingulate Attenuates Repetitive Behaviors in Shank3B Mice, Int. J. Neuropsychopharmacol. 23 (8) (2020) 533–542.
[21] S.O. Ogren, T.M. Eriksson, E. Elvander-Tottie, C. D'Addario, J.C. Ekstrom, P. Svenningsson, B. Meister, J. Kehr, O. Stiedl, The role of 5-HT(1A) receptors in learning and memory, Behav. brain Res. 195 (1) (2008) 54–77.
[22] A. Oblak, T.T. Gibbs, G.J. Blatt, Reduced serotonin receptor subtypes in a limbic and a neocortical region in autism, Autism Res. Off. J. Int. Soc. Autism Res. 6 (6) (2013) 571–583.
[23] A. Lefevre, R. Mottolese, J. Redoute, N. Costes, D. Le Bars, M.M. Geoffray, M. Leboyer, A. Sirigu, Oxytocin Fails to Recruit Serotonergic Neurotransmission in the Autistic Brain, Cereb. cortex 28 (12) (2018) 4169–4178.
[24] A. Lefevre, N. Richard, R. Mottolese, M. Leboyer, A. Sirigu, An association between serotonin 1a receptor, gray matter volume, and sociability in healthy subjects and in autism spectrum disorder, autism research: official journal of the international society for autism, Research 13 (11) (2020) 1843–1855.
[25] R.D. Todd, R.D. Ciaranello, Demonstration of inter- and intraspecies differences in serotonin binding sites by antibodies from an autistic child, Proc. Natl. Acad. Sci. USA 82 (2) (1985) 612–616.
[26] C.C. Wang, H.C. Lin, Y.H. Chan, P.W. Gean, Y.K. Yang, P.S. Chen, 5-HT1A-receptor agonist modified amygdala activity and amygdala-associated social behavior in a valproate-induced rat autism model, Int. J. Neuropsychopharmacol. 16 (9) (2013) 2027–2039.
[27] A.R. Brunoni, M. Lopes, F. Fregni, A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression, Int. J. Neuropsychopharmacol. 11 (8) (2008) 1169–1180.
[28] M. Nibuya, S. Morinobu, R.S. Duman, Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments, The, J. Neurosci.: Off. J. Soc. Neurosci. 15 (11) (1995) 7539–7547.
[29] T. Itoh, M. Tokumura, K. Abe, Effects of rolipram, a phosphodiesterase 4 inhibitor, in combination with imipramine on depressive behavior, CRE-binding activity and BDNF level in learned helplessness rats, Eur. J. Pharmacol. 498 (1–3) (2004) 135–142.
[30] Z. Rogoz, B. Legutko, Combined treatment with imipramine and metyrapone induces hippocampal and cortical brain-derived neurotrophic factor gene expression in rats, Pharm. Rep. 57 (6) (2005) 840–844.
[31] R. Hellweg, A. Ziegenhorn, I. Heuser, M. Deuschle, Serum concentrations of nerve growth factor and brain-derived neurotrophic factor in depressed patients before and after antidepressant treatment, Pharmacopsychiatry 41 (2) (2008) 66–71.
[32] H.Y. Lee, Y.K. Kim, Plasma brain-derived neurotrophic factor as a peripheral marker for the action mechanism of antidepressants, Neuropsychobiology 57 (4) (2008) 194–199.
[33] S. Sen, R. Duman, G. Sanacora, Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications, Biol. Psychiatry 64 (6) (2008) 527–532.
[34] C. Pittenger, R.S. Duman, Stress, depression, and neuroplasticity: a convergence of mechanisms, Neuropsychopharmacol.: Off. Publ. Am. Coll. Neuropsychopharmacol. 33 (1) (2008) 88–109.
[35] H.D. Schmidt, M. Banasr, R.S. Duman, Future antidepressant targets: neurotrophic factors and related signaling cascades, Drug Disco Today Ther. Strateg 5 (3) (2008) 151–156.
[36] C. Bjorkholm, L.M. Monteggia, BDNF - a key transducer of antidepressant effects, Neuropharmacology 102 (2016) 72–79.
