Analysis of BDNF in Brain Structures of Inbred Mice with Different Phenotypes of Mental and Stress Reaction

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The content of BDNF was measured in cerebral structures of intact C57Bl/6 and BALB/c mice in winter and spring. The level of cerebral neurotrophic factor in laboratory mice depended on genetic characteristics and chronobiological factors.

Key Words: *BDNF; inbred C57Bl/6, BALB/c mice; chronobiology*

Brain-derived neurotrophic factor (BDNF), specific for the brain, provides neuronal growth and differentiation and neuroplasticity of the brain [6]. Experimental studies demonstrated BDNF involvement into the mechanisms of stress response [5]. For example, immobilization decreases BDNF synthesis in rat hippocampus with restoration 2 h after discontinuation of the exposure [13,15]. Expression of BDNF in the hypothalamus increased under similar experimental conditions [7,12]. Aversive exposure (injection of hypertonic solution) decreased BDNF level in the hypothalamus [3], while electric pain exposure caused a decrease in BDNF production in the hippocampus [9]. Photo- and sensory stimulation increased the content of neurotrophin in the cortex [4,10].

Analysis of published data indicated the involvement of BDNF in the reaction to stress factors.

Possible causes of the detected opposite changes and different time-dependence of the effects can be genetic heterogeneity of stress responses in laboratory mice and chronobiological dependence of BDNF content. Mammals exposed to stress exhibit different behavioral strategies, the most pronounced of which is reaction with predominating exploratory activity and passive defense freezing reaction. Studies on inbred animals and selection by the inbreeding method proved the hereditary nature of these behavioral forms [11]. Inbred C57Bl/6 and BALB/c mice sharply differ by the main neurochemical and neurohumoral mechanisms of the stress response [11]. In turn, neurotransmitters and hormones involved in the formation of the stress reaction are essential for the formation of BDNF [14]. However, chronobiological studies revealed seasonal fluctuations in the content of endogenous regulators, which can also be responsible for the differences in BDNF levels detected during different seasons [1].

We studied BDNF levels in cerebral structures of C57Bl/6 and BALB/c mice in winter and spring.

MATERIALS AND METHODS

Experiments were carried out on inbred male C57Bl/6 and BALB/c mice (20 g) from Stolbovaya Breeding Center, Russian Academy of Medical Sciences. The animals were kept under standard vivarium conditions in V. V. Zakusov Institute of Pharmacology during at least 2 weeks before the experiment, 10 animals per cage, on common diets at normal 12:12 h day:night regimen. Experiment I was carried out in May, experi-

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TABLE 1. BDNF Content in the Cerebral Structures of BALB/c and C57Bl/6 Mice during Different Seasons (n=6; $M\pm Sd$)

Mouse strain		Hypothalamus	Hippocampus	Striatum	Cortex
BALB/c	December	0.28±0.02***$^{++}$	0.23±0.03	0.07±0.01*$^{+}$	0.07±0.01*$^{++}$
	February	0.52±0.11**	0.31±0.1**	0.14±0.04**	0.15±0.03***
	May	1.85±0.13*^{o}	1.05±0.15^{o}	0.27±0.02*^{o}	0.55±0.07^{o}
C57Bl/6	December	0.22±0.02^{++}	0.21±0.01^{++}	0.05±0.01	0.060±0.004
	February	0.30±0.04	0.10±0.05	0.05±0.02	0.060±0.004
	May	1.65±0.14^{o}	1.07±0.26^{o}	0.34±0.05^{o}	0.51±0.01^{o}

Note. *p<0.05, **p<0.01, ***p<0.001 *vs.* C57Bl/6, ^{+}p<0.01, ^{++}p<0.001 *vs.* February, ^{o}p<0.001 compared to December and February.

ment II in December, and experiment III in February. All experiments were carried out at 9.00-12.00.

The mice were decapitated immediately after removal from the cage. The hypothalamus, hippocampus, striatum, and cortex were isolated from the brain and stored at -70°C. The tissues were homogenized at 4°C by polytrone (Tissue Tearor™ Biospec Products, Inc.) in extraction buffer. BDNF concentration was evaluated by enzyme immunoassay (ELISA) according to the Promega Company protocol. Mouse behavior was evaluated in the open field test. The results were statistically processed using Jandel Scientific SigmaPlot and Statistica 6.0 software and Student's bilateral t test for independent samplings.

RESULTS

The content of BDNF in the cerebral structures of C57Bl/6 and BALB/c mice was significantly higher in May than in December and February (Table 1). Differences between the December and February values were less pronounced (Table 1). Seasonal fluctuations can be responsible for these facts. C57Bl/6 and BALB/c mice are widely used in physiological, genetic, and psychopharmacological studies as experimental models of active and passive behavior under conditions of mental stress. Their behavior changes in spring, which is caused by hormonal shifts [1]. Increased activity of BALB/c mice in spring in comparison with other seasons is confirmed in our study (Table 2). Differences in BDNF levels (Table 1) can be due to increase in testosterone production from winter to spring [1], as it is known that testosterone stimulates the production of BDNF [8].

