DOI 10.1007/s11055-016-0343-5
Neuroscience and Behavioral Physiology, Vol. 46, No. 9, November, 2016

Studies of Catalepsy and Other Forms of Behavior Using Recombinant Mouse Strains

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Translated from Rossiiskii Fiziologicheskii Zhurnal imeni I. M. Sechenova, Vol. 101, No. 6, pp. 670–677, June, 2015. Original article submitted March 10, 2015. Revised version received April 15, 2015.

Catalepsy is a passive defensive freezing reaction in response to threatening stimuli. In hypertrophied form it constitutes a symptom of impaired brain function. In mice, the main gene determining the predisposition to catalepsy is located in the distal 111.35–116.16 Mbp fragment of chromosome 13. This fragment was transferred by backcrossing from the genome of cataleptic CBS mice to the genome of the catalepsy-resistant strain C57BL/6J and two recombinant strains were obtained – C57BL6.CBA-D13Mit76C and C57BL6.CBA-D13Mit76B – carrying this fragment from CBA or C57BL/6J, respectively. The proportion of cataleptic animals among C57BL6.CBA-D13Mit76C mice was found to be greater than that among control C57BL6.CBA-D13Mit76B mice. Testing in the startle reaction and a social interaction paradigm revealed no differences in behavior. At the same time, C57BL6.CBA-D13Mit76C mice showed lower levels of exploratory activity in the open field test than C57BL6.CBA-D13Mit76B mice. The duration of immobility in C57BL6.CBA-D13Mit76C animals in the forced swimming test was also significantly shorter than that in C57BL6.CBA-D13Mit76B mice.

Keywords: recombinant mice, catalepsy, open field test, forced swimming, startle reaction, social interaction.

In light of the continuing development of personalized medicine, studies of the effects of genetic variation on behavior and the actions of various drugs are among the relevant problems in contemporary neuroscience. Genetic polymorphism can produce significant alterations to behavioral reactions to drugs via changes in the structures of proteins underlying drug delivery or signal transduction. The same genes on different genetic backgrounds can functional differently, which can lead to different responses to drug treatment [8]. The use of recombinant strains differing only in terms of a small genome fragment is a state-of-the-art approach to studying the genetic and molecular mechanisms regulating behavior and yields more precise identification of the effects of the transferred fragment [17].

Catalepsy consists of prolonged immobility with plastic muscle tone, characterized by the inability to alter an imposed posture; it is widespread in nature in various vertebrates. Catalepsy in birds and mammals is an evolutionarily fixed passive-defensive behavioral strategy and protects from predators as an alternative to combat, and is usually associated with fear or some other traumatizing stimulus [10]. Hypertrophied signs of catalepsy can represent a symptom of severe disorders of brain function such as schizophrenia, depression, and Parkinson's disease [21].

In mice, catalepsy can be induced by pinching the skin at the nape of the neck (pinch catalepsy) [9, 20]. However, pinch catalepsy is quite rare: of nine strains studied, prolonged freezing reactions were seen only in CBA/LacJ mice [5, 14]. Studies using multigenome QTL analysis [4] and prolonged breeding [11] located the main gene for the predisposition to catalepsy in the distal fragment of chromosome 13 [3, 4].

Studies to verify the location of the main gene for catalepsy in the distal fragment of chromosome 13 were performed at the Behavioral Neurogenomics Laboratory of the Institute of Cytology and Genetics, Russian Academy of Sciences and produced the congenic strain AKR.CBA-D13

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Mit76C by transfer of the 113.6–116.16 Mbp fragment of chromosome 13, labeled with the microsatellite D13Mit76, from strain CBA/LacJ to the genome of AKR/J mice by nine sequential backcrosses to strain AKR/J. Mice of the congenic strain AKR.CBA-D13Mit76C have a clearly apparent predisposition to catalepsy [12].

Mice of this strain have also been shown to be characterized by decreased exploratory behavior in the open field test and increased intermale aggression as compared with mice of the parental strain AKR. No differences in behavior were seen in the forced swimming test [2]. Apart from changes in behavior, AKR.CBA-D13Mit76C mice were found to be characterized by decreased expression of the genes of the serotonin system in the brain and in the functional activity of serotonin receptors as compared with mice of the parental strain AKR [18].

C57BL/6J mice provide the generally accepted standard genetic background for studies of gene transfer. The creation of new mouse strains carrying the CBA allele of the main gene for catalepsy in the C57BL/6J genome is of great theoretical and applied interest.