[37] S.J. Tsai, Is autism caused by early hyperactivity of brain-derived neurotrophic factor? Med. Hypotheses 65 (1) (2005) 79–82.
[38] D. Reim, M.J. Schmeisser, Neurotrophic factors in mouse models of autism spectrum disorder: focus on BDNF and IGF-1, Adv. Anat., Embryol., Cell Biol. 224 (2017) 121–134.
[39] K. Nishimura, K. Nakamura, A. Anitha, K. Yamada, M. Tsujii, Y. Iwayama, E. Hattori, T. Toyota, N. Takei, T. Miyachi, Y. Iwata, K. Suzuki, H. Matsuzaki, M. Kawai, Y. Sekine, K. Tsuchiya, G. Sugihara, S. Suda, Y. Ouchi, T. Sugiyama, T. Yoshikawa, N. Mori, Genetic analyses of the brain-derived neurotrophic factor (BDNF) gene in autism, Biochem. Biophys. Res. Commun. 356 (1) (2007) 200–206.
[40] N.K. Popova, V.S. Naumenko, Neuronal and behavioral plasticity: the role of serotonin and BDNF systems tandem, Expert Opin. Ther. Targets 23 (3) (2019) 227–239.
[41] D.T. Stephenson, S.M. O'Neill, S. Narayan, A. Tiwari, E. Arnold, H.D. Samaroo, F. Du, R.H. Ring, B. Campbell, M. Pletcher, V.A. Vaidya, D. Morton, Histopathologic characterization of the BTBR mouse model of autistic-like behavior reveals selective changes in neurodevelopmental proteins and adult hippocampal neurogenesis, Mol. Autism 2 (1) (2011) 7.
[42] G.G. Gould, J.G. Hensler, T.F. Burke, R.H. Benno, E.S. Onaivi, L.C. Daws, Density and function of central serotonin (5-HT) transporters, 5-HT1A and 5-HT2A receptors, and effects of their targeting on BTBR T+tf/J mouse social behavior, J. Neurochem. 116 (2) (2011) 291–303.
[43] A.Y. Rodnyy, E.A. Kulikova, E.M. Kondaurova, N. V.S, Serotonin 5-HT1A, 5-HT2A, and 5-HT7 Receptors in the Brain of the BTBR Mouse the Model of Autism, Neurochem. J. 15 (1) (2021) 42–49.
[44] U. Renner, A. Zeug, A. Woehler, M. Niebert, A. Dityatev, G. Dityateva, N. Gorinski, D. Guseva, D. Abdel-Galil, M. Frohlich, F. Doring, E. Wischmeyer, D. W. Richter, E. Neher, E.G. Ponimaskin, Heterodimerization of serotonin receptors 5-HT1A and 5-HT7 differentially regulates receptor signalling and trafficking, J. Cell Sci. 125 (Pt 10) (2012) 2486–2499.
[45] A.Y. Rodnyy, E.M. Kondaurova, D.V. Bazovkina, E.A. Kulikova, T.V. Ilchibaeva, A.I. Kovetskaya, I.A. Baraboshkina, E.Y. Bazhenova, N.K. Popova, V. S. Naumenko, Serotonin 5-HT7 receptor overexpression in the raphe nuclei area

produces antidepressive effect and affects brain serotonin system in male mice, J. Neurosci. Res. 100 (7) (2022) 1506–1523.
[46] V.S. Naumenko, N.K. Popova, E. Lacivita, M. Leopoldo, E.G. Ponimaskin, Interplay between serotonin 5-HT1A and 5-HT7 receptors in depressive disorders, CNS Neurosci. Ther. 20 (7) (2014) 582–590.
[47] D. Grimm, M.A. Kay, J.A. Kleinschmidt, Helper virus-free, optically controllable, and two-plasmid-based production of adeno-associated virus vectors of serotypes 1 to 6, Mol. Ther.: J. Am. Soc. Gene Ther. 7 (6) (2003) 839–850.
[48] B.M. Slotnick, C.M. Leonard, A stereotaxic atlas of the albino mouse forebrain, U. S. Dept. of Health, Education and Welfare, Rockville, Maryland, 1975.
