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Poster abstract	Remarks
1. Radiation damage to nervous system: Designing of optimal models for realistic neuron morphology in hippocampus	
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A quantitative study of early mechanisms of the central nervous system (CNS) disorders induced by high-energy heavy ions at the molecular and cellular levels is one of urgent problem of modern radiobiology. In order to investigate radiation-induced effects on the electrophysiology of neural network in rat hippocampus, we developed optimal models of hippocampal neurons under irradiation of charged particles. The simple neuron models constructed from realistic cell morphology with experimental data. The computations based on the Geant4-DNA Monte Carlo track structure code were performed using the previous modeling approach of initial radiation-induced events of heavy charged particles in the individual neuronal cells. We estimated and compared distribution of energy deposition events and production of reactive chemical species within developed models of CA3, CA1 pyramidal neurons, DG granule cells and interneurons in hippocampus. Similar distributions of energy deposition events were obtained in both simplified and realistic neuron models.	
2. Mathematical modeling of the electrophysiological activity of hippocampal CA3 pyramidal peurons	
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Modern experimental data suggest that the hippocampus is one of the most ionizing radiation-sensitive parts of central nervous system. Hippocampus with its retained capability of neurogenesis plays the key role in the formation of long-term memory as well as in the integration of information received by the brain and its distribution in other brain areas. The present work focuses at designing a branching mathematical model of hippocampal CA3 pyramidal neurons taking into account main membrane and biochemical processes underlying the generation of the action potential. The mechanisms responsible for the generation of action potential bursts were analyzed. An important feature of this study is that the spatial structure of a single neuron is reproduced, which opens prospects for the further development of neuroradiobiological aspects of the model using microdosimetry methods.	

3. Simulation of the energy deposition events induced by heavy charged particles in neuron synaptic receptors and voltage-gated ion channels

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When evaluating the risk associated with exposure to space-born heavy nuclei during an interplanetary flight, the possible development of the cosmonauts' central nervous system (CNS) disorders should be taken into account. In this study our special interest is calculation of molecular damage in the CNS critical structural sites induced by ionizing radiations of different quality. Accordingly, we develop a modeling approach designed for prediction of radiation-induced damages in the molecular structure of glutamate synaptic receptors and voltage-gated ion channels, which are considered to play an important role in generation of action potential spikes, regulation of learning and memory. The applied simulation technique is based on the Geant4-DNA toolkit. The computations were performed for protons, helium, carbon and iron ions of different energies within a relatively wide range of linear energy transfer values from a few to hundreds of keV/ μ m. In the result we obtained distributions of molecular hits by energy depositions within subunits of receptors under irradiation with selected particles track. The estimation of energy depositions and radiolytic species yields suggests that initial processes in heavy particles tracks may potentially lead to further dysfunction of neuron signaling and plasticity.

4. Neurochemical alterations in central nervous system of rodents after exposure to different radiation modalities

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Extending the use of hadron therapy for brain tumours together with issues of radiation protection to astronauts on future long-term space flights raise concerns for a normal functioning of the brain after radiation exposure. Last years, experiments with animals shown that the exposure to some radiation modalities, presumably high charge and high energy particles, induce early as well as prolonged deficit in the central nervous system (CNS). Although the recent studies revealed some mechanisms responsible for radiation-induced behavioural and cognitive deficits, the exact nature of the observed structural and functional CNS disorders remains mostly unclear. The dependence of the observed impairments on the physical parameters of radiations also remains an intriguing question. The present study is focused on the comparative evaluation of the neurotransmitter metabolism in brain regions of rats exposed to a single 1 Gy dose of ${}^{12}C$ ions, protons and γ -rays. Using the method of high performance liquid chromatography with electrochemical detection, concentrations of three key brain neurotransmitters (dopamine, serotonin and noradrenaline) and their metabolites were measured in the prefrontal cortex, nucleus accumbens, hypothalamus, hippocampus and striatum, the brain regions associated with the limbic system and responsible for memory, cognition, rewards and motivation. The neurotransmitter metabolism was evaluated 30 and 90 days after exposures to assess the dynamics of changes.