The aim of the present work was to study the effects of the telomeric region of chromosome 13 carrying the CBA allele of the main gene for catalepsy in mice with the C57BL/6J genotype on the extent of catalepsy and various types of behavior. The following tasks were addressed: 1) to create partially recombinant strains carrying the CBA or C57BL/6J alleles of the main gene for catalepsy in the C57BL/6J genome (C57BL6.CBA-D13Mit76C and C57BL6.CBA-D13Mit76B, respectively); 2) to compare the behavior of mice of these strains in tests for catalepsy, the startle reflex, the open field, forced swimming, and social interactions.

Methods

Animals. Experiments were performed at the "Conventional Animals Animal House" joint facility (RFMEFI61914 X0005 and RFMEFI62114X0010) of the Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, using male mice obtained for testing behavior at stage 3 of backcrossing of the two recombinant mouse strains created, i.e., C57BL6.CBA-D13Mit76C and C57BL6.CBAD13Mit76B.

Creation of strains. Strains were created by transfer of the chromosome 13 fragment containing the main gene for catalepsy from CBA/Lac mice to the genome of the noncataleptic strain C57BL/6J. Transfer was monitored using microsatellite markers D13Mit76 and D13Mit78.

Males of the congenic strain AKR.CBA-D13Mit76C created at the Behavioral Neurogenomics Laboratory [12] and C57BL/6J females were mated to obtain first-generation F1 hybrids. Male F1 hybrids were mated with females of the inbred mouse strain C57BL/6J. Backcrosses of animals heterozygous for the D13Mit76 and D13Mit78 markers were mated with the recipient strain C57BL/6J. This procedure was repeated a further two times. After the third backcross to C57BL/6J mice, heterozygous backcrosses for the D13Mit76 and D13Mit78 markers were mated together, and homozygous strains C57BL6.CBA-D13Mit76C and C57BL6.CBA-D13Mit76B, carrying the CBA and C57BL/6J alleles of the D13Mit76 and D13Mit78 markers, respectively, were identified among the offspring of this mating.

The parental mouse strains were inbred, while the recombinant strains C57BL6.CBA-D13Mit76C and C57BL6.CBA-D13Mit76B were at the third backcross. After weaning from mothers at age 30 days, males were kept in groups of 10 individuals in cages of size 40 × 25 × 12 cm. Experiments used animals aged 2–3 months and weighing 25 ± 1 g. Two days before testing started, animals were moved to individual changes of the same size with the aim of excluding group effects.

Catalepsy test. Catalepsy was tested in male C57BL6.CBA-D13Mit76C (n = 17) and C57BL6.CBA-D13Mit76B (n = 16) males as described previously [5, 13]. The test was regarded as positive if the mouse retained the imposed posture for at least 20 sec. Test duration was limited to 120 sec, after which the animal was returned to its cage. Animals freezing in at least 3/10 tests were regarded as cataleptic [5, 14].

Behavioral studies were performed in the open field test using male C57BL6.CBA-D13Mit76C (n = 13) and C57BL6.CBA-D13Mit76B (n = 16) mice, in the forced swimming test using male C57BL6.CBA-D13Mit76C (n = 12) and C57BL6.CBA-D13Mit76B (n = 15) mice, in the social interaction test using male C57BL6.CBA-D13Mit76C (n = = 13) and C57BL6.CBA-D13Mit76B (n = 16) mice, and in the startle reaction test using C57BL6.CBA-D13Mit76C (n = 13) and C57BL6.CBA-D13Mit76B (n = 16) mice.

Behavior in the open field, forced swimming, and social interaction tests was recorded on CD and analyzed using the computer program EthoStudio [6].

Open field test. The open field test was performed using a round arena (40 cm in diameter) delimited by a white plastic wall (25 cm high) and illuminated via a matte semi-transparent floor by two 12-W halogen lamps positioned 40 cm beneath the arena. This inverted illumination maximized the contrast between the arena and the animal [15]. The mouse was placed by the wall and behavior was recorded for 5 min using a digital video camera (Sony, Japan) positioned 80 cm from the arena. The arena was thoroughly washed after each test. Video data from the camera were analyzed frame-by-frame using the original program EthoStudio [15]. Path lengths (horizontal activity) were measured automatically, as was the time spent in the center of the arena (diameter 20 cm). The number of vertical rearings and the number of acts of washing were recorded manually.

