ISSN 0026-8933, Molecular Biology, 2018, Vol. 52, No. 2, pp. 212–221. © Pleiades Publishing, Inc., 2018.
Original Russian Text © A.S. Tsybko, T.V. Ilchibaeva, D.V. Bazovkina, V.S. Naumenko, 2018, published in Molekulyarnaya Biologiya, 2018, Vol. 52, No. 2, pp. 246–256.

MOLECULAR CELL BIOLOGY

UDC 577.25

Functional Responses to the Chronic Activation of 5-HT$_{1A}$ Receptors in Mice with Genetic Predisposition to Catalepsy

A. S. Tsybko*, T. V. Ilchibaeva, D. V. Bazovkina, and V. S. Naumenko

Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, 630090 Russia

**e-mail: antoncybko@mail.ru*

Received July 14, 2016; in final form, October 6, 2016

Abstract—The effects of chronic 5-HT$_{1A}$ receptor activation on the behavior, functional activity of 5-HT$_{1A}$ receptors, and expression of key genes of the brain 5-HT system were studied in mice of the catalepsy-prone CBA strain and the catalepsy-resistant C57BL/6 strain. Chronic treatment with 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (1.0 mg/kg i.p., 14 days) led to a significant decrease in the hypothermic response to acute administration of 8-OH-DPAT in CBA and C57BL/6 mice, which indicates the desensitization of 5-HT$_{1A}$ receptors in both strains. Pretreatment with the 5-HT$_7$ receptor agonist SB 269970 did not affect the hypothermic response to the acute administration of 8-OH-DPAT, which suggests an independent functional response of 5-HT$_{1A}$ receptors. The treatment did not induce any changes in the behavior in the open field paradigm in CBA mice, but significantly increased the total path, the time spent in the center, and the number of rearings in C57BL/6 mice, which indicates the enhancement of locomotor and exploratory activity in C57BL/6 mice. The chronic activation of 5-HT$_{1A}$ receptor downregulated 5-HT$_{1A}$ gene expression, as well as the expression of the gene that encodes tryptophan hydroxylase 2, a key enzyme of 5-HT biosynthesis, in the midbrain and the expression of the gene that encodes the 5-HT$_{2A}$ receptor in the frontal cortex of CBA, but not C57BL/6 mice. The obtained data provide a new evidence on the receptor–gene cross talk in the brain 5-HT system that may underlie the loss of pharmacological efficacy of 5-HT$_{1A}$ receptor agonists. In turn, the loss of the behavioral response and compensatory alterations in key genes of the brain 5-HT system in CBA mice suggests that catalepsy-prone and -resistant genotypes demonstrate different sensibility to the effects of drugs.

Keywords: genetic predisposition to catalepsy, chronic 8-OH-DPAT treatment, 5-HT$_{1A}$ receptors, key genes of the 5-HT system, behavior

DOI: 10.1134/S002689331705020X

INTRODUCTION

Catalepsy is a state of immobility due to the strongly pronounced plastic muscle tonus. In mammals, catalepsy represents a behavioral strategy of passive defense and serves to escape predation or to diminish attacks of aggressive counterparts [1]. In humans, the hypertrophic form of catalepsy is a symptom of severe schizophrenia and depression [2].

In mice, cataleptic immobility can be induced by repeated pinching of the skin in the nape area [3]; different strains exhibit significant variations in their predisposition to pinched-induced catalepsy [4]. For instance, C57BL/6J, AKR/J, DBA1/J, and CC57BR/Mv mice are not prone to catalepsy, while in strains C3H/HeJ, A/He, BALB/cLac, and DD/He, catalepsy can be induced in approximately 10% of animals. The strongest predisposition to catalepsy was observed in CBA/LacJ; more than 50% of CBA mice remain immobile for over 60 s after five or six pinches [4].

It is known that the major locus responsible for predisposition to catalepsy in mice is located in the distal part of chromosome 13 [5–7], which also contains the gene that encodes 5-HT$_{1A}$ receptor. The available data implicate these receptors in the regulation of catalepsy; their activation has an anticataleptic effect. Agonists of 5-HT$_{1A}$ receptors, such as 8-OH-DPAT, flesinoxan, tandospirone, and NLX-112, inhibit catalepsy induced by neuroleptic drugs [8–13] and decrease the duration of catalepsy in GC rats (genetic and catatonia) [14, 15], as well as in CBA and AKR.CBA-D13Mit76 mice [16, 17]. It was also found that selection for catalepsy leads to the elimination of the AKR allele of D13Mit76 linked to the 5-HT$_{1A}$ receptor gene and to its substitution by the CBA allele

Abbreviations: 5-HT, serotonin; 5-HT$_{1A}$ receptor, serotonin receptor subtype 1A; 5-HT$_{2A}$ receptor, serotonin receptor subtype 2A; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin; SB269970, (2R)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine hydrochloride); rPolII, RNA polymerase II; TPH-2, tryptophan hydroxylase 2; 5-HTT, serotonin transporter; RT, reverse transcription; PCR, polymerase chain reaction.

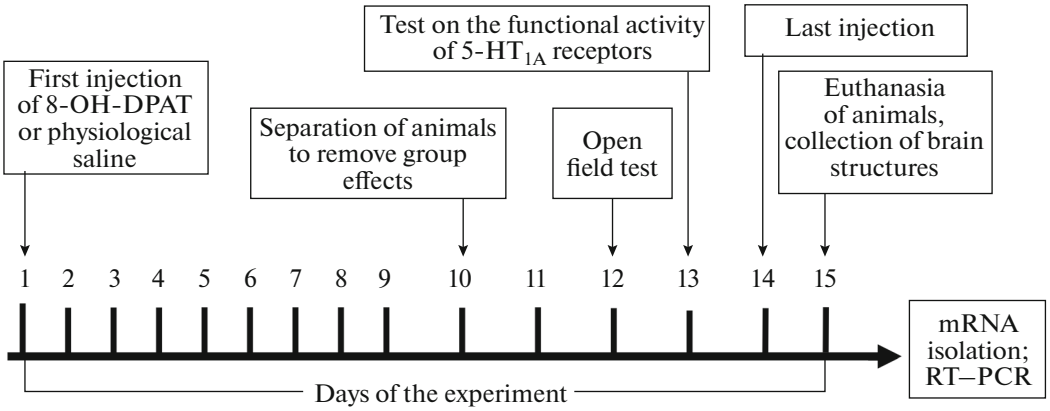

Fig. 1. Study design.

[18]. At the same time, the pattern of 5-HT_{1A} receptor activity observed in selected animals was similar to the pattern typical for CBA mice [18].

