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Molecular Neurobiology

ISSN 0893-7648
Volume 51
Number 3

Mol Neurobiol (2015) 51:1443-1451
DOI 10.1007/s12035-014-8821-7

Springer

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Received: 19 May 2014 / Accepted: 16 July 2014 / Published online: 2 August 2014

Abstract Mice were exposed to 1 month of spaceflight on Russian biosatellite BION-M1 to determine its effect on the expression of key genes in the brain dopamine (DA) and serotonin (5-HT) systems. Spaceflight decreased the expression of crucial genes involved in DA synthesis and degradation, as well as the D1 receptor. However, spaceflight failed to alter the expression of tryptophan hydroxylase-2, 5-HT transporter, 5-HT_{1A}, and 5-HT_3 receptor genes, though it reduced 5-HT_{2A} receptor gene expression in the hypothalamus. We revealed risk DA and 5-HT neurogenes for long-term spaceflight for the first time, as well as microgravity-responsive genes (tyrosine hydroxylase, catechol-O-methyltransferase, and D1 receptor in the nigrostriatal system; D1 and 5-HT_{2A} receptors in the hypothalamus; and monoamine oxidase A (MAO A) in the frontal cortex). Decreased genetic control of the DA system may contribute to the spaceflight-induced locomotor impairment and dyskinesia described for both humans and rats.

Keywords Spaceflight · Microgravity · Stress · Brain serotonergic system · Brain dopaminergic system · Gene expression · Mice

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Introduction

The effect of altered gravitation on the brain is a basic problem encountered with spaceflight. Living organisms on Earth evolved in a relatively constant gravitational environment, so the condition of microgravity is non-physiological. The milestone problem is the effect of long-term spaceflight on brain function. Taking into account the pivotal role of brain neurotransmitters in the regulation of mood, emotionality, and behavior, the effect of spaceflight on brain neurotransmitters arouses keen interest.

Serotonin (5-HT) is a classic neurotransmitter involved in the regulation of different kinds of behavior [1], sleep [2, 3], and the stress response [4]. Dysfunction of the brain 5-HT system is implicated in the mechanisms underlying severe neuropsychiatric disorders, including aggression, depression, and suicide [5–7], as well as schizophrenia [8–10], anxiety [11], substance abuse [12, 13] and drug addiction [14, 15], and Parkinson's [16] and Alzheimer's [17] diseases.

Brain dopamine (DA) represents another neurotransmitter that attracts attention given its well-defined role in the regulation of movement and muscle tone [18] and its implication in exercise-induced central fatigue [19], tardive dyskinesia [20], Parkinson's [21, 22] and Alzheimer's [23–25] diseases, major depression [26–28], and schizophrenia [29–32].

Data on the effect of actual spaceflight on the brain 5-HT and DA systems are scarce and limited to the hypothalamic area. The levels of 5-HT and DA and the activity of the main enzymes in DA metabolism, tyrosine hydroxylase (TH) and monoamine oxidase (MAO), have been determined in the hypothalamus. The functional significance ofin the hypothalamus. The functional significance of of rats after 19.5 days of spaceflight on board the biosatellites Cosmos 782 and 936 [33, 34]. No significant changes were found in the DA level or TH and MAO activity. In the space experiment Cosmos 1129, the concentrations of norepinephrine, DA, and 5-HT were

studied in isolated nuclei from the rat hypothalamus after 18.5 days of spaceflight. A reduced norepinephrine level was found in some hypothalamic nuclei, but no significant changes in DA levels were revealed [33]. The concentration of 5-HT was unchanged in the majority of hypothalamic nuclei, but an increase was found in supraoptic nuclei and a decrease in paraventricular nuclei. Long-term spaceflight and weightlessness were suggested to not represent a stressogenic factor with respect to the 5-HT system in the hypothalamus [34]. Notwithstanding the limitations of these data, these results decreased interest to the DA and 5-HT systems, and the effect of long-term spaceflight on the main central regulators of emotions, movement, and behavior remained unknown.

The aim of the present study was to evaluate the effect of actual long-term spaceflight on the expression of key genes in the DA and 5-HT systems. The candidate genes include (1) key genes in DA synthesis (TH) and degradation (catechol-O-methyltransferase (COMT)) as well as dopamine transporter (DAT), dopamine D1 and D2 receptors; (2) 5-HT transporter (5-HTT), 5-HT_{1A}, 5-HT_{2A}, and 5-HT_3 receptor genes and pivotal gene involved in 5-HT synthesis in the brain (tryptophan hydroxylase-2 (Tph-2)); and (3) principal genes involved in 5-HT and DA degradation: MAO A and MAO B.

