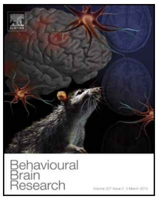
Behavioural Brain Research xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr



Research report

Combined effects of antiorthostatic suspension and ionizing radiation on the behaviour and neurotransmitters changes in different brain structures of rats

V.S. Kokhan[a,*], M.I. Matveeva[a], A.S. Bazyan[b], V.S. Kudrin[c], A. Mukhametov[d], A.S. Shtemberg[a]

[a] *Laboratory of Extreme Physiology, Institute of Medico-Biological Problems RAS, Moscow, Russia*
[b] *Institute of Higher Nervous Activity and Neurophysiology RAS, Moscow, Russia*
[c] *Zakusov Institute of Pharmacology RAMS, Moscow, Russia*
[d] *Institute of Physiologically Active Compounds RAS, Chernogolovka, Russia*

HIGHLIGHTS

- The study of influence of microgravity (model antiorthostatic suspension) and the combined irradiation (gamma-rays and protons in Bragg peak) revealed significant apologies psycho-emotional status and cognitive abilities of experimental animals—rats.
- Changes in the content of monoamines and their metabolites, and acetylcholine were detected in the hippocampus, hypothalamus and prefrontal cortex in response to the model of microgravity and ionizing radiation alone, but not in combination.
- We retrieved the first evidences that microgravity model's and ionizing radiation can act antagonistically regarding the psycho-emotional status and the cognitive abilities, which is also associated with a reduction of alterations in the monoamines content in the studied brain structures.

ARTICLE INFO

Article history:
Received 28 July 2016
Received in revised form 16 October 2016
Accepted 20 October 2016
Available online xxx

Keywords:
CNS risks
Antiorthostatic suspension
Ionizing radiation
Cognition
Neurotransmitters changes

ABSTRACT

Space flight factors (SFF) significantly affect the operating activity of astronauts during deep space missions. In contrast to an orbital flight, leaving the Earth's magnetic field is fraught with the dangers of exposure to ionizing radiation and more specifically, the high-energy nuclei component of galactic cosmic rays. Microgravity, just another critical non-radiation factor, significantly affects the normal functioning of the CNS. Some morphological structures of the brain, such as the prefrontal cortex and the hippocampus, that are rich in monoaminergic and acetylcholinergic neurones, are the most sensitive to the effects of ionizing radiation and non-radiation spaceflight factors (SFF). In this work we have studied the combined effects of microgravity (in antiorthostatic suspension model, AS) and irradiation (γ-ray and protons in spread-out Bragg peak) on the behaviour, cognitive abilities, and metabolism of monoamines and acetylcholine in the key structures of the rat's brain. Irradiation (as independently as combined with AS) resulted in the decrease of thigmotaxis in rats. Learning problems, caused by the malfunctioning of the working memory but not the spatial memory, were observed in response to AS as well as to the SFF in combination. Analysis of monoamines metabolism showed that the serotoninergic system was the most affected by the SFF. Concentration of acetylcholine in the hippocampus significantly increased in the groups of irradiated rats, and in the groups which were exposed to the SFF in combination, compared to the rats exposed only to AS.

1. Introduction

Problems in operating activity of astronauts, caused by defeats in the CNS, are among the main limiting factors during the deep space missions. Gravitational overloads, hypo-magnetic field and ionizing radiation create risks for the normal functioning of the CNS. Moreover, leaving the geomagnetic field is associated with a significant increase in radiation load, foremost due to the galactic cosmic ray's component—the heavy charged particles (HZE). Space flight factors in combination can potentiate the mutual effects; act as synergistically as antagonistically regarding the functions of the CNS. Presently, researchers mainly study the radiation factor. There

---

* Corresponding author.
E-mail address: viktor_kohan@hotmail.com (V.S. Kokhan).

http://dx.doi.org/10.1016/j.bbr.2016.10.032
0166-4328/

are almost no modern data on neurobiology effects caused by the combined action of SFF.

