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Antidepressant-Like Effects of Central BDNF Administration in Mice of Antidepressant Sensitive Catalepsy (ASC) Strain

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Abstract

Although numerous data evidence the implication of brain-derived neurotrophic factor (BDNF) in the pathophysiology of depression, the potential for BDNF to correct genetically defined depressive-like states is poorly studied. This study was aimed to reveal antidepressant-like effects of BDNF (300 ng, 2×, i.c.v.) on behavior and mRNA expression of genes associated with depression-like state in the brain in mice of antidepressant sensitive catalepsy (ASC) strain characterized by high hereditary predisposition to catalepsy and depressive-like features. Behavioral tests were held on the 7th-16th days after the first (4th-13th after the second) BDNF injection. Results showed that BDNF normalized impaired sexual motivation in the ASC males, and this BDNF effect differed, with advantageous effects, from that of widely used antidepressants. The anticataleptic effect of two BDNF injections was enhanced compared with a single administration. A tendency to decrease the immobility duration in tail-suspension test was observed in BDNF-treated ASC mice. The effects on catalepsy and sexual motivation were specific since BDNF did not alter locomotor and exploratory activity or social interest in the ASC mice. Along with behavioral antidepressant-like effects on the ASC mice, BDNF increased hippocampal mRNA levels of *Bdnf* and *Creb1* (cAMP response element-binding protein gene). BDNF also augmented mRNA levels of *Arc* gene encoding Arc (Activity-regulated cytoskeleton-associated) protein involved in BDNF-induced processes of neuronal and synaptic plasticity in hippocampus and prefrontal cortex. The data suggest that: [1] BDNF is effective in the treatment of some genetically defined behavioral disturbances; [2] BDNF influences sexually-motivated behavior; [3] *Arc* mRNA levels may serve as a molecular marker of BDNF physiological activity associated with its long-lasting behavioral effects; [4] ASC mouse strain can be used as a suitable model to study mechanisms of BDNF effects on hereditary-dependent behavioral disorders.

Key Words: BDNF, animal model, antidepressant-like activity, behavior, *Arc* mRNA, mice

Introduction

Neurotrophic factors are involved in growth, development and maintenance of the nervous system (7). Their deficit seems to play an important role in the pathogenesis while augmentation of concentrations of the factors is promising for the therapy of some diseases including depression (45, 48). Particular attention is paid to brain-derived neurotrophic factor (BDNF) since its serum level is a sensitive marker of the antidepressant treatment efficiency in depressive patients (10, 24, 38, 49). Moreover, effective antidepressive therapy (chronic treatment with antidepressants or electroconvulsive shock) increased BDNF expression in hippocampus and cerebral cortex of rats (27, 43, 46).

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However, there are only a few studies on the potential of exogenous BDNF to correct depressive-like behavior (21, 26, 50, 53). Antidepressant-like effects of central BDNF administration were demonstrated for the model of learned helplessness in rats (50). Recently, we found that BDNF corrected the genetically defined high levels of expression of catalepsy in mice of antidepressant sensitive catalepsy (ASC) strain (58).

Catalepsy or freezing reaction is a state of prolonged immobility with the inability to correct an externally imposed unnatural posture (47). In humans, catalepsy is a syndrome of grave mental illnesses such as schizophrenia and depressive disorders (17, 51, 55). In laboratory rodents, catalepsy is usually induced by administration of haloperidol, and is considered as an animal model of extrapyramidal syndrome (29, 47). In contrast to haloperidol catalepsy, which can be induced in almost any mouse or rat, nonpharmacological catalepsy is a very rare phenomenon (32, 33) and high predisposition to such a type of catalepsy indicates significant changes in the nervous system (32, 35). In rats and mice genetically predisposed to catalepsy, pronounced freezing can be induced by harmless low-stressful manipulations, such as lifting the forepaws of an animal with a stick (32) or pinching the scruff of its neck (33), respectively. Moreover, we found that selective breeding for high predisposition to catalepsy in rats and mice produced multiple behavioral, endocrine and neurochemical alterations similar to those observed in depressive patients (32, 35).

ASC/Icg mice were bred from catalepsy-prone CBA/Lac and catalepsy-resistant AKR/J inbred mouse strains (30, 31). Mice of ASC strain are characterized by extremely high predisposition to pinch-induced catalepsy (31) combined with a set of depressive-like behavioral and physiological features, including low locomotor and exploratory activity, increased immobility in the forced swim test and tail-suspension test (8), decreased sexual motivation (60), impaired extinction of a conditioned passive avoidance response (18, 52), decreased immune response (3) and considerable alterations in the brain serotonin system (30, 42). The genetically determined catalepsy in ASC mice is sensitive to chronic but not to acute administration of the classical tricyclic antidepressant imipramine (59) and SSRI (Selective Serotonin Reuptake Inhibitor) fluoxetine (57) that resembles the therapeutic effects of antidepressants indicating the predictive validity of this model. These findings support face, predictive and construct validity of this model as the model of depression and suggest that ASC mouse strain is an adequate model to study genetically defined mechanisms of depression and antidepressant effects on behavior while expression of catalepsy in ASC mice is a useful marker for antidepressant activity.