Importantly, selection for catalepsy also enhances depression-like behavior in mice [19] and induces considerable neuroanatomic changes in the brain similar to those observed in patients with depression [20]. At the same time, the administration of antidepressants [21] and brain-derived neurotrophic factor (BDNF) [22] has a pronounced anticataleptic and antidepressant effect in these mice. It is also noteworthy that numerous data implicate 5-HT_{1A} receptors in the mechanisms of anxiety, depression, suicide attempts, and in response to treatment with antidepressants [23–25]. Taking into account all of the above facts, investigation of functional responses of 5-HT_{1A} receptors in association with the genetic predisposition to catalepsy is of central importance and may contribute to the understanding of mechanisms of depression-like behavior. However, there have been no studies for comparing the effects of chronic 5-HT_{1A} receptor activation in mice with different hereditary predisposition to catalepsy. The role of genotype in the functional activity of 5-HT_{1A} receptors, as well as the involvement of these receptors in the control of catalepsy are far from being fully understood.

The objective of the present work was to investigate the effects of chronic 5-HT_{1A} receptor activation by their selective agonist 8-OH-DPAT on the functional activity of these receptors, on the expression of genes that encode 5-HT_{1A} and 5-HT_{2A} receptors, the serotonin transporter (5-HTT), and tryptophan hydroxylase 2 (TPH-2, a key enzyme of 5-HT biosynthesis) in the brain, as well as on the behavior of mice that differ in their predisposition to catalepsy.

EXPERIMENTAL

Animals. Experiments were performed on adult male mice of the CBA/Lac strain with hereditary predisposition to catalepsy and the noncataleptic strain C57BL/6J (18 mice of each strain). Animals were kept in plastic cages 40 × 30 × 15 cm under standard conditions (18–22°C; 50–60% relative humidity; natural illumination with a 12-h light and 12-h dark period) with ad libitum supply of standard feed and water. Two days before the experiment, mice were placed in individual cages to exclude group effects. Animal maintenance and experimental procedures conformed to the requirements of the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80023, 1996).

Drugs. 8-OH-DPAT (8-Hydroxy-2-(di-n-propylamino)tetralin; Sigma, United States) is an agonist of 5-HT_{1A} receptors (pKi = 8.82 ± 0.01 nM), which also exhibits considerable affinity to 5-HT_7 receptors (pKi = 6.07 ± 0.05 nM) [26, 27]. The compound was dissolved in saline and administered intraperitoneally in the dose of 1 mg/kg; animals of the control group received a single injection in the functional activity test, while the test group received chronic treatment during 14 days (Fig. 1). In this case, 8-OH-DPAT was administered after behavioral tests. Animals of the control group received injections of saline.

The open field test was performed in an open chamber (40 × 40 × 25 cm) made of nontransparent plastic. The bottom of the chamber was of semitransparent plastic and was positioned 40 cm above two 12-W halogenic lamps [28]. A mouse was placed by the middle of a chamber wall, and its behavior was analyzed for 5 min. The parameters registered included the total length of the path, time spent in the center, the number of washings, the number of rearings, and the number of defecations. All behavioral traits were registered using the EthoStudio software system [28].

Functional activity (sensitivity) of 5-HT_{1A} receptors was assessed on day 13 by measuring the magnitude of the hypothermic response to the acute administration of 8-OH-DPAT [29]. Although 5-HT_{1A} receptors are

located primarily in the brain [30], the drug (1 mg/kg, or 3.0 μmol/kg) was administered intraperitoneally, since it is known that 8-OH-DPAT can cross the blood–brain barrier. The body temperature was measured before and 20 min after the injection using a rectal sensor for mice and rats (Phymep, France) with a microprocessor-based thermometer (Hanna Instrument, Singapore). The difference between the final and the initial body temperature (delta t°C) was used to describe the magnitude of the hypothermic response.

Two days after the test, the animals were decapitated and the frontal cortex, the hippocampus, and the raphe nuclei of the midbrain were isolated on ice. Tissues were frozen in liquid nitrogen and stored at −70°C.

To verify that 5-HT_7 receptors did not contribute to the functional response of 5-HT_{1A} receptors, a preliminary experiment was performed where the functional response of 5-HT_{1A} receptors to acute administration of 8-OH-DPAT was evaluated after a prior injection of a selective antagonist of 5-HT_7 receptors, SB 269970 ((2R)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl) ethyl]pyrrolidine hydrochloride, pKi = 8.9) (Tocris Bioscience, United States). For this purpose, SB269970 (which cannot cross the blood–brain barrier) was dissolved in distilled water to the concentration of 0.025 nmol and injected into the left lateral brain ventricle of CBA/Lac mice (AP: 0.5; L: 1.6 mm; DV: 2 mm [31]). Prior to intraventricular drug administration, animals were anesthetized with diethyl ether for 20–30 s. Control animals were injected with an equal volume of sterile water (5 μL). At 15 min after the intraventricular injection, mice of both groups received an intraperitoneal injection of 8-OH-DPAT (1 mg/kg). The magnitude of the hypothermic response was determined as described above.

Gene expression was analyzed by quantitative RT–PCR using a protocol developed in our laboratory [32]. The method employs two types of standards, i.e., internal and external. The internal standard, mRNA of RNA polymerase II (rPol II) was used to control reverse transcription and to calculate the levels of target mRNAs. Mouse DNA with known concentration served as the external standard to control PCR and to determine the number of target gene copies in the specimens.

Total RNA was isolated using guanidine isothiocyanate, phenol, and chloroform; treated with RNase-free DNase (1 unit per specimen; 37°C, 10 min), and extracted again with guanidine isothiocyanate, phenol, and chloroform [32].

RT–PCR. Total RNA (8 μL or 1 μg) was mixed with 180 ng random hexamer primer (final primer concentration, 5 μM) and sterile KCl (2.25 μmol in 16 μL), denatured at 94°C for 5 min in a Hybaid Omn-E thermal cycler (Great Britain), and annealing was performed at 41°C for 15 min. Next, 15 μL of the reaction mixture that contained *M-MLV* reverse transcriptase (200 units), Tris–HCl (pH 8.3, 0.225 μmol), dNTPs (0.015 μmol each), DTT (0.225 μmol), and $MnCl_2$ (0.03 μmol) was added, and the resulting mixture (final volume, 31 μL) was incubated at 41°C for 60 min. The obtained cDNA was stored at −20°C.