The obtained results indicate the presence of changes in the dopamine-, serotonin- and noradrenaline-ergic systems of rats after exposure to all three radiation modalities. After exposure to ${}^{12}C$ ions, the most pronounced differences between irradiated and control animals were found in the prefrontal cortex, hypothalamus and nucleus accumbens that indicates the important role of these brain regions in late effects of heavy charged particles on the CNS functions. Comparison of neurochemical indices in animals of different age groups revealed a decrease in the intensity of temporal neurotransmitter changes in irradiated rats in all the analyzed structures except the striatum [1–3]. After irradiation with 60 Co γ -rays, the most significant modification of the dynamics of monoamine metabolism was observed in the prefrontal cortex, hypothalamus and hippocampus that indicates the sensitivity of these structures to γ -irradiation in doses of about 1 Gy. It was found that in the examined brain regions the effect of γ -rays has little effect on the overall direction of changes after irradiation, however, with respect to age, the nature of metabolism varies in many parameters. A comparison of the results obtained with ¹²C ions shows that the effect of γ -rays on the temporal dynamics of neurotransmitter metabolism is less significant compared with the action of heavy nuclei [4]. In the evaluated periods, proton irradiation caused pronounced changes in the hippocampus, nucleus accumbens and striatum, while in other structures they were less noticeable. At the same time, in the nucleus accumbens and striatum, according to some indices, the observed changes were similar to the effects of ¹²C ions, while in the hippocampus they differed from the effects of the other two types of irradiation.

The observed neurotransmitter metabolism patterns may indicate the presence of certain compensatory mechanisms being induced in response to irradiation and capable of partial restoration of limbic systems' functions. At higher LET values, the compensatory processes appear to be realized to a lesser degree, and the functional disturbances increase with time. At the same time, more significant alterations in the neurotransmitter appear to result in more intensive recovery processes, which may be the cause of changes in the normal dynamics of neurotransmitter metabolism.

References:

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5. HPRT mutation induction in V79 hamster cells after HZE ion exposure

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High charge and energy (HZE) ions have been used to study the induction of HPRT mutations in V79 Chinese hamster cells in prolonged times after irradiation. Heavy ions, for example in galactic cosmic rays, possess various energies and consequently various linear energy transfer (LET) values. Together with increased use of heavy ion therapy, the delayed effects of HZE ions are becoming more and more important.

In this study, we have compared the influence of three accelerated heavy ions: ${}^{18}O (E = 35.2 \text{ MeV/n})$, ${}^{20}Ne (E = 47.7 \text{ MeV/n} \text{ and } 51.8 \text{ MeV/n})$, and ${}^{11}B (E = 32.4 \text{ MeV/n})$ and gamma radiation of ${}^{60}Co$. LET of the HZE ions was in the range from 49 to 149 keV/µm. Induction of HPRT mutations was measured for the doses 0.5 - 2 Gy and 0.5 - 7 Gy for the heavy ions and gamma radiation respectively. The number of newly created HPRT mutations after irradiation was observed during every cell culture recultivation (in general every 3 days) - up to approximately 40 days (70 – 80 generations) after exposure.

It was found that the dependence of mutant fraction (MF) on expression time is a non-monotonic function with significant maximum for radiations with high LET (over 115 keV/ μ m); however, for low LET radiations, gamma rays in particular, this function is monotonically decreasing. The mutant fraction maximum was shifted towards longer expression times with higher LET. In the range from 49 to 149 keV/ μ m, the MF maximum position dependence on LET can be well described by an exponential function. The maximal value of MF was also increasing with increasing LET. The number of radiation induced mutants in different expression times was compared to the number and type of chromosomal aberrations in the same time periods. Some of the radiation induced mutants were selected for structural DNA analysis. First results suggest that around 30 % of samples contain deletion of at least 1 exon. Dose dependence of mutation yield and possible mechanisms explaining the effects of HZE ions are discussed as well.

6. Formation and repair of DNA double-strand breaks in brain cells of Spraque Dawley rats after gamma rays and proton irradiation

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Neurons are constantly exposed to endogenous and exogenous agents that induce potentially harmful DNA lesions. DNA double-strand breaks (DSBs) are widely considered the most cytotoxic form of DNA damage because their inefficient or inaccurate repair results in genetic rearrangements. Adult neurons can accumulate such unrepaired lesions during whole life, which can lead to brain dysfunctions and cognitive impairment. The aim of this research is to investigate DNA DSB repair in rat cerebellar cortex neurons. The cerebellar cortex has a unique, never repeating structure and is composed of several layers: the molecular cell layer, Purkinje cell layer, and granular cell layer. Each of these layers is formed by specific cell types involved in cerebellar circuits and is connected with the whole brain structure. The Purkinje neurons are one of the most important cell types in the cerebellar cortex which form the ultimate destination of the afferent pathways to the cerebellar cortex.

