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RESEARCH PAPER

Alterations in pharmacological and behavioural responses in recombinant mouse line with an increased predisposition to catalepsy: role of the 5-HT$_{1A}$ receptor

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BACKGROUND AND PURPOSE

One important syndrome of psychiatric disorders in humans is catalepsy. Here, we created mice with different predispositions to catalepsy and analysed their pharmacological and behavioural properties.

EXPERIMENTAL APPROACH

Two mouse lines, B6-M76C and B6-M76B, were created by transfer of the main locus of catalepsy containing the 5-HT$_{1A}$ receptor gene to the C57BL/6 genetic background. Behaviour, brain morphology, expression of key components of the serotoninergic system, and pharmacological responses to acute and chronic stimulation of the 5-HT$_{1A}$ receptor were compared.

KEY RESULTS

B6-M76B mice were not cataleptic, whereas 14% of B6-M76C mice demonstrated catalepsy and decreased depressive-like behaviour. Acute administration of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT resulted in dose-dependent hypothermia and in decreased locomotion in both lines. Chronic 8-OH-DPAT administration abolished the 5-HT$_{1A}$ receptor-mediated hypothermic response in B6-M76C mice and increased locomotor activity in B6-M76B mice. In addition, 5-HT metabolism was significantly reduced in the hippocampus of B6-M76C mice, and this effect was accompanied by an increased expression of the 5-HT$_{1A}$ receptor.

CONCLUSIONS AND IMPLICATIONS

Our findings indicate that transfer of the main locus of hereditary catalepsy containing the 5-HT$_{1A}$ receptor from CBA mice to the C57BL/6 genetic background led to increased postsynaptic and decreased presynaptic functional responses of the 5-HT$_{1A}$ receptor. This characteristic establishes the B6-M76C line as an attractive model for the pharmacological screening of 5-HT$_{1A}$ receptor-related drugs specifically acting on either pre- or postsynaptic receptors.

LINKED ARTICLES

This article is part of a themed section on Updating Neuropathology and Neuropharmacology of Monoaminergic Systems. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v173.13/issuetoc

Abbreviations

5-HIAA, 5-hydroxyindoleacetic acid; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino) tetralin; ASC, antidepressant-sensitive catalepsy; BDNF, brain-derived neurotrophic factor; EPM, elevated plus-maze test; FST, forced swim test; OF, open field test; Slc6a4, gene encoding 5-HT transporter; Tph2, tryptophan hydroxylase 2

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Tables of Links

TARGETS	
GPCRs[a]	**Enzymes**[c]
5-HT_{1A} receptor	MAO-A
5-HT_{2A} receptor	Tph2
Catalytic receptors[b]	**Transporters**[d]
p75 (TNFRSF16)	Slc6a4
TrkB	

LIGANDS	
5-HT	Haloperidol
8-OH-DPAT	Imipramine
BDNF	p-Chlorophenylalanine
Fluoxetine	

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 ([a,b,c,d]Alexander *et al.*, 2015a,b,c,d).

Introduction

Catalepsy (tonic immobility, immobility reflex and animal hypnosis) is characterized by muscular rigidity leading to prolonged immobility and an inability to correct an externally imposed awkward posture. Under physiological conditions, catalepsy can be obtained in some vertebrates as a kind of passive defensive behaviour against a predator (Dixon, 1998). In humans, excessive catalepsy-like dyskinesia is a pathological symptom occurring in schizophrenia, mood disorders (e.g. depression) and Parkinsons' disease (Klemm, 1989). It has been shown that the brain serotoninergic (5-HT) system is crucially involved in the mechanisms of catalepsy in mice. For example, pharmacological activation of the 5-HT_{1A} receptor with 8-OH-DPAT attenuates catalepsy induced by neuroleptics (Wadenberg and Hillegaart, 1995; Wadenberg, 1996) as well as hereditary catalepsy in rats and mice (Kulikov *et al.*, 1994; Popova *et al.*, 1994; Popova and Kulikov, 1995). In addition, decreased expression of the gene for the 5-HT_{2A} receptor was found in the frontal cortex of all mice predisposed to catalepsy when compared with catalepsy-resistant mice of the AKR strain (Naumenko *et al.*, 2010). Moreover, irreversible inhibition of tryptophan hydroxylase 2 (Tph2), the key enzyme for 5-HT synthesis, by *p*-chlorophenylalanine significantly reduces catalepsy in rodents (Popova and Kulikov, 1995; Wadenberg and Hillegaart, 1995; Popova, 1997). More recently, the involvement of brain-derived neurotrophic factor (BDNF) in the mechanisms of hereditary catalepsy in mice has also been demonstrated (Tikhonova *et al.*, 2009; Naumenko *et al.*, 2012; Naumenko *et al.*, 2014).

In rodents, catalepsy can be induced by administration of the antipsychotic drug haloperidol, and such animals can be used as an appropriate model for analysis of extrapyramidal dysfunctions (Wadenberg, 1996). In contrast, a drug-free or hereditary catalepsy is a relatively rare phenomenon, even though it might represent a suitable model for the analysis of genetic and molecular mechanisms involved in the psychopathology of catalepsy as well as for the development of novel anti-cataleptic pharmacological substances. In mice with predisposition to hereditary catalepsy, a cataleptic episode can be evoked by pinching the skin at the scruff of the neck (pinch-induced catalepsy) (Ornstein and Amir, 1981). It is noteworthy that the predisposition to pinch-induced catalepsy undergoes significant genetic variability. It has been shown that pinching never induces catalepsy in intact C57BL/6, DBA/2 and AKR mouse strains. In contrast, about 50% of CBA/Lac mice showed pronounced immobility for more than 60 s after 4–5 pinches (Kulikov *et al.*, 1989; Kulikov *et al.*, 1993). Previously, we demonstrated that hereditary catalepsy in CBA mice can be markedly increased by prolonged selective breeding of the backcrosses between the CBA and catalepsy-resistant AKR strain to produce mice with a high predisposition to catalepsy. About 80% of mice of the resulting Antidepressant-Sensitive Catalepsy (ASC) line demonstrated cataleptic immobility. In addition to increased catalepsy, ASC mice showed depressive-like behaviour and immune deficiency (Alperina *et al.*, 2007). It is noteworthy that chronic pharmacological treatment of ASC mice with classic antidepressants, including imipramine, fluoxetine and acute i.c.v. administration of BDNF, significantly reduced catalepsy, suggesting that ASC mice are a suitable model for verification of antidepressive drugs (Tikhonova *et al.*, 2006; Tikhonova *et al.*, 2010; Naumenko *et al.*, 2012).

Using a combination of selective breeding, genetic recombination and quantitative trait loci analysis, the main locus of predisposition to catalepsy was mapped on the105.89–118.83 Mbp fragment of mouse chromosome 13 (Kulikov *et al.*, 2003; Kondaurova *et al.*, 2006; Kulikov *et al.*, 2008a). Among others, this locus contains a gene encoding the 5-HT_{1A} receptor. Transfer of the CBA-derived 105.89–118.83 Mbp fragment of chromosome 13 to the genome of the AKR mouse line resulted in the creation of the recombinant AKR. CBA-D13Mit76 line demonstrating pronounced catalepsy (50%), a high level of intermale aggression and learning deficits in the Morris water maze (Kulikov *et al.*, 2014), which can be restored after an acute i.c.v. administration of BDNF (Kulikov *et al.*, 2014). In addition to changes in behaviour, AKR.CBA-D13Mit76 mice are characterized by decreased expressions of the key genes of the brain serotonergic system and reduced functional activity of 5-HT_{2A} receptors in comparison with mice of the parental AKR strain (Naumenko

et al., 2014). However, no significant changes in 5-HT levels or its metabolism in the brain of AKR.CBA-D13Mit76 mice were observed (Sinyakova *et al.*, 2014).

Even though the involvement of the 105.89–118.83 Mbp fragment of chromosome 13 in catalepsy was convincingly shown, the role of this locus in the regulation of brain function and pathological behaviour as well as underlying mechanisms are still not known. Therefore, the results obtained with the AKR.CBA-D13Mit76 line are difficult to compare with other animal models, because the AKR genotype is not generally utilized as a genetic background for transgenic mice. While the catalepsy-resistant C57BL/6 strain is widely used as a genetic background in numerous mouse models as well as in multiple pharmacological screenings, we decided to transfer the main locus of predisposition to catalepsy containing the 5-HT_{1A} receptor gene from the catalepsy-prone strain CBA to the genome of catalepsy-resistant C57BL/6 mice. To perform this task, we created the B6.CBA-D13Mit76C (B6-M76C) and B6.CBA-D13Mit76B (B6-M76B) recombinant lines on the genetic C57BL/6 background; these two lines were distinguished by the CBA-derived and C57BL/6-derived fragments of chromosome 13 containing the 5-HT_{1A} receptor gene. These lines were then compared for the occurrence of catalepsy, for behavioural differences as well as for their brain morphology. In addition, we compared these mouse lines with respect to their behavioural and hypothermic responses after acute and chronic pharmacological stimulation of 5-HT_{1A} receptors with the selective receptor agonist 8-OH-DPAT. Finally, we evaluated the expression of key genes of the brain serotonergic and BDNF systems as well as the brain level of 5-HT and its main metabolite 5-hydroxyindoleacetic acid (5-HIAA) in both lines.