Materials and Methods

Animals The experiments were carried out on adult (about 4 months old at the beginning of the experiments) weighted about 25±2-g male mice of C57BL/6 inbred strain. Forty-five mice were launched into space for 30 days on the Bion-M1 spacecraft, part of the Bion series of Russian space missions. The animal-carrying space capsule was launched into orbit on April 19, 2013 and returned to Earth on May 19, 2013.

The spaceflight and shuttle cabin control mice were kept three per cage in special chambers (shuttle cabins) described in detail by Sychev and coauthors [35] in a natural light-dark cycle (12 h light and 12 h dark). The mice of spaceflight and shuttle cabin control groups have access to paste-like food with 76–78 % water content ad libitum. The temperature in the chambers was 21 °C; relative humidity was 61 %. After spaceflight, mice did not differ significantly from control groups in body weight. For details see [36].

The vivarium control mice were kept three per cage in standard cages in a natural light-dark cycle (12 h light and 12 h dark). The mice have access to standard granulated food and water ad libitum. The room temperature was about 23 °C; relative humidity was about 50 %.

The mice were sacrificed by manual cervical dislocation followed by decapitation within 6 h after landing. The frontal cortex, visual cortex, hypothalamus, hippocampus, striatum, substantia nigra, and raphe nucleus area of the midbrain were dissected on ice and frozen in liquid nitrogen. The brain structures were dissected by the same researcher based on a mouse brain atlas [37]. For the frontal cortex, the following coordinates were used: anterior-posterior (AP) +1.6 to +2.8, lateral (L) −2 to +2; the thickness of the slice was about 1.5 mm. Visual cortex coordinates were AP −3.0 to −4.0, L −1 to +1; the thickness of the slice was about 1.5 mm. The hypothalamus was dissected using coordinates AP +0.3 to −2.9, L −1 to +1, dorsal-ventral (DV) 3.2 to 5.8. Both hippocampi were dissected from AP −0.8 to AP −2.9. Striatum coordinates were AP +1.3 to −1.0, L −2.4 to −3.8 and +2.4 to +3.8, DV 2.4 to 3.8. For the midbrain, a cranial section was made in front of the superior colliculi (AP −3) and a caudal section in front of the fossa rhomboidalis (AP −7.3), and then, the colliculi were removed. The substantia nigra was dissected using coordinates: AP −2.7 to −3.4, L −1.2 to −2.0 and +1.2 to +2.0, DV 3.6 to 4.4.

The same brain structures were dissected from mice of the ground control group. The brain structures from six mice from the spaceflight group and eight mice from the ground control group were transferred to the laboratory of Behavioral Neurogenomics, Novosibirsk, for further studies.

To differentiate the effect of microgravity from the effect of stress on the expression of the investigated genes, we performed a special series of experiments on seven mice that spent 1 month in the same capsules that were used for spaceflight but under conditions of gravitation (shuttle cabin control). A group of intact mice (n=7) was used as an additional control for the cabin control mice. The mice were sacrificed by manual cervical dislocation followed by decapitation and the same brain structures were dissected as described above.

All experimental procedures were in compliance with the Guidelines for the Use of Animals in Neuroscience Research, 1992. All efforts were made to minimize the number of animals used and their suffering.

RT-PCR Total RNA was extracted using TRIzol (Bio-Rad, USA) according to the manufacturer's instructions, treated with RNA-free DNAse (Promega, USA), and diluted to 0.125 μg/μl with DEPC-treated water. One microgram of total RNA was taken for complementary DNA (cDNA) synthesis with a random hexanucleotide mixture [38]. Genomic DNA contamination of the cDNA samples was tested using PCR with primers specific for the mouse tryptophan hydroxylase-1 gene [39, 40]. The concentration of genomic DNA in the cDNA samples did not exceed 30 copies per microliter. The number of copies of DNA-dependent RNA polymerase II (rPol II), TH, MAO A, MAO B, COMT, D1, D2 receptors, DAT, 5-HT_{1A}, 5-HT_{2A}, and 5-HT_3 receptors, Tph-2, and 5-HTT cDNA was evaluated by real-time quantitative PCR using selective primers (Table 1), SYBR Green I fluorescence detection (R-414 Master mix, Syntol, Moscow, Russia), and genomic DNA extracted from the livers of male C57BL/6 J mice as the external standard (200 copies per nanogram of genomic DNA). We used 50, 100, 200, 400, 800, 1,600,