[49] N.V. Khotskin, A.V. Plyusnina, E.A. Kulikova, E.Y. Bazhenova, D.V. Fursenko, I. E. Sorokin, I. Kolotygin, P. Mormede, E.E. Terenina, O.B. Shevelev, A.V. Kulikov, On association of the lethal yellow (A(Y)) mutation in the agouti gene with the alterations in mouse brain and behavior, Behav. brain Res. 359 (2019) 446–456.
[50] A.V. Kulikov, M.A. Tikhonova, V.A. Kulikov, Automated measurement of spatial preference in the open field test with transmitted lighting, J. Neurosci. Methods 170 (2) (2008) 345–351.
[51] K. Njung'e, S.L. Handley, Evaluation of marble-burying behavior as a model of anxiety, Pharmacol., Biochem., Behav. 38 (1) (1991) 63–67.
[52] R.M. Deacon, Digging and marble burying in mice: simple methods for in vivo identification of biological impacts, Nat. Protoc. 1 (1) (2006) 122–124.
[53] M. Yang, J.L. Silverman, J.N. Crawley, Automated three-chambered social approach task for mice, Current protocols in neuroscience Chapter 8 (2011) Unit 8 26.
[54] V.S. Naumenko, D.V. Bazovkina, A.A. Semenova, A.S. Tsybko, T.V. Il'chibaeva, E. M. Kondaurova, N.K. Popova, Effect of glial cell line-derived neurotrophic factor on behavior and key members of the brain serotonin system in mouse strains genetically predisposed to behavioral disorders, J. Neurosci. Res. 91 (12) (2013) 1628–1638.
[55] E.M. Kondaurova, A.Y. Rodnyy, T.V. Ilchibaeva, A.S. Tsybko, D.V. Eremin, Y. V. Antonov, N.K. Popova, V.S. Naumenko, Genetic background underlying 5-HT1A receptor functioning affects the response to fluoxetine, Int. J. Mol. Sci. 21 (22) (2020).
[56] V.S. Naumenko, R.V. Kozhemyakina, I.F. Plyusnina, A.V. Kulikov, N.K. Popova, Serotonin 5-HT1A receptor in infancy-onset aggression: comparison with genetically defined aggression in adult rats, Behav. brain Res. 243 (2013) 97–101.
[57] A.V. Kulikov, V.S. Naumenko, I.P. Voronova, M.A. Tikhonova, N.K. Popova, Quantitative RT-PCR assay of 5-HT1A and 5-HT2A serotonin receptor mRNAs using genomic DNA as an external standard, J. Neurosci. Methods 141 (1) (2005) 97–101.
[58] V.S. Naumenko, A.V. Kulikov, Quantitative assay of 5-HT(1A) serotonin receptor gene expression in the brain, Mol. Biol. 40 (1) (2006) 37–44.
[59] V.S. Naumenko, D.V. Osipova, E.V. Kostina, A.V. Kulikov, Utilization of a two-standard system in real-time PCR for quantification of gene expression in the brain, J. Neurosci. Methods 170 (2) (2008) 197–203.
[60] R.J. Carey, A. Shanahan, E.N. Damianopoulos, C.P. Muller, J.P. Huston, Behavior selectively elicited by novel stimuli: modulation by the 5-HT1A agonist 8-OHD-PAT and antagonist WAY-100635, Behav. Pharmacol. 19 (4) (2008) 361–364.
[61] S. Kelai, T. Renoir, L. Chouchana, F. Saurini, N. Hanoun, M. Hamon, L. Lanfumey, Chronic voluntary ethanol intake hypersensitizes 5-HT(1A) autoreceptors in C57BL/6J mice, J. Neurochem. 107 (6) (2008) 1660–1670.
[62] N.K. Popova, E.A. Ivanova, 5-HT(1A) receptor antagonist p-MPPI attenuates acute ethanol effects in mice and rats, Neurosci. Lett. 322 (1) (2002) 1–4.
[63] J.M. Mancilla-Diaz, R.E. Escartin-Perez, V.E. Lopez-Alonso, B. Floran-Garduno, J. B. Romano-Camacho, Role of 5-HT1A and 5-HT1B receptors in the hypophagic effect of 5-HT on the structure of feeding behavior, Med Sci. Monit. 11 (3) (2005) BR74–BR79.