Primers used to amplify fragments of target cDNAs (table) were designed based on sequences available from the EMBL Nucleotide database and synthesized by Biosan (Russia). The PCR mixture contained 1 μL cDNA, 2 μL PCR buffer, 0.3 μL $MgCl_2$ (0.1 M), 1 μL dNTPs (4 mM), 2.5 μL of primer mixture (2 μM each), 1 unit of *Taq* polymerase, and sterile water to the final volume of 20 μL. PCR was performed in an Eppendorf Master Cycler (Eppendorf, Germany) using the following protocol: 2 min at 94°C; target-specific number of cycles composed of 10 s at 94°C, 30 s at the annealing temperature (Table 1), and 15 s at 72°C; 2 min at 72°C. The number of reaction cycles was 25 for 5-HTT in the midbrain, 26 for rPolII in the hippocampus, 27 for rPolII, TPH-2, and 5-HT_{1A} receptor in the midbrain and the frontal cortex, 28 for 5-HT_{1A} receptor in the hippocampus, and 30 for 5-HT_{2A} receptor in all brain structures studied. The optimal number of cycles that correspond to the exponential phase of amplicon accumulation was determined in a series of preliminary experiments.

PCR was performed at the same time and under the same conditions, with a series of mouse genomic DNA dilutions used as the external standard (5, 10, 20, and 30 ng, two replicates of each). The negative control contained no template. PCR products were separated by electrophoresis in 2% agarose gel stained with ethidium bromide and photographed using a digital camera. The intensity of fluorescence of PCR products synthesized from cDNA specimens and genomic DNA standards was measured using the Scion Image software (Scion Corporation, www.sciocorp.com) Next, the data were calibrated using the standard curve obtained by amplification of a series of genomic DNA dilutions (5, 10, 20, and 30 ng genomic DNA) and showing the amplification intensity as a function of genomic DNA concentration, which enabled us to determine the number of target cDNA copies in our specimens. The expression levels were presented as the number of target cDNA copies relative to 100 copies of the internal standard cDNA (rPol II).

Statistical analysis. Data were presented as $m \pm SEM$ and compared using two-way ANOVA with subsequent Fisher's post-hoc test.

RESULTS

It was found that the drug, as well as the drug × strain interaction had a significant effect on the total path length ($F_{1,34} = 10.4$; $P < 0.01$ and $F_{1,34} = 6.3$; $P < 0.05$, respectively). 8-OH-DPAT significantly increased the path length in C57BL/6 mice ($P < 0.001$; Fig. 2a). Both the drug administration ($F_{1,34} = 13.9$; $P < 0.001$)

Table 1. Nucleotide sequences and characteristics of the primers used

Gene	Primer sequence	Annealing temperature, °C	PCR product size, bp
5-HT_{1A}	F 5'-gactgccaccctctgccctatatc-3' R 5'-tcagcaaggcaaacaattccag-3'	62	199
5-HT_{2A}	F 5'-agaagccaccttgtgtgtga-3' R 5'-ttgctcgttgctgatggact-3'	61	169
TPH-2	F 5'-cattcctcgcacaattccagtcg-3' R 5'-agtctacatccatcccaactgctg-3'	61	239
5-HTT	F 5'-aagccccaccttgactcctcc-3' R 5'-ctccttcctctcctcacatatcc-3'	58	198
rPol II	F 5'- gttgtcgggcagcagaatgtag -3' R 5'-tcaatgagaccttctcgtcctcc-3'	63	188

and the drug × strain interaction ($F_{1,34} = 5.3$; $P < 0.05$) also affected the time spent in the center of arena, which increased significantly in C57BL/6 mice ($P < 0.001$; Fig. 2b). Chronic administration of 8-OH-DPAT increased the number of rearings in C57BL/6 mice ($P < 0.001$), but not in CBA animals (Fig. 3a). 8-OH-DPAT did not affect the number of groomings in either strain, although there were interstrain differences, with a higher number of groomings in CBA mice than in C57BL/6 mice ($P < 0.05$; Fig. 3b).

As expected, the chronic administration of 8-OH-DPAT, a selective agonist of 5-HT_{1A} receptors, decreased their functional activity ($F_{1,36} = 16.8$, $P < 0.001$; Fig. 5b). In particular, the magnitude of the hypothermic response to acute 8-OH-DPAT administration diminished threefold in experimental CBA mice ($P < 0.05$) and twofold in experimental C57BL/6 mice ($P < 0.01$). At the same time, a preliminary experiment showed that inhibition of 5-HT_7 receptors by the SB 269970 antagonist did not affect the magnitude of the hypothermic response induced by 8-OH-DPAT ($F_{1,16} = 0.43$; $P = 0.52$; Fig. 4).

Two-way ANOVA revealed the effects of strain ($F_{1,33} = 4.17$; $P < 0.05$) and the drug × strain interaction ($F_{1,33} = 7.14$; $P < 0.05$) on the level of 5-HT_{1A} receptor transcripts in the midbrain (Fig. 5a). A post-hoc test showed that the chronic administration of 8-OH-DPAT significantly downregulated the levels of 5-HT_{1A} receptor mRNA in CBA mice ($P < 0.01$), but not in C57BL/6 animals. The level of 5-HT_{1A} receptor

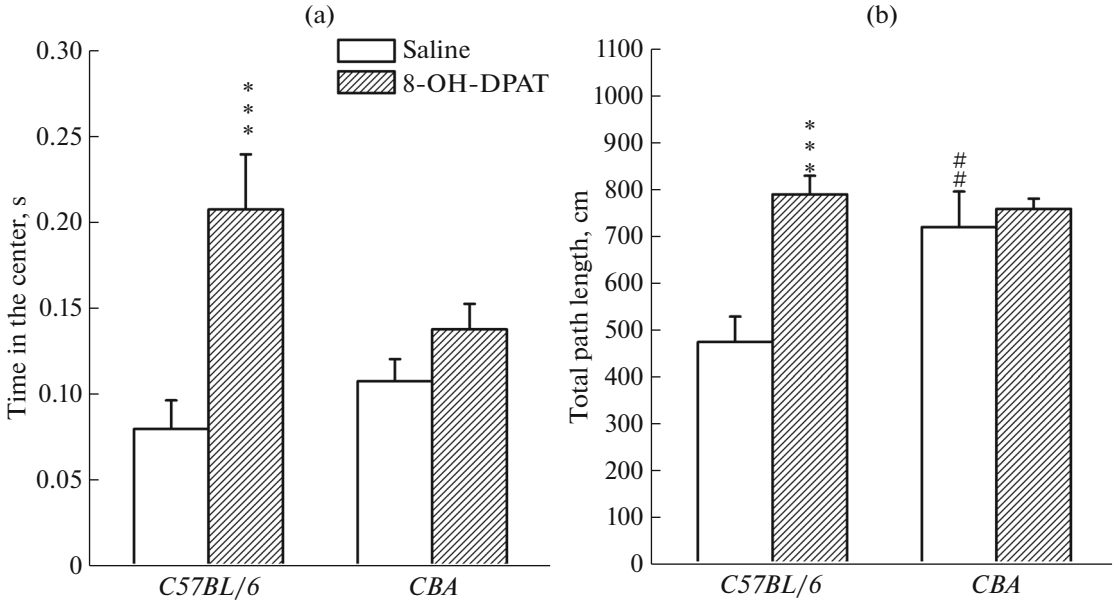

Fig. 2. Effect of chronic 8-OH-DPAT administration on the time spent in the center of the arena (a) and on the total path length (b) in the open field test in CBA and C57BL/6 mice; $n \geq 8$. ***$P < 0.001$ in comparison to control animals of the same strain; ##$P < 0.01$ compared to the C57BL/6 control.