Table 1 The primer sequences, annealing temperatures and PCR product lengths

Gene	Sequence	Annealing temperature, °C	Product length, bp
D1	F5′-GGAAACCCTGTCGAATGCTCTC-3′ R5′-CCAGCCAAACCACACAAATACATCG-3′	64	222
D2	F5′-TCCGCCACTTCTTGACATACATTG-3′ R5′-CCCATCCACAGCCTCCTCTAAG-3′	65	203
TH	F5′-CCGTACACCCTGGCCATTGATG-3′ R5′-ATGAAGGCCAGGAGGAATGCAGG-3′	64	320
COMT	F5′-GACTTCCTGGCGTATGTGAG-3′ R5′-AGAGTGAGTGTGTGTCATCG-3′	60	199
MAO B	F5′-ACGACCCATTACCAGTACCTT-3′ R5′-AAGCCTTTGAGACACACTGAAT-3′	62	198
MAO A	F5′-AATGAGGATGTTAAATGGGTAGATGTTGGT-3′ R5′-CTTGACATATTCAACTAGACGCTC-3′	62	138
DAT	F5′-TTCACTGTCATCCTCATCTCTTTC R5′-TCAAAATACTCAGCAGCGGGTG	63	224
5-HT1A	F5′-GACTGCCACCCTCTGCCCTATATC-3′ R5′-TCAGCAAGGCAAACAATTCCAG-3′	62	200
5-HT2A	F5′-AGAAGCCACCTTGTGTGTGA-3′ R5′-TTGCTCATTGCTGATGGACT-3′	61	169
5-HT3	F5′-CTATCCTCCATCCGCCACTTC-3′ R5′-CCCACTCAAGAATAATGCCAAATG-3′	63	179
Tph-2	F5′-CATTCCTCGCACAATTCCAGTCG-3′ R5′-AGTCTACATCCATCCCAACTGCTG-3′	61	239
5-HTT	F5′-AAGCCCCACCTTGACTCCTCC-3′ R5′-CTCCTTCCTCTCCTCACATATCC-3′	57	198
rPol II	F5′-GTTGTCGGGCAGCAGAATGTAG-3′ R5′-TCAATGAGACCTTCTCGTCCTCC-3′	63	188

3,200, and 6,400 copies of genomic DNA as external standards for all studied genes. Reagent controls were carried out under the same conditions but without the template. Gene expression was evaluated as the number of cDNA copies with respect to 100 copies of rPol II cDNA [38–40]. Melting curve analysis was performed at the end of each run for each primer pair, allowing us to control amplification specificity.

Statistical Analysis The results were presented as mean± SEM and compared using one-way ANOVA followed by a post hoc Fisher test.

Results

Spaceflight considerably affected the expression of key genes of the brain dopaminergic and serotonergic systems. The expression of D1 receptor was significantly lower in the hypothalamus ($F_{1,11}$=4.7; p<0.05) and striatum ($F_{1,10}$=6.2; p<0.05) of spaceflight mice compared to ground control (Fig. 1a). At the same time, spaceflight failed to alter D1 receptor gene expression in other investigated brain structures.

Spaceflight significantly reduced the expression of the gene encoding key enzyme for DA biosynthesis—TH ($F_{1,11}$=9.9; p<0.05) in substantia (s.) nigra but not in the midbrain area ($F_{1,12}$=2.9; p>0.05) (Fig. 2) and the expression of the COMT gene in the striatum ($F_{1,11}$=4.9; p<0.05) (Fig. 1c). Some increase in COMT gene expression in the hippocampus of spaceflight mice was shown, however, it was below the significance threshold ($F_{1,12}$=3.9; p=0.07). Expression of the COMT gene in other five investigated brain structures was unaltered.

Spaceflight reduced the expression of genes encoding both main enzymes for DA and 5-HT catabolism. MAO A gene expression was significantly decreased in the frontal cortex ($F_{1,11}$=5.7; p<0.05) (Fig. 3a) and in the striatum ($F_{1,9}$=4.7; p=0.05). The reduction of MAO B gene expression in the raphe nucleus area of the midbrain ($F_{1,12}$=4.5; p<0.05) was shown (Fig. 3c). Spaceflight failed to alter the expression of MAO A and MAO B in other investigated brain structures.

The expression of genes encoding D2 receptor and DAT in all investigated brain structures of spaceflight mice was unaltered (Fig. 1b, d).

Spaceflight considerably decreased the expression of 5-HT_{2A} receptor in the hypothalamus ($F_{1,12}$=4.9; p<0.05) compared to control mice (Fig. 4b). The reduction of 5-HT_{2A} receptor gene expression in the striatum was below the significance threshold