Forced swimming test. The mouse was placed in a transparent plastic box (30 × 30 × 30 cm) filled to 3/4 full with water at a temperature of 25°C. After a 1-min adaptation period, the total duration of freezing was measured over period of 4 min.

Social interaction test. A mongrel white male (the intruder, aged one month) was placed in the test mouse's home

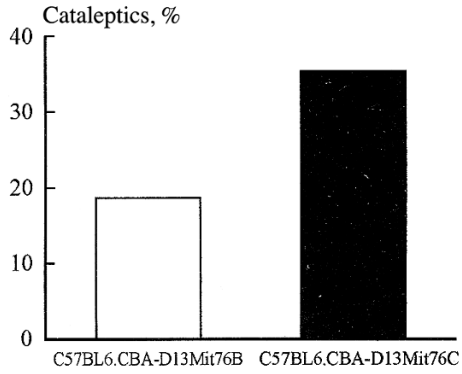

Fig. 1. Proportions (%) of cataleptics among C57BL6.CBA-D13Mit76B and C57BL6.CBA-D13Mit76C mice in the pinch catalepsy test.

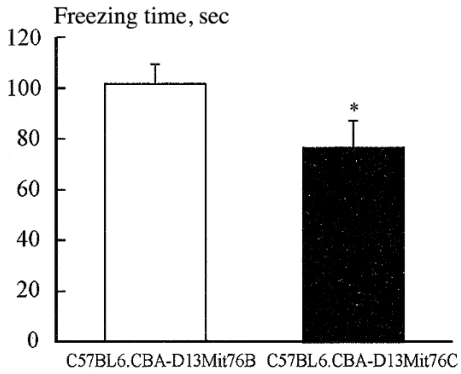

Fig. 2. Freezing times in the forced swimming test in C57BL6.CBA-D13 Mit76B and C57BL6.CBA-D13Mit76C mice in the pinch catalepsy test; *$p < 0.05$ compared with C57BL6.CBA-D13Mit76B.

cage. The number of social contacts by the test male (sniffing and pursuing the intruder) was measured, along with the duration of social contacts (sec). Each intruder was used no more than five times. Behavior was recorded for 10 min using a digital video camera (Sony, Japan) positioned 80 cm from the cage.

Startle reaction. The startle reaction arising in response to delivery of a sound signal was studied in a TSE apparatus (Germany). After switching the apparatus on, white nose (at 65 dB) was generated in the chamber. After a 3-min adaptation period for mice in the chamber, a sound signal was presented every 15 sec, with alternation of a single standard signal and pairs consisting of a warning + standard signal using a (PP + P) × 4 regime – prestimulus inhibition with PP at 75 dB + 110 dB separated by 100 msec followed by a sound signal at 85 dB. A total of eight signals were used [7]. Muscular startle reactions were recorded using a computer connected to the apparatus and expressed in TSE apparatus units. Mean acoustic startle amplitudes were determined in response to the standard signal (P), as well as the mean value of the reaction to the warning + standard signal combination (PP) for four presentations. Prestimulus inhibition (PPI) was then calculated as

$$PPI = [(P - PP)/P] \cdot 100\%.$$

Mouse care and all experimental procedures were performed in compliance with guidelines for the care and use of laboratory animals of the National Institutes of Health (NIH publication No. 80-23), 1996.

Statistics. Proportions of animals with marked catalepsy were compared using Fisher's angular transformation after conversion of percentages to radians. Other behavioral parameters were expressed as means ± standard error and were analyzed in Statistica 6.0 by unifactorial analysis of variance (ANOVA) and post hoc multiple comparisons using the Fischer test.

Results

Testing for predisposition to catalepsy showed that the proportion of cataleptic animals among C57BL6.CBA-D13Mit76C mice was 35%, compared with 18% among C57BL6.CBA-D13Mit76B mice (Fig. 1).

Studies in the open field test revealed significant differences in vertical movement activity between C57BL6.CBA-D13Mit76C and C57BL6.CBA-D13Mit76B mice ($F_{1,27} = 10.46$, $p < 0.003$). The number of vertical rearings was smaller in C57BL6.CBA-D13Mit76C mice than C57BL6.CBA-D13Mit76B animals ($p < 0.01$). There was no difference between the strains in terms of characteristics such as horizontal movement activity ($F_{1,27} = 2.37$, $p = 0.14$) and the time spent in the center ($F_{1,27} < 1$; Table 1).

