Neuroscience Research 117 (2017) 14–21

Contents lists available at ScienceDirect

Neuroscience Research

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

Alteration of the brain morphology and the response to the acute stress in the recombinant mouse lines with different predisposition to catalepsy

E.A. Kulikova*,[1], D.V. Bazovkina[1], Y.V. Antonov, A.E. Akulov, A.V. Kulikov, E.M. Kondaurova

Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Science, Novosibirsk, Russia

ARTICLE INFO

Article history:
Received 4 August 2016
Received in revised form
15 November 2016
Accepted 18 November 2016
Available online 21 November 2016

Keywords:
Recombinant lines
Catalepsy
MRI
Brain
Open field
Spatial learning
Mice
Stress

ABSTRACT

Catalepsy is an inability to correct an externally imposed awkward posture; it is associated with schizophrenia and depression in human. We created new recombinant B6.CBA-D13Mit76C and B6.CBA-D13Mit76B mouse lines on the C57Bl/6 genome, carrying the 102.73–110.56 Mbp fragment of chromosome 13 derived from the catalepsy-prone CBA strain and catalepsy-resistant C57BL/6 strain, respectively. We compared the behavior and brain morphology (11.7T BioSpec 117/16 USR tomograph, Germany) in these lines. The effects of acute emotional stress on corticosterone's level in the blood and mRNA expression of *Bdnf* and *Arc* genes in the brain were investigated. The B6.CBA-D13Mit76B mice were non-cataleptic, while about 17% of B6.CBA-D13Mit76C mice demonstrated catalepsy-like immobility. No difference between these lines was revealed in the open field and social interaction tests. In the Morris water maze test, both lines effectively found the platform on the fourth day; however B6.CBA-D13Mit76B mice achieved significantly better results than cataleptic-prone animals. B6.CBA-D13Mit76C mice were characterized by decreased volume of the total brain and reduced sizes of striatum, cerebellum and pituitary gland. The both lines showed the similar basal and stress-induced levels of corticosterone, while the brain expression of *Bdnf* and *Arc* genes was more vulnerable to stress in the catalepsy-prone B6.CBA-D13Mit76C line.

1. Introduction

Catalepsy (tonic immobility, animal hypnosis) represents a kind of passive defensive behavior and it is found in many species of vertebrates. It is characterized by a prolonged immobility, an inability to correct an externally imposed awkward posture. In birds and mammals, catalepsy is a passive defensive freezing strategy in response to the appearance of predator or other threatening stimulus. Catalepsy is associated with symptoms of schizophrenia, depression, Parkinson's disease in human (Klemm, 1989; Dixon, 1998).

Drug-free catalepsy can be induced in mice by pinching the skin at the scruff of the neck (Ornstein and Amir, 1981). Significant genetic variability in the predisposition to catalepsy was found: C57BL/6, DBA/2J and AKR mice never displayed cataleptic response, while about 50% of CBA mice showed pronounced immobility continuing more than 60 s after 4–5 pinches (Kulikov et al., 1993). The QTL-analysis, selective breeding and genetic recombination mapped the main gene of predisposition to catalepsy on 102.73-110.56 Mbp fragment of mouse chromosome 13 that coding about 20% of the trait penetrance (Kulikov et al., 2003, 2008a; Kondaurova et al., 2006).

The recombinant AKR.CBA-D13Mit76C line was created by transferring of this CBA-derived fragment of chromosome 13 to the genome of catalepsy-resistant AKR strain. About 50% of AKR.CBA-D13Mit76C mice showed pronounced catalepsy, demonstrating the AKR-like level of locomotor activity in the open field test. Moreover, AKR.CBA-D13Mit76C mice were characterized by high level of intermale aggression (Kondaurova et al., 2010) and learning deficit in the Morris water maze test (Kulikov et al., 2014) compared to AKR strain. This profound alteration in the behavior can be explained by a neurochemical imbalance induced by transfer of 102.73-110.56 Mbp fragment of the chromosome 13. Moreover, magnetic

* Corresponding author at: Researcher, Department of Behavioral Neurogenomics, Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Science, Lavrentyeva av. 10, Novosibirsk 630090, Russia.
E-mail addresses: kulikova@bionet.nsc.ru, lisa_kulikova@ngs.ru (E.A. Kulikova).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.neures.2016.11.009
0168-0102/

resonance imaging showed that the catalepsy-prone AKR.CBA-D13Mit76C mice were characterized by the smaller size of the pituitary gland compared to the parental AKR strain (Tikhonova et al., 2013). This brain structure is known to be involved in mechanisms of schizophrenia, catatonia and depression (Kooy et al., 2001; Upadhyaya et al., 2007). Besides, restraint stress-induced corticosterone elevation was diminished in the cataleptic AKR.CBA-D13Mit76C mice (Tikhonova et al., 2013). Thus, the association between the hereditary catalepsy, neuroanatomical characteristics, and neurochemical responses to emotional stress could be suggested. However, it is difficult to compare the results obtained from the AKR.CBA-D13Mit76C mice with other animal models because numerous transgenic mice used as models of behavioral and brain dysfunctions are created using C57BL/6 (B6) genetic background.

