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Research report

Hereditary catalepsy in mice is associated with the brain dysmorphology and altered stress response

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HIGHLIGHTS

- Catalepsy-prone mice had the reduced pituitary gland and enlarged thalamus.
- Restraint stress facilitates catalepsy.
- Stress-induced corticosterone elevation was diminished in catalepsy-prone mice.
- Stress increased 5-HT metabolism in catalepsy-prone mice.

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ABSTRACT

Catalepsy is a passive defensive strategy in response to threatening stimuli. In exaggerated forms it is associated with brain dysfunctions. The study was aimed to examine (1) possible association of the hereditary catalepsy with neuroanatomical characteristics and (2) sensitivity of the catalepsy expression, HPA and brain serotonin (5-HT) systems to restraint stress (for one hour) in mice of catalepsy-prone (CBA/Lac, ASC (Antidepressant Sensitive Catalepsy), congenic AKR.CBA-D13M76) and catalepsy-resistant (AKR/J) strains. Magnetic resonance imaging showed that the catalepsy-prone mice were characterized by the smaller size of the pituitary gland and the larger size of the thalamus. In ASC mice, diencephalon region (including hypothalamus) and striatum were significantly reduced in size. Restraint stress provoked catalepsy in AKR mice and enhanced it in the catalepsy-prone mice. Stress-induced corticosterone elevation was diminished, while 5-HT metabolism (5-HIAA level or 5-HIAA/5-HT ratio) in the midbrain was significantly augmented by stress in the catalepsy-prone mice. The multivariate factor analysis revealed interactions between the basal levels and the stress-induced alterations of 5-HT metabolism in the hippocampus and midbrain suggesting the interaction between multiple alterations in 5-HT neurotransmission in several brain structures in the regulation of hereditary catalepsy.

The study indicated an association between the hereditary catalepsy, neuroanatomical characteristics, and neurochemical responses to emotional stress. The catalepsy-prone genotypes seem to be more susceptible to stress that suggests them as the adequate models to study the genetic predisposition to stress-based neuropathology. The data support the association of hereditary catalepsy with the inherited brain dysfunction of a neurodegenerative nature.

1. Introduction

Catalepsy is a natural passive defensive strategy [1]. An exaggerated form of catalepsy is observed as a prolonged immobility with plastic muscle tonus and an inability to correct an externally imposed awkward posture, and is associated with neurological and mental disorders [2,3]. Catalepsy is often a negative consequence of antipsychotic treatment [4,5]. In rodents, catalepsy induced by antipsychotic haloperidol (haloperidol-induced catalepsy) serves as a model of extra pyramidal dysfunction [6,7].

Drug-free catalepsy can be induced by pinching mice at the scruff of their neck [8]. This pinch-induced catalepsy is very rare

Abbreviations: ASC, Antidepressant Sensitive Catalepsy mouse strain; 5-HT, serotonin; 5-HIAA, 5-hydroxyindolacetic acid; MRI, magnetic resonance imaging; HPA axis, hypothalamic-pituitary-adrenal axis.

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phenomenon that is not found in mice of the most common inbred strains, such as C57BL/6J, DBA/2, AKR/J, etc. But about 50% of CBA/Lac mice showed catalepsy after several pinches [9]. The hereditary predisposition to catalepsy of CBA/Lac strain was markedly increased with the prolonged selection, and ASC/Icg (Antidepressant Sensitive Catalepsy) strain was created [10,11]. About 80–85% of ASC mice displayed catalepsy and a set of characteristics similar to those observed in depressive patients [10,12–18]. The main gene for catalepsy was mapped to the 61–70-cM fragment of the chromosome 13 [11]. Then, the congenic AKR.CBA-D13Mit76 strain with the CBA-derived fragment of chromosome 13 containing the major gene of catalepsy transferred to the AKR genome was created. About 50% of mice of the AKR.CBA-D13Mit76 strain showed pronounced catalepsy [11]. Intracerebral administration of neurotrophic factor BDNF [19,20] decreased catalepsy manifestation in ASC and CBA mice suggesting the association between catalepsy and neurodegeneration in certain brain structures. Brain serotonin system was found to play an essential role in the regulation of hereditary defined catalepsy [16,21] and anticataleptic effect of BDNF [20].