Mechanisms of long-lasting behavioral effects and antidepressant properties of BDNF remain unclear. Chronic treatment with classical antidepressants is known to increase expression of the *Bdnf* or *Creb1* genes, encoding BDNF and cAMP response element-binding protein (CREB), respectively, in the hippocampus and prefrontal cortex (6, 11, 43, 56). Activity-regulated cytoskeleton-associated (Arc) protein also attracts particular attention in this respect since up-regulation of the *Arc* gene mRNA after antidepressant treatment in hippocampus and prefrontal cortex has been shown (2, 13, 16, 37, 44). Moreover, Arc is involved in the mechanisms of BDNF-induced neuronal and synaptic plasticity (54).

The aims of this study are to examine whether [1] the effects of BDNF on catalepsy in ASC mice are enhanced by repeated administration of the drug; [2] BDNF administration influences other depressive-like behavioral features in ASC mice (depressive-like immobility in the tail-suspension test, manifestation of sexual motivation); [3] BDNF evokes long-term alterations in hippocampal *Bdnf*, *Creb1* and *Arc* mRNA expression levels related to changes in brain physiological activity and behavior in ASC mice.

Materials and Methods

Animals

The experiments were performed on adult three-month old male mice of ASC strain weighing 25 ± 0.5 g. ASC strain was produced in our laboratory (Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia) using selective breeding for high predisposition to catalepsy from the CBA × (CBA × AKR) backcross population between catalepsy-prone CBA/LacJ and catalepsy-resistant AKR/J inbred strains and maintained by brother-sister inbreeding for at least 30 generations (31). After weaning, the mice were separated by sex and kept by ten mice per cage (40 × 25 × 15 cm) under standard conditions (temperature: 18-22°C, relative humidity: 50-60%, standard food and water *ad libitum*). Then mice were divided into two weight-balanced experimental groups (control and BDNF-treated) and kept in cages of the same size by five mice per cage during the treatment period. Two days before behavioral testing, the animals were isolated in cages of the same size to minimize group effects. Experimental sessions were conducted between 12:00 and 15:00 h; the tests for sexual motivation and social interest were carried out between 14:00 and 17:00 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.

Drugs and Drug Administration

Human recombinant BDNF was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). It was diluted in saline and injected at a dose of 300 ng/mouse (in a volume of 5 μl) into the left lateral brain ventricle (AP: -0.5 mm, L: -1.6 mm, DV: 2 mm); after three days, the injection was repeated at the same dose and volume into the right lateral brain ventricle (AP: -0.5 mm, L: +1.6 mm, DV: 2 mm). Before central drug administration, the animals were anesthetized during 20-30 s with diethyl ether. The control group received saline of the same volume.

To induce estrus in female mice, β-estradiol (Sigma-Aldrich Co.) diluted in peach oil was administered subcutaneously at 48 h before testing with a dose of 40 μg/animal. Progesterone ("Dal'chimpharm", Khabarovsk, Russia) diluted in peach oil was injected at 2-4 h before testing in a dose of 500 μg/animal.

Behavioral Tests

Behavioral testing was started seven days after the first BDNF injection in the ASC mice since in the model of learned helplessness with stress-induced depressive-like state, BDNF corrected altered behavior several days after administration (50) but had no effects several hours after treatment (22).

Open-field test. The test was carried out on the 7th day after the first drug administration. Mice were tested in a round (40 cm in diameter) arena. The plastic walls were 25 cm high. The floor of the arena was made of mat and semitransparent plastic. The arena was placed on the mount 40 cm above two halogen lamps of 12 W each. The light from the lamps diffused by the semitransparent floor was transmitted through the arena to the objective of a digital camera (Panasonic) placed 80 cm above the arena. The camera was connected to a PC-compatible computer through IEEE1396 interface. A mouse was placed at a wall of the arena, and its position and movements were tracked for 5 min. The arena was carefully cleaned after each test. The video stream from the camera was analyzed frame-by-frame using the original EthoStudio software (36). Distance travelled (cm) by a mouse was detected automatically while the number of rearing, grooming bouts and defecation was measured by an observer blind to experimental design.