Methods

Breeding of mouse lines

Two recombinant lines were generated by transferring the CBA-derived fragment 105.89–118.83 Mbp of chromosome 13 including the main locus of catalepsy to the C57BL/6 (B6) genome (Kulikov *et al.*, 2003). Males generated of this catalepsy-prone AKR.CBA-D13Mit76 recombinant mouse line containing the CBA-derived fragment in the AKR genome were mated with females of inbred mouse strain B6 to obtain the F1 hybrids (Kulikov *et al.*, 2008a). The latter were used to create the recombinant line. After eight successive backcrossings of the F1 hybrids to the B6 strain, the heterozygous backcrosses were intercrossed to generate B6.CBA-D13Mit76C (B6-M76C) and B6.CBA-D13Mit76B (B6-M76B), containing the CBA-derived and B6-derived alleles of D13Mit76 and AKR-derived and B6-derived alleles of D13Mit78 in the B6 genome respectively.

Table 1

Primer sequences, annealing temperatures and PCR product length

Gene	Sequence	Annealing temperature, °C	Product length, bp
D13Mit76 marker	F5′-atgcacctgtctaaatgtgtgc-3′ R5′-agagggactgtgggactgtg-3′	60	109
D13Mit78 marker	F5′-acagcacgggtttatcatcc-3′ R5′-tatgcctgccaggcttctat-3′	60	229
BDNF	F5′- tagcaaaaagagaattggctg −3′ R5′- tttcaggtcatggatatgtcc −3′	59	255
TrkB	F5′-cattcactgtgagaggcaacc-3′ F5′-atcagggtgtagtctccgttatt-3′	63	175
p75	F5′- acaacacccagcacccagga −3′ R5′-cacaaccacagcagccaaga −3′	62	171
MAO-A	F5′-aatgaggatgttaaatgggtagatgttggt-3′ R5′-cttgacatattcaactagacgctc-3′	62	138
5-HT_{1A} receptor	F5′-gactgccaccctctgccctatatc-3′ R5′-tcagcaaggcaaacaattccag-3′	62	200
5-HT_{2A} receptor	F5′-agaagccaccttgtgtgtga-3′ R5′-ttgctcattgctgatggact-3′	61	169
Tph2	F5′-cattcctcgcacaattccagtcg-3′ R5′-agtctacatccatcccaactgctg-3′	61	239
Slc6a4	F5′-aagccccaccttgactcctcc-3′ R5′-ctccttcctctcctcacatatcc-3′	57	198
Polr2a	F5′-gttgtcgggcagcagaatgtag-3′ R5′-tcaatgagaccttctcgtcctcc-3′	63	188

Genotyping

DNA samples were extracted from tail tip tissue as previously described (Kulikov *et al.*, 2003). Briefly, a piece of tail tip was digested overnight with 1 mL of buffer containing Tris–HCl, pH 7.9, 0.1 M, NaCl, 0.1 M, EDTA, 0.1 M, 0.5% SDS and 100 mg·mL^{-1} proteinase K, and DNA was extracted with 1 mL of phenol–chloroform mixture (1:1) and kept at −20°C. DNA samples were genotyped by PCR with two polymorphic microsatellites D13Mit76 and D13Mit78, mapped at 110.56 and 118.83 Mbp chromosome 13 respectively. Genomic DNA (50 ng in 5 μL) was mixed with 5 μL of corresponding primers (to a final concentration of 250 pM for each) (Table 1), 1 U of TaqDNA-polymerase (Sibenzyme, Novosibirsk, Russia) and PCR buffer was added to make up to 20 μL, the final reaction volume. The PCR protocol was (a) 96°C, 5 min, (b) 35 cycles (92°C, 40 s; 60°C, 30 s; and 72°C, 30 s) and (c) 72°C, 2 min (Moisan *et al.*, 1996). Mouse genotype was determined by electrophoreses of corresponding PCR products on 3% agarose gels. In order to generate B6-M76C, the line of mice carrying the D13Mit76 marker *c*/*c* genotype (*c*-allele derived from CBA) and D13Mit78 marker *a*/*a* genotype (*a*-allele derived from AKR) were chosen. For B6-M76B, line was generated from mice carrying D13Mit76 marker *b*/*b* genotype (*b*-allele derived from C57BL/6) and genotype D13Mit78 marker *b*/*b* were chosen (Figure 1A and B).

Animals and experimental procedures

The breeding of new lines was performed in the frame of the basic research project no. 0324–2015-0004 and conducted in the Centre for Genetic Resources Laboratory Animals (RFMEFI61914X0005 and RFMEFI62114X0010). Adult male mice of B6-M76C and B6-M76B lines were used for behavioural and pharmacological experiments. Animals were housed in groups of 7–8 per cage under standard conditions (20–22°C, free access to food and water, 12 h light/dark cycle). Two days before the experiments, mice were weighed and isolated in individual cages to remove the group effect. In all experiments, mice of different genotypes were aligned by weight. All experiments were performed in a double-blind fashion. The brain morphology of the B6-M76C (n = 5) and B6-M76B (n = 6) mice was compared by MRI tomography.

In behavioural experiments, B6-M76C (n = 16) and B6-M76B (n = 9) mice were tested in the open field (OF), elevated plus-maze (EPM) and forced swim tests (FST) and then for the occurrence of pinch-induced catalepsy (see discussion below). Two days later, randomly chosen animals of each genotype (n = 8) were decapitated; the frontal cortex, hippocampus and midbrain area were dissected, frozen in liquid nitrogen and stored at −80°C until RNA isolation. An increased number of mice of B6-M76C genotype (n = 16) was used for more precise evaluation of the percentage of cataleptics in this line.

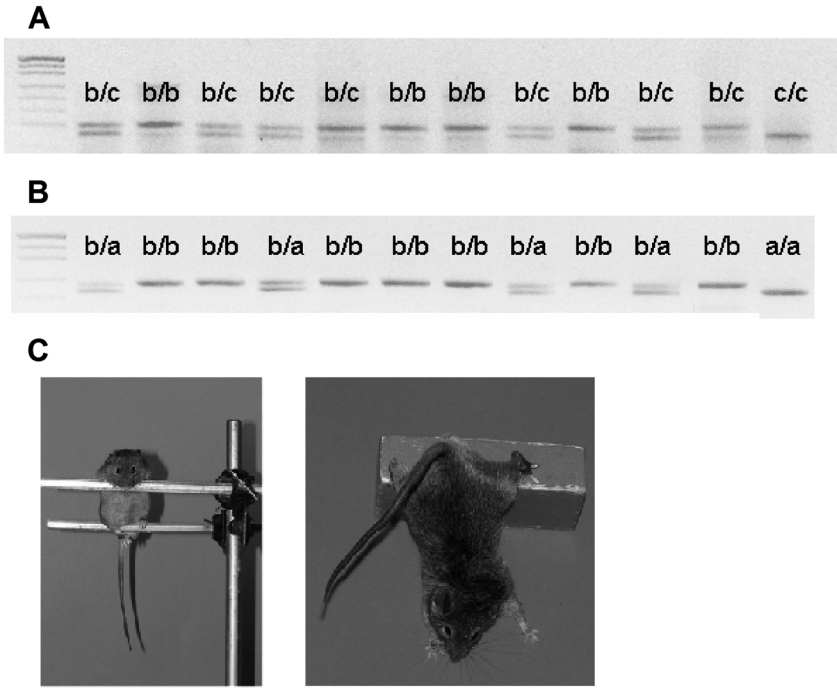

Figure 1

(A) Representative picture of the gel after electrophoresis of PCR products amplified with primers for D13Mit76 microsatellite used for selection of B6-M76C mice carrying the *c*/*c* genotype marker (where *c* means *c*-allele derived from CBA) and B6-M76B mice carrying the *b*/*b* genotype marker (where *b* means *b*-allele derived from C57BL/6). (B) Electrophoresis of PCR products amplified using primers for D13Mit78 microsatellite used for selection of B6-M76C mice carrying the *a*/*a* genotype marker (where *a* means *a*-allele derived from AKR) and B6-M76B mice carrying the *b*/*b* genotype marker. (C) Representative photographs showing mice of the B6-M76C line during a catalepsy episode (see also Supporting Information Videos S1 and S2).