Studies in the forced swimming test showed that the duration of immobility in recombinant C57BL6.CBA-D13Mit76C mice was significantly shorter than that in the control strain C57BL6.CBA-D13Mit76B ($F_{1,25} = 4.12$, $p = 0.05$; Fig. 2).

The social interaction test revealed no differences in the latent period of the first social approach ($F_{1,27} < 1$), the number of approaches ($F_{1,27} = 1.15$, $p > 0.05$), or the total duration of social contacts ($F_{1,27} = 1.54$, $p > 0.05$). There were also no differences in measures of aggressive behavior: the latent period of the first aggressive contact ($F_{1,27} < 1$), the number of attacks ($F_{1,27} < 1$), and the duration of aggression ($F_{1,27} < 1$) (Table 2).

Results obtained from testing the startle reflex did not identify any differences between the mean level of prestimulus inhibition for C57BL6.CBA-D13Mit76C animals (60.81 ± 7.67) and C57BL6.CBA-D13Mit76B animals (50.55 ± 6.22) ($F_{1,27} = 1.10$, $p > 0.05$). There was also no difference in the magnitudes of the startle reflex in the two strains ($F_{1,27} < 1$), (Table 3).

Discussion

The studies reported here were performed using mice o t recombinant strain C57BL6.CBA-D13Mit76C, which differ from the recipient stain C57BL/6J in terms of a fragment of chromosome 13 located in the 111.35–116.16 Mbp and

TABLE 1. Behavior of C57BL6.CBA-D13Mit76B and C57BL6.CBA-D13Mit76C Mice in the Open Field Test

Behavioral parameter	C57BL6.CBA-D13Mit76B	C57BL6.CBA-D13Mit76C
Path length, cm	868.7 ± 82.0	698.8 ± 69.2
Time spent in center, %	9.7 ± 2.4	8.1 ± 2.0
Number of vertical rearings	13.8 ± 2.2	4.9 ± 1.4**

Note. $^{**}p < 0.01$ compared with C57BL6.CBA-D13Mit76B mice.

TABLE 2. Behavior of C57BL6.CBA-D13Mit76B and C57BL6.CBA-D13Mit76C Mice in the Social Interaction Test

Behavioral parameter	C57BL6.CBA-D13Mit76B	C57BL6.CBA-D13Mit76C
Number of social contacts	12.0 ± 1.5	14.7 ± 2.2
Duration of social contacts, sec	120.2 ± 16.2	154.1 ± 22.9
Number of aggressive attacks	10.8 ± 2.4	9.1 ± 3.5
Duration of aggressive attacks, sec	41.0 ± 16.5	41.1 ± 22.1

TABLE 3. Behavior of C57BL6.CBA-D13Mit76B and C57BL6.CBA-D13Mit76C Mice in the Startle Reflex Test

Behavioral parameter	C57BL6.CBA-D13Mit76B	C57BL6.CBA-D13Mit76C
Startle amplitude	321.8 ± 39.8	321.3 ± 41.6
Prestimulus inhibition, %	50.6 ± 6.2	60.8 ± 7.7

from C57BL6.CBA-D13Mit76B mice, prepared by the same method as C57BL6.CBA-D13Mit76C mice, though they did not differ from C57BL/6J mice in terms of the chromosome 13 fragment.

The genetic structure of inherited catalepsy in mice is determined by a main gene located on chromosome 13 at 111.35–116.16 Mbp, which determines about 20% of the penetrance of the characteristic, as well as by 29 other loci widely disseminated throughout the genome, with a total contribution of about 30% to the penetrance of the characteristic [3, 12]. The minor proportion of catalepsy (35%) among C57BL6.CBA-D13Mit76C mice is in good agreement with previous results [3, 12] and supports the localization of the main gene for catalepsy to the 111.35–116.16 Mbp fragment. On the other hand, 18% of C57BL6.CBA-D13Mit76B mice were cataleptics. The presence of cataleptics among these mice can be explained in terms of the fact that at this stage of production of recombinant strains, the genome of strain C57BL6.CBA-D13Mit76B still contains a quite large proportion of the genome of strain C57BL6. CBA-D13Mit76C, of which about 50% are cataleptic. Thus, strain C57BL6.CBA-D13Mit76B still includes sufficient modifier genes from strain C57BL6.CBA-D13Mit76C to support the appearance of this characteristic.