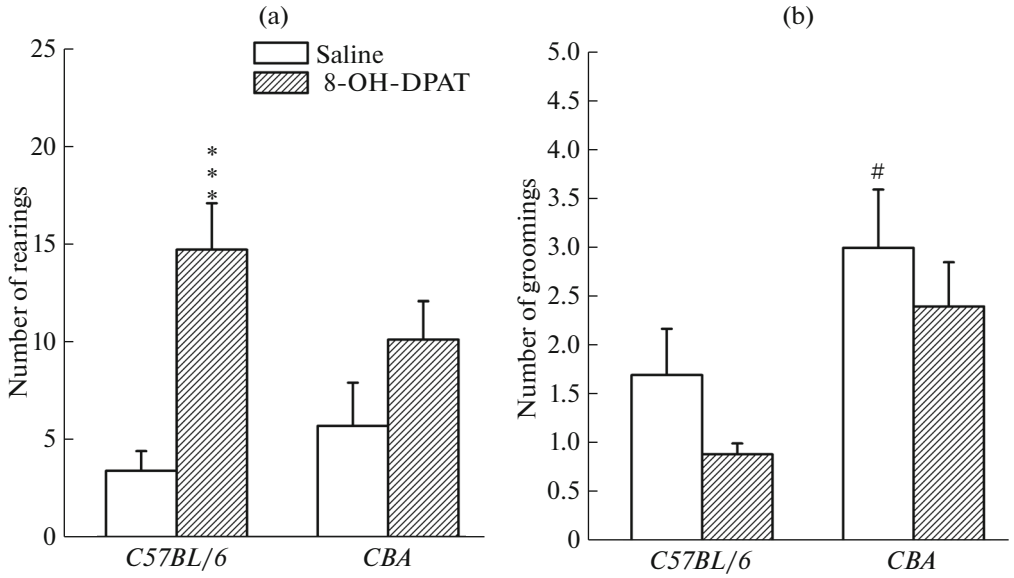

Fig. 3. Effect of chronic 8-OH-DPAT administration on the number of rearings (a) and groomings (b) in the open field test in CBA and C57BL/6 mice; $n \geq 8$. ***$P < 0.001$ compared to control animals of the same strain; $^{\#}P < 0.05$ compared to the C57BL/6 control.

mRNA in the hippocampus was strongly affected by the strain factor ($F_{1,29} = 17$; $P < 0.001$); in control CBA mice, it was two times higher than in C57BL/6 control mice ($P < 0.001$). In both strains, the administration of 8-OH-DPAT did not affect the levels of 5-HT_{1A} receptor transcripts in the frontal cortex. At the same time, the levels of 5-HT_{2A} receptor transcripts in the frontal cortex were affected by the strain ($F_{1,25} = 54$; $P < 0.001$) and the drug × strain interaction ($F_{1,25} = 4.24$; $P < 0.05$), and in the hippocampus, they were affected by the strain ($F_{1,30} = 36$; $P < 0.001$). The chronic administration of 8-OH-DPAT led to a decrease in the levels of 5-HT_{2A} receptor transcripts in the frontal cortex of CBA mice ($P < 0.05$), but not of C57BL/6 mice (Fig. 6). In addition, a post-hoc test showed that CBA mice had lower levels of 5-HT_{2A} receptor mRNA both in the frontal cortex and in the hippocampus compared to C57BL/6 animals ($P < 0.001$ and $P < 0.01$, respectively; Fig. 6).

Chronic administration of 8-OH-DPAT significantly decreased the levels of TPH-2-encoding mRNA in the midbrain of CBA mice ($P < 0.05$; Fig. 7a). A post-hoc test showed that these levels depended on the strain effect ($F_{1,30} = 4.51$; $P < 0.05$); CBA mice had significantly lower levels of TPH-2 mRNA than C57BL/6 mice ($P < 0.001$). The administration of 8-OH-DPAT did not affect the level of 5-HTT mRNA in the midbrain of experimental animals (Fig. 7b). At the same time, the levels of these transcripts were significantly higher in control CBA mice than in control C57BL/6 mice ($P < 0.001$).

DISCUSSION

Chronic activation of 5-HT_{1A} receptors by the 8-OH-DPAT agonist led to significant changes in behavior in the open field test in C57BL/6 mice, but not in CBA animals. In particular, there was a significant increase in the time spent in the center of arena , in the total path length, and in the number of rearings. The observed changes in the behavior of C57BL/6 mice indicate that long-term 8-OH-DPAT administration had an anxiolytic effect. At the same time, single-time administration of 8-OH-DPAT resulted in a

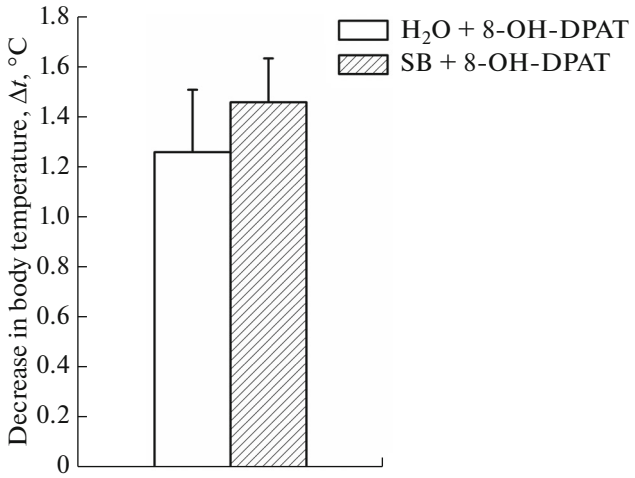

Fig. 4. Changes in the body temperature 20 min after administration of 8-OH-DPAT (1 mg/kg) in CBA mice who received a preliminary intraventricular injection of SB 269970 (0.025 nmol) or sterile water; $n \geq 8$.

