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Original paper

Neurochemical insights into the radiation protection of astronauts: Distinction between low- and moderate-LET radiation components

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ABSTRACT

Radiation protection of astronauts remains an ongoing challenge in preparation of deep space exploratory missions. Exposure to space radiation consisting of multiple radiation components is associated with a significant risk of experiencing central nervous system (CNS) detriments, potentially influencing the crew operational decisions. Developing of countermeasures protecting CNS from the deleterious exposure requires understanding the mechanistic nature of cognitive impairments induced by different components of space radiation. The current study was designed to identify differences in neurochemical modifications caused by exposure to low- and moderate-LET radiations and to elucidate a distinction between the observed outcomes. We exposed rats to accelerated protons (170 MeV; 0.5 keV/μm) or to carbon ions (^{12}C; 500 MeV/u; 10.5 keV/μm) delivered at the same dose of 1 Gy. Neurochemical alterations were evaluated 1, 30, and 90 days after exposure via indices of the monoamine metabolism measured in five brain structures, including prefrontal cortex, hypothalamus, nucleus accumbens, hippocampus and striatum. We obtained the detailed patterns of neurochemical modifications after exposure to the mentioned radiation modalities. Our data show that the enhancement in the radiation LET from relatively low to moderate values leads to different neurochemical outcomes and that a particular effect depends on the irradiated brain structure. We also hypothesized that exposure to the moderate-LET radiations can induce a hyperactivation of feedback neurochemical mechanisms, which blur metabolic deviations and lead to the delayed impairments in brain functions. Based on our findings we discuss possible contribution of the observed changes to behavioural impairments.

1. Introduction

Radiation damage to the central nervous system (CNS) has become an ongoing health challenge of last decades largely due to the issues of radiation hazards of human missions to other planets and asteroids [1–4]. In such a mission, for example to Mars, the crew would be exposed to continuous Galactic Cosmic Rays (GCRs), and potentially large bursts of Solar Energetic Particles (SEPs) [5,6]. These components of space radiation both include a fraction of high-energy particles capable to disrupt normal functioning of the brain.

GCRs are high energy particles composed of roughly 89–90% protons, ~10–13% helium ions, ~1% of electrons and ~1% heavier nuclei [7,8]. Because of their high energies, GCRs are difficult to shield against, both during a Mars cruise and at the Martian surface. SEPs are dominantly protons with fractions of helium ions and heavier nuclei, which spectra may vary significantly in individual SEP event both with time and with energy [9]. Most of the SEPs are potentially risking mainly during a Mars cruise, however, in “hard spectrum” events, ions can be accelerated to energies well above 150 MeV/u with substantial fluxes reaching the Martian surface.

Ground-based simulation of CNS effects, which can be induced by components of space radiation shows that the exposure to high-energy particle beams leads to a variety of neural malfunctions in laboratory animals, including both acute and late outcomes. The most reported effects being observed within a relatively short period after irradiation embrace altered cognitive function, manifesting via detriments in short-

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term memory, reduced motor function and behavioural changes related to motor behaviour, amphetamine-mediated test of aversive learning, and operant conditioning [10]. Late alterations may be represented by a number of CNS disorders such as prolonged deficits in long-term memory, degenerative changes stimulating the aging and other cognitive impairments [1–3,11,12]. Despite an intensive study of these abnormalities at the levels of behaviour, cognition and specific cellular responses, the neurochemical mechanisms and the most relevant brain regions associated with the observed alterations have not been clearly identified.

Evaluation of the brain biogenic amines' metabolism provides an important information on the current neurochemical state of CNS. Dopamine- (DA), serotonin- (or 5-hydroxytryptamine, 5-HT) and noradrenaline (NA)-ergic systems [13–18] are the brain pathways essential for the formation of emotional and motivational states and behaviour control through the hierarchical neural networks [19]. Most of the neurological disorders are connected with deviations in DA-, 5-HT- or NA-ergic regulation.

Available evidences link radiation-induced CNS impairments with damage to some structures of the brain mesolimbic and mesocortical dopaminergic systems, which have been recognized for their central role in motivated behaviours, various types of reward, emotional state, as well as in cognitive processes and executive functions [20,21]. We and other authors have reported radiation-induced alterations in the prefrontal cortex, nucleus accumbens, striatum, hippocampus, and hypothalamus [13–15], the structures controlling the behaviour of animals via modification of the activities of the NA, DA, and 5-HT systems [20–22]. The prefrontal cortex is known to play a key role in learning as well as short-term and long-term memory processes [23,24]; the hypothalamus contains the centres of food, drink, and sexual behaviour [25–27]; the striatum performs motor control and determines complex behaviour [28,29]; the hippocampus is the functional centre of the brain responsible for learning and memory [11,30–32], cognitive map of brain [33,34], spatial orientation [35–37], and the context of the environment [19]; the nucleus accumbens is the center of integration of information between the prefrontal cortex, hypothalamus, amygdala, habenula, and the hippocampus [25,38]. Despite the observations reporting disruption in the activity of these structures, additional pieces of data are needed to conclude on the role of each of them in a particular physiology outcome of radiation exposure.

Clinically significant CNS risks and their dependences on the dose, dose-rate and radiation quality are also poorly understood at this time. This contributes to uncertainties in predicting the total radiation risk to the crew in Martian mission as well as creates difficulties for developing potential medical countermeasures.

An effective dose of 1 Gy is found to be a total mission dose equivalent for a round trip Mars surface mission with 180 days (each way) cruise, and 500 days on the Mars [39]. On the other hand, 1 Gy is a career limit dose for astronauts being used by several space agencies, including Russian Space Agency (Roscosmos), European Space Agency (ESA), and Japan Aerospace Exploration Agency (JAXA), while NASA uses an individual risk-based system for radiation protection assuming even lower dose limits [40,41]. This motivates for a more detailed investigation of health effects caused by doses of about 1 Gy, especially for prediction of the health injury at different phases of the mission as well as in the case if a large SEP burst occurred during a transit and the crew received a sufficient dose [42].

Protons and heavy ions of a high linear energy transfer (LET) are widely discussed in connection with the radiation protection of space travellers. Among the heavy ions, Fe particles of around 150–170 keV/μm are frequently used in ground-based experiments to simulate the hard spectrum component of space radiation. At the same time, a cumulative health effect of high-energy ions comprising the C-O group with LET of about 10s keV/μm could also be significant due to their even higher abundance in the GCR flux than is observed for Fe particles [7]. In this context, the energy range of 10^2–10^3 MeV/u is of interest because it corresponds to the maximal differential flux of particles in cosmic rays spectra.

To date, few data are available in literature about the mechanistic nature of brain disorders induced by particles of a moderate and low LET, especially in the context of CNS-related space radiation risk. Thus, this study was designed to evaluate specific brain responses to irradiation with 10.6 keV/μm carbon ions (500 MeV/u) and 0.5 keV/μm proton (170 MeV), attempting to identify the brain regions and neurochemical mechanisms vulnerable to these radiation modalities.

