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Spatial learning in the Morris water maze in mice genetically different in the predisposition to catalepsy: The effect of intraventricular treatment with brain-derived neurotrophic factor

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ABSTRACT

Hereditary catalepsy in mice is accompanied with volume reduction of some brain structures and high vulnerability to inflammatory agents. Here an association between hereditary catalepsy and spatial learning deficit in the Morris water maze (MWM) in adult mouse males of catalepsy-resistant AKR, catalepsy-prone CBA and AKR.CBA-D13Mit76 (D13) strains was studied. Recombinant D13 strain was created by means of the transfer of the CBA-derived allele of the major gene of catalepsy to the AKR genome. D13 mice showed a low MWM performance in the acquisition test and high expression of the gene coding proinflammatory interleukin-6 (*Il-6*) in the hippocampus and cortex compared with mice of the parental AKR and CBA strains. An acute ivc administration of 300 ng of brain derived neurotrophic factor (BDNF) normalized the performance in the MWM, but did not decrease the high *Il-6* gene expression in the brain of D13 mice. These results indicated a possible association between the hereditary catalepsy, MWM performance, BDNF and level of *Il-6* mRNA in the brain, although the relation between these characteristics seems to be more complex. D13 recombinant mice with deficit of spatial learning is a promising model for study of the genetic and molecular mechanisms of learning disorders as well as for screening potential cognitive enhancers.

1. Introduction

Catalepsy is a state of pronounced motor inhibition and its exaggerated forms frequently accompany schizophrenia, Parkinsonism and extrapyramidal dysfunctions (Caroff et al., 2000; Daniels, 2009; Lee, 2007, 2010; Paparrigopoulos et al., 2009; Sanberg et al., 1988; Weder et al., 2008). Hereditary catalepsy is very rare phenomenon and it is not found in mice of the most common inbred strains, such as C57BL/6J, DBA/2, or AKR/J. At the same time, about 50% of CBA/Lac mice are predisposed to catalepsy (Kulikov et al., 1993). The main locus for catalepsy was mapped to 111–116 Mb fragment of chromosome 13 (Kulikov et al., 2003, 2008a). The CBA-derived 111–116 Mb fragment of chromosome 13 was transferred to the AKR genome and the recombinant AKR.CBA-D13Mit76 (D13) strain was created. About 50% of D13 mice showed pronounced catalepsy (Kulikov et al., 2008a).

There is evidence of an association between hereditary catalepsy, neuroinflammation and neurodegeneration. Hereditary catalepsy is accompanied with reduction of volume of the pituitary gland, hypothalamus and striatum (Tikhonova et al., 2013). Proinflammatory agents such as bacterial lipopolysaccharide (LPS) (Bazhenova et al., 2013; Kulikov et al., 2010b) and interleukin-6 (IL-6) (Bazovkina et al., 2011) produced catalepsy in mice.

Brain derived neurotrophic factor (BDNF) is critical for neuronal development, survival and plasticity (Binder and Scharfman, 2004; McAllister et al., 1999; Thoenen, 1995). Neurodegeneration was associated with deficit of BDNF (Duman, 2002). An anticataleptic effect of a single icv administration of BDNF in mice was revealed (Naumenko et al., 2012; Tikhonova et al., 2012).

The Morris water maze (MWM) is frequently used to study the mechanisms and disorders of spatial learning and memory (D'Hooge and De Deyn, 2001). Proinflammatory agents such as lipopolysaccharide (LPS) and interleukin (IL)-1β disrupt learning in the MWM (Arai et al., 2001; Oitzl et al., 1993; Sparkman et al., 2005a, 2005b). Another proinflammatory cytokine IL-6 attenuates acquisition (Wei et al., 2012) and facilitates LPS-induced disruption of working memory (Sparkman et al., 2006) in the MWM. Although BDNF is involved in

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the long-term potentiation and long-term memory (Lu et al., 2008; Waterhouse and Xu, 2009), the data on the effect of BDNF on the acquisition or memory retention in the MWM are contradictory. On the one hand, no effect of intracranial BDNF, antibody to BDNF (Cirulli et al., 2000) or antisense olygodeoxynucleotides to BDNF (Tandon et al., 1999) on MWM performance was shown. Chronic intracranial administration both relatively low (1.2 μg/day) or high (12 μg/day) of BDNF failed to restore the MWM learning deficit resulting from traumatic brain injury (Blaha et al., 2000). On the other hand, a high dose (24 μg) of BDNF injected after the last acquisition trial improved the memory retention on the consecutive day (Cirulli et al., 2004).

The aim of the present study was to test an association between hereditary catalepsy, spatial learning in the MWM, BDNF and *Il-6* mRNA level in the brain. For these purposes it was intended 1) to compare learning performance in the MWM and *Il-6* mRNA level in the brain of catalepsy-resistant AKR and catalepsy-prone CBA and D13 mice as well as 2) to study effect of a single icv administration of BDNF on the MWM learning and *Il-6* mRNA level in the brain of catalepsy-prone mice.