[64] J.B. Fregoneze, H. Ferreira, T. Soares, C.P. Luz, C. Bulcao, T. Nascimento, C. A. Marinho, C. Sarmento, I.R. De-Oliveira, M. Cunha, et al., SDZ 216-525, a selective 5-HT1A receptor antagonist, reverts zinc-induced inhibition of water intake in dehydrated rats, Braz. J. Med. Biol. Res. = Rev. Bras. De. Pesqui. Med. e Biol. 28 (6) (1995) 711–714.
[65] S.M. Rawls, H. Shah, G. Ayoub, R.B. Raffa, 5-HT(1A)-like receptor activation inhibits abstinence-induced methamphetamine withdrawal in planarians, Neurosci. Lett. 484 (2) (2010) 113–117.
[66] R.J. Carey, G. DePalma, E. Damianopoulos, A. Shanahan, C.P. Muller, J. P. Huston, Evidence that the 5-HT1A autoreceptor is an important pharmacological target for the modulation of cocaine behavioral stimulant effects, Brain Res. 1034 (1–2) (2005) 162–171.
[67] C.P. Muller, J.P. Huston, Determining the region-specific contributions of 5-HT receptors to the psychostimulant effects of cocaine, Trends Pharmacol. Sci. 27 (2) (2006) 105–112.
[68] R.J. Carey, G. Depalma, E. Damianopoulos, C.P. Muller, J.P. Huston, The 5-HT1A receptor and behavioral stimulation in the rat: effects of 8-OHDPAT on spontaneous and cocaine-induced behavior, Psychopharmacology 177 (1–2) (2004) 46–54.
[69] B. Song, W. Chen, J.C. Marvizon, Inhibition of opioid release in the rat spinal cord by serotonin 5-HT(1A) receptors, Brain Res. 1158 (2007) 57–62.
[70] Z.R. Donaldson, D.A. Piel, T.L. Santos, J. Richardson-Jones, E.D. Leonardo, S. G. Beck, F.A. Champagne, R. Hen, Developmental effects of serotonin 1A autoreceptors on anxiety and social behavior, Neuropsychopharmacol.: Off. Publ. Am. Coll. Neuropsychopharmacol. 39 (2) (2014) 291–302.
[71] R.H. Larke, N. Maninger, B.J. Ragen, S.P. Mendoza, K.L. Bales, Serotonin 1A agonism decreases affiliative behavior in pair-bonded titi monkeys, Horm. Behav. 86 (2016) 71–77.
[72] Y. Mao, Y. Xing, J. Li, D. Dong, S. Zhang, Z. Zhao, J. Xie, R. Wang, H. Li, Guanosine ameliorates positive symptoms of schizophrenia via modulating 5-HT1A and 5-HT2A receptors, Am. J. Transl. Res. 13 (5) (2021) 4040–4054.
[73] Y. Glikmann-Johnston, M.M. Saling, D.C. Reutens, J.C. Stout, Hippocampal 5-HT1A Receptor and Spatial Learning and Memory, Front. Pharmacol. 6 (2015) 289.
[74] O. Stiedl, E. Pappa, A. Konradsson-Geuken, S.O. Ogren, The role of the serotonin receptor subtypes 5-HT1A and 5-HT7 and its interaction in emotional learning and memory, Front. Pharmacol. 6 (2015) 162.
[75] L.R. Bader, J.D. Carboni, C.A. Burleson, M.A. Cooper, 5-HT1A receptor activation reduces fear-related behavior following social defeat in Syrian hamsters, Pharmacol., Biochem., Behav. 122 (2014) 182–190.
[76] M. Toth, 5-HT1A receptor knockout mouse as a genetic model of anxiety, Eur. J. Pharmacol. 463 (1–3) (2003) 177–184.
[77] M. Bove, S. Schiavone, P. Tucci, V. Sikora, S. Dimonte, A.L. Colia, M.G. Morgese, L. Trabace, Ketamine administration in early postnatal life as a tool for mimicking Autism Spectrum Disorders core symptoms, Prog. neuro-Psychopharmacol. Biol. Psychiatry 117 (2022), 110560.