In pharmacological experiments, the effects of acute i.p. administration of 0.1 and 1 mg·kg^{-1} of 8-OH-DPAT on the behaviour in the OF as well as on the hypothermic response (see below) were investigated in males divided into six groups (three groups for each genotype) and treated with vehicle, 0.1 mg·kg^{-1} and 1 mg·kg^{-1} of 8-OH-DPAT (n = 8 per genotype per drug). In addition, the effects of chronic administration of 8-OH-DPAT (14 days, 1 mg·kg^{-1}, i.p.) on the behaviour in the OF and 5-HT$_{1A}$ receptor-mediated hypothermic responses were investigated in male groups (n = 8 per genotype per drug).

Moreover, 5-HT metabolism in the frontal cortex, hippocampus, striatum and midbrain area of B6-M76C and B6-M76B mice (n = 8 per genotype) was studied using HPLC. All experimental procedures were in compliance with the EC Directive 86/609/EEC for animal experiments and were approved by the Institutes' Ethics Committee. All efforts were made to minimize the number of animals used and their suffering. The researchers were blind to the protocol of the experiments. Animal studies are reported in compliance with the ARRIVE guidelines (Kilkenny *et al.*, 2010; McGrath & Lilley, 2015).

Pharmacological analysis of the 5-HT$_{1A}$ receptor functional activity

The functional activity of 5-HT$_{1A}$ receptors was estimated by quantification of the hypothermic response obtained after acute administration of the 5-HT$_{1A}$ agonist 8-OH-DPAT (1 mg·kg^{-1}, i.p.) (Overstreet *et al.*, 1996; Naumenko *et al.*, 2011). The body temperature was measured by means of a KJT thermocouple (Hanna Instruments, Singapore) with Cooper Constantan Rectal Probes for mice (Physitemp Instruments, Clifton NJ, USA) 20 min after drug or saline administration. For estimation of hypothermic effect after chronic 8-OH-DPAT administration, two temperature values were analysed; the first one was measured in animals undergoing a 14 day treatment procedure before and the second one 20 min after acute 8-OH-DPAT administration to mice of experimental and sham groups.

Pinch-induced catalepsy

The catalepsy test was performed according to the procedure described by Kulikov *et al.* (1989, 1993). Animals were firmly pinched between two fingers for 5 s at the scruff of their neck, placed on parallel bars with the forepaws 5 cm above the hind legs and then gently released. The catalepsy duration was recorded in s from the moment when an animal was released to the moment when the animal shifted its front paws from the initial position on the upper bar or made gross body or head movements. The trial ended either when a mouse started to move or after 120 s of immobility. Each animal was submitted to 10 successive trials with 2 min intervals. Mice were kept in the home cages between the trials. The mouse was considered to be cataleptic if the time of immobility was longer than 20 s in at least three of the 10 trials.

Open field

The OF test was carried out in a circle arena (40 cm in diameter) surrounded by a white plastic wall (25 cm high) and illuminated through the mat and semi-transparent floor with two halogen lamps of 12 W each placed 40 cm under the floor (Kulikov *et al.*, 2008b). The mouse was placed near the wall, and its movements were tracked for 5 min with a digital camera (Sony, Tokyo, Japan). The area was carefully cleaned after each test. The video stream from the camera was analysed frame by frame using the original EthoStudio software (Kulikov and Popova, 2008). The horizontal locomotor activity (distance run) and time in the centre were measured automatically. The number of rearing events was measured manually.

Elevated plus-maze

The EPM was performed using a device consisting of two crosswise open and closed arms (35 × 7 cm). The closed arms had side walls 30 cm in length and 20 cm high. The mouse was placed in the centre of the maze facing an enclosed arm. Behaviour was tracked for 5 min with a digital camera. The time spent in the closed arms, the time spent in the centre, the number of times it entered the closed and open arms and the number of peeks and head dippings were measured.

Forced swim test

Mice were placed in a clear plastic box (30 × 30 × 30 cm) filled with water at a temperature of 25°C. Total immobility time was recorded after 2 min of adaptation during 4 min.

Real-time PCR

Total RNA was extracted with Trizol (Bio Rad, Hercules, CA, USA), and 1 μg of the total RNA was taken for cDNA synthesis with a random hexanucleotide mixture (Kulikov *et al.*, 2005). The number of copies of 5-HT$_{1A}$, 5-HT$_{2A}$ receptors, MAO A (MAO-A), Tph2, genes encoding the 5-HT transporter (*Slc6a4*), BDNF, TrkB and p75 receptor cDNAs was evaluated with SYBR Green real-time quantitative PCR using selective primers (Table 1). The 50, 100, 200, 400, 800, 1600, 3200 and 5000 copies of genomic DNA were used as external standards for all the genes studied. The gene expression was presented as a relative number of cDNA copies compared with 100 copies of *Polr2a* cDNA (Kulikov *et al.*, 2005; Naumenko and Kulikov, 2006; Naumenko *et al.*, 2008).

HPLC

Concentrations of 5-HT and 5-HIAA were assessed with HPLC. The chromatography system contained the following: Lune C 18(2) column (5 μm particle size, L × i.d. 150 × 2 mm; Phenomenex, Torrance, CA, USA) and Nucleosil C 8 guard column (10 × 4.6 mm; Superlco Analytical, Sigma-Aldrich, St. Louis, MO, USA), electrochemical detection (500 mV, Coulochem III; ESA Inc., Chelmsford, MA, USA), flowcell (BAS Inc., Lafayette, IN, USA), delivery module LC-20AD (Shimadzu Corporation, Kyoto, Japan) and autosampler Optimas (Spark, Emmen, Holland). The mobile phase contained KH_2PO_4 (100 mM, pH = 4.5), 0.1 mM Na_2EDTA, 1.4 mM 1-octanesulfonic acid sodium salt (Sigma, St. Louis, MO, USA) and methanol (8% vol; Vekton Ltd., Voronezh, Russia). The flow rate was 0.6 mL·min^{-1}. Tissue samples were homogenized in 200 μL of 0.8 M $HClO_4$ (Sigma). The homogenates were centrifuged for 15 min at 15 000× g (4°C), and the supernatant was transferred to a clear tube and was diluted twice in Milli-Q H_2O. The pellet was diluted in 0.1 M NaOH (Vekton Ltd.,Voronezh, Russia), and protein concentration was estimated by the Bradford protein assay. Solutions containing 2 ng of 5-HT and 5-HIAA were used as an external standard. The concentration of biogenic amines in samples was estimated by comparison of the magnitudes of corresponding picks with the respective

external standards using the MultiChrome v.1.5 software (Ampersand Ltd., Moscow, Russia) and expressed in ng·mg^{-1} of the total protein in sample estimated using the Bradford assay. The intensity of 5-HT metabolism was evaluated by the ratio of 5-HIAA/5-HT.

MRI

MRI experiments were performed on a horizontal 11.7 T magnet (BioSpec 117/16 USR; Bruker, Karlsruhe, Germany). During the MRI experiment, each mouse received isoflurane in oxygen mix (1.5%, flow rate 200 mL·min^{-1}) for anaesthesia. Animals were placed in the prone position on the animal bed, which was then slid into the magnet bore. A respiratory pillow placed underneath the lower torso was used to monitor respiration (SA Instruments, Stony Brook, NY, USA). All images of the mouse brain were obtained with a transmitter volume (500.3 MHz; distribution, 72/89 mm) and receiver surface (500.3 MHz; 20 × 20 mm) 1H radiofrequency coils. High-resolution T2-weighted images of the mouse brain (slice thickness = 0.5 mm, inter-slice gap = 0.5 mm, field of view = 2 × 2 cm, matrix = 256 × 256, number of averages = 2 and scan duration = 3 min and 44 s) were recorded by TurboRARE (rapid with relaxation enhancement) with the following pulse sequence parameters: TE = 11 ms, TEeff = 33 ms, TR = 3.5 s and RARE factor = 8. The T2-weighted images were obtained in three plane orientations: axial, sagittal and coronal. For quantification, one or two slices from each projection were used: the axial orientation (−0.28 and −2.3 mm to bregma), the sagittal orientation (level of bregma) and the coronal orientation (2.5 mm from the dorsal surface of the brain). The brain structures were restricted using the manufacturers' Region of Interest Tool software in ParaVision 5.0 and a standard mouse brain atlas by Hof *et al.*,(2000). The areas of all structures and the brain size in each slice were calculated by one slice as the number of pixels multiplied by the size of one pixel in mm^2. For measurements, the sizes of the hippocampus, midbrain and cortex in the coronal slice the sum of right and left parts of these structures were used. Total volume of the brain was estimated using 17 slices of sagittal orientation (slice thickness: 0.5 mm, inter-slice gap: 0 mm) and calculated as a sum of the areas of slices multiplied by 0.5 mm.