2. Materials and methods

2.1. Animals

Sixty male Sprague-Dawley rats (HSD:SD; certified by Harlan Sprague Dawley, Inc.) weighing 195–200 g were delivered from the Pushchino Animal Breeding Facility of the Institute of Bioorganic Chemistry (Pushchino, Russia). The rats were placed to the animal facility of the Joint Institute for Nuclear Research (JINR) and acclimatized for 7 days prior to irradiation. All the animals were maintained in plastic cages (maximum five rats per cage) under constant temperature conditions (20 ± 2 °C), fed with autoclaved rat chow and water by bottle *ad libitum* and kept under a 12-h light/12-h dark cycle. At the time of irradiation the rats were 7.5–8.0 weeks old.

In order to evaluate the effects of exposure to protons and ^{12}C particles, two groups of animals (30 rats each) were used. 15 animals of each group were irradiated with protons or ^{12}C ions, while the remaining 15 rats were used as controls, i.e. each of the irradiated groups had its own sham-irradiated control. Neurochemical analyses were performed 24 h, 30 days and 90 days after exposures. For each of these three testing periods 5 irradiated and 5 control animals were examined.

All animal procedures were performed in accordance with the regulations and principles expressed in the Russian Guidelines for Animal Experiments [43] and Sanitary Regulations for the Organization, Equipment, and Maintenance of Animal Facilities for Experimental Biology (Vivaria) [44], which meet the basic requirements of EU Directive 2010/63/EU for animal experiments. All protocols were employed in compliance with the rules approved by the bioethical committees of the Institute of Biomedical Problems and the Institute of Higher Nervous Activity and Neurophysiology of the Russian Academy of Sciences.

2.2. Irradiation procedure

All irradiated animals were exposed to a single dose of 1 Gy of protons or ^{12}C particles in order to have a total Martian mission dose [39]. The exposure to 170 MeV protons occurred at the Phasotron facility of the Medical and Technical Complex of JINR's Dzhelepov Laboratory of Nuclear Problems. The animals were fixed in well-ventilated 8.5 × 8.5 × 20-cm irradiation jigs made of mouldable plastic and adapted for simultaneous keeping of 4 animals. Each jig was placed at the centre of a 9.0 × 9.0-cm squared proton beam having a track-averaged LET of 0.5 keV/μm. No any materials were located between the beamline output and the target. The rats received a total body irradiation at a nominal dose rate of 1 Gy/min. The LET variation through the rat brain was less than 5%. The beam dosimetry calibration was performed using a clinical dosimeter PTW UNIDOS-E according to guidelines of the International Atomic Energy Agency [45]. The uniformity of the dose cross-section and energy of the proton beam was online controlled with multiwire ionization chambers and Si detectors [46]. The beam uniformity was also visually controlled with the Gafchromic RTQA-1010P dosimetry film which was placed behind the irradiated animals. The total error of the absorbed dose measurements did not exceed 10%. The control rats were sham-irradiated by being placed in irradiation jigs and kept the same time in the preparation room of the Phasotron facility without exposure to protons.

The irradiation with 500 MeV/u ^{12}C particles was performed at the Nuclotron facility of JINR's Veksler and Baldin Laboratory of High Energy Physics. The rats were fixed in 6.5 × 6.5 × 16.5-cm acrylic irradiation jigs with multiple ventilation holes and placed sequentially per 2 jigs at the centre of a 5-cm-diameter circle ^{12}C ion beam with a particles' LET of 10.6 keV/μm. The absorbed dose was measured by a free-air ionization chamber with a 4.5 × 4.5-cm sensitive area placed upstream of the target. No other materials were located before the target. A quasi-uniform radiation field was created by defocusing of the ion beam with a magnetic lens before the point of exposure. The inhomogeneity of the field along the *X* and *Y* axes did not exceed ± 5% within the exposure area. The total error of the absorbed dose measurements did not exceed 10%. The LET variation through the rat brain was less than 6%. The control rats were taken to the Nuclotron facility but were not exposed to the beam. The jigs with control rats were sham-irradiated by remaining in the preparation room, while their counterparts were taken into the irradiation vault of the accelerator.

After all the exposures are completed, the rats were returned to the animal facility of JINR and maintained until the neurochemical studies.

The animal work procedures and other parameters of the experiment, except the radiation modality used, were kept as close as possible between all the groups of rats.

2.3. Analysis of neuromodulator concentrations

At the day of testing the rats were decapitated and their brains were removed and placed onto an ice-cold surface. The prefrontal cortex, nucleus accumbens, hypothalamus, hippocampus and striatum were dissected, rapidly frozen in the liquid nitrogen, and weighed. The pieces of dissected brain structures were then homogenized at +4 °C in the glass tube of a Schuett homgen^{plus} semi-automatic homogenizer (Schuett-biotec, GmbH, Göttingen, Germany) with a Teflon pestle (clearance between the pestle and tube 0.2 mm) at the pestle rotation rate of 3000 rpm. Homogenization and extraction were done in a 0.1 M $HClO_4$ solution supplemented with the internal standard DOBA (3,4-dioxybenzylamine, a catecholamine drug which is absent in the native tissue; the concentration was 0.5 nmol/mL). Tissues from the nucleus accumbens were homogenized in 40-fold volumes, while the pieces of remaining brain areas were processed in a 20-fold volume of the extraction medium. The homogenates were then centrifuged at +4 °C and 10,000*g* for 15 min. Supernatants were collected and used for measuring the contents of NA, DA with its metabolites 3,4-dioxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT), and 5-HT with its metabolite 5-hydroxyindoleacetic acid (5-HIAA). Concentrations of these substances were then used for evaluation of the DOPAC/DA, HVA/DA, and 5-HIAA/5-HT ratios, characterizing the monoamine turnover.

The concentrations of the substances (in nmol/mg of tissue) were measured with the LC304T high-performance liquid chromatography (HPLC) system (Bioanalytical Systems, Inc., West Lafayette, IN, USA) with a Rheodyne 7125 syringe loading injector and a 20 μL loop for the application of samples. The investigated substances were separated on a ReproSil-Pur ODS − 3 reversed-phase column, 3 μm, 100 × 4 mm (Dr. Maisch HPLC GmbH, Ammerbuch-Entringen, Germany). A PM − 80 pump (Bioanalytical Systems, Inc., West Lafayette, IN, USA) with the mobile phase elution rate of 1.0 mL/min at a pressure of 200 atm (20.265 MPa) was used. The mobile phase was a 0.1 M citrate–phosphate buffer, which contained 1.1 mM octanesulfonic acid, 0.1 mM EDTA, and 9% acetonitrile (pH 3.0). The flow rate was 1 mL/min. The measurements were performed using an LC − 4B electrochemical detector (Bioanalytical Systems, Inc., West Lafayette, IN, USA) with a glassy carbon electrode (+0.85 V) and an Ag/AgCl reference electrode. The data recording was performed with the MULTICHROM 1.5 hardware-software complex (Ampersand Ltd., Moscow, Russia). The chromatography system was calibrated using a standard solution of measured substances at the concentration of 500 pM/mL.