Test for catalepsy. The test was carried out on the same day right after the open-field test. It was performed according to the previously described procedure (33). Animals were firmly pinched between two fingers for 5 s at the scruff of a neck, then placed on parallel bars, with the forepaws at 5 cm above the hind legs, and were then released gently. The duration of catalepsy was measured 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 trials. The mouse was considered as cataleptic if the time of immobility was above 20 s in no less than three of ten trials. Catalepsy was estimated by the share of cataleptic animals and mean immobility in the three trials with maximal duration of cataleptic freezing.

Tail-suspension test. Tail-suspension test is a widely used screening method for antidepressants in mice (15). On the 12th day after the first BDNF injection, mice were suspended by the tail with adhesive tape to a horizontal bar 30 cm above the table. Mouse behavior was recorded with the digital video camera for 6 min and immobility time (in second) was recorded by an observer blind to experimental design.

Test for sexual motivation. To evaluate the behavioral component of female-induced sexual motivation, the partition test was used. This test was validated for measuring the sexual arousal in naÏve (5) and sexually experienced (4) rats and mice. Each male mouse was placed in a 29 × 15 × 10-cm experimental metal cage divided by a perforated transparent partition into two compartments for two days for adaptation prior to experimenting. Experimental trials were held on the 14th day after the first BDNF administration.

The perforated transparent partitions were designed so that the males could smell, see and hear receptive females but were not able to establish any physical contact associated with mating; the holes in partition were 10 mm in diameter and the partition thickness was about 3 mm. The animals were given 5 min for adaptation; then their activity was recorded with a digital video camera and processed by a trained observer who was blind to the experimental design. Spontaneous activity at the partition was assessed for 10 min. Each animal was then exposed to a receptive female. The amount of time a test male spent actively exploring the partition over 10 min (partition time) and the number of approaches it made to the partition over this interval were recorded. The time and approaches were counted whenever the male contacted the partition with its nose or one or two forepaws (5). Estrus in the random-bred three-month old albino female test animals was induced by β-estradiol and progesterone. Estrus was confirmed by vaginal smears and lordosis reaction of the female animals.

Table 1. Sequences and annealing temperatures of primers used for quantitative RT-PCR

Gene	Sequence	Annealing Temperature (°C)	PCR Product Size (bp)
Tph1	F 5'-gcttcaaagacaatgtctatcgtagaag-3'	60	164
	R 5'-ggcgtgggtcgggtagagtttgttt-3'		
Polr2a	F 5'-gttgtcgggcagcagaatgtag-3'	63	188
	R 5'-tcaatgagaccttctcgtcctcc-3'		
Arc	F 5'-tgtcccagatccagaaccac-3'	62	234
	R 5'-cagcgtccacatacagtgtc-3'		
Creb1	F 5'-ctgcctcaggcgatgtacaa-3'	63	145
	R 5'-ttcattagacggacctctctcttc-3'		
Bdnf	F 5'-tagcaaaaagagaattggctg-3'	59	255
	R 5'-tttcaggtcatggatatgtcc-3'		

Social interaction test. To prove the specificity of the differences in the expression of sexually motivated behavior in the test for sexual motivation, the expression of social interest was evaluated in the social interaction test with a social partner (a juvenile male). After the test for sexual motivation, the experimental male mice were placed into individual home cages of 40 × 25 × 15 cm in size for two days. On the 16th day after the first drug administration, they were tested for social interest when a juvenile (five-week old) male of A/He inbred strain was introduced into the home cage of the test subject for a 10-min period. Behavior was recorded with a digital video camera and processed by a trained observer who was blind to the experimental design. Social interest was assessed by the duration (in second) of social interactions (*e.g.* sniffing the partner's head, body, genitals and tail; trailing and grooming) with the juvenile (25, 61). The number and duration of aggressive attacks were also recorded.

RT-PCR Assay

To verify whether BDNF had a physiological effect on the brain of BDNF-treated ASC mice, the expression levels of the *Bdnf*, *Creb1* and *Arc* genes were determined in the hippocampus and prefrontal cortex of the animals. On the 21st day after the first drug administration, the animals were decapitated, their brains were removed on ice, prefrontal cortices and hippocampi were dissected, frozen with liquid nitrogen and then stored at -70°C until RNA extraction. After total RNA extraction with phenol, chloroform and guanidinium thiocyanate, reverse transcription (RT) reaction was carried out with random hexanucleotide mixture as a primer (40). Genomic DNA contamination in the cDNA samples was tested in PCR specific for mouse tryptophan hydroxylase 1 coding gene (*Tph1*) primers (Table 1) (40). No trace of genomic DNA in the cDNA samples was detected at 36 cycles of amplification.