2. Methods

2.1. Animals and treatments

The experiments were carried out on adult male mice of catalepsy-resistant AKR/J and catalepsy-prone CBA/Lac and AKR.CBA-D13Mit76 (D13) strains. The recombinant D13 strain was created by means of the transfer of 111–116 Mb fragment of chromosome 13 marked with microsatellite D13Mit76 from CBA strain to the AKR genome (Kulikov et al., 2008a). All mice were 8 weeks old, weighted about 25 ± 2 g and were housed under standard laboratory conditions at natural light–dark cycle (12 h light and 12 h dark), ambient temperature 22 ± 0.2 °C and free access to water and food. Three days before the experiment the mice were isolated into individual cages to remove the group effect. All experimental procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize the number of animals used and their suffering.

For central administration human recombinant BDNF (5 μg, Sigma-Aldrich, USA) was diluted in 85 μl of sterile saline and injected under short term light diethyl ether anesthesia (the time between onset and offset of the ether vapour was 40–50 c) at the dose of 300 ng in 5 μl into the left lateral ventricle. The control animals were injected with sterile saline into the left lateral ventricle (Naumenko et al., 2012; Tikhonova et al., 2012). A 10 μl hamilton syringe was used for ivc injection. The needle length was limited to 2.5 mm with a piece of 48.5 mm of polypropylene tube (outer diameter 5 mm, inner diameter 0.9 mm). The injection accuracy was controlled by the needle trace position when the animals were decapitated and the cortex and hippocampus were dissected for the *Il-6* mRNA level estimation (see below). In 82% of mice the needle hit the left ventricle. The mice with verified ivc injection were taken for further statistical calculations.

2.2. Experimental design

In the first experiment 10 mouse males of each AKR, CBA and D13 strains were compared for the learning performance (acquisition and retention) in the MWM test.

In the second experiment other intact mouse males of AKR/J (n = 15), CBA (n = 16) and D13 (n = 15) strains were decapitated their brains were removed on ice and the hippocampus and hypothalamus were dissected, frozen with liquid nitrogen and stored at −70 °C until mRNA extraction.

In the third experiment 26 D13 mice were ivc injected with BDNF (n = 14) or saline (n = 12). Seven days after injection the learning performance of the animals were assayed in the MWM test and 14 days after injection all these animals were decapitated, their brains were removed on ice and the hippocampus and hypothalamus were dissected, frozen with liquid nitrogen and stored at −70 °C until mRNA extraction.

2.3. Morris water maze

Since AKR and D13 mice were white and, therefore, were hardly automatically tracked due to a poor contrast with the white background, we tracked all animals using transmitted (inverted) lighting technique developed for open field (Kulikov et al., 2008b) and forced swim (Kulikov et al., 2010a) tests. Our MWM apparatus with inverted lighting consisted of a round white plastic tank (70 cm diameter, 25 cm high) with mat and semitransparent floor. The tank was placed on the mount at 40 cm above four halogen lamps of 12 wt each and filled up to 15 cm with water at 25 °C. The water was rendered opaque with powdered milk. The surface of the tank was virtually divided by four quadrants. A glass escape round platform (5 cm diameter and 14.5 cm height) was located 0.5 cm below the water surface near the center of one quadrant of the maze (target quadrant I). Mice were tracked automatically with a digital video camera (Sony) connected with a PC-compatible computer via IEEE1394 interface. Data was analyzed with program EthoStudio (Kulikov et al., 2008b).

Mice received three acquisition trials per day for 4 consecutive days. For each trial the mouse was place in the water at the fixed start position near the wall at the boundary between quadrants II and III (counting clockwise from the quadrant I). A maximum 60 s was allowed, during which the mouse had to find the platform and climb onto it. If the animal failed to find the platform, it was guided with a sieve to the platform. Training trials were separated with 60 s intertrial intervals. Parameters measured during an acquisition trial included: escape latency time (s) spent to reach the platform location, distance run (cm) and cumulative distance (cm) to platform location (Dalm et al., 2000, 2009; Gallagher et al., 1993). The mean values of the three day trials were calculated.

On the fifth consecutive day the retention test was performed. The platform was removed and a mouse was released in the maze near the wall at the boundary between quadrants II and III. During three 60 s trials separated with 60 s intertrial intervals the time spent (%) and the distance run (%) in the quadrants as well as cumulative distance (cm) to the prior platform position in the target quadrant were measured. The mean values of the three trials were calculated. Some scientists place animals randomly at one of the four different locations and chose the quadrant III as the opposite to the target quadrant (Dalm et al., 2000; Andero et al., 2012). Since in our experiment mice were always placed at the boundary between quadrants II and III, these two quadrants had an advantage over quadrants I and IV. Therefore, in our case the quadrant III was not the correct opposite quadrant. That is why, in the present study the quadrant IV was chosen as the opposite to the target quadrant (I).

2.4. Real-time RT-PCR

Total RNA was isolated with Trizol (Bio-Rad, USA) according to the manufacturer's protocol diluted with DEPC-treated water to 0.125 μg/μl and stored at −70 °C.

One microgram aliquot of the total RNA (8 μl) was mixed with 180 ng of a random hexanucleotide mixture, 2.25 μl of sterile KCl (1 M) and sterile water to a final volume of 16 μl, denaturated at 94 °C for 5 min and allowed to anneal at 41 °C for 15 min. After that 15 μl of mixture containing reverse transcriptase M-MLV (200 U, Biosan, Novosibirsk, Russia), Tris–HCl (pH 8.3, 0.225 μmol), dNTPs (0.015 μmol), DTT (0.225 μmol)