Two datasets concerned 30- and 90-day results of the neurochemical study following the exposure to ^{12}C ions were obtained in our previous work and reused in the current study to provide the reference data for the moderate-LET radiation [13]. Other data sets were not shown before.

2.4. Statistical analysis

The concentrations of all substances were normalized to the sham-irradiation values, which were set to 100%. The data were expressed as the normalized mean ± the standard error of the mean (SEM). The results of the measurements were analysed using a one-way Analysis of Variance (ANOVA) [47]. *Post-hoc* comparisons were performed with Fisher's least significant difference (LSD) test [48]. To establish the general direction of changes, a two-tailed Wilcoxon matched pairs test was used [49]. All statistical analyses were performed using STATISTICA software (StatSoft, Inc., Tulsa, OK). Results were considered significant if the significance level was $p < 0.05$. If the observed level was $p < 0.1$, a trend toward statistical significance was noted. A special assessment of neurochemical differences between sham-irradiated groups in proton and ^{12}C-ion experiments was made, and no significant differences or trends toward statistical significance were found.

3. Results

3.1. Overall map of brain neuromodulator alterations

The brain regions, where the neuromodulator metabolism was affected by radiation exposures, are summarized in Fig. 1. In the acute period (24 h) after proton irradiation, statistically significant alterations were observed in the prefrontal cortex, nucleus accumbens, hippocampus and striatum. At that time the first three of the regions were also affected by ^{12}C particles while no considerable changes were observed in the striatum.

30 days after exposure, the patterns of radiation-affected sites of the brain were equivalent for both types of exposure. In this period the changes persisted in the nucleus accumbens and hippocampus, and they were also detected in the striatum. 90-day analysis revealed statistically significant effects of both radiations in the nucleus accumbens and hypothalamus and the effect of the proton exposure in the hippocampus.

Overall, the mapping in Fig. 1 suggests that in some brain areas both radiation modalities can induce neuromodulator alterations in a time-dependent manner. The prefrontal cortex, in particular, is shown to be associated with the acute outcomes while the hypothalamus exhibits significant changes in late periods. At the same time, there are regions, which hold early metabolic changes through the months. In the present study such manner of alterations was observed in the nucleus accumbens and hippocampus. In the former structure the changes persisted up to 90 days in all irradiated groups; in the latter one, metabolic deviations were also detected in all exposed animals with the exception of 90-day group irradiated with ^{12}C particles.

Finally, although the same brain regions were eventually affected by both radiations, the exact metabolic changes in them varied considerably depending on the type of exposure.

3.2. Prefrontal cortex

Neurochemical studies revealed differences in the acute response of the prefrontal cortex to proton and ^{12}C-ion irradiation. 24 h after exposure, protons were found to induce changes in the metabolism of DA and 5-HT (Fig. 2a). There was a significant increase in the concentrations of DA and DOPAC to 177.2 and 144.8%, respectively (the corresponding *p*-values are 0.035 and 0.018), relative to sham-irradiated controls. There were also trends to the reduction of the HVA/DA ratio to 51.8% ($p = 0.062$) and to the increase of the 5-HT level to 116.0%

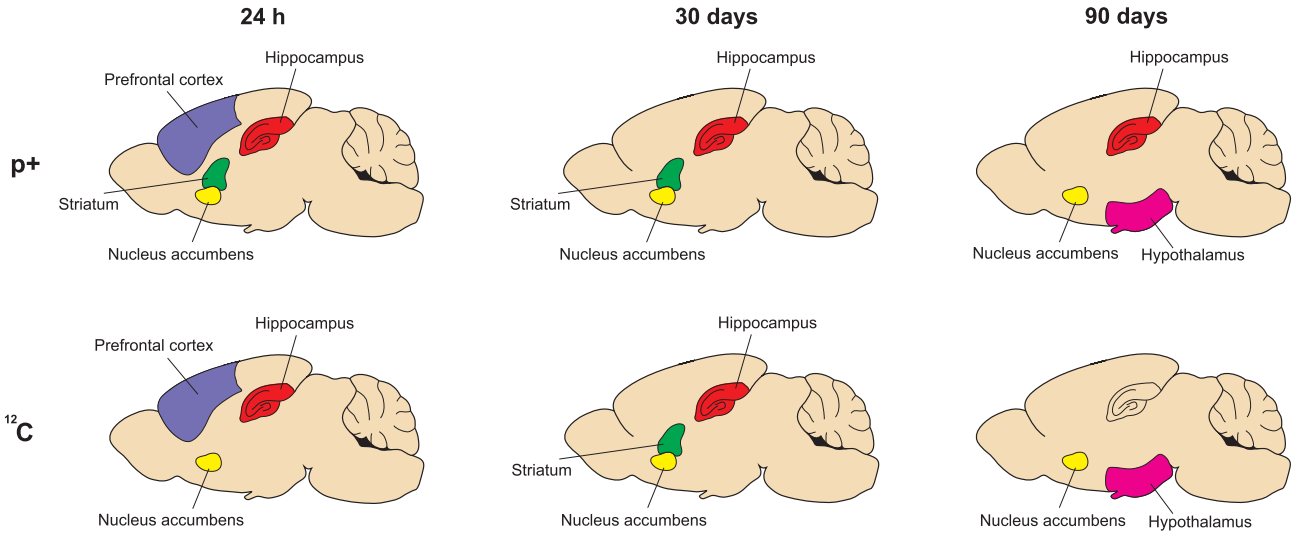

Fig. 1. The overall map of brain neuromodulator alterations observed after exposure to 170 MeV protons and 500 MeV/u ^{12}C particles at the dose of 1 Gy. Each particular graph depicts the brain regions where statistically significant changes and trends toward statistical significance were detected.

($p = 0.082$), revealed simultaneously.

These endpoints of the proton exposure were compared to 24-h results of irradiation with ^{12}C ions (Fig. 2b). The comparison revealed a difference between metabolic patterns of the two types of exposure. Irradiation with ^{12}C ions resulted in a significant decrease in the concentrations of DOPAC, 5-HT and 5-HIAA to 69.2, 68.6 and 75.3%, respectively (the corresponding p-values were 0.015, 0.018, 0.021 and 0.050). There was also a trend to reduction in the level of NA to 70.6% ($p = 0.050$). Furthermore, the Wilcoxon matched pairs test indicated a unidirectional decrease in the levels of the majority of the studied indices after irradiation with ^{12}C particles ($p = 0.028$).