Statistical analysis

All experimental results are presented as mean ± SEM and compared by one-way or two-way ANOVA. The percentage of cataleptic mice was compared using Fishers' arcsine conversion. In all statistical analyses, $P < 0.05$ was considered as a threshold for statistical significance. When F achieved $P < 0.05$ and there was no significant variance in homogeneity, statistically significant effects were analysed using *post hoc* Fishers' test for group comparison. The Dixon's Q-test was used for identification and rejection of outliers. The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis *et al.*, 2015).

Results

Transferring the distal fragment of chromosome 13 results in increased catalepsy

A drug-free hereditary catalepsy represents a promising model for investigating the mechanisms underlying different kinds of catalepsy-associated psychopathology (Kulikov *et al.*, 2006; Kulikov *et al.*, 2008b). In order to create a novel mouse model of catalepsy, we transferred of CBA-derived 105.89–118.83 Mbp fragment of chromosome 13 to the genome of catalepsy-resistant C57BL/6 mice. This mouse strain was selected because the C57BL/6 genome is the most widely used genetic background for numerous mouse models. The transferring of the CBA-derived fragment of chromosome 13 to the B6 genome was controlled using two polymorphic microsatellites D13Mit76 (110.56 Mbp) and D13Mit78 (118.83 Mbp) (Figure 1A and B). The heterozygous backcrosses of the eighth generation were intercrossed to generate B6-M76C and B6-M76B containing the CBA-derived and B6-derived alleles of D13Mit76 and AKR-derived and B6-derived alleles of D13Mit78 in the B6 genome respectively.

Both new lines (i.e. B6-M76C and B6-M76B) were subjected to the catalepsy test. In the case of the B6-M76B line, we did not obtain any cataleptic response, while 14% of animals from the B6-M76C line showed pronounced catalepsy (Figure 1C and Supporting Information Videos S1 and S2). These data confirm that the transfer of the main catalepsy locus located in the 105.89–118.83 Mbp fragment of chromosome 13 to the catalepsy-resistant line leads to the development of cataleptic behaviour.

Behavioural effects of transferring the distal fragment of chromosome 13

To study the possible relationship between catalepsy and different kinds of behaviour, we performed detailed analysis of various behavioural aspects, in particular those used as read-outs for psychiatric and neurological disorders. For that, mice of both lines were subjected to the OF test, EPM test and FST.

The OF test is widely used to evaluate locomotor activity (total distance traveled in the OF arena) (van Gaalen and Steckler, 2000; Prut and Belzung, 2003) and exploratory behaviour (number of rearing events) in rodents (Crusio, 2001). The results of the OF test revealed that B6-M76B and B6-M76C mouse lines did not differ by the distance travelled, by the number of rearings and time in the centre in the OF (Table 2).

The EPM test is routinely applied to study the response of animals to stimuli that cause fear and anxiety. It is considered that animals avoid entering the open arms of the maze because they are normally afraid of the open and illuminated areas. Therefore, the number of entries into the open arms as well as the time spent in them is utilized as parameters

Table 2

Behaviour analysis of B6-M76B and B6-M76C mice in the OF test

Behaviour parameter	B6-M76B (n = 9)	B6-M76C (n = 16)
Distance run, cm	1136.56 ± 115.86	1197.14 ± 110.35
Time in the centre, %	0.11 ± 0.03	0.08 ± 0.01
Number of rearings	13.22 ± 2.90	16.69 ± 2.61

Data are presented as a mean ± SEM.
No significant differences between B6-M76C and B6-M76B lines were found.

Table 3

Behaviour analysis of B6-M76C and B6-M76B mice in the EPM

Behavior parameter	B6-M76B (n = 9)	B6-M76C (n = 16)
Number entering in the open arms	3.6 ± 1	1.9 ± 0.6
Time in the open arms, s	48.5 ± 27.0	97.0 ± 29.3
Number entering in the closed arms	*7.1 ± 1.0*	*3.1 ± 0.6***
Time in the closed arms, s	210.7 ± 31.2	173.5 ± 32.0
Number of peeks	1.8 ± 0.5	1.2 ± 0.4
Number head deeps	7.9 ± 1.9	6.0 ± 1.6

Data are presented as a mean ± SEM.
**$P < 0.02$ compared with B6-M76B (presented in italics).

reflecting the degree of anxiety (Pellow *et al.*, 1985; Ramos *et al.*, 1997). In the EPM test, mice of the lines investigated did not differ by number of entries in the open arms, time spent in the open and in the closed arms, by number of peeks and by number of head dippings. These results suggest that B6-M76B and B6-M76C lines are similar in their manifestation of anxiety. In contrast, the number of entries in the closed arms was significantly reduced in the B6-M76C mice in comparison with the B6-M76B mice (Table 3).

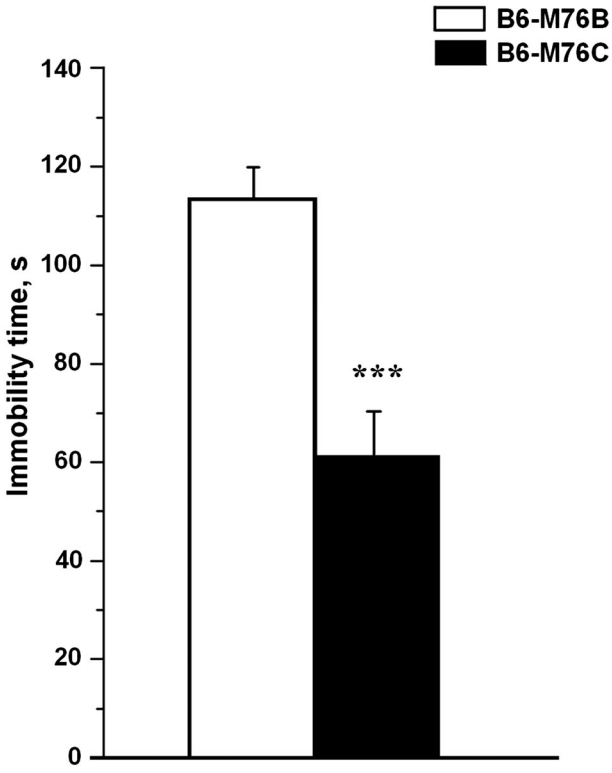

Figure 2

Immobility time in the FST in B6-M76B (n = 9) and B6-M76C (n = 16) males. Data are presented as mean ± SEM. ***$P < 0.001$.

To evaluate the possible association between the chromosome 13 fragment with depressive-like behaviour, the B6-M76B and B6-M76C lines were subjected to the FST. Increased immobility in the FST is a generally accepted as an indicator of increased depressive-like behaviour. This idea is mainly based on the observation that classical antidepressants decrease the immobility time in this test (Steru *et al.*, 1985; Borsini and Meli, 1988). Results obtained in the FST revealed that mice of the recombinant B6-M76C line demonstrate significantly decreased immobility time in comparison with animals of the B6-M76B line (Figure 2), indicating a decreased depressive-like behaviour in B6-M76C mice.

These results show that the transferring of the main locus of catalepsy to the B6 background did not alter either the locomotor and exploration activities or the anxiety state in mice. In contrast, B6-M76C mice demonstrated decreased depressive-like behaviour.