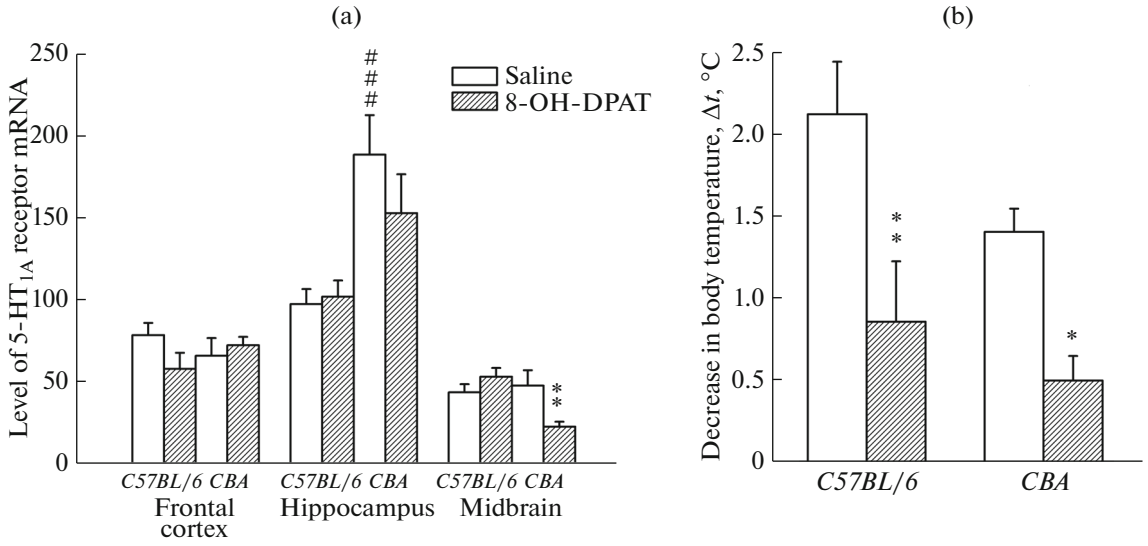

Fig. 5. Effect of chronic 8-OH-DPAT administration on the transcript level (a) and functional activity (b) of 5-HT_{1A} receptors in brain structures of C57BL/6 and CBA mice. The number of cDNA copies that encode 5-HT_{1A} receptor was determined per 100 copies of rPol II cDNA; $n \geq 8$, * $P < 0.05$; ** $P < 0.01$ compared to control animals of the same strain; ### $P < 0.001$ compared to the C57BL/6 control.

pronounced decrease of motor activity [33]. The latter effect may be due to suppressed excitation of serotonergic neurons, or to a significant decrease of 5-HT concentration in the synaptic cleft, or to the hypothermic response, or to a combination of these factors associated with the acute activation of 5-HT_{1A} receptors [34]. The observed interstrain differences (such as the greater path length and larger number of rearings in CBA mice) agree with the results of a previous comparative study which, however, compared these strains not between themselves, but with the DBA/2J strain [35].

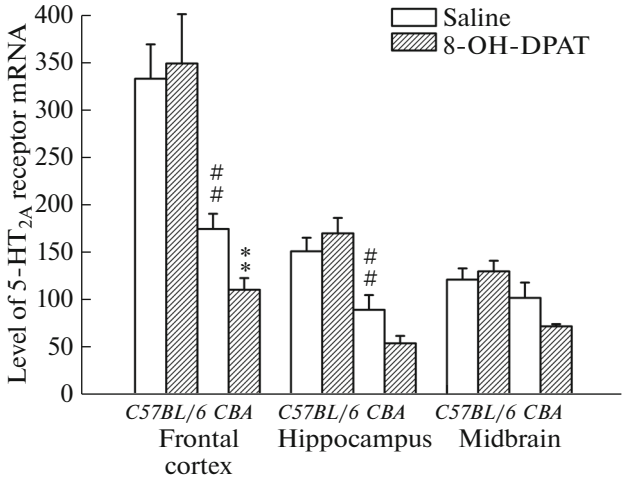

Fig. 6. Effect of chronic 8-OH-DPAT administration on the level of 5-HT_{2A} receptor transcripts in brain structures of C57BL/6 and CBA mice. Number of cDNA copies that enode 5-HT_{2A} receptor was determined per 100 copies of rPol II cDNA; $n \geq 8$, ** $P < 0.01$ compared to control animals of the same strain; ## $P < 0.01$ compared to C57BL/6 control.

The pronounced decrease in the hypothermic response observed in both CBA and C57BL/6 mice indicates that the chronic activation of 5-HT_{1A} receptors by the selective agonist 8-OH-DPAT led to a decrease in their functional activity (desensitization). This result in a good concordance with the data published by other groups [36, 37], as well as with our previous results [38]. It should be noted that, as shown in the preliminary experiment, the inhibition of 5-HT_7 receptors did not affect the magnitude of the hypothermic response to acute 8-OH-DPAT administration. This piece of evidence suggests that 5-HT_7 receptors are not involved in the hypothermic reaction induced by 8-OH-DPAT. We previously showed that central 5-HT_7 receptors participate in temperature regulation independently from 5-HT_{1A} receptors [39]. These data, along with those obtained in the present work, make us conclude that functional effects of 8-OH-DPAT are mediated by the activation of 5-HT_{1A} receptors independently from 5-HT_7 receptors, despite that 8-OH-DPAT also shows an affinity to the latter group of receptors.

CBA mice treated with 8-OH-DPAT exhibited the decreased expression of the 5-HT_{1A} receptor gene in the midbrain, where these receptors are located on the body and on presynaptic endings of 5-HT neurons and perform an autoregulatory function. On the one hand, this downregulation may be a result of desensitization and have a compensatory nature. On the other hand, it was only observed in CBA mice, which indirectly suggests that these cataleptic animals have some spe-