In this context, the goal of the present study was to transfer of CBA-derived 102.73-110.56 Mbp fragment of mouse chromosome 13 to catalepsy-resistant B6 genome and evaluate the effect of this transfer on predisposition to catalepsy, locomotor activity, spartial learning, social behavior, brain morphology and sensitivity to acute stress. For this purpose, 1) the B6.CBA-D13Mit76C and B6.CBA-D13Mit76B recombinant lines differing only in this distal region of mouse chromosome 13 were bred; 2) mice of these lines were tested in the pinch-induced catalepsy, open field, social interaction and Morris water maze tests; 3) the sizes of brain structures in mice of these lines were estimated by Magnetic Resonance Imaging (MRI) 4) the influence of acute restraint stress on the *Arc* and *Bdnf* genes expression in the brain was determined in the B6.CBA-D13Mit76C and B6.CBA-D13Mit76B recombinant lines.

2. Materials and methods

2.1. Breeding of lines

Two recombinant lines were created by transferring the CBA-derived fragment 102.73–110.56 Mbp of chromosome 13 including the main locus of catalepsy to the B6 genome. Males of generated in the Laboratory of Behavioral Neurogenomics (Novosibirsk, Russia) catalepsy-prone AKR.CBA-D13Mit76C recombinant line containing the CBA-derived fragment in the AKR genome (Kulikov et al., 2008a) and females of inbred mouse strain B6 were mated to obtain the F1 hybrids. The latter was used for the recombinant line creation. The recombinant lines were produced by eight successive backcrossing of the F1 hybrids to B6 strain. The transfer of the CBA-derived fragment of chromosome 13 to the B6 genome was controlled using three polymorphic microsatellites D13Mit287 (102.73 Mbp), D13Mit76 (110.56 Mbp) and D13Mit78 (118.83 Mbp). The heterozygous backcrosses of the eighth generation were intercrossed to generate B6.CBA-D13Mit76C (B6-M76C) and B6.CBA-D13Mit76B (B6-M76B) containing respectively the CBA-and B6-derived alleles of D13Mit287 and D13Mit76 and AKR- and B6-derived alleles of D13Mit78 in the B6 genome. The genomic profile of recombinant lines: for the B6-M76B line – it is almost the same as B6 genome and for the B6-M76C line – it is 99% genome of B6 except the distal fragment of chromosome 13 marked by earlier described microsatellites (see Fig. S1 in the online version at DOI: http://dx.doi.org/10.1016/j.neures.2016.11.009). The breeding of B6-M76C and B6-M76B lines were 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 RFMEFI6211X0010).

2.2. Animals

Adult (10–12 weeks old) male mice of B6-M76C and B6-M76B lines were used. Animals were housed in groups of 7–8 per cage 40 cm x 25 cm x 15 cm under standard conditions (20–22 °C, free access to food and water, 12 h light/dark cycle). Two days before the experiments, the mice were weighed (about 23 g) and were isolated into individual cages to remove the group effect.

In the first experiment, B6-M76C (n = 12) and B6-M76B (n = 12) mice were tested in the pinch-induced catalepsy, open field and social interaction tests. In the second experiment, spatial learning was compared in the recombinant lines in Morris water maze test (n = 8, of each genotype). Mice were tracked automatically with digital video camera (Sony) connected with a PC-compatible computer via IEEE1394 interface. Data were analyzed with program EthoStudio (Kulikov et al., 2008b). In the third experiment, the brain morphology of B6-M76C (n = 6) and B6-M76B (n = 5) mice was investigated by MRI. In the fourth experiment, the mice of each genotype were derived into two groups (control and stress) (n = 6-10 per group). The mice of the control group were sacrificed right after taking them out of their individual home cage, while the mice of stress group were exposed to emotional stress for 40 min and then they were sacrificed. The blood samples were collected for measuring the plasma corticosterone level. The structures of the brain (hippocampus, striatum and hypothalamus) were collected for measuring the influence of the stress and genotype on the genes expression.

All experimental procedures were in compliance with the EC Directive 86/609/EEC for animal experiments and were approved by the Institute's ethics committee. All efforts were made to minimize the number of animals used and their suffering.

2.3. Pinch-induced catalepsy

Catalepsy test was performed according to early described procedure (Kulikov et al., 1993). Animals were firmly pinched between two fingers for 5 s at the scruff of the neck, placed on the parallel bars, with the forepaws at 5 cm above the hind legs and then were released gently. The catalepsy duration was recorded in seconds from the instant when an animal was released to the instant 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. Every 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 as cataleptic if the time of immobility was above 20 s in no less than 3 of 10 trials.

2.4. Open field

The open field test (OF) was carried out on a circle arena (40 cm in diameter) bordered with white plastic wall (25 cm high) and illuminated through the mat and semitransparent floor with two halogen lamps of 12 W each placed 40 cm under the floor (Kulikov et al., 2008b). Mouse was placed near the wall and tested for 5 min. The distance traveled, time spent in the center of arena and the number of rearings were measured.

2.5. Social interaction test

One month old male of CD line (the intruder) was placed in the home cage of investigated male mice. The number of social contacts (sniffing, prosecution intruder), duration of social contacts (sec), number and duration of fights were measured during 10 min.

2.6. Morris water maze

The experiment was conducted, as previously described (Kulikov et al., 2014). A circular white plastic tank (100 cm diameter, 40 cm high) with mat and semitransparent floor was used.