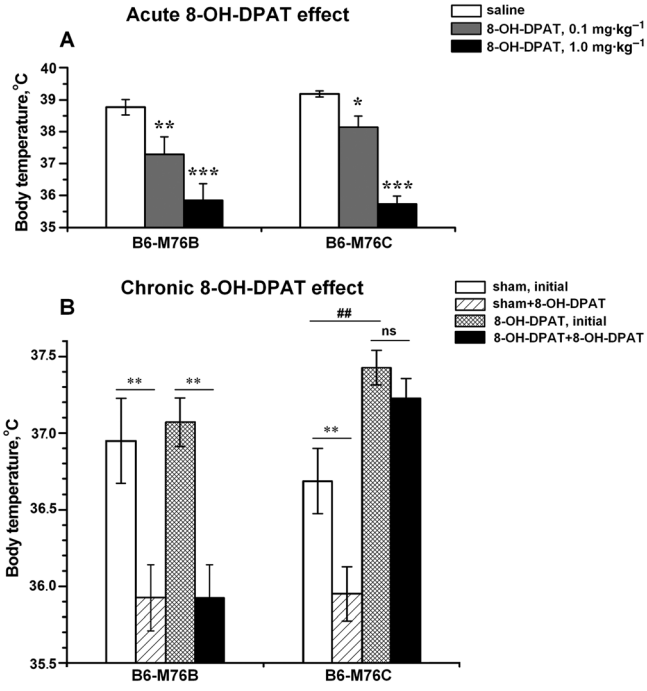

Figure 3

Effect of (A) acute (0.1 and 1 mg·kg^{-1}, i.p.) and (B) chronic (1 mg·kg^{-1}, i.p., 14 days) administration of 5-HT$_{1A}$ receptor agonist 8-OH-DPAT on the body temperature in B6-M76B and B6-M76C mice. Data are presented as mean ± SEM. In experiments for analysis of the acute 8-OH-DPAT effects (A), body temperature was measured 20 min after 8-OH-DPAT or saline administration (n = 8 per genotype per drug). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ versus saline group. In the experiment for the chronic 8-OH-DPAT effects (B), mice were treated with 8-OH-DPAT (1 mg·kg^{-1} – 8-OH-DPAT group) or saline (sham group) for 14 days. To reveal the effect of chronic 8-OH-DPAT treatment on the 5-HT$_{1A}$ receptor-mediated hypothermic response, after 14 days of treatment, all groups received a single i.p. injection of 8-OH-DPAT (1 mg·kg^{-1}), and the body temperature was measured before and 20 min after this injection (n = 8 per genotype per drug). **$P < 0.01$, ##$P < 0.01$ versus initial temperature of sham group. ns, non-significant differences.

Effects of acute and chronic 8-OH-DPAT treatment on 5-HT$_{1A}$ receptor-mediated hypothermic response

The main locus of catalepsy transferred to the C57BL/6 mice contains a gene encoding for the 5-HT$_{1A}$ receptor. Among other effects, stimulation of this receptor is known to induce pronounced hypothermia (Naumenko *et al.*, 2011). Next we compared the effect of acute and chronic administration of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT on the hypothermic response in both recombinant mouse lines. A single i.p. administration of 8-OH-DPAT at concentration of 0.1 or 1 mg·kg^{-1} (acute stimulation) produced a significant dose-dependent hypothermic effect in mice of both lines, with no significant interline differences (Figure 3A). In contrast, chronic administration of 8-OH-DPAT resulted in considerable differences in the hypothermic response induced by the subsequent acute 5-HT$_{1A}$ receptor stimulation between B6-M76B and B6-M76C mice (Figure 3B). It is noteworthy that the initial body temperature of the B6-M76C animals chronically treated with 8-OH-DPAT was significantly higher than that of the animals in the control group. In contrast, no differences in the initial body temperature between chronically treated and non-treated B6-M76B were observed (Figure 3B). Significant differences in the hypothermic response between the two lines were also found after acute injection of 1 mg·kg^{-1} 8-OH-DPAT to the chronically treated animals: mice of the B6-M76B line showed a pronounced decrease in body temperature, while this hypothermic response was completely abolished in the catalepsy-prone B6-M76C line.

Effects of acute and chronic 8-OH-DPAT treatment on locomotor and exploratory activity

Having demonstrated an interline difference in the 5-HT$_{1A}$ receptor-induced hypothermia, we next compared the behavioural effects of acute and chronic administration of 8-OH-DPAT between B6-M76B and B6-M76C mice using the OF test. As shown in Figure 4A and B, acute i.p. administration of 8-OH-DPAT (0.1 or 1 mg·kg^{-1}) resulted in a dose-dependent decrease in locomotor (distance run) as well as in exploratory (number of rearings) activities in both lines, although the changes in the explorative activity (in particular at the lower dosage of 8-OH-DPAT) were more noticeable in the B6-M76C mice (Figure 4B). It is noteworthy that the acute administration of 8-OH-DPAT does not affect the time spent by animals of either line in the centre of the arena (not shown).

Then we investigated whether the prolonged treatment of B6-M76B and B6-M76C animals with 8-OH-DPAT (14 days, 1 mg·kg^{-1}, i.p. injections) has line-specific behavioural consequences. In these experiments, we observed that chronic 8-OH-DPAT administration significantly increases the locomotor activity only in B6-M76B mice, while no changes in the distance run were observed in animals of the B6-M76C line (Figure 4C). More importantly, the increase in locomotor activity induced by chronic 8-OH-DPAT treatment was significantly higher in the B6-M76B line than in the B6-M76C animals (Figure 4C). In contrast, chronic 8-OH-DPAT treatment did not significantly modify the explorative behavior of either line. Also, no interline

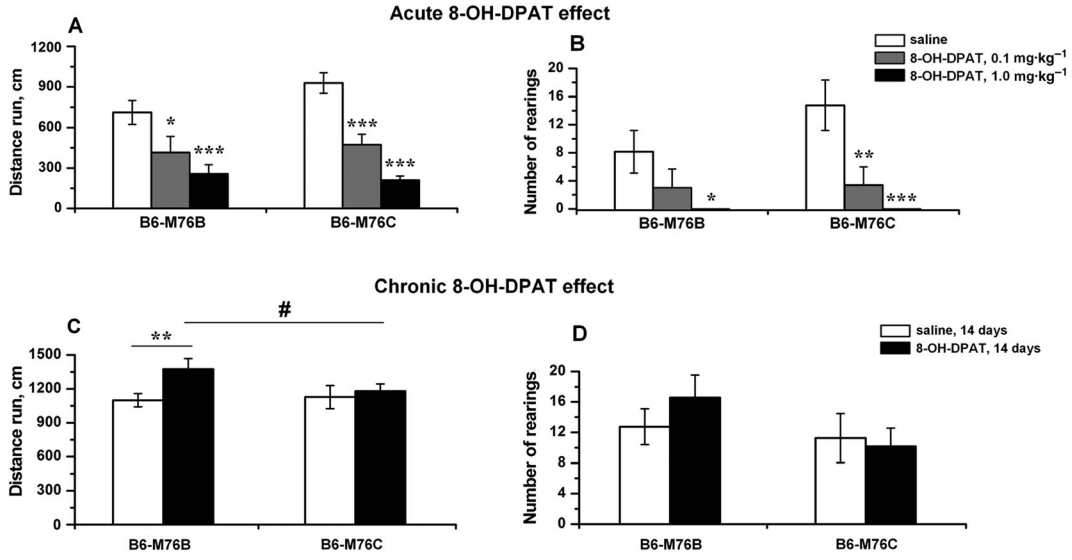

Figure 4

Effect of acute (A and B: 0.1 and 1 mg·kg^{-1}, i.p.) and chronic (C and D: 1 mg·kg^{-1}, i.p., 14 days) administration of 5-HT$_{1A}$ receptor agonist 8-OH-DPAT on locomotor activity (distance run) (A, C) and exploration activity (number of rearings) (B, D) in the OF test in B6-M76B and B6-M76C mice. Data are presented as mean ± SEM. In the experiment for the analysis of the acute 8-OH-DPAT effects, the behaviour of mice in the OF test was estimated 20 min after 8-OH-DPAT or saline administration (n = 8 per genotype per drug). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ versus saline group. In experiments analysing the effects of chronic 8-OH-DPAT administration, the OF test was performed after daily injection of 8-OH-DPAT (1 mg kg^{-1} – 8-OH-DPAT group) or saline (sham group) for 14 days (n = 8 per genotype per drug); $^{**}P < 0.01$ versus saline (sham) group; $^{\#}P < 0.05$ versus B6-M76B mice of 8-OH-DPAT group.

differences were obtained on analysis of the number of rearings (Figure 4D). The effects of genotype and drug were also not significant for the time spent in the centre of the arena (not shown).