Stress-responses are composed of alterations in behavior, autonomic function and the secretion of multiple hormones and brain monoamines including 5-HT and its metabolism [22,23]. Excessive stress can be extremely harmful. Stressful events could precipitate or exacerbate some of mental disorders [24,25].

The purpose of this study was to examine (1) the neuroanatomical characteristics; (2) effect of restraint stress on catalepsy, plasma corticosterone level, concentration of 5-HT and its main metabolite 5-HIAA in the hypothalamus, hippocampus, and midbrain in catalepsy-resistant and catalepsy-prone mice.

2. Materials and methods

2.1. Animals

The experiments were carried out on adult male mice of AKR/J and CBA/Lac inbred strains, mice of ASC/Icg (Antidepressant Sensitive Catalepsy) strain and mice of congenic AKR.CBA-D13Mit76/Icg strain. The inbred ASC mouse strain was created in the Laboratory of Behavioral Neurogenomics, Institute of Cytology and Genetics SB RAS (Novosibirsk, Russia) as the result of prolonged selective breeding for high predisposition to catalepsy from the (CBAx(CBAxAKR)) backcross population between catalepsy-prone CBA and catalepsy-resistant AKR strains [10,11]. The congenic AKR.CBA-D13Mit76 mouse line was created in the Laboratory of Behavioral Neurogenomics, Institute of Cytology and Genetics SB RAS (Novosibirsk, Russia) as the result of transferring the 55–70-cM fragment of chromosome 13 containing the major gene of high predisposition to catalepsy from CBA strain genome to AKR genetic background [10,11,26]. Mice of CBA, ASC, and AKR.CBA-D13Mit76 strains are catalepsy-prone (genetically predisposed to manifest catalepsy reaction in response to pinching the scruff of their neck), while mice of AKR strain are catalepsy-resistant and never display cataleptic freezing under normal conditions. The mice were about 15 weeks old and weighing 25 ± 2 g After weaning, the mice were separated by sex and kept 10 per 40 cm × 25 cm × 15 cm cage under standard conditions (a natural light–dark cycle (16 h light and 8 h dark), temperature: 18–22 °C, relative humidity: 50–60%, standard food and water ad libitum). Two days before the experiment animals were isolated in cages of the same size to minimize group effects. Experimental manipulations in the first experimental series (for measuring the monoamine and corticosterone levels) were performed between 10:00 and 12:00 h. MRI was performed between 10:00 and 14:00 h. Experimental manipulations and behavioral tests in the second experimental series were carried out between 12:00 and 15:00 h.

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.

2.2. Experimental design

Three experimental series were held. In the first series intact mice of each strain were divided into two groups, mice were sacrificed right after taking them out their individual home cage ("Control" group) or after an hour of emotional (restriction) stress ("Stress" group) (n = 9–11 per group). Brain and blood samples were collected for measuring the brain levels of 5-HT and its metabolism and plasma corticosterone levels. In the second experimental series, mice of each strain were also divided into two groups, the test for catalepsy was carried out in intact mice and in mice right after 1 h-exposure to emotional stress (n = 8–10 per group). In the third experimental series, brains of intact mice of each strain were examined using magnetic resonance imaging (MRI) (n = 8–11 per group).

2.3. Emotional stress procedure

For the induction of emotional stress, mice were restricted for 60 min in small cylindrical metal cages (8.5 cm × 2.5 cm) with 48 ventilating openings of 0.4 cm in diameter. The restriction device allowed mice to stretch their legs but not to move within the tube.

2.4. Test for catalepsy

Catalepsy was tested according to earlier described and verified protocol [9,11]. An animal was firmly pinched between two fingers for 5 s at the scruff of the neck, placed on parallel bars, with the forepaws at 5 cm above the hind legs and then was released gently. The catalepsy duration was timed from the instant the animal was released to the instant the animal shifted its front paws from its initial position on the upper bar or made gross body or head movements. A trial ended either when the animal started to move or after 120 s of freezing. Immobility time of more than 20 s was considered as positive (cataleptic) response. Every animal was successively tested with 2-min intervals (a mouse was placed in its home cage between the trials) until three positive responses were achieved, but no more than 10 times. Catalepsy time (s) was evaluated as the means of the three trials with the maximal immobility duration.