DNA DSB induction and repair were investigated in paraffin-embedded rat cerebellum tissues by immunohistochemical staining of DSB markers (γ H2AX and 53BP1 proteins) after exposure to ⁶⁰Co γ -rays and 170 MeV protons. Adult Sprague Dawley rats received cranial irradiation with ⁶⁰Co γ -rays or whole-body proton irradiation. For the dose correlation after irradiation with ⁶⁰Co γ -rays, the rats received doses of 1, 3, and 5 Gy and were analyzed 1 h post-irradiation (PI). For the time correlation after irradiation with ⁶⁰Co γ -rays at a dose of 3 Gy, the rats were analyzed 1, 4, and 24 h (PI); and for the time correlation after exposure to 1 Gy of protons, analysis was performed 1, 24 h, and 30 days PI. The kinetics of the formation and repair of radiation-induced colocalized γ H2AX/53BP1 foci was measured in Purkinje neurons of the rat cerebellar cortex. It is shown that the effective elimination of the induced foci occurred 24 hours after gamma and proton irradiation. The quantity of 53BP1/ γ H2AX foci decreased exponentially with time PI both for γ -rays and for protons. Also, a statistically significant difference in the 53BP1/ γ H2AX foci size after proton and γ -ray exposure has been observed: the proton-induced 53BP1/ γ H2AX foci are statistically larger than the γ -induced ones. The dose dependence of radiation-induced colocalized γ H2AX/53BP1 foci yield has been studied and found to be linear.

7. Formation of direct and enzymatic double-strand breaks of DNA under the influence of repair inhibitors after exposure to ionizing radiation of different quality

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Ionizing radiation induces a wide spectrum of DNA damage in living cells. The DNA double-strand breaks (DSBs) are the heaviest lesions of genetic structures. It is well known that as a result of energy deposition in molecular structures, DSBs of different types are induced, including direct and clustered ones. During the repair of different types of DNA lesions (base damage, single-strand breaks, AP sites), enzymatic DSB form from the single-strand breaks that appeared as an intermediate of the repair process.

Dose dependences of DNA DSB yield have been obtained for γ -rays and accelerated ¹¹B and ¹⁵N ions. Their linear character continues even in the presence of DNA repair inhibitors. Higher DSB yields were observed after irradiation by accelerated boron ions. It is shown that the number of DSBs reduces with post-irradiation time for all types of radiation. However, significant differences in DSB repair were observed in the presence of inhibitors. After γ -irradiation, the number of DSBs significantly increases for the samples with inhibitors. The modification ratio after irradiation with boron and nitrogen ions is considerably lower. It is shown that the repair curves largely depend on ionizing radiation LET. At low LET values, an increase in the DNA DSB level was observed; with increasing LET, the DSB level is reduced.

The results are also confirmed when calculating the radiation-induced foci (RIF) amount after γ -irradiation, both under normal conditions and in the presence of inhibitors. It is shown that under normal conditions, the maximum RIF amount is achieved 1 h after irradiation; after 4 h, most of the foci (~ 80%) are eliminated. In the presence of repair inhibitors, the RIF amount increases linearly.

8. Induction and repair of clustered DNA double-strand breaks after lowenergy ions irradiation

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The (in)ability of normal cells to repair DNA damage is largely dependent on the initial extent of DNA damage. Energy and charge of the particle play the principal role in spatial distribution of ionizations, and thus in the DNA damage. In presented study, we evaluated impact of different track structure of various low-energy ions with similar LET on DNA double-strand breaks (DSBs) induction and repair.