The observed metabolic alterations most probably suggest that the exposure to protons leads to the deficit in the pathway $\mathrm{DA} \overset{\mathrm{MAO}}{\rightarrow} \mathrm{DOPAC} \overset{\mathrm{COMT}}{\rightarrow} \mathrm{HVA}$ in this period. A simultaneous increase in DA and DOPAC levels together with the reduction of HVA/DA ratio indicates possible inhibition of the reaction dependent on the catechol-O-methyl transferase (COMT) activity and shift in the DA metabolism towards the transformation associated with the monoamine oxidase (MAO). The irradiation with ^{12}C particles, in contrast to this, induces a deficit in DA synthesis rather than in DA metabolism. This evidence is supported by the simultaneous decrease in the concentrations of DA and NA, parallel to unidirectional reduction in the majority of the evaluated metabolic parameters. Regarding alterations in the DA metabolism, the observed changes are likely caused by inhibition of the reaction $\mathrm{DA} \overset{\mathrm{MAO}}{\rightarrow} \mathrm{DOPAC}$, but not $\mathrm{DOPAC} \overset{\mathrm{COMT}}{\rightarrow} \mathrm{HVA}$ or $\mathrm{DA} \overset{\mathrm{COMT}}{\rightarrow} \mathrm{3\text{-}MT} \overset{\mathrm{MAO}}{\rightarrow} \mathrm{HVA}$.

Similar differences between proton and ^{12}C particle exposures take place in relation to the 5-HT metabolism. An elevated level of 5-HT following the proton irradiation presumably indicates an inhibition in one of the transformations within the chain $\mathrm{5\text{-}HT} \overset{\mathrm{MAO}}{\rightarrow} \mathrm{5\text{-}HIAL} \overset{\mathrm{ALDR2}}{\rightarrow} \mathrm{5\text{-}HIAA}$, where 5-HIAL is the 5-hydroxyindole acetaldehyde and ALDR2 is the aldehyde dehydrogenase (type 2). Alternatively, the exposure to ^{12}C particles apparently causes a deficit

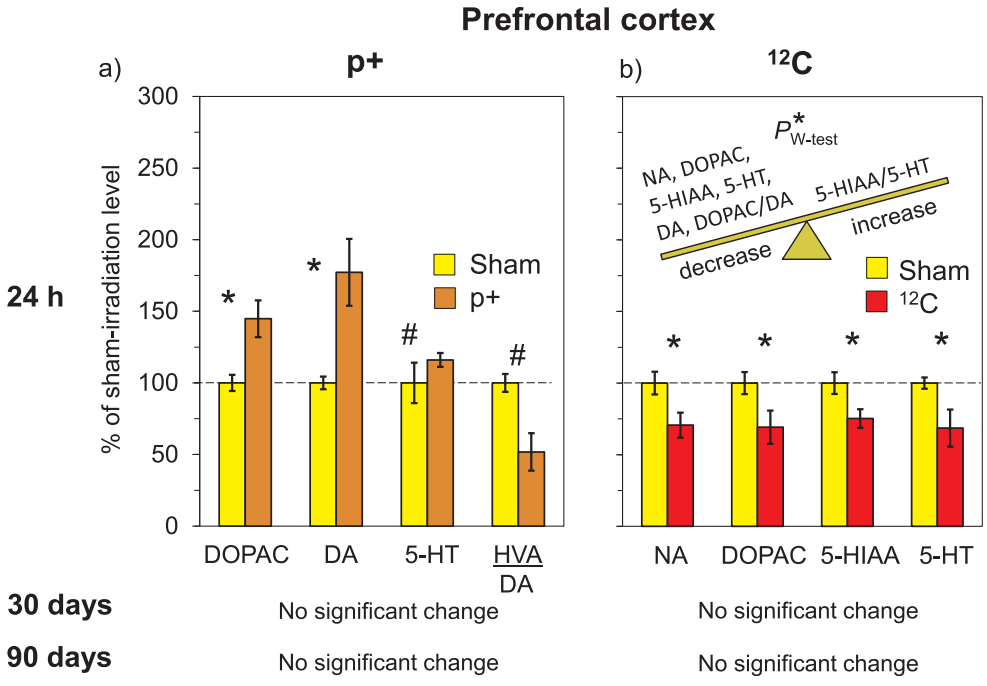

Fig. 2. Effects of 170 MeV protons (*a*) and 500 MeV/u ^{12}C particles (*b*) on the monoamine metabolism in the rat prefrontal cortex, as measured 24 h, 30 days, and 90 days postirradiation. The columns indicate the concentrations of monoamines and their metabolites scaled per levels of these substances in sham-irradiated controls. The data is only presented for substances that demonstrate statistically significant changes and trends towards the significance. The error bars represent the standard errors of the mean. (*) $p < 0.05$ and (#) $p < 0.1$ between the exposed and sham-irradiated rats. The inner plot designates a general direction of changes in sets of the indices after radiation exposure (indices demonstrating decrease vs. indices demonstrating increase). "$P_{\text{W-test}}$" indicates the statistical significance of differences between sets of neuromodulator indices measured in irradiated and control animals (according to the Wilcoxon matched pairs test).

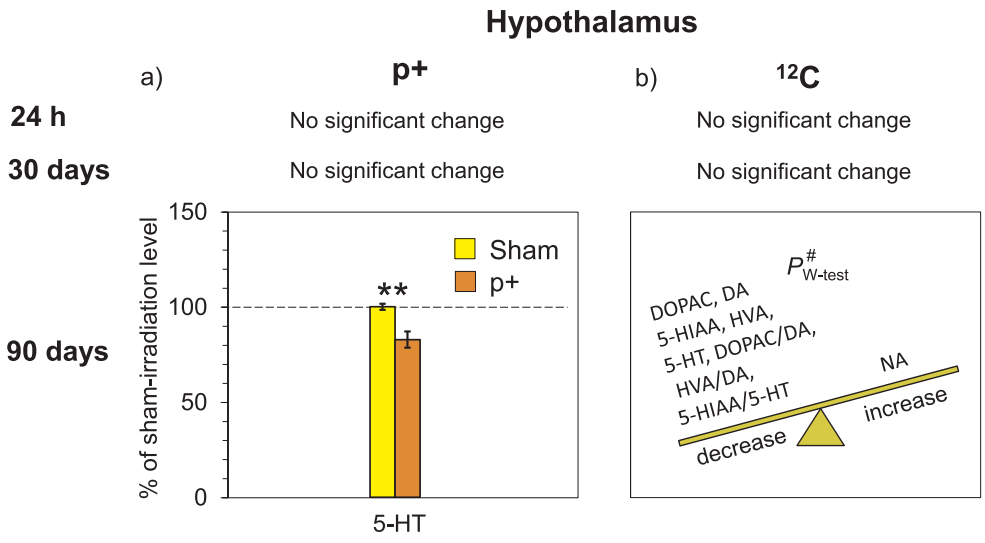

Fig. 3. Effects of 170 MeV protons (*a*) and 500 MeV/u ^{12}C particles (*b*) on the monoamine metabolism in the rat hypothalamus, as measured 24 h, 30 days, and 90 days postirradiation. The columns indicate the concentrations of monoamines and their metabolites scaled per levels of these substances in sham-irradiated controls. The data is only presented for substances that demonstrate statistically significant changes and trends towards the significance. The error bars represent the standard errors of the mean. (**) $p < 0.01$ and (#) $p < 0.1$ between the exposed and sham-irradiated rats. The inner plot designates a general direction of changes in sets of the indices after radiation exposure (indices demonstrating decrease vs. indices demonstrating increase). "$P_{W\text{-test}}$" indicates the statistical significance of differences between sets of neuromodulator indices measured in irradiated and control animals (according to the Wilcoxon matched pairs test).

in 5-HT synthesis that results in a simultaneous reduction in the concentrations of 5-HT and 5-HIAA.