For quantification of *Bdnf*, *Creb1* and *Arc* gene expression levels, quantitative PCR assay was used as described in details earlier (34, 40, 41). In the present study, mRNA of DNA-dependent RNA-polymerase 2A (*Polr2a*) was used as an internal standard and the mouse genomic DNA of known concentration as an external standard. All primers used in the present study were based on sequences published in the Ensembl Nucleotide database and synthesized by the Biosset Company (Novosibirsk, Russia). The primer sequences, annealing temperatures and product lengths are presented in Table 1. For BDNF, the primers were identical to a region in exon 9 of the gene (1).

A 1 µl aliquot of cDNA was mixed with 2 µl of PCR buffer, 0.3 µl of $MgCl_2$ (0.1 M), 1 µl of dNTPs (4 mM), 2.5 µl of mixture of forward and reverse primers (2 µM of each; Table 1), 1 U of Taq polymerase and sterile water was added to make up to a final volume of 20 µl. PCR was carried out according to the following protocol: 3 min at 94°C for 1 cycle; 10 s at 94°C, 30 s at corresponding annealing temperature (Table 1), 15 s at 72°C for 27 cycles for *Arc*, or 28 cycles for *Polr2a* or *Bdnf*, or 29 cycles for *Creb1*; 2 min at 72°C for 1 cycle. Simultaneously, mouse genomic DNA samples in different dilutions used as an external standard were amplified in separate tubes. Concentrations of 2.5, 5, 10, 20 and 30 ng/µl, corresponding to 500, 1000, 2000, 4000 and 6000 copies/µl, respectively, were used to evaluate the number of copies of the target genes in the samples. PCR-products of samples and standards were separated by electrophoresis in 1.5% agarose gel stained with ethidium bromide and photographed with a digital camera. Fluorescence intensity of the PCR-product bands was measured using Scion Image software (Scion Corporation; Frederick, Maryland, USA; www.scioncorp.com). PCR products of cDNA were calibrated ac-

cording to corresponding standard curves which allowed determination of the number of *Bdnf*, *Creb1*, *Arc* and *Polr2a* cDNA copies in 1 µl of total cDNA. The expression of *Bdnf*, *Creb1* and *Arc* genes was presented as the number of a target gene cDNA copies with respect to 100 cDNA copies of *Polr2a*.

Statistical Analysis

The data are presented as means ± SEM for quantitative attributes and as percentage for qualitative ones. Differences between experimental groups were analyzed by Fisher exact two-tailed criterion (2 × 2 contingency table) for categorical variables (share of cataleptics or aggressive males) and Repeated Measures ANOVA followed by Newman-Keuls *post-hoc* comparison (indices in the test for sexual motivation) or one-way ANOVA (indices in the other behavioral tests and mRNA levels) for numerical variables.

Results

In this study, BDNF administration was found to influence some types of behavior in ASC mice. BDNF reduced catalepsy manifestation decreasing both the percentage of cataleptics ($P < 0.05$; Fig. 1A) and mean immobility time ($F_{(1,35)} = 6.5$, $P < 0.05$; Fig. 1B). A tendency to decrease immobility time in the tail-suspension test in the BDNF-treated group was found (145.5 ± 13.8 s in the saline-treated control and 111.4 ± 11.6 s in the BDNF-treated mice, $F_{(1,21)} = 3.5$, $P = 0.074$).

Expression of sexual motivation was also significantly improved by the BDNF injections in the ASC males. Repeated Measures ANOVA revealed significant effects of BDNF administration ($F_{(1,21)} = 8.0$, $P < 0.05$), the presence of a receptive female ($F_{(1,21)} = 396.3$, $P < 0.001$) and the interaction between these two factors ($F_{(1,21)} = 19.9$, $P < 0.001$) on the main index of sexual motivation, the time spent by a male at the partition. Number of approaches to the partition was also significantly influenced by the presence of a receptive female ($F_{(1,21)} = 181.8$, $P < 0.001$) and the interaction between the factors of the presence of a receptive female and BDNF administration ($F_{(1,21)} = 6.8$, $P < 0.05$). Significant effect of the interaction between the factors studied on sexually motivated behavior indicated that the difference between control males and experimental males treated with BDNF depended on the presence or absence of a receptive female behind the partition. While spontaneous activity towards empty compartment did not differ between controls and the BDNF-treated groups ($P > 0.05$), BDNF-treated males made more approaches to the partition ($P < 0.05$) and spent significantly more time at the partition ($P < 0.001$) than the control mice in the presence of a receptive female (Fig. 2).