Comparison of the brain morphology between B6-M76B and B6-M76C mice using MRI

In order to reveal possible mechanisms underlying the aforementioned differences between B6-M76B and B6-M76C lines, we first compared their brain morphology. MRI-based measurement of brain structures is an important indicator of pending cognitive decline in humans suffering from neurodegenerative diseases. Also in mouse models of neurodegenerative disorders, neuroimaging is considered to be a useful tool for phenotyping specific diseases and monitoring disease progression (Kooy *et al.*, 2001; Waerzeggers *et al.*, 2010). In the present study, morphology of the following brain structures was investigated: thalamus, corpus callosum, diencephalon region (including thalamus and hypothalamus), hippocampus (right and left parts), ventricle system, midbrain (right and left parts) and cortex (right and left parts) (Figure 5). Results of the MRI experiments are also summarized in Table 4. Detailed morphological analysis of MRI data revealed that the diencephalon of the B6-M76C mice is significantly smaller than in the B6-M76B mice. In addition, we found that the total volume of the brain as well as of the brain area in the axial orientations (−2.3 mm from bregma) were reduced in B6-M76C mice when compared with B6-M76B mice. At the same time, thalamus, hippocampus, midbrain, corpus callosum and brain areas in coronal (2.5 mm from the surface of brain) as well as in sagittal orientations (bregma) did not significantly differ between these lines. Taken together, these results demonstrate that the transferring of the main catalepsy locus produces brain shrinkage associated with a profound decrease in the size of the diencephalon, a brain structure implicated in mechanisms of psychiatric disorders.

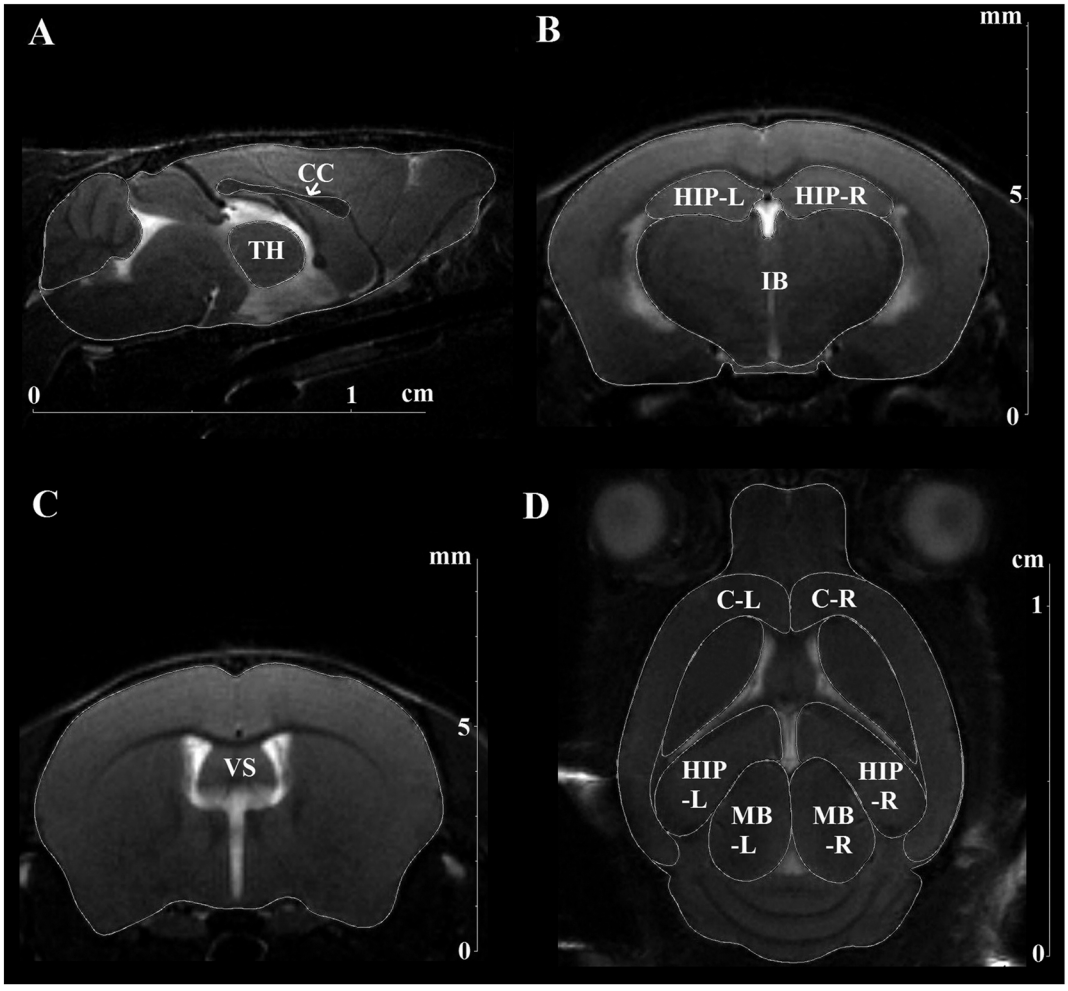

Figure 5

The T2-weighted MRI images of mouse brain obtained using 11.7T BioSpec 117/16 USR (Bruker) tomograph. The brain structures investigated in the study are designated as follows: (A) sagittal slice (bregma). Thalamus (TH), corpus callosum (CC). (B) Axial slice (−2.3 mm to bregma). Diencephalon region (IB), hippocampus (right/left parts) (HIP-R, HIP-L). (C) Axial slice (−0.28 mm to bregma). Ventricle system (VS). (D) Coronal slice (−2.5 mm from the dorsal surface of the brain). Cortex (C-R, C-L), hippocampus (HIP-R, HIP-L) and midbrain (MB-R, MB-L). $n = 5$ for B6-M76C and $n = 6$ for B6-M76B mice.

Table 4

Sizes of the brain structures measured using MRI in the B6-M76B and B6-M76C lines

Slice	Bregma (mm)	Structure (mm^2)	B6-M76B Non-cataleptic	B6-M76C Cataleptic	$F_{1,9}$	P
Sagittal	0	Brain	67.186 ± 0.777	66.554 ± 0.224	0.51	
		Thalamus	4.102 ± 0.070	4.059 ± 0.055	0.213	
		Corpus callosum	1.433 ± 0.056	1.331 ± 0.079	1.165	>0.05
Axial	−2.3	*Brain*	*50.834 ± 0.318*	*49.103 ± 0.347*	*13.53*	*<0.01*
		Diencephalon total	*17.207 ± 0.123*	*16.380 ± 0.105*	*24.99*	*<0.001*
		Hippocampus right	2.050 ± 0.105	2.105 ± 0.054	0.189	
		Hippocampus left	1.943 ± 0.137	2.016 ± 0.030	0.228	
	−0.28	Brain	43.070 ± 0.671	41.568 ± 0.332	3.52	>0.05
		Ventricle	1.738 ± 0.085	1.825 ± 0.166	0.244	>0.05
Coronal	−2.5	Brain	91.518 ± 0.942	88.447 ± 1.107	4.52	>0.05
		Hippocampus sum	12.972 ± 0.568	12.752 ± 0.277	0.106	
		Midbrain sum	12.831 ± 0.265	12.256 ± 0.293	2.124	>0.05
		Cortex sum	24.228 ± 0.454	23.639 ± 0.309	1.050	>0.05
Sagittal		*Volume (mm^3)*	*429.427 ± 2.990*	*413.806 ± 2.452*	*15.42*	*<0.01*

Data are presented as the means ± SEMs of the values obtained in an independent group of animals ($n = 5$ for B6-M76C and $n = 6$ for B6-M76B mice). The sizes of brain structures (area, mm^2) were calculated in the one slice of axial, sagittal and coronal orientations with different instance from bregma. Total volume of the brain was estimated using 17 slices of sagittal orientation (volume, mm^3). Significant differences are in italics.

Molecular effects of transferring the main cataleptic locus

The pharmacological experiments described above revealed significant interline differences in hypothermic response as well as in locomotor activity after chronic stimulation of the 5-HT_{1A} receptor with 8-OH-DPAT. Therefore, we next compared the brain level of 5-HT and its main metabolite 5-HIAA in B6-M76B and B6-M76C mice. The 5-HT level in the cortex, midbrain, hippocampus and striatum did not differ significantly between the lines investigated. The level of 5-HIAA was also similar in all structures tested (Figure 6). However, the 5-HIAA/5-HT ratio, which reflects the efficiency of 5-HT metabolism was significantly decreased in the hippocampus, but not in the midbrain and striatum of B6-M76C line in comparison with B6-M76B animals (Figure 6E).