Despite the intensive studies, pathophysiological picture of radiation injury of the CNS has not yet been well understood. According to the observations, irradiation of rodents with $^{56}Fe^{26+}$ (1 GeV, 0.1–2 Gy) led to learning problems in tests for evaluation of the spatial memory [1,2] and the working memory [3]. These results were confirmed in the irradiation of rats with other heavy ions—$^{28}Si^{14+}$ (1 GeV/1 Gy) and $^{48}Ti^{22+}$ (1.1 GeV/0.5 and 1 Gy), which led to postponed cognitive impairments [4]. Irradiation of mice with $^{12}C^{6+}$ (284.7 MeV, LET = 25.6 keV/μm, 4 Gy) caused impairment of spatial learning in Morris water maze [5]. However, other researchers [1,6] did not find any deficiencies in cognitive functions, or the observed effects were controversial. As well, any impairments in hippocampal-independent learning were not revealed after the irradiation by $^{56}Fe^{26+}$ (1 GeV/0.1 and 1 Gy) and $^{56}Fe^{26+}$ (0.6 GeV/0.1–0.5 Gy). Moreover, irradiation of rats with protons (165 MeV, 1.5 Gy) caused an improvement of the working memory; however, this effect inverted when the absorbed dose increased up to 3 Gy [7]. Irradiation by protons in spread-out Bragg peak, in the absorbed dose of 2 Gy but not 1 Gy, affected the reproduction of a memory trace; though this effect was controversial in different behavioural models [8].

The structures of the brain which are rich in monoaminergic neurones and synapses – the hippocampus, the striatum, the prefrontal cortex and others – were the most sensitive to the HZE [9]. Dopamine easily oxidizes to form a number of toxic products: DHBT-1 and other benzothiazines [10,11], leykoaminohrom-o-semiquinone and products of its conjugation [12]. This suggestion has been supported by the data from the behavioural analysis detecting the problems primarily in the spatial memory [13,14], which requires an intactness of the limbic system of the CNS that is rich in dopaminergic neurons. Study of the monoamines content in the selected brain structures showed an increase in the content of 3-methoxytyramine (3-MT) (3 Gy) and a decrease for serotonin (5-HT) (2 Gy) in the prefrontal cortex in response to irradiation by protons of 165 MeV. Also, a reduction in the content of 3-MT was observed in the hippocampus [7]. Irradiation by protons in spread-out Bragg peak led to more significant changes. Authors noticed a reduction of dopamine and noradrenaline content in the prefrontal cortex, as well as 3-MT content in the striatum [8]. In response to the exposure to HZE—$^{12}C^{6+}$ (1 Gy, 500 MeV), changes in the content of monoamines and metabolites were more pronounced, compared to the effect of the protons. Thus, drastically reduced the content of 3-MT, 3,4-dihydroxyphenylacetic acid, 5-hydroxyindoleacetic acid and 5-HT in the prefrontal cortex of the Wistar rats. At the same time, the significant neurochemical changes in the hippocampus, the hypothalamus and the striatum were not observed [15]. Significantly less is known about the role of other neurotransmitter systems. Thus, it was demonstrated that selective potentiation of acetylcholine receptor a7nAChR causes a radioprotective effect that substantially increases the survival level of mice with a sublethal dose of irradiation [16]. Irradiation of NTera2-derived neurons and astrocytes (as pure as mixed cultures) by the selected single-type charged particles (protons 250 MeV, $^{12}C^{6+}$ 290 MeV, or $^{56}Fe^{26+}$ 1000 MeV) in absorbed doses of 0.1, 0.5 and 2 Gy led to reciprocal effect against the glutamate uptake; thus, an uptake activity in neurons increased after the irradiation (3 h, 2 days and 7 days post exposure) but an astrocytes transporter activity decreased (only on 7 day, only $^{12}C^{6+}$ and $^{56}Fe^{26+}$) [17].