The content of BDNF was different in different cerebral structures. Our results are in line with published data indicating higher concentrations of the neurotrophin in the hypothalamus and hippocampus in comparison with the striatum and cortex [14]. Despite seasonal fluctuations in BDNF content, the ratio of its concentrations in cerebral structures remains unchanged (Table 1).

In the majority of experiments BDNF level was somewhat higher in BALB/c mice (Table 1). These differences in cerebral structures were recorded in February; in December the only exclusion was the hippocampus, while in spring the differences leveled (Table 1).

The phenotypes of C57Bl/6 and BALB/c mice behavior under conditions of mental stress formed

TABLE 2. Differences in the Behavior of BALB/c and C57Bl/6 Mice in Illuminated Open Field Test during Different Months ($M\pm Sd$)

Mouse strain		Total motor activity	Peripheral activity	Central activity	Number of excursions to the center	Vertical activity
BALB/c	December (n=15)	17.5±9.9^{oo}	17.4±9.9^{o}	0.1±0.2^{oo}		
	February (n=10)	23.3±2.5	21.9±2.1	1.0±0.7***		0.4±0.7
	May (n=6)	76.2±57.3^{++}	60.3±51.5^{+}	15.7±12.4^{++}	0.2±0.4	
C57Bl/6	December (n=15)	107.3±41.1	69.0±26.3	21.5±25.2	6.1±6.6	10.8±14.8
	February (n=10)	96.1±3.6	67.9±2.6	21.6±1.7		6.6±1.1
	May (n=6)	108.0±21.1	63.8±7.1	26.7±7.9	1.3±1.2	16.2±8.2^{++}

Note. *p<0.001 *vs.* December, ^{+}p<0.05, ^{++}p<0.01 compared to February, ^{o}p<0.01, ^{oo}p<0.001 compared to May.

during different shifts in neurotransmitter and hormone levels [2]. Some parameters did not differ in intact mice of the two strains and were observed only during the development of stress response, while, *e. g.*, the levels of ACTH and corticosterone differed sharply in animals not exposed to stress [11]. Our findings indicate that the level of BDNF in cerebral structures is specific of animals of the studied genotypes.

Hence, changes in BDNF content in brain structures of laboratory animals should be analyzed with consideration for the genetic characteristics of mice and chronobiological factors.

REFERENCES

1. E. B. Arushanyan, *Chronopharmacology on the Brink of Two Centuries* (in Russian], Stavropol (2005).
2. S. B. Seredenin, T. A. Voronina, G. G. Neznamov, *et al.*, *Vestn. Rossiisk. Akad. Med. Nauk*, No. 11, 3-9 (1998).
3. E. Castren, H. Thoenen, and D. Lindholm, *Neuroscience*, **64**, 71-80 (1995).
4. E. Castren, F. Zafra, H. Thoenen, and D. Lindholm, *Proc. Natl. Acad. Sci. USA*, **89**, 9444-9448 (1992).
5. R. S. Duman, J. Malberg, S. Nakagawa, and C. D'Sa, *Biol. Psychiatry*, **48**, 732-739 (2000).
6. G. R. Lewin and Y. A. Barde, *Annu. Rev. Neurosci.*, **19**, 289-317 (1996).
7. F. Rage, L. Givalois, F. Marmigere, *et al.*, *Neuroscience*, **112**, No. 2, 309-318 (2002).
8. S. Rasika, A. Alvarez-Buylla, and F. Nottebohm, *Neuron*, **22**, No. 1, 53-62 (1999).
9. A. M. Rasmusson, L. Shi, and R. Duman, *Neuropsychopharmacology*, **27**, No. 2, 133-142 (2002).
10. N. Rocamora, E. Welker, M. Pascual, and E. Soriano, *J. Neurosci.*, **16**, 4411-4419 (1996).
11. S. B. Seredenin, *Psychopharmacol. Biol. Narcol.*, **1**, 494-509 (2003).
12. M. A. Smith, S. Makino, S. Y. Kim, and R. Kvetnansky, *Endocrinology*, **136**, 3743-3750 (1995).
13. M. A. Smith, S. Makino, R. Kvetnansky, and R. M. Post, *J. Neurosci.*, **15**, 1768-1777 (1995).
14. L. Tapia-Arancibia, F. Rage, L. Givalois, and S. Arancibia, *Front. Neuroendocrinol.*, **25**, 77-107 (2004).
15. T. Ueyama, Y. Kawai, K. Nemoto, *et al.*, *Neurosci. Res.*, **28**, 103-110 (1997).