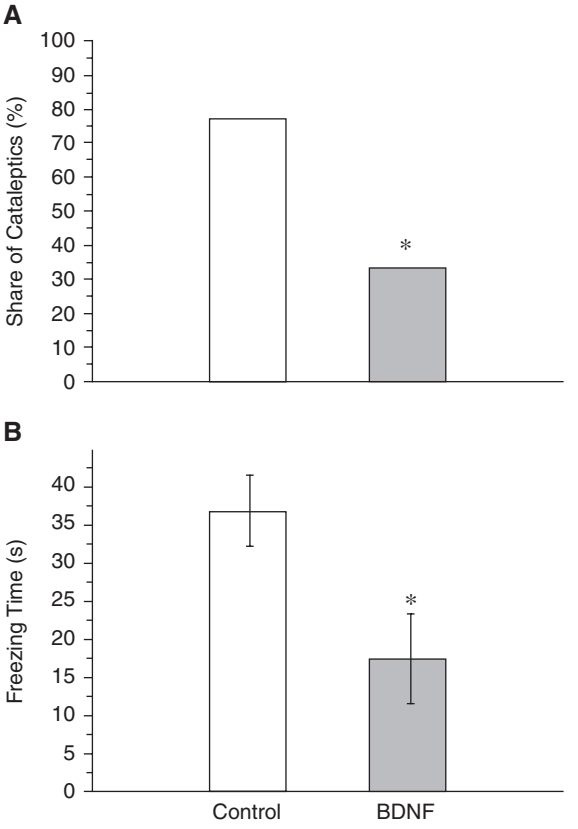

Fig. 1. Effects of central BDNF administration (300 ng/mouse, 2×, i.c.v.) on share of cataleptic animals (A) and time of cataleptic freezing (s) (B) in ASC mice. Total number of animals tested was 22 for the control group and 15 for the BDNF-treated group. The data are presented as percentage for share of cataleptic animals and as means ± SEM for time of cataleptic freezing. Differences between the experimental groups were analyzed by Fisher exact two-tailed criterion (2 × 2 contingency table) for categorical variables (share of cataleptics) and one-way ANOVA for numerical variables (time of cataleptic freezing). Statistically significant differences with respect to the control group treated with saline: *$P < 0.05$.

At the same time, no significant effect of BDNF administration on the time ($F_{(1,21)} < 1$) of social interactions was demonstrated (Fig. 3). The level of aggressiveness was very low: only two mice out of 23 tested attacked the juvenile intruder. Experimental groups did not differ significantly in the share of males manifested aggression towards the juvenile male (0% in control and 18.2% in BDNF-treated group, $P > 0.05$). The low number of the aggressive episodes prevented statistical analysis of quantitative parameters of aggression (number and duration of the attacks).

BDNF treatment did not influence locomotor (distance travelled) or exploratory (number of rearing)

Table 2. Effects of central BDNF administration on behavior of ASC mice in the open-field test

Index	Control	BDNF	F, P
Locomotor activity (path length, cm)	713.1 ± 51.9	776.1 ± 52.5	$F_{(1,20)} < 1$
Exploratory activity (no. of rearings)	7.1 ± 1.3	9.4 ± 2.5	$F_{(1,20)} < 1$
Emotionality (no. of fecal boluses)	1.6 ± 0.3	2.1 ± 0.3	$F_{(1,20)} = 2.1$, $P > 0.05$
Emotionality (no. of grooming bouts)	2.2 ± 0.4	1.8 ± 0.3	$F_{(1,20)} < 1$

The dosage of BDNF administered was 300 ng/mouse, twice and by i.c.v. Data are presented as the means ± SEM of the values obtained in an independent group of animals (n = 11 per group).