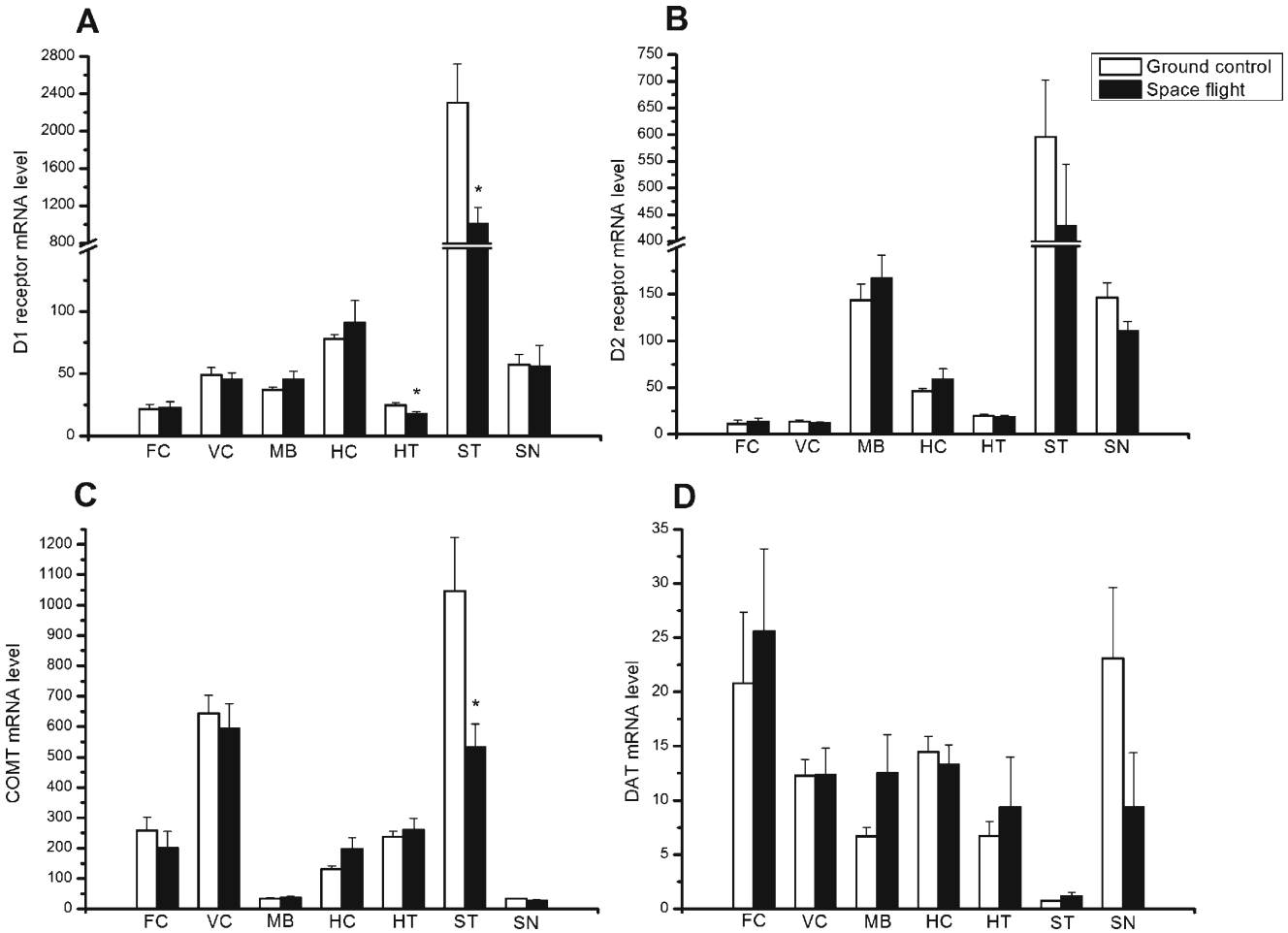

Fig. 1 Effect of spaceflight on D1 (**a**), D2 (**b**) receptors and COMT (**c**) and DAT (**d**) gene expression in mouse brain. *FC* frontal cortex, *VC* visual cortex, *MB* midbrain, *HC* hippocampus, *HT* hypothalamus, *ST* striatum, *SN* substantia nigra. Gene expression is presented as the number of gene cDNA copies with respect to 100 cDNA copies of rPol II. All magnitudes are presented as mean±SEM of at least six animals. *$p<0.05$ versus ground control

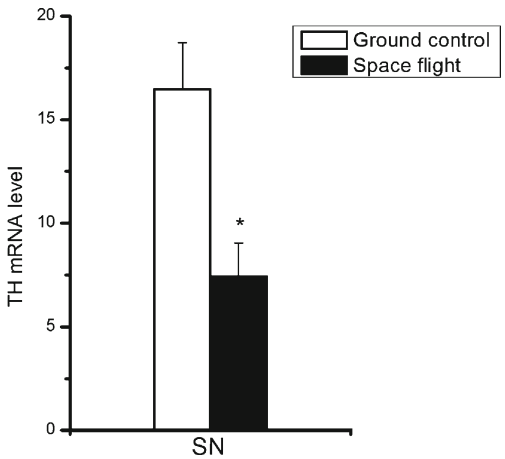

Fig. 2 Effect of spaceflight on TH gene expression in substantia nigra. Gene expression is presented as the number of gene cDNA copies with respect to 100 cDNA copies of rPol II. All magnitudes are presented as mean±SEM of at least six animals. *$p<0.05$ versus ground control

($F_{1,11}$=3.9; p=0.08). The increase of 5-HT_3 receptor gene expression in spaceflight mice was observed as tendency ($F_{1,12}$= 3.9; p=0.07) only in the raphe nucleus area of the midbrain (Fig. 4c).