2.5. Assay for corticosterone level

Blood samples were collected into heparinazed tubes, centrifuged for 20 min at 3000 rpm at +4 °C, plasma was stored at −24 °C until assay. Corticosterone levels (pg/ml) were detected in diluted plasma samples (1:30) using Correlate-EIA™ Corticosterone Enzyme Immunoassay Kit (Assay Designs Inc., USA) according to the manufacturer's instructions.

2.6. High performance liquid chromatography

Hypothalamus, hippocampus, and midbrain were rapidly dissected, weighed and frozen in liquid nitrogen, and stored at −70 °C until the following procedures. Tissue samples were homogenized in 200 μl of buffer containing 0.4 M $HClO_4$ (Sigma–Aldrich, USA), 0.27 mM EDTA (Ameresco, USA) and 100 ng/ml 3,4-dihydroxybenzylamine (DHBA; Sigma–Aldrich, USA) as the internal standard. The homogenates were centrifuged for 5 min at 15,000 × g (4 °C) and supernatant was filtered through Whatman GF/C fiberglass filters (Whatman Int. Ltd., UK). Levels of 5-HT and 5-HIAA were then evaluated by HPLC on Nucleosil C8 column (Nucleosil C8 column, 3 μm particle size, L × I.D. 100 mm × 4.6 mm, Sigma–Aldrich, USA) with electrochemical detection (500 mV, Coulochem III, ESA, Inc., USA) and flowcell (BAS-Inc, USA) using solvent delivery module LC-20AD (Shimadzu Corporation, Japan). The mobile phase was monobasic potassium phosphate (100 mM, pH 4.5) buffer containing 0.1 mM Na_2EDTA, 1.4 mM 1-Octanesulfonic acid sodium salt (Sigma, USA) and methanol (4 volume percent; Vekton Ltd., Russia) with a flow rate of 0.6 ml/min.

Standard solution containing 2 ng of 5-HT and 5-HIAA was repeatedly assayed throughout the entire procedure. The magnitudes of 5-HT and 5-HIAA peaks were estimated using MutiChrom v.1.5 software (Ampersand Ltd., Russia) and calibrated against internal (DHBA) and corresponding external standards. Monoamine/metabolite content was expressed in μg per g of tissue sample.

2.7. MRI procedure

We used a 11.7 T BioSpec 117/16 USR (Bruker, Germany) tomograph for experimental studies in vivo.

The T2-weighed images (TR = 2000 ms, TE = 15.6 ms, number of averages: 1, number of slices: 9, slice orientation: axial, slice thickness: 0.5 mm, inter-slice gap: 1.5 mm, field of view: 2 cm × 2 cm, matrix: 256 × 256, scan duration: 6 min 40 s) for axial slice were obtained using spin echo method of multi slice scanning MSME (Multi Slice Multi spin Echo). The T2-weighed images (TR = 2500 ms, TE = 11 ms, TEeff = 33 ms, number of averages: 2, number of slices: 9, slice orientation: coronal, sagittal, slice thickness: 1.0 mm, inter-slice gap: 1.5 mm, field of view: 2 cm × 2 cm, matrix: 256 × 256, scan duration: 2 min 40 s) for coronal and sagittal slices were obtained using RARE (rapid-acquisition relaxation-enhancement) pulse sequence. The sizes of brain structures (area, cm^2) were calculated only in the one slice of each projection.

Slice in the axial orientation corresponded to a level of approximately −2.3 mm to bregma. Slice in the coronal orientation was the fourth from the dorsal surface of the brain. Slice in the sagittal orientation corresponded to a level of bregma.

The brain structures were restricted using the manufacturer's Region of Interest (ROI) Tool software and a standard mouse brain atlas by Hof and Young [27].

High-resolution MR imaging clearly shows not only the gross anatomy of the mouse brain architecture, but also allows the visualization of small structures. The