Studies of the behavior of C57BL6.CBA-D13Mit76C and C57BL6.CBA-D13Mit76B mice in the open field test did not identify any differences in behavioral parameters such as horizontal activity or time spent in the center due to transfer of the fragment of chromosome 13, though the number of vertical rearings in animals of the cataleptic strain C57BL6.CBA-D13Mit76C was significantly smaller than that in C57BL6.CBA-D13Mit76B mice. This can be interpreted as a decrease in exploratory activity in C57BL6. CBA-D13Mit76C animals. These data are consistent with reported decreases in these parameters in ASC mice (Antidepressant-Sensitive Cataleptic) bred for a strong predisposition to catalepsy [1] and recombinant AKR.CBA-D13Mit76C mice [2].

Studies in the forced swimming test demonstrated a significant decrease in the duration of immobility in C57BL6. CBA-D13Mit76C mice, carrying the CBA allele of the D13Mit76 marker, as compared with C57BL6.CBA-D13Mit76B mice. Previous studies have demonstrated fixation of long periods of immobility in the forced swimming test during breeding for catalepsy in ASC mice [1] and GC (genetic catalepsy) rats [16]. On the one hand, this was interpreted as evidence of a link between inherited catalepsy and "depressivity." On the other hand, results obtained from C57BL6.CBA-D13Mit76C animals provide evidence of an association between catalepsy and the duration of immobility in the forced swimming test. It was suggested that the 111.35–116.16 Mbp fragment of chromosome 13 has no effect on the extent of immobility in the forced swimming test, while prolonged selection for catalepsy covers modifier genes increasing the "depressive" characteristics of behavior [2]. Results obtained from testing mice of the new recombi-

nant strain C57BL6.CBA-D13Mit76C lead to the conclusion that the 111.35–116.16 Mbp fragment of chromosome 13 transferred from the CBA genome to the genetic background of strain C57BL/6J weakens the depressive characteristics as compared with C57BL6.CBA-D13Mit76B mice.

Transfer of the distal fragment of chromosome 13 to the C57BL/6J genome had no effect on social and aggressive behavior in the social interaction test in C57BL6.CBA-D13 Mit76C mice as compared with C57BL6.CBA-D13Mit 76B mice, while transfer of this fragment to the AKR genome significantly increased the proportion of aggressive animals in the cataleptic strain AKR.CBA-D13Mit76C as compared with the recipient strain AKR. It may be that the increase in aggression in AKR.CBA-D13Mit76C mice is associated with a neurochemical imbalance induced by transfer of the 111.35–116.16 Mbp fragment of chromosome 13 [2]. The results presented here suggest the conclusion that the effect of a small part of the chromosome on behavior depends on the genetic background on which it operates. In this case, on the background of the genes of the inbred strain C57BL/6J, the part of chromosome 13 transferred from the CBA genome had no effect on aggressive behavior. This region of chromosome 13 also had no effect on the acoustic startle reaction or prestimulus inhibition.

As individual genetic changes can lead to unexpected behavioral reactions and, sometimes, impairments to brain functions, the creation of a new recombinant mouse strain is a potential direction in studies of normal and pathological behavior, changes in gene functions in a novel genetic environment, and the effects of these genes on the operation of other genes, as well as the actions of various drugs. Thus, AKR.CBA-D13Mit76C mice have been shown to differ from the parental mouse strain AKR in having reduced brain serotonin system activity. AKR.CBA-D13Mit76C mice were also found to have a significant alteration in the response of the brain serotonin system and behavior in response to administration of brain-derived neurotrophic factor (BDNF) as compared with the parental strain AKR [19].

Thus, the influence of chromosome 13 region 111.35–116.16 Mbp on the severity of catalepsy was confirmed in C57BL6.CBA-D13Mit76C mice, in which 35% displayed freezing. A decrease in exploratory behavior was found in the open field test. The forced swimming test showed weakening of depression-like behavior in C57BL6.CBA-D13Mit76C mice.

This study was supported by finance project VI.53.2.2.