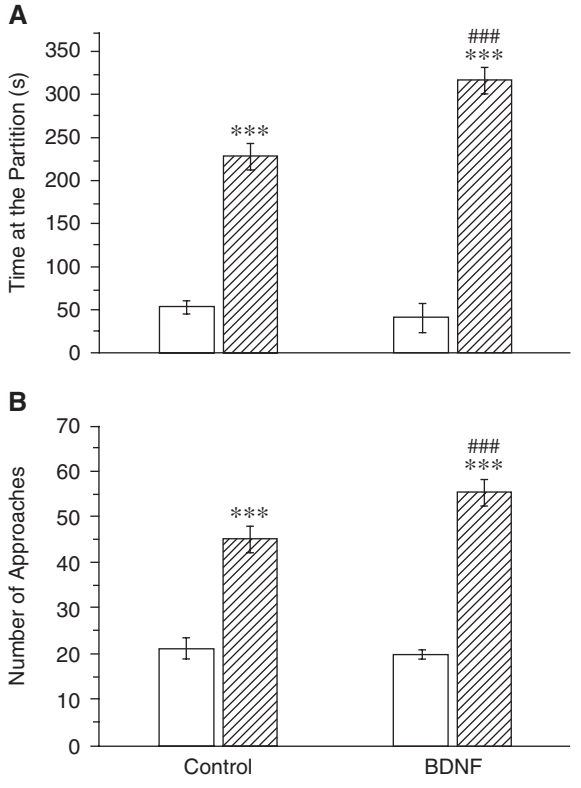

Fig. 2. Effects of central BDNF administration (300 ng/mouse, 2×, i.c.v.) on manifestation of sexual motivation in ASC males. Manifestation of sexual motivation was estimated in the partition test by the time a male spent actively exploring the perforated transparent partition separating it from a receptive female. Time spent at the partition (s) (A) and number of approaches to the partition (B) separating empty compartments are displayed as open bars, the alterations in behavioral activity after a receptive female introduction are presented as hatched bars. The total number of animals tested was 12 for the control group and 11 for the BDNF-treated group. Statistically significant differences: $^{***}P < 0.001$ *vs.* empty compartment; $^{###}P < 0.001$ *vs.* the control group treated with saline.

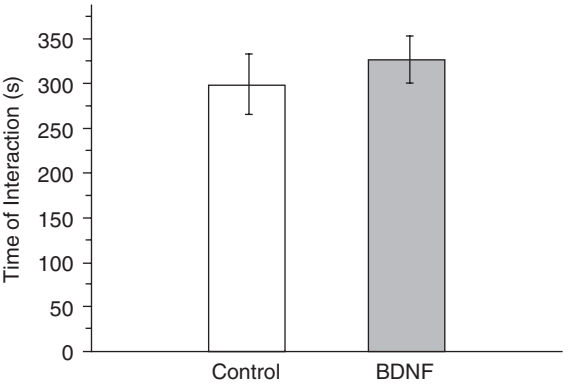

Fig. 3. Expression of social interest (time of interaction, s) measured in the social interaction test with juvenile male in control and BDNF-treated ASC males (300 ng/mouse, 2×, i.c.v.). Each bar and vertical line represent the means ± SEM of the values obtained in an independent group of animals (n = 11-12 per group).

activities, or emotionality (grooming bouts and defecations) of the ASC mice in the open-field (Table 2).

No differences between controls and the BDNF-treated mice in the mRNA levels of the "house-keeping" gene *Polr2a* were found in the prefrontal cortex (2010.4 ± 239.2 cDNA copies in the control and 2457.4 ± 302.7 cDNA copies in the BDNF-treated mice; $F_{(1,19)} = 1.4$, $P > 0.05$) and the hippocampus (2776.2 ± 268.1 cDNA copies in the control and 2702.1 ± 240.4 cDNA copies in the BDNF-treated mice; $F_{(1,14)} < 1$). On the other hand, more than two-fold increase in the level of *Arc* gene mRNA in the BDNF-treated ASC mice compared with the saline-treated controls was shown in 21 days after the first BDNF administration both in the hippocampus ($F_{(1,18)} = 34.6$, $P < 0.001$; Fig. 4A) and the prefrontal cortex ($F_{(1,18)} = 6.4$, $P < 0.05$; Fig. 4A). Up-regulation of *Bdnf* ($F_{(1,14)} = 13.3$, $P < 0.01$) and *Creb1* ($F_{(1,14)} = 8.5$, $P < 0.05$) genes was detected in the hippocampus but not in the prefrontal cortex ($F_{(1,14)} < 1$) of the BDNF-treated group (Figs. 4B and 4C).

Discussion

ASC mice are characterized by the very high predisposition to catalepsy. Two intracerebroventricular BDNF injections attenuated significantly catalepsy manifestation in the ASC mice. This result