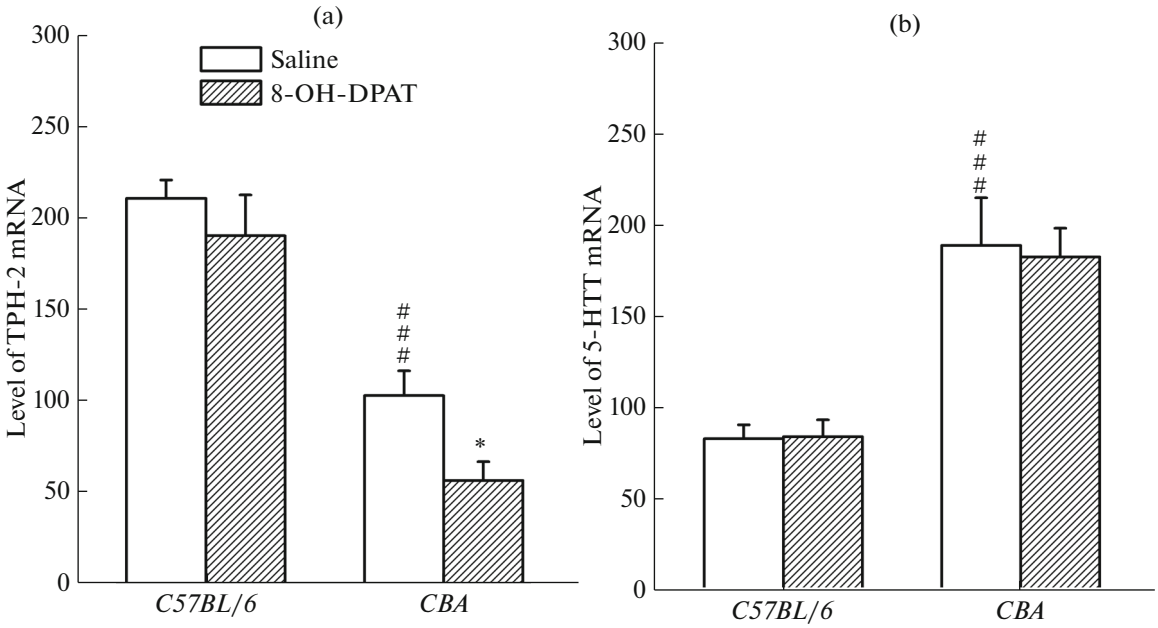

Fig. 7. Levels of mRNAs of (a) TPH-2 and (b) 5-HTT in the midbrain of C57BL/6 and CBA mice who received chronic treatment with 8-OH-DPAT. Number of TPH-2 and 5-HTT cDNA copies was determined per 100 copies of rPol II cDNA; $n \geq 8$. $^{*}P < 0.05$ compared to control animals of the same strain; $^{\#\#\#}P < 0.001$ compared to C57BL/6 control.

cific alterations in the system of 5-HT_{1A} autoreceptors. It is known that the anxiolytic effect of 8-OH-DPAT is mediated by presynaptic 5-HT_{1A} receptors [40]; therefore, the fact that behavior of CBA mice did not change in response to chronic 8-OH-DPAT administration can also be associated with specific properties of 5-HT_{1A} autoreceptors. It is also possible that postsynaptic 5-HT_{1A} receptors also play a role in this phenomenon: their expression in the hippocampus was significantly higher in CBA mice than in C57BL/6 mice. Interestingly, De Vry et al. showed that the overexpression of postsynaptic 5-HT_{1A} receptors in the cortex and the hippocampus diminished locomotor activity (especially in the presence of 8-OH-DPAT) [41]. It can be supposed that the enhanced expression of 5-HT_{1A} receptors in the hippocampus of CBA mice may significantly delay or attenuate their behavioral response to chronic 8-OH-DPAT administration.

We also found that the chronic administration of 8-OH-DPAT downregulated the expression of the 5-HT_{2A} receptor gene in the frontal cortex of CBA mice. This fact agrees well with the data obtained previously [38]. At the same time, the expression of the 5-HT_{2A} receptor gene was found to exhibit significant variation between strains. In particular, the expression of this gene in the frontal cortex and the hippocampus was significantly lower in cataleptic CBA mice than in C57BL/6 animals. We previously showed that all cataleptic mouse strains were characterized by decreased functional activity and the expression of 5-HT_{2A} receptors in the frontal cortex [42]. it is noteworthy that patients with depression also exhibit a decrease in the 5-HT_{2A} receptor density in the hippocampus [43]. Furthermore, our data indicate the ability of 5-HT_{1A} receptors to regulate 5-HT_{2A} receptors on the level of gene expression, which is in agreement with the notion about the two-way functional interaction between these two receptor subtypes. Previously, it was shown that the activation of 5-HT_{1A} receptors by the 8-OH-DPAT agonist caused a significant and dose-dependent decrease in the number of head shakes mediated by 5-HT_{2A} receptors [44]. The opposite is also true; the activation of 5-HT_{2A} receptors reversed the cataleptic immobility and significantly decreased the functional activity of 5-HT_{1A} receptors [45], whereas the inhibition of 5-HT_{2A} receptors enhanced the hypothermic response mediated by 5-HT_{1A} receptors [46].

A further result that deserves the discussion is the diminished expression of TPH-2, a key enzyme of 5-HT biosynthesis in the brain, in CBA mice that received chronic 8-OH-DPAT injections. At the same time, the compound did not affect the expression of the 5-HTT gene in both mouse strains. However, the control mice of the CBA strain significantly exhibited the decreased expression of the TPH-2 gene and, at the same time, significantly elevated the expression of the 5-HTT gene in comparison to control C57BL/6 mice. The latter phenomenon may be an adaptation aimed to enhance the efficiency of 5-HT reuptake from the synaptic cleft in the situation where TPH-2 activity is decreased.

The molecular mechanism by which 8-OH-DPAT suppresses the expression of genes that encode 5-HT_{1A} and 5-HT_{2A} receptors, as well as TPH-2, remains unclear. However, it is known that 5-HT_{1A} receptors regulate 5-HT secretion [44] and that acute administration of 5-HT_{1A} receptor agonists inhibits excitation of 5-HT neurons and, as a result, diminishes the 5-HT secretion in the projection areas. In contrast, the repeated activation of 5-HT_{1A} autoreceptors, which leads to their desensitization is known to increase the 5-HT levels [45]. The impaired transcriptional regulation of 5-HT_{1A} autoreceptors, which prevents desensitization, leads to depression and insensitivity to antidepressants [23]. The currently accumulated body of data suggests that 5-HT is an important inducer of early response genes, such as *c-Fos* or *Rac1* [46, 47]; therefore, there can be no doubt that it is involved in the regulation of gene expression. It can be supposed that the chronic activation of 5-HT_{1A} autoreceptors modulates the expression of 5-HT_{2A} receptor and TPH-2 genes by increasing 5-HT levels. The available data support the hypothesis that 5-HT may play a central role in the regulation of 5-HT_{1A} receptor gene expression.