In addition to the direct measurement of 5-HT and 5-HIAA concentrations using HPLC, we compared the expression of the key genes of the brain serotonergic system, including the 5-HT_{1A} receptor, 5-HT_{2A} receptor, 5-HT transporter (*Slc6a4*), Tph2, the rate-limiting enzyme for 5-HT biosynthesis in the brain, and MAO-A, an enzyme that degrades monoaminergic neurotransmitters, such as dopamine, noradrenaline and 5-HT. The expression of these genes was determined in the frontal cortex, hippocampus and midbrain using quantitative real-time PCR. These experiments demonstrated that the expression of the 5-HT_{1A} receptor gene was significantly higher in the hippocampus of the B6-M76C mice, while the amount of 5-HT_{1A} receptor mRNA in the cortex and midbrain was similar in both lines (Figure 7A). Also, the level of the 5-HT_{2A} receptor mRNA was similar in both lines in all brain regions investigated (Figure 7B). In addition, the gene expression analysis revealed that the 5-HT transporter *Slc6a4* mRNA level in the midbrain of B6-M76C mice was significantly decreased in comparison with the B6-M76B animals (Figure 7C). The expression of *Tph2* and *MAO-A* genes was also significantly reduced in the midbrain of B6-M76C mice (Figure 7C). Decreased expression levels of *Slc6a4*, *Tph2* and *MAO-A* genes in the midbrain in combination with a reduced 5-HIAA/5-HT ratio in the hippocampus suggest a reduced functionality of the brain serotonergic system in B6-M76C. At the same time, an increase in the *5-HT_{1A} receptor* gene expression observed in the hippocampus of B6-M76C mice seems to represent a compensatory mechanism for enhancing this dysregulated 5-HT signalling.

Finally, we analysed the effect of the transfer of the distal fragment of chromosome 13 on the expression of the genes involved in neurotrophic signalling. In this experiment, we did not find any significant changes in the expression of BDNF or in the expression levels of the main BDNF receptors TrkB and p75 in the brain of B6-M76B and B6-M76C mice (not shown).

Discussion

The main locus of a predisposition to catalepsy is mapped in the distal fragment 105.89–118.83 Mbp of chromosome 13, and its CBA-derived allele determines about 20% of this trait penetrance, while other 30% of trait penetrance is defined by 29 polygenes wildly distributed in the whole genome

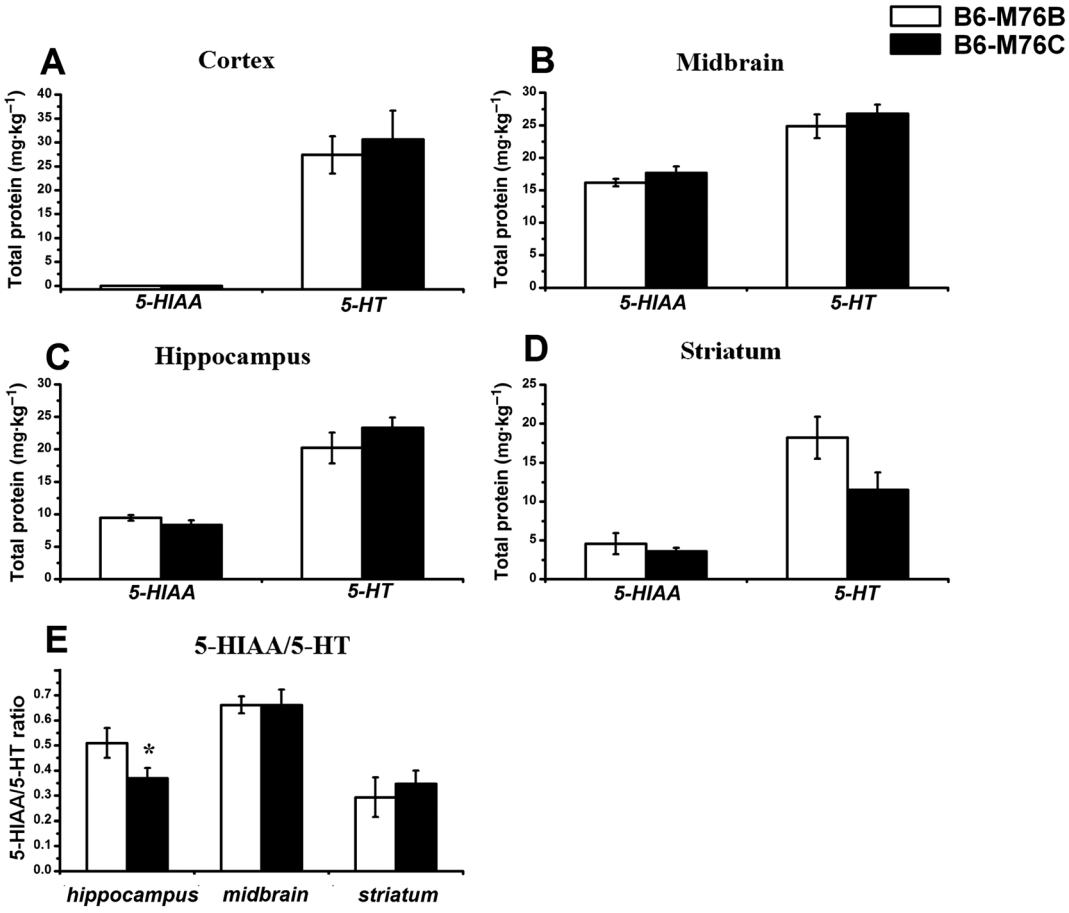

Figure 6

Concentrations of 5-HT and its main metabolite 5-HIAA in the different brain areas of B6-M76C and B6-M76B lines are shown. (A) cortex, (B) midbrain, (C) hippocampus and (D) striatum. (E) 5-HIAA/5-HT ratio reflecting the efficiency of 5-HT metabolism. Data are presented as mean ± SEM (n = 8 per genotype). $^{*}P < 0.05$ versus B6-M76B mice.

(Kulikov *et al.*, 2003; Kulikov *et al.*, 2008a). In the present study, we showed that the transfer of this fragment from CBA mice to the catalepsy-resistant B6 genetic background led to the development of catalepsy in the resulting B6-M76C line. It is noteworthy that the transfer of the 105.89–118.83 Mbp fragment of chromosome 13 derived from B6 animals resulted in the catalepsy-resistant B6-M76B line. These findings provide additional experimental confirmation for the critical involvement of the distal fragment of chromosome 13 in the development of gene-based catalepsy. Interestingly, that 14% of B6-M76C mice demonstrated cataleptic-like immobility tightly correlated with the 20% of the trait penetrance proposed for the CBA-derived allele. Previously, it was shown that transferring the CBA-derived fragment of chromosome 13 containing the main locus of catalepsy to the genetic background of the AKR strain also results in a mouse line with catalepsy. The resulting AKR.CBA-D13Mit76 mice showed trait penetrance for cataleptics (approximately 50%), is similar to the parental strain CBA. This can be explained by the fact that the AKR genome contains additional genes enhancing the effect of the main genes of catalepsy in the CBA-derived allele (Kulikov *et al.*, 2008a).

Predisposition to catalepsy is often associated with profound changes in behaviour (Kolpakov *et al.*, 2004; Bazovkina *et al.*, 2005; Kondaurova *et al.*, 2010). Our observation that mice of the B6-M76B and B6-M76C recombinant lines did not differ with regard to the distance travelled, time in the centre and the number of vertical postures in the OF test suggests that a predisposition to catalepsy in the B6-M76C line is not associated with deficits in locomotor activity. In addition, time spent in the open arms in the EPM test did not differ significantly between these two lines, demonstrating that transfer of the main locus of predisposition to catalepsy to the B6 background does not produce any changes in the exploratory activity or in anxiety state under basal conditions. These data are in accordance with the results obtained in catalepsy-prone AKR.CBA-D13Mit76 mice (Bazovkina *et al.*, 2005; Kondaurova *et al.*, 2010). Interestingly, we observed a reduction in the number of entries in the closed arms in the EPM test in B6-M76C mice, which can be an indirect indication of increased susceptibility to stress. At the same time, animals of the B6-M76C line demonstrated a significantly shorter immobility time in the FST, a generally accepted indicator of depressive behaviour used to investigate the activity of antidepressant drugs (Steru *et al.*, 1985; Borsini and Meli,