[78] C.H. Tsai, K.L. Chen, H.J. Li, K.H. Chen, C.W. Hsu, C.H. Lu, K.Y. Hsieh, C. Y. Huang, The symptoms of autism including social communication deficits and repetitive and restricted behaviors are associated with different emotional and behavioral problems, Sci. Rep. 10 (1) (2020) 20509.
[79] M.A. Shillingsburg, B. Hansen, M. Wright, Rapport building and instructional fading prior to discrete trial instruction: moving from child-led play to intensive teaching, Behav. Modif. 43 (2) (2019) 288–306.
[80] R. Benno, Y. Smirnova, S. Vera, A. Liggett, N. Schanz, Exaggerated responses to stress in the BTBR T+tf/J mouse: an unusual behavioral phenotype, Behav. brain Res. 197 (2) (2009) 462–465.
[81] J. De Vry, R. Schreiber, C. Melon, M. Dalmus, K.R. Jentzsch, 5-HT1A receptors are differentially involved in the anxiolytic- and antidepressant-like effects of 8-OH-DPAT and fluoxetine in the rat, Eur. Neuropsychopharmacol.: J. Eur. Coll. Neuropsychopharmacol. 14 (6) (2004) 487–495.
[82] R. Schreiber, J. De, Vry, Neuronal circuits involved in the anxiolytic effects of the 5-HT1A receptor agonists 8-OH-DPAT ipsapirone and buspirone in the rat, Eur. J. Pharmacol. 249 (3) (1993) 341–351.
[83] P. Celada, A. Bortolozzi, F. Artigas, Serotonin 5-HT1A receptors as targets for agents to treat psychiatric disorders: rationale and current status of research, CNS Drugs 27 (9) (2013) 703–716.
[84] H.F. Wu, Y.J. Chen, M.C. Chu, Y.T. Hsu, T.Y. Lu, I.T. Chen, P.S. Chen, H.C. Lin, Deep brain stimulation modified autism-like deficits via the serotonin system in a valproic acid-induced rat model, Int. J. Mol. Sci. 19 (9) (2018).
[85] H. Ueno, Y. Takahashi, S. Murakami, K. Wani, Y. Matsumoto, M. Okamoto, T. Ishihara, Effect of simultaneous testing of two mice in the tail suspension test and forced swim test, Sci. Rep. 12 (1) (2022) 9224.
[86] A. Can, D.T. Dao, C.E. Terrillion, S.C. Piantadosi, S. Bhat, T.D. Gould, The tail suspension test, J. Vis. Exp.: JoVE 59 (2012), e3769.
[87] E.M. Kondaurova, D.V. Bazovkina, V.S. Naumenko, 5-HT1A/5-HT7 receptor interplay: Chronic activation of 5-HT7 receptors decreases the functional activity of 5-HT1A receptor and its content in the mouse brain, Mol. Biol. 51 (1) (2017) 157–165.
[88] F. Kobe, D. Guseva, T.P. Jensen, A. Wirth, U. Renner, D. Hess, M. Muller, L. Medrihan, W. Zhang, M. Zhang, K. Braun, S. Westerholz, A. Herzog, K. Radyushkin, A. El-Kordi, H. Ehrenreich, D.W. Richter, D.A. Rusakov, E. Ponimaskin, 5-HT7R/G12 signaling regulates neuronal morphology and function in an age-dependent manner, J. Neurosci.: Off. J. Soc. Neurosci. 32 (9) (2012) 2915–2930.
[89] J.C. Beique, B. Campbell, P. Perring, M.W. Hamblin, P. Walker, L. Mladenovic, R. Andrade, Serotonergic regulation of membrane potential in developing rat prefrontal cortex: coordinated expression of 5-hydroxytryptamine (5-HT)1A, 5-HT2A, and 5-HT7 receptors, The, J. Neurosci.: Off. J. Soc. Neurosci. 24 (20) (2004) 4807–4817.
[90] P. Rumajogee, A. Madeira, D. Verge, M. Hamon, M.C. Miquel, Up-regulation of the neuronal serotoninergic phenotype in vitro: BDNF and cAMP share Trk B-dependent mechanisms, J. Neurochem. 83 (6) (2002) 1525–1528.