Microgravity also affects the normal functioning of the CNS. Retaining the human in a weightlessness condition leads to disturbances in attention, space orientation and performance in tests for abstract thinking [18]. However, care should be taken when interpreting these data; they do not allow to distinguish the microgravity effects from the ones of the other SFF; and even those effects can be caused by problems in the vestibular apparatus. Up to date, there is no any direct evidence of disturbances in the cognitive abilities in response to the effects of microgravity. During the American space mission *STS-54*, it was demonstrated that microgravity (10^{-6}G) causes a decrease in expression and activity of tyrosine-hydroxilase in rats [19]. Another big study revealed that long-term stay of mice in microgravity environment (up to 91 days) causes a decrease in expression level of NGF in the hippocampus, cortex, and adrenal gland [20]. The last data from the orbital program of *BION* satellites showed a decrease in the expression level of mRNA for such neurotropic factors as GDNF and CDNF in dopaminergic neurones of the nigrostriatal system in experiments on mice [21]. At the same time, a long-term space flight did not affect the expression of genes encoding BDNF as well as its receptors (TrkB and p75) and did not cause any dysregulation in genetic control of the neuronal apoptosis [22].

Up to date, researchers have not yet fully studied the combined effects of SFF in a ground-based simulation. At the same time, the orbital missions on *ISS* (humans) and *BION* satellites (rats, mice, turtles, geckos and other) cannot serve as a complete model of a deep space flight. Data on the neurobiological effects of the combined action of radiation and non-radiation SFF are extremely scarce. Results of studies of cognitive abilities of model animals after the exposure to the combined action of SFF are ambiguous. HZE in combination act synergistically, potentiating the effects of each other. Thus, in three months post-irradiation, the mice exposed to either protons (150 MeV, 0.1 Gy) or combined proton and $^{56}Fe^{26+}$ (600 MeV, 0.5 Gy) irradiation show an impaired novel object recognition, which was not observed in the mice irradiated with $^{56}Fe^{26+}$ only [23]. In our previous work, in course of discriminant training of Wistar rats in Y-maze (conditioned stimulus: the sound of different frequency), any significant change was not revealed. However, there was a trend in better dynamics of learning in rats exposed to the combination of AS and radiation [7]. Molecular mechanisms underlying effects of the combined action of the SFF have almost not been studied. Only a change in the content of monoamines and their metabolites in the selected morphological structures of the brain were shown. Thus, the γ-irradiation (3 Gy, ^{137}Cs) in combination with the antiorthostatic suspension (AS) led to normalization in the content of 5-HIAA in the striatum, which content increased due to the effects of AS only [24]. Recently, it was shown that a prolonged exposure to AS (21 days) and γ-irradiation (122 keV, 40 mGy) causes an increase in the content of oxidative stress biomarkers, thus increasing the likelihood of brain injury and reduction in the antioxidant system [25].

In this context, the current study is dedicated to elucidation of the effects of microgravity (in AS model) and irradiation (γ-ray and protons in spread-out Bragg peak) as separately as in combination, on psycho-emotional status, cognitive abilities and their neurochemical background in the ground-based experiment. The purpose was to determine if there is an interaction or an additive effect between the results of exposure to γ-ray and protons irradiation in microgravity condition (in AS model). Finally, we determined whether the radiation- and AS-induced behavioural effects resulted from the changes in the content of monoamines, their metabolites, and also of acetylcholine in the morphological brain structures that are the most sensitive to ionizing radiation.

2. Materials and methods

2.1. Animals

Outbred male Wistar rats of 130–150 g body weight (6 weeks old) were used. Control and experimental groups were housed together except the time when the rats treated by AS were kept

Please cite this article in press as: V.S. Kokhan, et al., Combined effects of antiorthostatic suspension and ionizing radiation on the behaviour and neurotransmitters changes in different brain structures of rats, Behav Brain Res (2016), http://dx.doi.org/10.1016/j.bbr.2016.10.032