Spaceflight failed to cause any changes in 5-HT_{1A} receptor gene expression in all seven investigated brain structures (Fig. 4a). There were no changes in the expression of gene encoding the key enzyme for 5-HT biosynthesis in the brain, Tph-2, and 5-HTT in the raphe nucleus area of the midbrain of spaceflight mice (Fig. 4d, e).

To elucidate the role of stress in the inhibitory effect of the spaceflight on some genes, we used shuttle cabin (the same environment, normal gravitation) and ground control groups to investigate the expression of response to spaceflight genes. It was found that stress considerably reduced the expression of MAO B in the midbrain ($F_{1,12}$=21.4; $p<0.001$) (Fig. 3d) and MAO A in the striatum ($F_{1,12}$=35.4; $p<0.001$) (Fig. 3b) that coincides with the results for the spaceflight group (Fig. 3a, c).

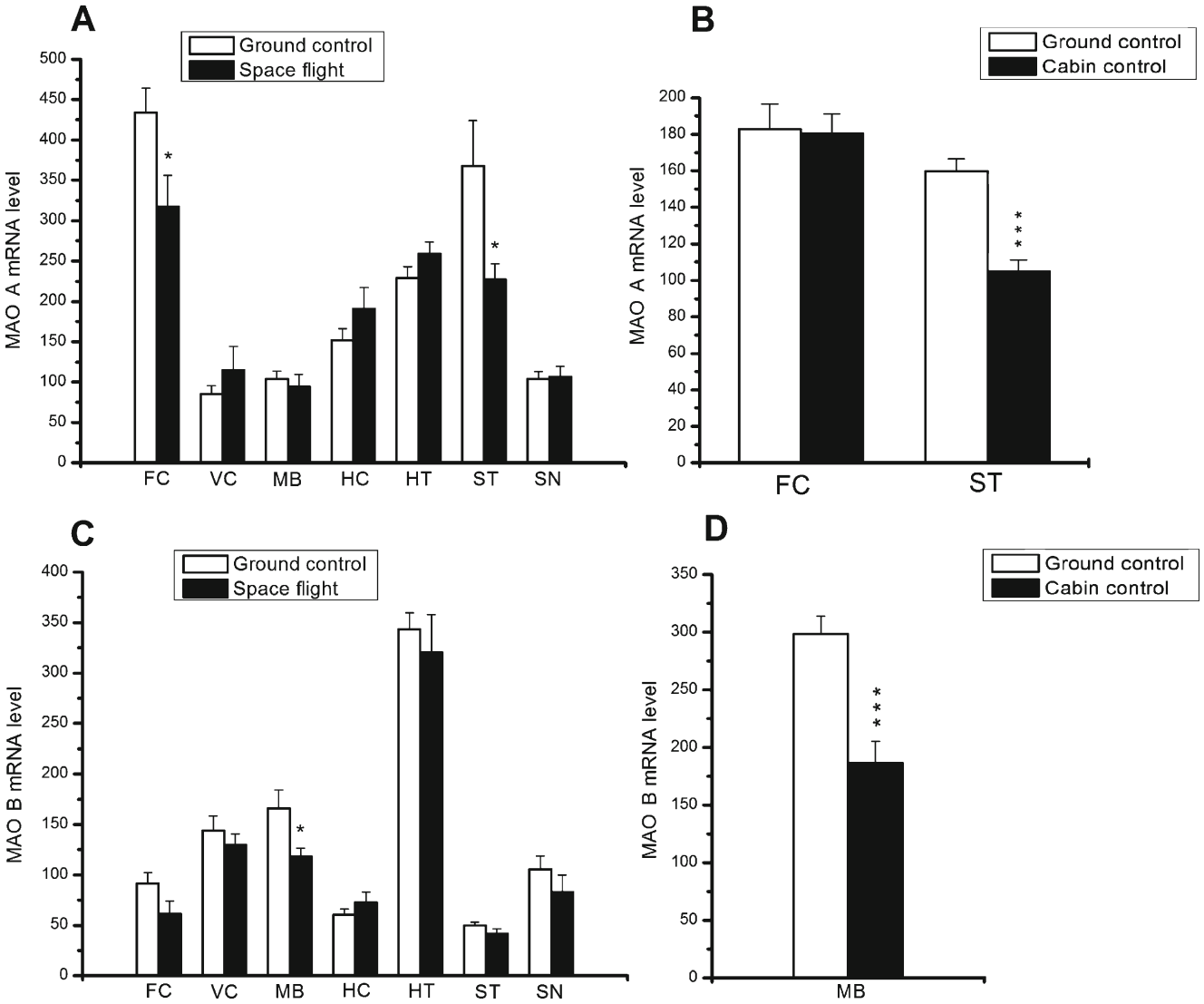

Fig. 3 MAO A and MAO B gene expression after spaceflight (**a**, **c**) and shuttle cabin housing (**b**, **d**). *FC* frontal cortex, *VC* visual cortex, *MB* midbrain, *HC* hippocampus, *HT* hypothalamus, *ST* striatum, *SN* substantia nigra. Gene expression is presented as the number of gene cDNA copies with respect to 100 cDNA copies of rPol II. All magnitudes are presented as mean±SEM of at least six animals. *$p<0.05$; ***$p<0.001$ versus corresponding ground control

At the same time, the expression of other response to spaceflight genes (TH in s. nigra, COMT in the striatum, D1 receptor in the striatum and hypothalamus, and 5-HT_{2A} receptor in the hypothalamus) in the mice of shuttle cabin control group was not significantly different from the mice of ground control group (Figs. 5 and 6).

Discussion

The 5-HT and DA brain systems responded to actual spaceflight with decreased gene expression in some brain regions. Significant changes were found in the genetic control of the DA system. Long-term spaceflight decreased the expression of genes encoding enzymes for both DA biosynthesis and degradation. Reduced expression of the gene encoding a key enzyme in DA biosynthesis in the main area of DA synthesis in the brain, the substantia nigra, was shown after spaceflight. DA catabolism in the brain occurs via two pathways: oxidative deamination by MAO A and MAO B and O-methylation by COMT. MAO B expression in the midbrain and MAO A and COMT expression in the striatum were decreased after spaceflight. Taken together with the decreased expression of dopamine D1 receptor in the striatum and hypothalamus, our data indicate a substantial attenuating effect of long-term spaceflight on the genetic control of the brain DA system. Importantly, the changes were found in the nigrostriatal DA system, which is considered the center of sensorimotor integration [41], regulating the tone and contraction of skeletal muscle [18].