At 30th and 90th days, no significant differences between control and irradiated rats were observed for any of the two types of exposure.

The results indicate that proton irradiation leads mainly to increase in DA and 5-HT metabolic indices, while the exposure to ^{12}C particles results in DA and 5-HT depletion. These differences may explain an absence of strong alterations in the elaboration and recall of the passive avoidance reflex after the exposure to protons [50] and the presence of such impairments after irradiation with high-LET particles like ^{56}Fe, ^{48}Ti, and ^{28}Si [3].

3.3. Hypothalamus

In contrast to the prefrontal cortex, significant changes in the hypothalamus were observed only 90 days following the exposures. Fig. 3a shows that the irradiation with protons induced a significant decrease in the level of 5-HT to 82.8% ($p = 0.009$). A different pattern of alterations was found after the exposure to ^{12}C particles, although there was no significant reduction in the levels of particular substances, a trend towards the unidirectional decrease of the measured indices was detected ($p = 0.059$) (Fig. 3b). In this case, 9 of 10 evaluated metabolic parameters were shown to decrease, compared to the controls. In the earlier periods (24 h and 30 days) after irradiation, no significant changes were observed in the hypothalamic monoamine metabolism.

The findings illustrate an exacerbated reduction in overall hypothalamic monoamine metabolism after the exposure to ^{12}C particles that is not observed following irradiation with protons. Assuming that the decrease in 5-HT concentration can be associated with depletion of either 5-HT precursor or 5-HT transporter, proton irradiation may inhibit, in particular, the release of glucocorticoids, such as corticosterone/cortisol, into the bloodstream [51–54]. The irradiation with ^{12}C particles, associated with an overall decrease in the 5-HT and DA metabolic indices, apparently leads to a more pronounced effect on the neuroendocrine regulation, potentially relevant to behavioural disorders observed in our previous study [13].

3.4. Nucleus accumbens

In the nucleus accumbens, the levels of the monoamine neuromodulators and their metabolites showed a manifold pattern of changes following both radiation exposures; in both cases, different periods after irradiation were associated with alterations in different neuromodulator pathways (Fig. 4). After exposure to protons, early changes (24 h postirradiation) were found in the metabolism of 5-HT (Fig. 4a). It was shown that the levels of 5-HIAA and 5-HIAA/5-HT increased to 137.5 and 139.3% respectively (the corresponding *p* values are 0.027 and 0.098) in irradiated rats compared to controls. Late responses of the nucleus accumbens were associated with alterations in the metabolism of DA and NA. At 30 days postirradiation, there was a significant ($p = 0.021$) reduction in the HVA/DA ratio, an index of DA turnover, to 71.0% and a trend towards significant decrease in the HVA level (to 66.4%, $p = 0.098$). The Wilcoxon matched pairs test also revealed a significant ($p = 0.028$) unidirectionality of reduction in levels of the assessed metabolic indices after irradiation, which is represented by simultaneous decrease in all evaluated values except the concentration of 5-HT. At 90 days postirradiation, there was a slight increase in the concentration of NA to 136.5% ($p = 0.070$) and a trend towards the unidirectional elevation of the metabolic indices ($p = 0.093$ in the Wilcoxon matched pairs test). At large, the observed pattern of changes suggests that the most pronounced effect of proton irradiation is associated with 30-day period.

Compared to proton irradiation, the exposure to ^{12}C particles was found to induce a different pattern of time-depending alterations (Fig. 4b). 24 h after exposure, rats irradiated with carbon ions showed a significant ($p = 0.048$) decrease in the concentration of DA to 45.3%. The measurements performed at 30th day revealed a pronounced reduction in the level of 5-HIAA to 72.8% ($p = 0.049$). In these periods, none of other neuromodulator indices showed a statistically significant change. At 90 days postirradiation, there was a pronounced decrease in the concentration of NA to 25.5% ($p = 0.018$). Moreover, the Wilcoxon matched pairs test showed a statistically significant ($p = 0.013$) difference between the sets of neuromodulator indices measured in ^{12}C-exposed and control rats. In this case, most of the assessed metabolic parameters, except HVA, DOPAC/DA and HVA/DA, decreased in irradiated animals. The overall patterns of changes observed in the nucleus accumbens shows that, in contrast to protons, the most apparent effect of ^{12}C particles occurs at 90 day postirradiation.

All these findings suggest that exposure to protons results in a fast hyperactivation of 5-HT-pathways of the nucleus accumbens with the following overall slowdown in monoamine metabolism (mostly in DA turnover) and subsequent general enhancement in the neurochemical machinery of this brain structure. The moderate-LET ^{12}C particles induces, perhaps, a delayed neurochemical response in this brain area that results in an overall decrease in monoamine indices shifted towards 90 days. In this case, a general enhancement in neurochemical activity