Immunostaining of yH2AX and 53BP1 proteins (markers of DSBs) was carried out on normal human fibroblasts irradiated with low-LET y-rays and three representatives of high-LET radiations: ¹¹B (LET = 138 keV/ μ m, E = 8 MeV/n), ¹¹B $(\text{LET} = 45 \text{ keV}/\mu\text{m}, \text{E} = 32 \text{ MeV/n})$, and ²⁰Ne $(\text{LET} = 132 \text{ keV}/\mu\text{m}, \text{E} = 47 \text{ MeV/n})$. By quantifying the current number of yH2AX/53BP1 foci the curve of formation and disappearance of foci that reflects the DSBs rejoining kinetics was measured. Our results showed that repair kinetics is highly dependent on the radiation quality – the higher the LET, the slower yH2AX/53BP1 foci elimination was observed, indicating production of complex DSBs that are more difficult to repair. Comparison of radiations with similar LET values (~135 keV/µm) - 47 MeV/n neon and 8 MeV/n boron ions revealed formation of significantly more persistent foci after neon in comparison to boron ions irradiation. More detailed studies of yH2AX/53BP1 foci morphology were done on cells irradiated at low angle of 10° to maximize the resolution power of foci separation by confocal microscopy. The analysis of yH2AX/53BP1 foci size, circularity $[\pi^*(\text{area/perimeter}^2)]$, and complexity correlated with the above described results and further characterized induced DNA damage. Foci induced by ions were bigger, less circular, and composed of more closely spaced (sub)foci within one γ H2AX/53BP1 foci cluster. All the parameters were time dependent as well, and they were reaching the maximum usually at 4 h post-irradiation. Depending on the physical parameters of radiation (energy, charge of particle), the different microdosimetric patterns of energy dissipation along the particle track affected the extent and arrangement of DNA damage (complexity) and thus also its reparability.

9. Structure Induction and repair of DNA double-strand breaks in hippocampal neurons of mice of different age after exposure to ⁶⁰CO γrays *in vivo* and *in vitro*

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One of the central problems of modern radiobiology is the study of mechanisms of induction and repair of DNA damages in central nervous system cells, in particular, in hippocampal cells. The study of the mechanisms of formation and repair of molecular damage in the hippocampus nerve cells is of special interest, because these cells, unlike most cells of the central nervous system, keep proliferative activity, i.e. ability to neurogenesis. It was found that the hippocampus plays a key role in the formation of long-term memory, in the integration of brain information and its distribution in the higher brain regions. Age-related changes in hippocampus play an important role, which could lead to a changes of radiosensitivity in neurons to the ionizing radiation exposure.

With a DNA comet assay, regularities have been studied in the induction and repair of DNA double-strand breaks (DSBs) in hippocampal neurons of mice of different age *in vivo* and *in vitro* after exposure to 60 Co γ -rays. The obtained dose dependences of DNA DSB induction are linear both *in vivo* and *in vitro*. It is established that in young animals' neurons, the degree of DNA damage is higher than in older animals. It is shown that repair kinetics is basically different for exposure *in vivo* and *in vitro*.

10. Semax neuropeptide effects on behavioral trying reaction and skeletal musculature strength indicators in proton-exposed mice

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Outbred ICR (CD-1) female mice of the SPF category were exposed to natural Bragg peak protons at doses of 2.3 and 3 Gy. The Semax preparation, introduced in multiple doses during 7 days, statistically significantly retains the indicator intensity of the orientative-trying reaction (OTR), which was disordered in animals exposed to accelerated protons at a dose of 3 Gy. The preparation also normalizes the emotional status (ES) indicator on the 7th day after exposure at both doses. After exposure, the preparation statistically significantly retained the proportion of the central nervous system excitation and inhibition processes, which was evaluated by the OTR/ES ratio.

It has been found that irradiation at doses of 2.3 and 3 Gy decreases the skeletal musculature strength indicator to 81.3—84.6% of the control level, and using Semax recovers the indicator up to 93.1—101.1% of the control level.

11. Computer simulation of DNA damage induction after heavy ion irradiation

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Studying of DNA damage induction after heavy charged particle irradiation in mammalian cells is one of the topical problems in radiobiology. The application of computer simulation in this field assists in better understanding of key molecular interactions underlying cell response to the ionizing radiation. In this work we proposed a model approach to simulate the induction of different types of DNA damages in the cell after exposure to heavy ions. The spatial structure of charged particles tracks was simulated using Geant4-DNA software toolkit. The distributions of energy deposition events and absorbed dose on the scale of the cell nucleus after the action of different types of heavy ions in a wide range of LET were calculated. A quantitative estimation of primary DNA lesions induction and their spatial distribution according to the particle track structure was obtained.

12. Morphological changes in purkinje cells of rat's cerebellum cortex following irradiation with carbon ¹²C ions

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Investigations into the effects of heavy charged particles (HCPs) of galactic space radiation on the central nervous system (CNS) of cosmonauts are crucial for evaluation of radiation risks in exploration class missions. Cytological studies of rat's cerebellum irradiated with carbon ¹²C heavy ions with the energy of 500 MeV/nucleon were performed as part of a model experiment at the Joint Institute for Nuclear Research (JINR) in Dubna. Exposure of animals to HCPs increased reliably the relative number of destructively modified neurons in the cerebellum cortex. Dynamics of morphological changes points to more severe CNS effects from heavy ions.