Importantly, homologous effects of the chronic activation of 5-HT_{1A} receptors (limited to the same receptor system, e.g., desensitization), as well as its heterologous effects (interaction of different receptor systems), were only observed in mice with genetic predisposition to catalepsy, but not in animals with normal genotypes, such as those of the C57BL/6 strain. Apparently, the differences in the functional response to 8-OH-DPAT administration are due to specific features of 5-HT_{1A} receptors themselves. Another possible explanation for the different responses to the chronic activation of 5-HT_{1A} receptors is the formation of heterodimers of 5-HT_{1A} and 5-HT_7 receptors. Recent data indicate that 5-HT_{1A}/5-HT_7 heterodimerization is a prerequisite for 5-HT_{1A} receptor internalization, which significantly depends on the 5-HT_7 receptor density [48, 49]. Presumably, the higher concentration of 5-HT_{1A}/5-HT_7 heterodimers on presynaptic neurons compared to postsynaptic neurons underlies the different sensitivity of pre- and postsynaptic 5-HT_{1A} receptors and the regional variations in their desensitization [50]. The obtained results suggest that animals genetically predisposed to catalepsy are, in general, characterized with a complex pattern of 5-HT system autoregulation involving heterologous gene–receptor interactions among its key genes.

Thus, the results of our study provide new evidence of gene–receptor interactions in the 5-HT system of the brain, which may underlie the loss of pharmacological efficacy of anxiolytics. Furthermore, the lack of behavioral response and the compensatory changes in the expression of key genes of 5-HT system in CBA mice suggest that the cataleptic and the noncataleptic genotypes are characterized with different drug sensitivity.

ACKNOWLEDGMENTS

This work was financially supported by the Russian Science Foundation (project no. 14-15-00025). Maintenance of laboratory animals was supported by the budget project no. 0324-2016-0002.

REFERENCES

1. Popova N.K. 2004. The role of brain serotonin in the expression of genetically determined defensive behavior. *Russ. J. Genet.* **40** (6), 624–630.
2. Weder N.D., Muralee S., Penland H., et al. 2008. Catatonia: A review. *Ann Clin Psychiatry*. **20**, 97–107.
3. Amir S., Brown Z.W., Amir Z., et al. 1981. Body-pinches induced long lasting cataleptic-like immobility in mice: Behavioral characterization and the effects of naloxone. *Life Sci*. **10**, 1189–1194.
4. Kulikov A.V., Kozlachkova E.Y., Maslova G.B., et al. 1993. Inheritance of predisposition to catalepsy in mice. *Behav. Genet*. **23,** 379–384.
5. Kulikov A.V., Bazovkina D.V., Moisan M.P., et al. 2003. The mapping of the gene of susceptibility to catalepsy in mice using polymorphic microsatellite markers. *Dokl. Biol. Sci*. **393**, 531–534.
6. Kulikov A.V. 2004. Hereditary catalepsy: genetic and molecular mechanisms of catalepsy in mice. *Russ. J. Genetics*. **40,** 631–637.
7. Kulikov A.V., Bazovkina D.V., Kondaurova E.M., et al. 2008. Genetic structure of hereditary catalepsy in mice. *Genes Brain Behav*. **7**, 506–512.
8. Neal-Beliveau B.S., Joyce J.N., Lucki I. 1993. Serotonergetic involvement in haloperidol-induced catalepsy. *Exp. Ther*. **265**, 207–217.
9. Wadenberg M.L. 1996. Serotonergic mechanisms in neuroleptic-induced catalepsy in the rat. *Neurosci. Biobehav. Rev*. **20**, 325–339.
10. Prinssen E.P., Colpaert F.C., Koek W. 2002. 5-HT_{1A} receptor activation and anti-cataleptic effects: High-efficacy agonists maximally inhibit haloperidol-induced catalepsy. *Eur. J. Pharmacol*. **453**, 217–221.
11. Ohno Y., Shimizu S., Imaki J. 2009. Effects of tandospirone, a 5-HT_{1A} agonistic anxiolytic agent, on haloperidol-induced catalepsy and forebrain Fos expression in mice. *J. Pharmacol. Sci*. **109**, 593–599.
12. Newman-Tancredi A. 2010. The importance of 5-HT_{1A} receptor agonism in antipsychotic drug action: Rationale and perspectives. *Curr. Opin. Investig*. **11**, 802–812.
13. Iderberg H., McCreary A.C., Varney M.A., et al. 2015. NLX-112, a novel 5-HT_{1A} receptor agonist for the treatment of l-DOPA-induced dyskinesia: Behavioral and neurochemical profile in rat. *Exp. Neurol*. **271**, 335–350.
14. Kulikov A.V., Kolpakov V.G., Maslova G.B., et al. 1994. Effect of selective 5-HT_{1A} agonists and 5-HT_2

antagonists on inherited catalepsy in rats. *Psychopharmacology*. **114**, 172–174.