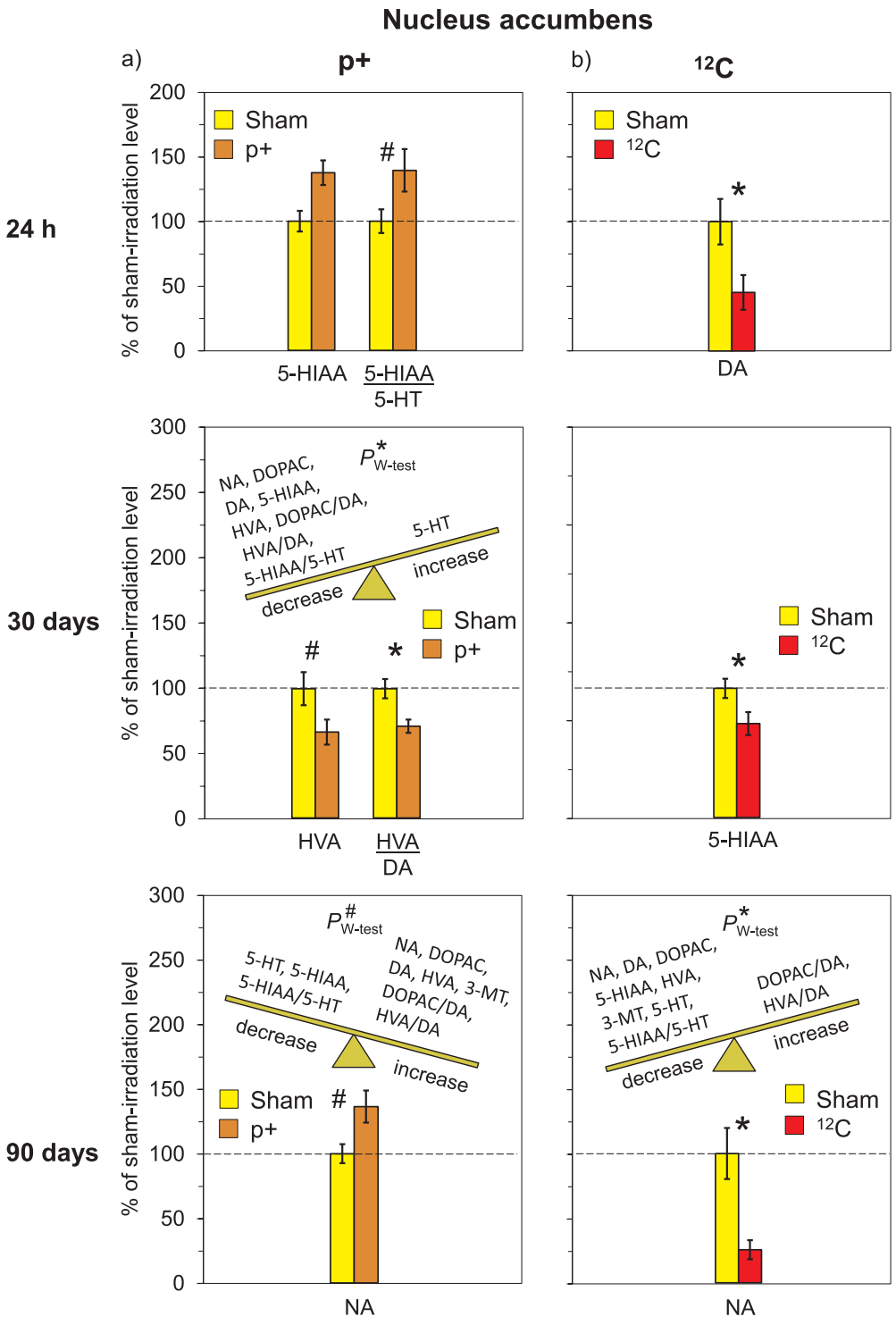

Fig. 4. Effects of 170 MeV protons (*a*) and 500 MeV/u ^{12}C particles (*b*) on the monoamine metabolism in the rat nucleus accumbens, as measured 24 h, 30 days, and 90 days postirradiation. The columns indicate the concentrations of monoamines and their metabolites scaled per levels of these substances in sham-irradiated controls. The data is only presented for substances that demonstrate statistically significant changes and trends towards the significance. The error bars represent the standard errors of the mean. (*) $p < 0.05$ and (#) $p < 0.1$ between the exposed and sham-irradiated rats. The inner plots designate general directions of changes in sets of the indices after radiation exposure (indices demonstrating decrease vs. indices demonstrating increase). "$P_{W\text{-test}}$" indicates the statistical significance of differences between sets of neuromodulator indices measured in irradiated and control animals (according to the Wilcoxon matched pairs test).

of the nucleus accumbens should be expected beyond this period.

3.5. Hippocampus

In the hippocampal neuromodulator metabolism, there were also differences between the effects induced by proton and ^{12}C particle irradiations. After exposure to protons, early outcomes (24 h post-irradiation) included a significant reduction in the concentrations of NA (to 72.9%, $p = 0.021$) and 5-HT (to 63.7%, $p = 0.011$), and pronounced increase in 5-HT turnover rate 5-HIAA/5-HT (to 136.0%, $p = 0.021$) (Fig. 4a). Results of the 30-day measurements showed only a significant decrease in the concentration of hippocampal DA (to 41.7%, $p = 0.018$). 90 days after exposure significant alterations were observed only in the metabolism of 5-HT that was reflected in considerable increase in the level of 5-HT itself (to 125.6%, $p = 0.040$) and a pronounced reduction in the 5-HIAA/5-HT ratio (to 76.3%, $p = 0.029$).

In contrast with the proton irradiation, the exposure to ^{12}C particles did not result in any change in the 24-h-levels of particular metabolic indices. In this period, the Wilcoxon matched pairs test only revealed a trend towards a statistically significant difference between sets of metabolic parameters in irradiated and control rats ($p = 0.086$). The mentioned difference in this case consisted in reduction in the majority