[91] J. Amigo, A. Diaz, F. Pilar-Cuellar, R. Vidal, A. Martin, V. Compan, A. Pazos, E. Castro, The absence of 5-HT4 receptors modulates depression- and anxiety-like responses and influences the response of fluoxetine in olfactory bulbectomised mice: Adaptive changes in hippocampal neuroplasticity markers and 5-HT1A autoreceptor, Neuropharmacology 111 (2016) 47–58.
[92] T.W. Kim, B.V. Lim, K. Kim, J.H. Seo, C.J. Kim, Treadmill exercise alleviates stress-induced impairment of social interaction through 5-hydroxytryptamine 1A receptor activation in rats, J. Exerc. Rehabil. 11 (4) (2015) 192–197.
[93] S.J. Lee, T.W. Kim, H.K. Park, S. Yoon, A.H. You, E.J. Moon, D.H. Shin, H. Cho, Postnatal treadmill exercise alleviates prenatal stress-induced anxiety in offspring rats by enhancing cell proliferation through 5-hydroxytryptamine 1A receptor activation, Int. Neurourol. J. 20 (Suppl 1) (2016) S57–S64.
[94] Y.C. Wu, R.A. Hill, M. Klug, M. van den Buuse, Sex-specific and region-specific changes in BDNF-TrkB signalling in the hippocampus of 5-HT1A receptor and BDNF single and double mutant mice, Brain Res. 1452 (2012) 10–17.
[95] L. Liu, M. Liu, W. Zhao, Y.L. Zhao, Y. Wang, Tetrahydropalmatine Regulates BDNF through TrkB/CAM Interaction to Alleviate the Neurotoxicity Induced by Methamphetamine, ACS Chem. Neurosci. 12 (18) (2021) 3373–3386.
[96] M.S. Kang, T.Y. Choi, H.G. Ryu, D. Lee, S.H. Lee, S.Y. Choi, K.T. Kim, Autism-like behavior caused by deletion of vaccinia-related kinase 3 is improved by TrkB stimulation, The, J. Exp. Med. 214 (10) (2017) 2947–2966.

[97] B. Zorner, D.P. Wolfer, D. Brandis, O. Kretz, C. Zacher, R. Madani, I. Grunwald, H. P. Lipp, R. Klein, F.A. Henn, P. Gass, Forebrain-specific trkB-receptor knockout mice: behaviorally more hyperactive than "depressive", Biol. Psychiatry 54 (10) (2003) 972–982.

[98] P. Mohseni-Moghaddam, M. Dogani, M. Hatami, S. Roohollahi, A. Amiresmaeli, N. Askari, A behavioral and molecular study; ameliorated anxiety-like behavior and cognitive dysfunction in a rat model of chronic unpredictable stress treated with oregano extract, Brain Behav. 12 (8) (2022), e2727.

[99] Y.L. Jiang, X.S. Wang, X.B. Li, A. Liu, Q.Y. Fan, L. Yang, B. Feng, K. Zhang, L. Lu, J.Y. Qi, F. Yang, D.K. Song, Y.M. Wu, M.G. Zhao, S.B. Liu, Tanshinone IIA improves contextual fear- and anxiety-like behaviors in mice via the CREB/BDNF/TrkB signaling pathway, Phytotherapy research: PTR (2022).

[100] L. Tang, S. Li, J. Yu, Y. Zhang, L. Yang, D. Tong, J. Xu, Nonylphenol induces anxiety-like behavior in rats by regulating BDNF/TrkB/CREB signal network, Food Chem. Toxicol.: Int. J. Publ. Br. Ind. Biol. Res. Assoc. 166 (2022), 113197.

[101] X. Sun, H.F. Zhang, C.L. Ma, H. Wei, B.M. Li, J. Luo, Alleviation of anxiety/depressive-like behaviors and improvement of cognitive functions by lactobacillus plantarum WLPL04 in chronically stressed mice, Can. J. Infect. Dis. Med. Microbiol. = J. Can. Des. Mal. Infect. Et. De. la Microbiol. Med. 2021 (2021) 6613903.