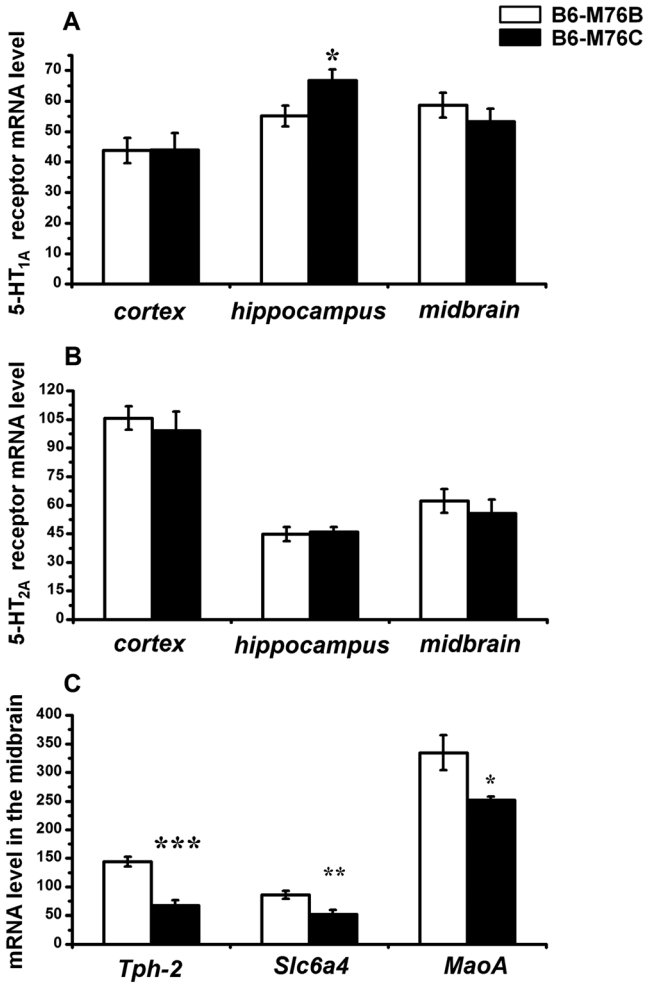

Figure 7

Expression of genes encoding for the 5-HT_{1A} (A) and 5-HT_{2A} (B) receptors in the frontal cortex, hippocampus and midbrain of B6-M76B and B6-M76C mice. Expression of genes encoding for Slc6a4, Tph2 and MAO-A in the midbrain (C) of B6-M76B and B6-M76C animals. Gene expression is presented as the number of cDNA copies with respect to 100 cDNA copies of *Polr2a*. All values are presented as mean ± SEM ($n = 8$/ per genotype). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ versus B6-M76B mice.

1988), suggesting a decreased depressive-like behaviour in B6-M76C mice.

Because the main locus of predisposition to catalepsy contains a gene encoding for the 5-HT_{1A} receptor, animals of the B6-M76C and B6-M76B lines should contain different 5-HT_{1A} receptor gene alleles derived from the CBA and C57BL/6 strain respectively. It has been shown that acute stimulation of the 5-HT_{1A} receptor with a selective agonist 8-OH-DPAT significantly attenuate catalepsy and locomotor activity in rodents (Kulikov *et al.*, 1994; Bazovkina *et al.*, 2010; Naumenko *et al.*, 2012) and produce a strong hypothermic effect (Naumenko *et al.*, 2010). In the present study, we found that acute administration of 8-OH-DPAT produced a strong dose-dependent hypothermia in both mouse lines. In contrast, the intensity of the hypothermic response evoked by a single 8-OH-DPAT injection in mice chronically treated with 8-OH-DPAT was significantly lower in B6-M76C than in B6-M76B animals. These results are in accordance with the data obtained in cataleptic-prone strain CBA (Popova *et al.*, 2010) and can be explained by the more effective desensitization of the postsynaptic 5-HT_{1A} receptors in B6-M76C line. Taken together with the observed central role of postsynaptic 5-HT_{1A} receptors in mediating the 8-OH-DPAT-induced hypothermia (Blier *et al.*, 2002), our data suggest that in the B6-M76C line, postsynaptic 5-HT_{1A} receptors undergo a more effective desensitization.

At the same time, chronic 8-OH-DPAT produced a significant increase in 5-HT_{1A} receptor-mediated locomotor activity only in B6-M76B mice. These results are in line with the well-known anxiolytic effect of 8-OH-DPAT (Overstreet *et al.*, 1996). In contrast, no changes in locomotor activity were observed in B6-M76C mice after prolonged 8-OH-DPAT treatment, suggesting the existence of compensatory and/or adaptive mechanisms in this line. Similar effects were also observed in CBA mice (Popova *et al.*, 2010). Taking into consideration the important role of presynaptic 5-HT_{1A} receptors in the regulation of locomotor activity (Faccidomo *et al.*, 2008) and catalepsy (Naumenko *et al.*, 2010), our data suggest presynaptic 5-HT_{1A} receptors have a reduced sensitivity in B6-M76C mice. Thus, transfer of the fragment containing the 5-HT_{1A} receptor from CBA mice to the C57BL/6 genetic background led to an increase in postsynaptic and decrease in presynaptic functional responses mediated by 5-HT_{1A} receptors in B6-M76C mice. This interesting feature sites B6-M76C line as an attractive model for the pharmacological screening of 5-HT_{1A} receptor-related drugs specifically acting on either the pre- or postsynaptic receptors.

The brain serotonergic system plays an important role in the regulation of catalepsy. It has been demonstrated that stimulation of the 5-HT_{1A} receptor with different agonists reduces haloperidol- and morphine-induced catalepsy (VanderWende and Spoerlein, 1979) as well as hereditary catalepsy in rats and mice (Popova and Kulikov, 1995; Popova, 1997). Moreover, irreversible inhibition of Tph2, the rate-limiting enzyme for 5-HT biosynthesis in the brain by *p*-chlorophenylalanine, attenuates both haloperidol-induced and hereditary catalepsy in rats (Kostowski *et al.*, 1972). Hereditary catalepsy in rats and CBA mice has been also shown to be associated with increased Tph2 activity and decreased 5-HT_{2A} receptor density in the striatum (Kulikov *et al.*, 1992; Kulikov *et al.*, 1995; Popova and Kulikov, 1995). The results of the present study provide additional evidence for the close association between catalepsy and the serotonergic system. We have found that the expression of the key genes involved in either synthesis or metabolism of 5-HT, including *Tph-2, MAO-A* and the 5-HT transporter *Slc6a4*, was significantly reduced in the midbrain of B6-M76C mice. Unexpectedly, concentrations of 5-HT and its main metabolite 5-HIAA in this brain structure were not changed in B6-M76C animals, suggesting the existence of compensatory mechanisms responsible for the stable biosynthesis of 5-HT. Correspondingly, the metabolism of 5-HT in the midbrain calculated as a ratio between 5-HT and 5-HIAA levels was also unaffected in B6-M76C mice. This result is in agreement with the findings that CBA, ASC, AKR.CBA-D13Mit76 and catalepsy-resistant AKR mice did not differ with regard to the levels of 5-HT and its metabolites in the midbrain

(Sinyakova *et al.*, 2014; Tikhonova *et al.*, 2013). At the same time, 5-HT metabolism was significantly lower in the hippocampus of B6-M76C mice, the brain structure strongly supplied by multiple serotonergic projections, and this effect was accompanied by an increased expression of the 5-HT_{1A} receptor gene. An increased expression of 5-HT_{1A} receptors in the hippocampus of B6-M76C mice paralleled by increased functional activity of the postsynaptic 5-HT_{1A} receptors (see discussion earlier) could be the main reason for the reduced depressive-like behaviour observed in this line. Indeed, it has been shown that a decreased level of postsynaptic 5-HT_{1A} receptors in combination with impaired receptor-mediated signalling is associated with depression (van Praag, 2004). In addition to 5-HT, neurotrophic signalling, and in particular BDNF, was shown to be involved in the mechanism of catalepsy (Tikhonova *et al.*, 2009; Naumenko *et al.*, 2012; Naumenko *et al.*, 2014). However, in the present study, we did not find any alteration in the levels of mRNA encoding BDNF and its main receptors TrkB and p75 in different brain regions of the B6-M76C mice. This finding suggests that the development of catalepsy evoked by transferring the CBA-derived fragment of chromosome 13 to a B6 genetic background is independent of the neurotrophic system.

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Author contributions

K.E.A. wrote the paper and performed MRI analysis and statistical analysis; B.D.V. performed behavioural studies, pharmacological studies, mRNA isolation and reverse transcription reaction; A.A. E. performed MRI analysis; T.A.S. performed pharmacological studies; F.D.V. made HPLC; K.A.V. is in experimental design and the creation of the lines, N.V.S. in general supervision and proofreading of the manuscript, P.E. in general supervision, writing and proofreading the manuscript and K.E.M. in the creation of mouse lines, experimental design, genes expression estimation, statistical analysis and proofreading of the MS.

Conflict of interest

The authors declare no conflicts of interest.

Declaration of transparency and scientific rigour

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research recommended by funding agencies, publishers and other organizations engaged with supporting research.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

http://dx.doi.org/10.1111/bph.13484

Video S1 Mice of catalepsy-prone B6-M76C line during catalepsy episode.
Video S2 Mice of catalepsy-resistant B6-M76B line in test for catalepsy.