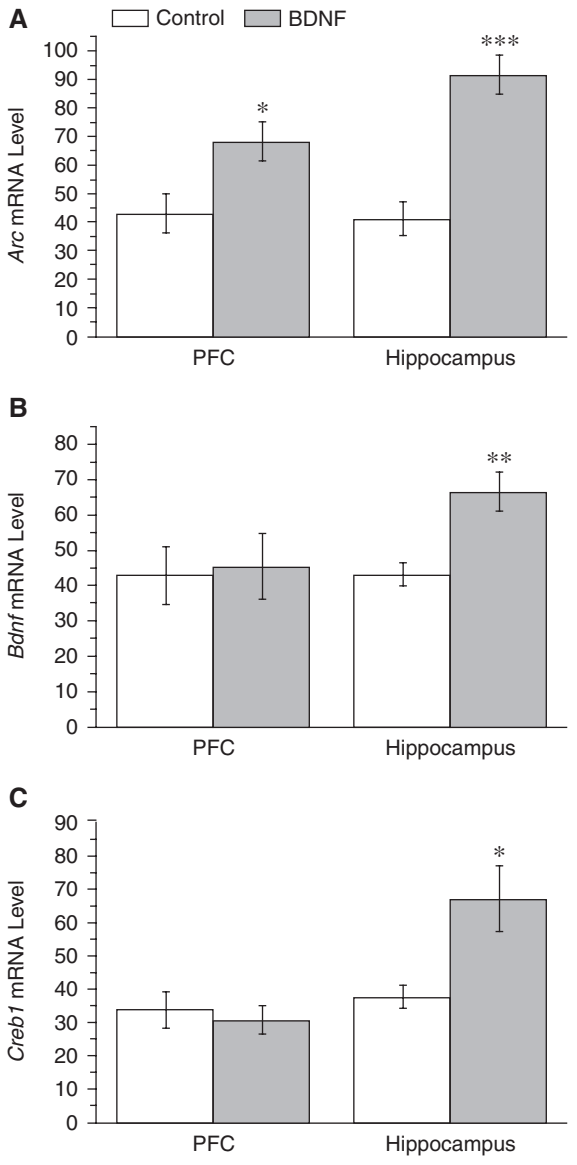

Fig. 4. mRNA levels of *Bdnf*, *Creb1* and *Arc* in the prefrontal cortex (PFC) and hippocampus in control and BDNF-treated (300 ng/mouse, 2×, i.c.v.) ASC mice. *Arc* (A), *Bdnf* (B) and *Creb1* (C) expression was evaluated as the number of a target gene cDNA copies with respect to 100 cDNA copies of "house-keeping" gene *Polr2a*, and is presented in arbitrary units (Y-axis). Each bar and vertical line represent the means ± SEM of the values obtained in an independent group of animals (n = 8-10 per group). Statistically significant differences: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ *vs*. the control group treated with saline.

agrees with our previous data that a single BDNF intracerebroventricular administration reduced catalepsy in mice of this strain (58). Noteworthy, the effect of two injections on catalepsy was more pronounced than that of a single injection: two injections of the drug reduced significantly not only the number of cataleptic animals but also the time of cataleptic freezing. Thus, repeated administration of BDNF enhanced the effect of the drug on catalepsy expression.

The anticataleptic effect of BDNF was specific and did not result from any inhibition of locomotion. Indeed, BDNF administration did not affect horizontal (distance traveled) and vertical (number of rearings) activities, or emotional behavior (grooming and defecation) in the open-field test.

BDNF treatment normalized some altered behavioral features associated with depressive-like state in the ASC mice. There was a tendency to decrease immobility time in the tail-suspension test, an analogue of forced swim test which is widely used for antidepressant screening in mice. In earlier studies, antidepressant-like effect of BDNF was observed in forced swim test: the peptide decreased significantly immobility time and enhanced active swimming in rats (26, 50). In general, our findings are in good agreement with these studies, although more pronounced effect of BDNF on immobility time in forced swim test in the studies on rats might be attributed to higher doses being used (1 μg/animal) (26), or to a different administration strategy (local injection into hippocampus) (50).

Reduced sexual motivation (sexual desire, libido) is often observed in patients with depressive disorders (14). However, widely used antidepressants that improve general state of the patients do not cure sexual dysfunctions. Moreover, effective antidepressants SSRIs frequently aggravate clinical symptoms of sexual disorders (23). Previously, we demonstrated a significant decrease in the expression of sexual motivation in ASC mice which was insensitive to chronic fluoxetine treatment (60). In contrast to fluoxetine, BDNF exerted beneficial effects on the expression of sexual motivation in ASC mice. It is noteworthy that this study is the first demonstration of BDNF effect on sexually-motivated behavior. The expression of sexual motivation was increased up to values observed in mice of the parental CBA strain with normal levels of sexual motivation (5, 60).

BDNF had no influence on the manifestation of social interest in the social interaction test. This fact confirmed the specificity of the BDNF effect on sexually-motivated behavior in ASC mice. It should be also noted that expression of social interest in the ASC mice did not differ from that of "normal" mice of parental strains (60). Thus, BDNF administration appeared to have no effect on "normally" expressed social interest while improving compromised sexual motivation.