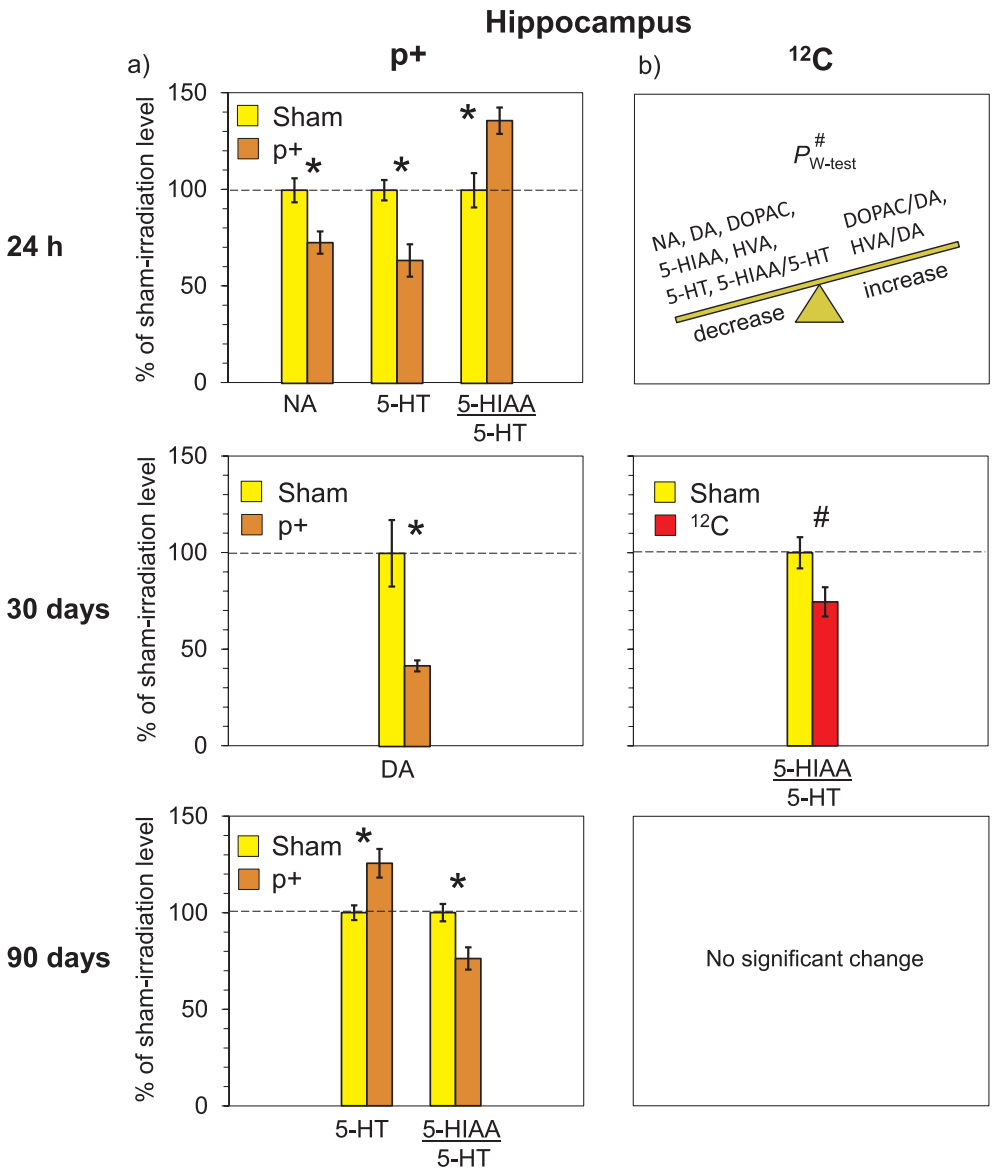

Fig. 5. Effects of 170 MeV protons (*a*) and 500 MeV/u ^{12}C particles (*b*) on the monoamine metabolism in the rat hippocampus, as measured 24 h, 30 days, and 90 days postirradiation. The columns indicate the concentrations of monoamines and their metabolites scaled per levels of these substances in sham-irradiated controls. The data is only presented for indices that demonstrate statistically significant changes and trends towards the significance. The error bars represent the standard errors of the mean. (*) $p < 0.05$ and (#) $p < 0.1$ between the exposed and sham-irradiated rats. The inner plot designates a general direction of changes in sets of the indices after radiation exposure (indices demonstrating decrease vs. indices demonstrating increase). "$P_{W\text{-}test}$" indicates the statistical significance of differences between sets of neuromodulator indices measured in irradiated and control animals (according to the Wilcoxon matched pairs test).

of the assessed indices, except DOPAC/DA and HVA/DA, after exposure. At 30 days postirradiation, there was only a slight decrease in the 5-HIAA/5-HT ratio (to 74.6%, $p = 0.074$), which characterizes turnover of 5-HT (Fig. 5b). At 90 days postirradiation, no statistically significant differences between irradiated and control rats were detected.

These results generally suggest that early effects of the proton exposure are associated with reduction in synthesis of NA, probably due to a lower yield of the reaction DA $\xrightarrow{\text{DBH}}$ NA, where DBH is the dopamine beta-hydroxylase, and enhanced metabolism of 5-HT, assuming an unchanged synthesis of this neuromodulator. Later on, the alterations touch the DA level and, finally, lead to a slowdown in the metabolism of 5-HT upon its unaltered synthesis. Contrary to low-LET protons, moderate-LET ^{12}C particles most likely induce a fast overall reduction in monoamine exchange with the earlier slowdown in 5-HT metabolism and faster recovery due to more stimulated compensatory processes.

The hippocampus is densely innervated by serotonergic fibres making it sensitive to changes in 5-HT-dependent metabolic pathways. Although reaction of the hippocampus to the presence of 5-HT is complicated and depends on many factors, like local 5-HT concentration, the ratio of different 5-HT receptor subtypes expressed, and the density of 5-HT receptors in a particular population of cells, most of the present day data suggest an inhibitory function of 5-HT [28]. To this extent, elevated 5-HT level observed at 90th day after proton irradiation may suggest inhibition of pyramidal neurons' function by hyperpolarizing their membrane potential and increasing local GABA transmission [55–59]. On the other hand, decrease in 5-HT level and enhancement of 5-HT turnover revealed 24 h following the proton exposure most probably indicates an acute stress reaction on irradiation. This change, however, may not result in considerable behavioural impairment, since 5-HT released from the projecting neurons in the raphe nuclei cell body can attenuate stress-induced activation by an auto-inhibitory feedback loop [60,61]. In contrast to proton irradiation, general decline in metabolic indices observed after ^{12}C particle exposure brings to the conclusion that the increase in damaging capacity of radiation leads to a more complex hippocampal metabolic profile, making difficult the identification of particular outcomes related to individual neuromodulators. The same metabolic pattern in the prefrontal cortex and hypothalamus suggests that in all these structures the moderate-LET radiations induce mixed metabolic impairments rather than particular ones.

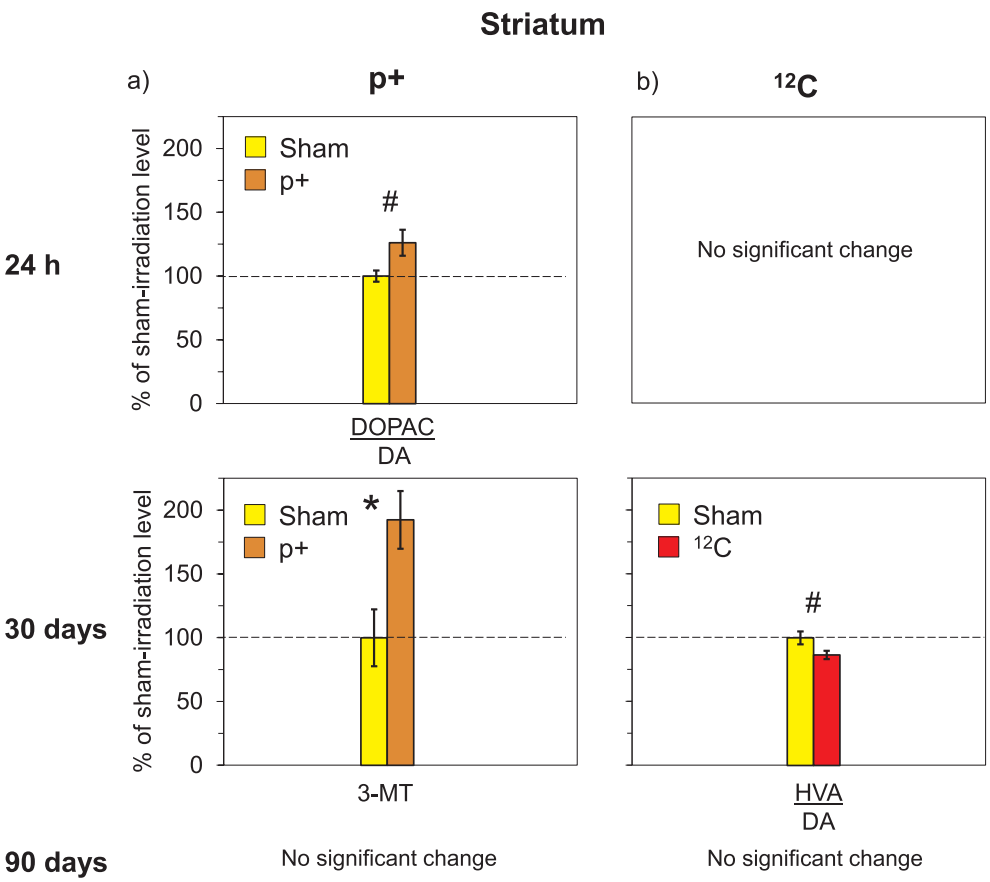

Fig. 6. Effects of 170 MeV protons (*a*) and 500 MeV/u ^{12}C particles (*b*) on the monoamine metabolism in the rat striatum, as measured 24 h, 30 days, and 90 days post-irradiation. The columns indicate the concentrations of monoamines and their metabolites scaled per levels of these substances in sham-irradiated controls. The data is only presented for indices that demonstrate statistically significant changes and trends towards the significance. The error bars represent the standard errors of the mean. (*) $p < 0.05$ and (#) $p < 0.1$ between the exposed and sham-irradiated rats.