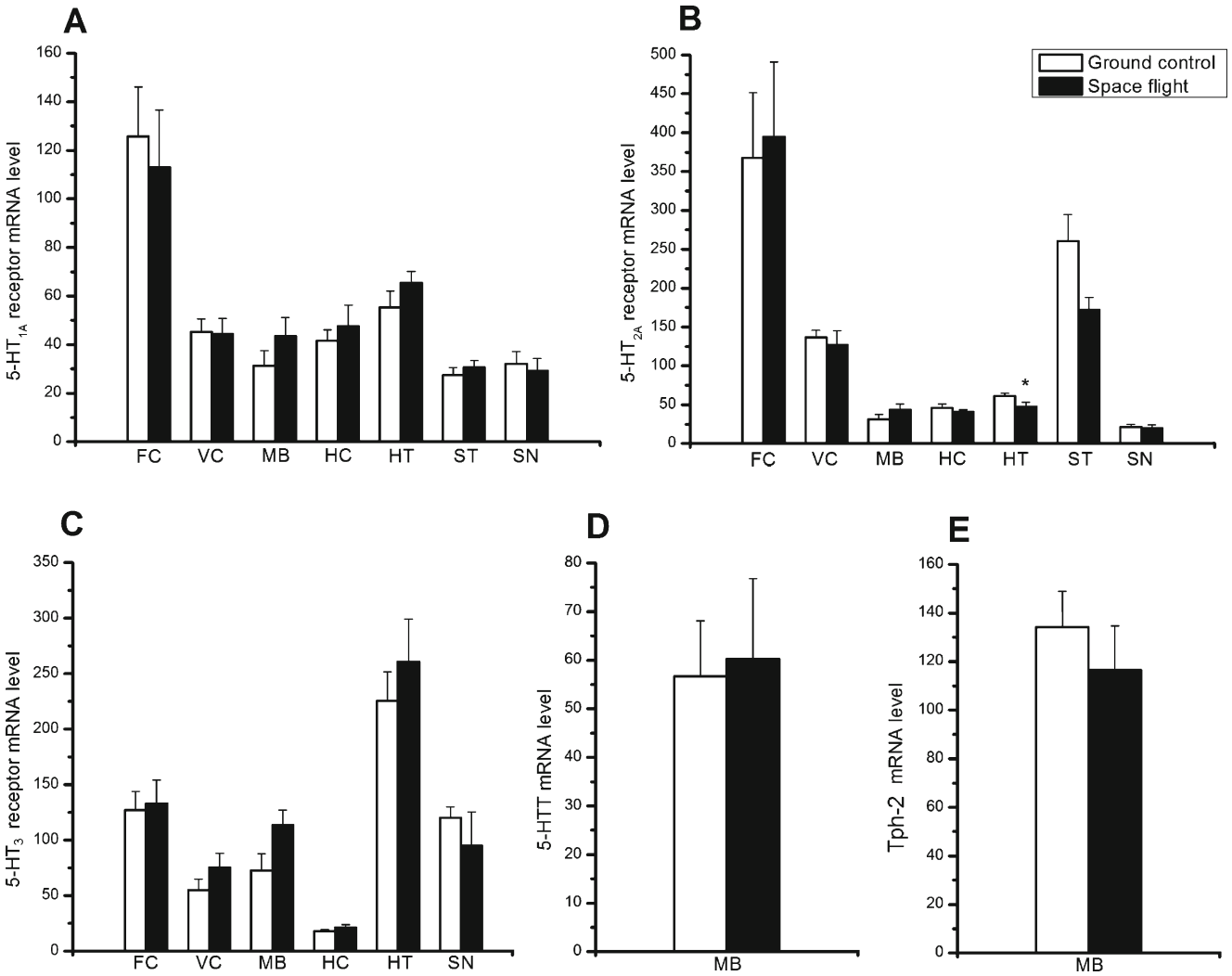

Fig. 4 Effect of spaceflight on 5-HT_{1A} (**a**), 5-HT_{2A} (**b**), and 5-HT_3 (**c**) receptors and 5-HTT (**d**) and Tph-2 (**e**) gene expression in mouse brain. *FC* frontal cortex, *VC* visual cortex, *MB* midbrain, *HC* hippocampus, *HT* hypothalamus, *ST* striatum, *SN* substantia nigra. Gene expression is presented as the number of gene cDNA copies with respect to 100 cDNA copies of rPol II. All magnitudes are presented as mean±SEM of at least six animals. *$p<0.05$ versus ground control

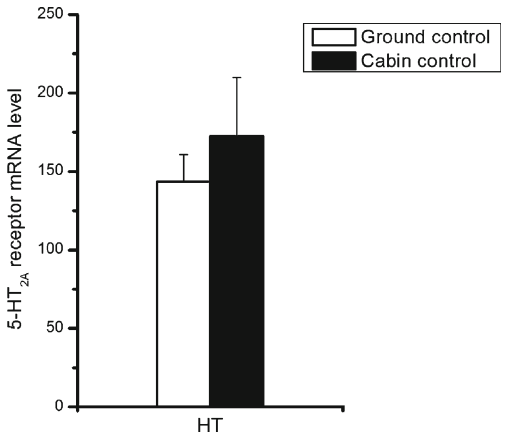

Fig. 5 Effect of shuttle cabin housing on 5-HT_{2A} receptor gene expression in the hypothalamus. Gene expression is presented as the number of gene cDNA copies with respect to 100 cDNA copies of rPol II. All magnitudes are presented as mean±SEM of at least six animals

One of the major problems of space travel is the deleterious effect of microgravity on bones [42] and skeletal muscle [43, 44]. Microgravity leads to a loss of calcium from weight-bearing bones and an increased risk of fractures and premature osteoporosis in later life [42]. Studies of the effect of microgravity on both rats and humans have demonstrated severe atonia, impaired postural and locomotor activity, rapid loss of muscle and fiber mass, reduced peak power, muscle atrophy, and increased rate of fatigue [45–49]. Our data suggest that damaging effects of space travel on skeletal muscle, as well as increased rates of fatigue, can be attributed not only to local changes in the substrates for muscle fiber metabolism and defective microcirculation following spaceflight [47, 50] but also to decreased nigrostriatal dopaminergic control. At the same time, one cannot exclude that muscle structure changes could lead to alteration in the brain DA system.