15. Popova N.K., Kulikov A.V. 1995. On the role of brain serotonin in expression of genetic predisposition to catalepsy in animal models. *Am. J. Med. Genet. Neuropsychiatric Gene)*. **60**, 214–220.
16. Popova N.K., Kulikov A.V., Avgustinovich D.F., et al. 1994. Influence of brain 5-HT_{1A} serotonin receptors in the regulation of inherited catalepsy. *Bull. Exp. Biol. Med*. **118**, 633–635.
17. Bazovkina D.V., Terenina E.E., Kulikov A.V. 2010. Effect of selective agonist of serotonin 5-HT_{1A} receptors on defensive behavior in mice with different predisposition to catalepsy. *Bull. Exp. Biol. Med*. **150**, 225–228.
18. Kondaurova E.M., Bazovkina D.V., Kulikov A.V., et al. 2006. Selective breeding for catalepsy changes the distribution of microsatellite D13Mit76 alleles linked to the 5-HT serotonin receptor gene in mice. *Genes Brain Behav*. **5**, 596–601.
19. Bazovkina D.V., Kulikova A.V., Kondaurova E.M., et al. 2005. Selection for the predisposition to catalepsy enhances depressive-like traits in mice. *Russ. J. Genetics*. **41,** 1002–1007.
20. Tikhonova M.A., Kulikov A.V., Bazovkina D.V., et al. 2013. Hereditary catalepsy in mice is associated with the brain dysmorphology and altered stress response. *Behav. Brain Res*. **243**, 53–60.
21. Tikhonova M.A., Al'perina E.L., Tolstikova T.G., et al. 2009. Effects of chronic fluoxetine treatment on catalepsy and immune response in mice genetically predisposed to freezing reaction: The role of 5-HT1A and 5-HT2A receptors and tph2 and SERT genes. *Zh. Vyssh. Nerv. Deiat. im. I. P. Pavlova*. **59**, 237–244.
22. Naumenko V.S., Kondaurova E.M., Bazovkina D.V., et al. 2012. Effect of brain-derived neurotrophic factor on behavior and key members of the brain serotonin system in genetically predisposed to behavioral disorders mouse strains. *Neuroscience*. **214**, 59–67.
23. Albert P.R., François B.L. 2010. Modifying 5-HT_{1A} receptor gene expression as a new target for antidepressant therapy. *Front. Neurosci*. **4**, 35. doi 10.3389/fnins.2010.00035
24. Popova N.K., Naumenko V.S. 2013. 5-HT_{1A} receptor as a key player in the brain 5-HT system. *Rev. Neurosci*. **24**, 191–204.
25. Celada P., Bortolozzi A., Artigas F. 2013. Serotonin 5-HT_{1A} receptors as targets for agents to treat psychiatric disorders: rationale and current status of research. *CNS Drugs*. **27**, 703–716.
26. Watson J., Collin L., Ho M., et al. 2000. 5-HT(1A) receptor agonist–antagonist binding affinity difference as a measure of intrinsic activity in recombinant and native tissue systems. *Br. J. Pharmacol*. **130**, 1108–1114.
27. Assie M.B., Koek W. 2000. [(3)H]-8-OH-DPAT binding in the rat brain raphe area: Involvement of 5-HT(1A) and non-5-HT(1A) receptors. *Br. J. Pharmacol*. **130**, 1348–1352.
28. Kulikov A.V., Tikhonova M.A., Kulikov V.A. 2008. Automated measurement of spatial preference in the open field test with transmitted lighting. *J. Neurosci. Meth*. **170**, 345–351.
29. Overstreet D.H. Rezvani A.H., Knapp D.J., et al. 1996. Further selection of rat lines differing in 5-HT-1A receptor sensitivity: Behavioral and functional correlates. *Psychiat. Genet*. **6**, 107–117.
30. Barnes N.M., Sharp T. 1999. A review of central 5-HT receptors and their function. *Neuropharmacology*. **38**, 1083–1152.
31. Slotnick B.M., Leonard C.M. 1975. *A Stereotaxic Atlas of the Albino Mouse Forebrain*. Rockville, MD: U.S. Dept. of Health, Education and Welfare.
32. Naumenko V.S., Kulikov A.V. 2006. Quantitative assay of 5-HT(1A) serotonin receptor gene expression in the brain. *Mol. Biol.* (Moscow). **40**, 30–36.
33. Brookshire B.R., Jones S.R. 2009. Direct and indirect 5-HT receptor agonists produce gender-specific effects on locomotor and vertical activities in C57 BL/6J mice. *Pharmacol. Biochem. Behav*. **94**, 194–203.
34. Piñeyro G., Blier P. 1999. Autoregulation of serotonin neurons: Role in antidepressant drug action. *Pharmacol. Rev.* **51**, 533–591.
35. Popova N.K., Naumenko V.S., Tibeikina M.A., et al. 2009. Serotonin transporter, 5-HT_{1A} receptor, and behavior in DBA/2J mice in comparison with four inbred mouse strains. *J. Neurosci. Res*. **87**, 3649–3657.
36. Martin K.F., Phillips I., Hearson M., et al. 1992. Characterization of 8-OH-DPAT-induced hypothermia in mice as a 5-HT_{1A} autoreceptor response and its evaluation as a model to selectively identify antidepressants. *Br. J. Pharmacol*. **107**, 15–21.
37. Blier P., Seletti B., Gilbert F., et al. 2002. Serotonin 1A receptor activation and hypothermia in humans: Lack of evidence for a presynaptic mediation. *Neuropsychopharmacology*. **27**, 301–308.
38. Popova N.K., Naumenko V.S., Cybko A.S., et al. 2010. Receptor-genes cross-talk: Effect of chronic 5-HT(1A) agonist 8-hydroxy-2-(di-n-propylamino)tetralin treatment on the expression of key genes in brain serotonin system and on behavior. *Neuroscience*. **169**, 229–235.
39. Naumenko V., Kondaurova E.M., Popova N.K. 2011. On the role of brain 5-HT_7 receptor in the mechanism of hypothermia: Comparison with hypothermia mediated via 5-HT_{1A} and 5-HT_3 receptor. *Neuropharmacology*. **61**, 1360–1365.
40. De Vry J., Schreiber R., Melon C., et al. 2004. 5-HT_{1A} receptors are differentially involved in the anxiolytic- and antidepressant-like effects of 8-OH-DPAT and fluoxetine in the rat. *Eur. Neuropsychopharmacology*. **14**, 487–495.
41. Bert B., Fink H., Hörtnagl H., et al. 2006. Mice overexpressing the 5-HT(1A) receptor in cortex and dentate gyrus display exaggerated locomotor and hypothermic response to 8-OH-DPAT. *Behav. Brain Res*. **167**, 328–341.
42. Naumenko V.S., Bazovkina D.V., Kondaurova E.M., et al. 2010. The role of 5-HT_{2A} receptor and 5-HT_{2A}/5-HT_{1A} receptor interaction in the suppression of catalepsy. *Genes Brain Behav*. **9**, 519–524.

43. Mintun M.A., Sheline Y.I., Moerlein S.M., et al. 2004. Decreased hippocampal 5-HT_{2A} receptorbinding in major depressive disorder: In vivo measurement with [18F]altanserin positron emission tomography. *Biol. Psychiatry*. **55**, 217–224.

44. Naumenko V.S., Bazovkina D.V., Kondaurova E.M. 2015. On the functional cross-talk between brain 5-HT_{1A} and 5-HT_{2A} receptors. *Zh. Vyssh. Nerv. Deiat. im. I. P. Pavlova*. **65**, 240–247.

45. Pineyro G., Blier P. 1999. Autoregulation of serotonin neurons: Role in antidepressant drug action. *Pharmacol. Rev*. **51**, 533–591.

46. Blier P., de Montigny C. 1994. Current advances and trends in the treatment of depression. *Trends Pharmacol. Sci*. **15**, 220–226.

47. Goppelt-Struebe M., Hahn A., Stroebel M., et al. 1999. Independent regulation of cyclo-oxygenase 2 expression by p42/44 mitogen-activated protein kinases and Ca2/calmodulin-dependent kinase. *Biochem. J*. **339**, 329–334.

48. Simon A.R., Severgnini M., Takahashi S., et al. 2005. 5-HT induction of *c-fos* gene expression requires reactive oxygen species and Rac1 and Ras GTPases. *Cell Biochem. Biophys*. **42**, 263–276.

49. Naumenko V.S., Popova N.K., Lacivita E., et al. 2014. Interplay between serotonin 5-HT_{1A} and 5-HT_7 receptors in depressive disorders. *CNS Neurosci. Ther*. **20**, 582–590.

50. Popova N.K., Ponimaskin E.G., Naumenko V.S. 2015. Cross-talk between 5-HT_{1A} and 5-HT_7 receptors: Role in the autoregulation of the brain serotonin system and in mechanism of antidepressants action. *Ross. Fiziol. Zh. im. I.M. Sechenova*. **101**, 1270–1278.

Translated by D. Timchenko