13. The effect of low doses of ionizing radiation on the mouse retina

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The retina is characterized by relatively high resistance to radiation, which is provided by its ability to repair damages caused by environmental factors. It is known that the effect of accelerated protons in doses more than 25 Gy lead to noticeable morphological and functional changes in the retina of the eye.

Researching of the mechanisms of exposure of various types of ionizing radiation on the mouse retina is fundamental importance for investigating the danger in the course of prolonged manned spaceflight. Flying to Mars, astronauts will be affected by various radiation sources - solar and galactic cosmic rays. In this case, the reaction of the retina to the radiation exposure may not appear immediately, but in length of time. In this connection, the effect of small doses of ionizing radiation (neutron fields of the phasotron, accelerated protons and gamma-rays) on the retina of the mouse eye in a remote period of time after irradiation was studied. It was shown that irradiation with accelerated protons, gamma-rays and in neutron fields of the phasotron at a dose of 1 Gy doesn't lead to a functional change in the retina of the mouse eye (the observation period is up to 6 months). It is assumed that with an increase the dose (5 Gy) and the observation time (8 months) an important role in the radiation effect on the retina plays the radiative damage of the blood vessels.

14. Microfossils in the Orgueil (CI1), Murchison (CM2) and Polonnaruwa (C-Ungrouped) meteorites imaged at the astrobiology sector, Laboratory of Radiation Biology, JINR

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The Tescan Vega 3 Scanning Electron Microscope of the Astrobiology Sector of Laboratory of Radiation Biology of the Joint Institute for Nuclear Research is now operational. This new instrument has been used to continue the search for evidence of the fossilized remains of indigenous microorganisms in carbonaceous meteorites. Samples that were initially studied include the Orgueil CI1 carbonaceous meteorite (France, May 14, 1864); Murchison CM2 (Australia, Sept. 18, 1969) and the Polonnaruwa (C-Ungrouped) stones that were observed to fall in central Sri Lanka on Dec. 29, 2012 and collected in-situ by R. Hoover soon after the fall. These meteorites have been found to contain a wide variety of well-preserved remains of cyanobacteria, diatoms, prasinophytes, tasmanites, and other microorganisms, some of which could be identified to genus and species. During the initial checkout phase of the Tescan Vega 3, the frustules of pennate diatoms were found in a freshly fractured interior surfaces of the Orgueil meteorite. This represents the first detection of diatoms in the Orgueil meteorite. Cyanobacterial filaments, prasinophyte algae, possible tasmanites, framboids and stacked platelets of magnetite were also found in Orgueil. Modern terrestrial epiphytic diatoms collected by R. Hoover in California in 1972 and cleaned by A. L. Brigger were imaged and analysed to evaluate the imaging properties of the new Tescan Vega 3 SEM and extremely high quality 2-D and 3-D images of these spectacular marine diatoms were obtained.

15. Induction of forward gene mutations, base-pair substitutions, rearrangements of chromosome and deletion of plasmid DNA by heavy ions ¹¹B in eukaryotic yeast cells

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In connection with the active space exploration the studies of the effects of heavy ions are currently of particular interest. In this work the heavy-ion accelerator U-400M (Dubna, JINR) was the source of accelerated ions. Cells have been irradiated by accelerated boron ions with the energy of 12 MeV/n (LET 101 keV/ μ m), 22 MeV/n (LET 61 keV/ μ m), and 34 MeV/n (LET 42 keV/ μ m) and also by the γ -rays ⁶⁰Co. For analysis of mutagenic action of ionizing radiation on eukaryotic cells we used the set of yeast genetic systems which allowed us to detect different types of gene or structural mutations.

In the case of eukaryotic yeast cells the relative biological effect (RBE) depended on linear energy transfer (LET) as a function with a local maximum. The heavy ion irradiation with a LET~60-80 keV/ μ m turned out to be most efficient for wide spectrum of mutations. The maximum of RBE depended from the nature of events. For lethal effect the maximum of RBE (~3) corresponded to LET~80 keV/ μ m. For ectopic recombination and deletion in plasmid the maximal RBE (~2 and ~7) corresponded to LET~60 keV/ μ m. The point mutations had either maximal value (~2) for base pair substitution at LET~60 keV/ μ m or plateau with following decreasing in the case of forward gene mutation (Can^R).