REFERENCES

1. D. V. Bazovkina, A. V. Kulikov, E. M. Kondaurova, and N. K. Popova, "Breeding for predisposition to catalepsy increases depression-like behavior in mice," *Genetika*, **41**, No. 9, 1222–1228 (2005).
2. E. M. Kondaurova, D. V. Bazovkina, and A. V. Kulikov, "Studies of the interaction between catalepsy and anxiety, aggression, and depression-like behavior using congenic mouse strains," *Ros. Fiziol. Zh.*, **96**, 464–471 (2010).
3. A. V. Kulikov and D. V. Bazovkina, "Testing of the linkage hypothesis in hybridological analysis of alternative behavioral signs with incomplete penetrance," *Genetika*, **39**, No. 8, 1066–1072 (2003).
4. A. V. Kulikov, D. V. Bazovkina, M. L. Moisan, and L. Mormede, "Mapping of the gene for the predisposition to catalepsy in mice using polymorphic microsatellite markers," *Dokl. Akad. Nauk.*, **293**, No. 1, 134–137 (2003).
5. A. V. Kulikov, E. Yu. Kozlachkova, and N. K. Popova, "Genetic control of catalepsy in mice," *Genetika*, **25**, No. 8, 1402–1408 (1989).
6. A. V. Kulikov, V. A. Kulikov, and D. V. Bazovkina, "Digital processing of visual information in behavioral experiments," *Zh. Vyssh. Nerv. Deyat.*, **55**, No. 1, 116–122 (2003).
7. N. K. Popova, N. N. Barykina, I. F. Plyusnina, et al, "Expression of the startle reaction in rats genetically predisposed to different types of defensive behavior," *Ros. Fiziol. Zh.*, **85**, No. 1, 99–104 (1999).
8. A. Abbott, "With your genes? Take one of these, three times a day," *Nature*, **425**, 760–762 (2003).
9. S. Amir, Z. V. Brown, Z. Amir, and K. Ornstein, "Body-pinches induces long lasting cataleptic like immobility in mice: Behavioral characterization and the effects of naloxone," *Life Sci.*, **10**, 1189–1194 (1981).
10. A. K. Dixon, "Ethological strategies for defence in animals and humans: their role in some psychiatric disorders," *Br. J. Med. Psychol.*, **71**, 417–445 (1998).
11. E. M. Kondaurova, D. V. Bazovkina, A. V. Kulikov, and N. K. Popova, "Selective breeding for catalepsy changes the distribution of microsatellite D13Mit76 alleles linked to the 5-HT1A serotonin receptor gene in mice," *Genes Brain Behav.*, **5**, 596–601 (2006).
12. A. V. Kulikov, D. V Bazovkina, E. M. Kondaurova, and N. K. Popova, "Genetic structure of hereditary catalepsy in mice," *Genes Brain Behav.*, **7**, 506–512 (2008).
13. A. V. Kulikov, E. Y. Kozlachkova, N. N. Kudryavtseva, and N. K. Popova, "Correlation between tryptophan hydroxylase activity in the brain and predisposition to pinch-induced catalepsy in mice," *Pharmacol. Biochem. Behav.*, **50**, 431–435 (1995).
14. A. V. Kulikov, E. Y. Kozlachkova, G. B. Maslova, and N. K. Popova, "Inheritance of predisposition to catalepsy in mice," *Behav. Genet.*, **23**, 379–384 (1993).
15. A. V. Kulikov, M. A. Tikhonova, and V. A. Kulikov, "Automated measurement of spatial preference in the open field test with transmitted lighting," *J. Neurosci. Meth.*, **170**, No. 2, 345–351 (2008).
16. A. V. Kulikov, N. K. Popova, and V. G. Kolpakov, "The genetic cataleptic (GC) rat strain as a model of depressive disorders," in: *Animal Models in Biological Psychiatry*, A. Kaluev (ed.), Nova Science Publishers, New York (2006), pp. 59–73.
17. M.-P. Moisan, "From QTL detection to gene identification," in: *Neurobehavioral Genetics. Methods and Applications*, B. Jones and P. Mormede (eds.), CTC Press, Boca Raton (1999), pp. 77–91.
18. V. S. Naumenko, E. M. Kondaurova, D. V. Bazovkina, et al., "On the role of 5-HT(1A) receptor gene in behavioral effect of brain-derived neurotrophic factor," *J. Neurosci. Res.*, **92**, 1035–1043 (2014).
19. V. S. Naumenko, E. M. Kondaurova, D. V. Bazovkina, et al., "Effect of BDNF on behavior and key members of the brain serotonin system in genetically predisposed to behavioral disorders mouse strains," *Neuroscience*, **214**, 59–67 (2012).
20. K. Ornstein and S. Amir, "Pinch-induced catalepsy in mice," *J. Comp. Physiol. Psychol.*, **95**, 827–835 (1981).
21. B. Singerman and R. Raheja, "Malignant catatonia – a continuing reality," *Ann. Clin. Psychiatry*, **6**, 259–266 (1994).