3.6. Striatum

After exposure to protons, acute striatal alterations observed 24 h postirradiation included only a slight increase in the DA turnover (to 126.2%, $p = 0.068$) assessed as the DOPAC/DA ratio (Fig. 6a). By the 30th day, the changes in the metabolism of DA persisted in the form of the increased concentration of 3-MT (to 192.4%, $p = 0.031$). Finally, 90-day measurements revealed no statistically significant metabolic alterations in irradiated rats.

The exposure to ^{12}C particles was proven to induce only a slight decrease in the HVA/DA ratio (to 86.6%, $p = 0.081$) at the 30th day postirradiation (Fig. 6b). No other striatal alterations were detected in ^{12}C-exposed animals.

In general, the changes induced by protons point to a switching between hyperactivation of MAO- and COMT-dependent DA oxidation occurring in the respective reactions DA $\overset{MAO}{\rightarrow}$ DOPAC and DA $\overset{COMT}{\rightarrow}$ 3 - MT with time. This pattern is not observed after exposure to ^{12}C particles which leads only to an overall slight decrease in the DA metabolism affecting probably all pathways of DA degradation.

4. Discussion

Radiation protection in deep space journeys remains an important scientific issue of the present-day space medicine. One of the forefront challenge in the field is the radiation-induced alterations in CNS, which may became an origin of a behaviour dysfunction impeding the safe operation of crewmembers. The majority of the recent studies, attempting to elucidate the known behaviour impairments, are focused mainly on morphometric and structural alterations to neurons, while little is known about the neurochemical nature for radiation-induced neurocognitive outcomes as well as on the earliest stages of radiation interaction with neural cells [1–3,13]. Meanwhile, the insights being gaining in identification of radiation effects in such brain systems as DA, 5-HT, and NA, offer potentially new opportunities for preventing neurodegenerative disorders via application of pharmaceutical substances relevant to these systems. Concentrating on examining the specificity of CNS response to low- and moderate-LET radiation exposures, the current study also provides an analysis of changes induced in DA-, 5-HT-, and NA-related processes, which play crucial role in controlling numerous cognitive functions [62].

Overall comparison of the outcomes induced by protons and ^{12}C particles suggests a different sensitivity of DA, 5-HT, and NA systems to low- and moderate-LET radiations. Moreover, these differences are shown to be coupled with the variability of brain regions in their response to irradiation. This may suggest that potential pharmaceutical countermeasures for prevention of radiation-induced memory deficits during space missions should probably include application of substances having a combined activity on DA-, 5-HT-, and NA-related brain mechanisms.

The present study indicated that low-LET protons induce changes presumably in particular indices of the brain monoamine metabolism, while ^{12}C particles of the moderate LET tend to stimulate more general alterations affecting metabolic parameters unidirectionally. Such pattern was clearly seen at least in the prefrontal cortex, hypothalamus, and hippocampus suggesting an enhanced sensitivity of these brain regions to moderate-LET radiations than to low-LET ones. These observations further imply that therapeutic countermeasures protecting astronauts from the space radiation should differ at particular mission stages due to a diversity in the composition of the radiation environment.

Obtained results show that some brain structures demonstrate a delayed metabolic response to moderate-LET irradiations comparing to low-LET ones. In the present study such effect is most evident in the nucleus accumbens and partially in the striatum. These findings support the notion that the delay in response to moderate-LET irradiation may

occur via enhanced feedback reaction of certain compensatory mechanisms, which do not allow stable metabolic changes be formed immediately after exposure. In terms of therapeutic countermeasures, this fact indicates that potential substances be suggested as radioprotectors for CNS should demonstrate a specific dynamics of action, which corresponds to the time-course of development of the delayed neurochemical outcomes.

In the current study, an overall map of radiation-induced neurochemical modifications in the brain was obtained for the fist time. This map provides a clear comparison between zones affected by low- and moderate-LET exposures. In contrast to our previous work [13], the current study demonstrates an original option for investigation of radiation injury to integrative brain pathways linking several brain structures at once. Our current results bring to a conclusion that the phenomenon of radiation damage to CNS, in parallel with identification of the local impairments in particular brain structures, also needs to be examined at the level of larger systems responsible for interconnecting pathways in the brain. This may uncover potentially new patterns of CNS reaction to radiation exposure.

Up to date, most of the data on radiation-induced CNS disorders have obtained following the exposure to particles with relatively high LET of about 150–170 keV/μm. Fewer amounts of data exist on moderate LET values in the area of tens of keV/μm. At the same time, the exposure to particle radiations of very low LET and LET close to the demarcation value between low and high LET, which is accepted to be 10 keV/μm [63], helps one to identify whether the absence of neurochemical alterations sometimes observed after heavy ion exposure is caused by insensibility of a brain region to ionizing radiation or it is a result of hyper-activation of feedback mechanisms which blur metabolic deviations. The present study provides an evidence for both of these possibilities depending on the particular brain structure under consideration. With regards to the space radiation hazard, these patterns of brain reaction may be an origin of complication in diagnosing CNS disorder at early stages after exposure, when application of protective measures is expected to be still possible. It is especially make sense when the irradiation is concentrated within a particular period of time much shorter than the duration of a deep space mission, for example, during the SEP events, when a significant fraction of the exposure can occur over a period of a few hours.

5. Conclusion

In the deep space travel, different mission phases imply exposure of a crew to different radiation modalities. Considering the data reported in the current study, this fact may additionally contribute to the variability of the astronauts' CNS state together with other stressing space-travel factors.

Our findings suggest that there is an enough noticeable difference between neurochemical alterations induced by protons and ^{12}C ions. The proton irradiation impairs only particular brain metabolic indices, while exposure to ^{12}C ions leads to an overall change in the monoamine metabolism process. The change in the quality of radiation via enhancement of its LET from relatively low (about 0.5 keV/μm) to moderate values (about 10 keV/μm) is therefore expected to stimulate different patterns of functional outcomes in CNS.

These observations, together with a detailed analysis of neurochemical mechanisms altered by different radiation modalities motivate for the development of a more precise radiation protection policy for the deep space missions, on the one hand, and provide a basis for creating additional options for therapeutic countermeasures on the other hand.

Conflict of interest

The authors disclose any actual or potential conflict of interest, including financial, personal, or other issues.

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