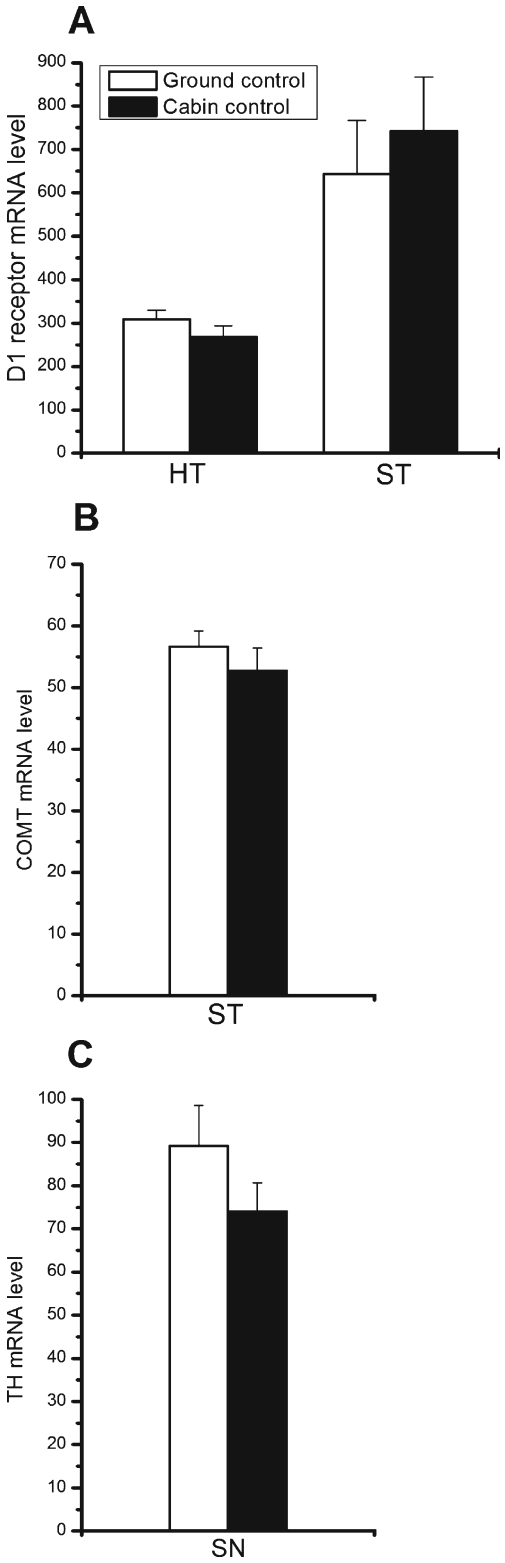

Fig. 6 D1 receptor (**a**) and COMT (**b**) and TH (**c**) gene expression after 1 month of shuttle cabin housing. *HT* hypothalamus, *ST* striatum, *SN* substantia nigra. Gene expression is presented as the number of gene cDNA copies with respect to 100 cDNA copies of rPol II. All magnitudes are presented as mean±SEM of at least six animals

A more limited effect of actual spaceflight was found on the genetic control of the 5-HT system. In contrast to the DA system, no changes were found in the expression of genes encoding the main regulators of 5-HT functional activity: Tph-2, 5-HTT, and 5-HT_{1A} receptors. Importantly, Tph-2 is a rate-limiting enzyme in 5-HT synthesis and the only really specific enzyme in 5-HT brain metabolism. The 5-HT_{1A} receptor is a key player in the autoregulation of the 5-HT brain system [51]. Spaceflight did not cause any significant changes in the expression of the 5-HT_3 receptor gene. The only specific 5-HT system change was found in the gene encoding the 5-HT_{2A} receptor; long-term spaceflight decreased the expression of 5-HT_{2A} receptor gene in the hypothalamus. The functional significance of this effect of spaceflight is not clear, but taking into account that 5-HT_{2A} receptors are implicated in the regulation of a wide range of physiological functions, including sleep, cognition, and memory [52, 53], the 5-HT_{2A} receptor is worthy of future investigations.

Along with DA, MAO catalyzes the oxidative deamination of 5-HT. MAO A has a higher affinity for 5-HT than MAO B and is considered the principle enzyme of 5-HT degradation. Therefore, the decreased MAO A expression in the frontal cortex and striatum can also be attributed to the brain 5-HT system.

A series of additional control mice spent 1 month on Earth in a shuttle cabin and exposed to the same experimental environment with the exception of microgravity, allowing us to reveal the changes in neurotransmitters produced by the lack of gravitation. The data attribute the decrease in the expression of key genes in the DA system, serotonergic 5-HT_{2A} receptor gene in the hypothalamus and MAO A in the frontal cortex, to the effect of microgravity. In contrast, the changes in MAO B in the midbrain and MAO A in the striatum seem to be associated with the effect of environmental stress.

Our results elucidated DA and 5-HT genes and brain areas that are sensitive and resistant to spaceflight. In contrast to the decreased expression of MAO A, COMT, and D1 receptor genes in the striatum and 5-HT_{2A} and D1 receptor genes in the hypothalamus, no changes were found in the expression of 5-HT or DA gene families in the hippocampus. *Locus minoris resistentiae* includes genes involved in the regulation of DA metabolism as well as the D1 receptor. The implication of the DA system in the regulation of movement and muscle tone suggests that decreased genetic control of the nigrostriatal DA system may contribute to the deleterious effect of spaceflight on skeletal muscle tone and locomotor activity described for both rats and humans.

Acknowledgments The study was supported by the Russian Foundation for Basic Research (grant number 14-04-00173) and the Program of the Russian Academy of Science "Molecular and Cell Biology" (grant number 6.7).

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