Furthermore, along with behavioral effects, BDNF administration produced long-lasting up-regulation of *Bdnf*, *Creb1* and *Arc* in the brain of the ASC mice. The observed enhancing effects of BDNF

on the *Bdnf*, *Creb1* and *Arc* mRNA levels were similar to those observed after chronic antidepressant treatment (2, 6, 11, 13, 16, 37, 43, 44, 56). Our results are evidence that BDNF reproduces not only behavioral effects of chronic antidepressant treatment but also induces similar molecular events.

The transcription factor CREB is a critical mediator for BDNF-induced gene expression and is crucial for the long-lasting synaptic effects of BDNF (20). The MAPK-CREB signaling pathway is known to be involved in the transcription of the immediate early genes, and *Arc* gene in particular (63). In turn, the immediate early gene *Arc* is involved in the regulation of neuronal and synaptic (long-term potentiation, long-term depression, and homeostatic) plasticity (9). Previous studies showed that BDNF administration up-regulated levels of the *Arc* gene mRNA in the hippocampus (39, 54, 62) and Arc protein levels in hippocampal cultures (28). However, these effects were observed within 2-4 h (9, 28, 62), while gradual mode of BDNF administration was needed to exert persistent elevation of the protein expression (up to 8 h) (28). Here, we found that augmented *Arc* mRNA level was observed in about three weeks after the BDNF treatment. It is the first demonstration of such long-term alterations in the gene expression induced by BDNF administration. We suggest that long-lasting *Arc* up-regulation in ASC mice was produced by sustained activation of endogenous BDNF and CREB pathway as mRNA levels of the *Bdnf* and *Creb1* genes were augmented as well. It should be noted that up-regulation of *Bdnf* was also shown earlier *in vitro* for neuron cutures 120 min after exposure to BDNF (19). The data of the present study suggest that up-regulation of *Bdnf* observed at the "immediate early gene" period may persist and play a crucial role in antidepressant-like effects on the expression of BDNF-related genes including *Arc*.

We studied the effects of BDNF in the hippocampus and prefrontal cortex since these brain structures are characterized by the highest levels of TrkB receptors, the main type of receptors for mediating BDNF effects, and are associated with high levels of neuronal and synaptic plasticity. Moreover, signs of neurodegenerative processes are often observed in these brain regions in depressed patients while successful antidepressant treatment attenuates the alterations (12). Here, the pronounced effect on *Bdnf*, *Creb1* and *Arc* gene expression was observed in the hippocampus while only mRNA level of *Arc* was also altered in the prefrontal cortex. Since BDNF was injected into lateral ventricles and then freely diffused into brain structures, the prevalence of BDNF-induced effects in the hippocampus could be attributed to its close anatomical proximity to lateral ventricles. Thus, we showed that BDNF triggers long-lasting changes of physiological activity in the brain which may underlie BDNF-induced alterations in behavior, and expression of *Arc* gene may serve as a useful marker of these processes.

Stress-evoked depressive-like behavior is associated with degenerative processes in the brain since stress induces neurodegeneration which can be normalized by BDNF treatment (45). Here, we demonstrated that BDNF administration was effective for genetically defined depressive-like state since it improved compromised neuro-psychic functions of ASC mice. Our results suggest that neurodegenerative alterations play critical role for developing the abnormal predisposition to catalepsy and impaired sexual motivation in ASC mice. The present study supports the notion of neurotrophic deficit in depressive disorders and indicates that BDNF may be effective in therapy of different forms of depression including endogenous genetically determined kind.

One of the serious problems for using BDNF in therapy of CNS disorders is its inability to cross blood-brain barrier. Hence, novel methods of BDNF delivery into CNS and/or modifications of BDNF molecules to improve its pharmacokinetical and pharmacodynamical properties are needed (48). For effective screening of the novel approaches, animal models with defined and reproducible markers of BDNF activity should be used. Model of genetically determined high predisposition to catalepsy in ASC mice meets these requirements and can be recommended for these purposes.

Like classic antidepressants, BDNF attenuated catalepsy expression in ASC mice. This effect was achieved after two injections of BDNF while classic antidepressants imipramine and fluoxetine required chronic treatment to exert anticataleptic effects (57, 59). Moreover, we showed that BDNF augmented expression of decreased sexual motivation in male ASC mice. This property of BDNF differs advantageously from the action of widely used antidepressants which have no effect on, or even aggravate, clinical course of sexual dysfunctions accompanying depressive disorders. The BDNF-induced behavioral alterations were accompanied by the significant increase in mRNA levels of *Bdnf*, *Creb1* and *Arc* in the hippocampus that indicated sustained activation of the endogeneous BDNF-related pathways.

The data of this study suggest that BDNF represents an antidepressant of a new generation, and ASC mouse strain can be used as a suitable and adequate model to study the mechanisms of BDNF effects on hereditary-dependent behavioral alterations.

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