Radiation Neuroscience

Radiobiological Research at JINR Accelerators

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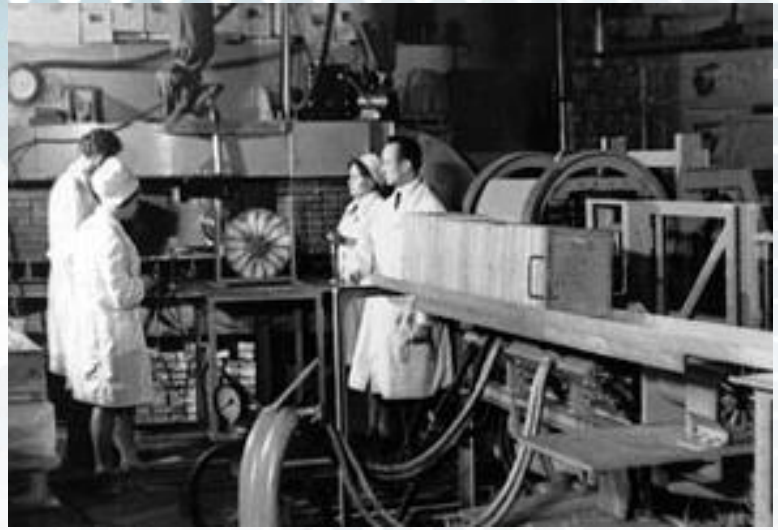
JINR and LRB

www.lrb.jinr.ru

JINR

- 1956 - JINR established
- 7 laboratories
- 18 collaborating countries and 6 states

Founder: acad. Prof. E. A. Krasavin



LRB

- 1959 - first radiobiological experiments (synchrocyclotron, LNP)
- 1978 - Biological Research Sector
- 1988 - Biological Division at DLNP
- 1995 - The Department of Radiation and Radiobiological Research
- 2005 - Laboratory of Radiation biology

Director: Prof. Dr. A. N. Bugay

Main theme of research:

The biological action of heavy charged particles of different energies

- ***Department of Radiation Biology and Physiology:***
- Molecular Radiobiology Sector
- Radiation Cytology Sector
- Radiation Physiology Sector
- Radiation Neurochemistry Sector
- Mathematical Modeling Sector
- Radiation Genetics Group

- ***Department of Radiation Research:***
- Group for Modeling of Ionizing Radiation Interaction with Matter
- Group for Studying Radiation Fields of JINR's Basic Facilities and Environment

- ***Astrobiology Sector***

Website: **lrb.jinr.ru**
E-mail: **lrb@jinr.ru**

Accelerators used in radiobiological research

Phasotron, protons 170 MeV

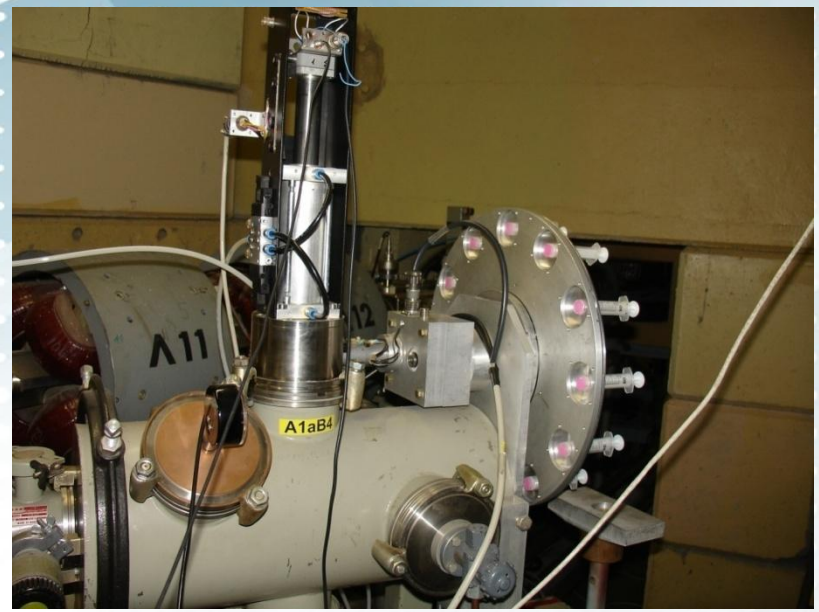
Nuclotron, ^{12}C 500 MeV/amu

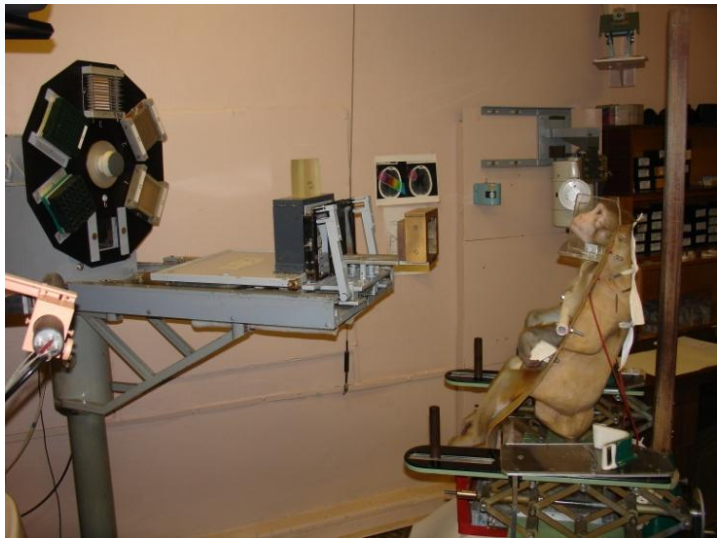
U-400M, up to 50 MeV/u Li, B, Ne ions

Radiobiological experiments:

X-rays – cell and animal irradiators (LRB)
Protons – phasotron (DLNP)
Neutrons –IBR2 reactor (FLNP)
Low energy heavy ions - U-400M cyclotron (FLNR)
High energy heavy ions –Nuclotron (VBLHEP)

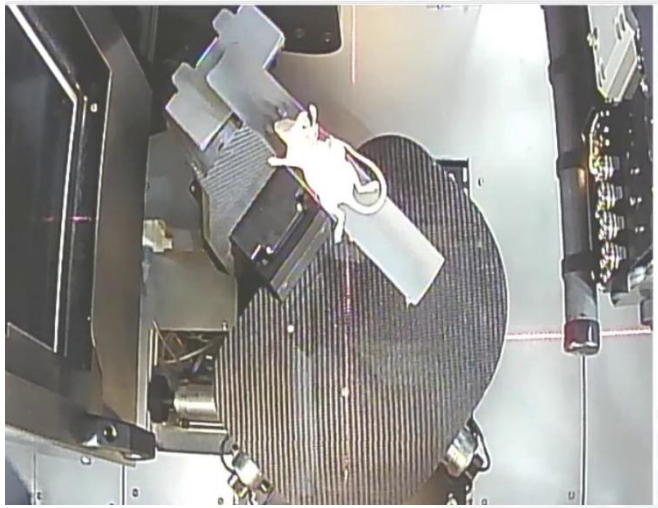
Stationary setup ("Genom") at the U-400M cyclotron for fast automatic irradiation of thin biological samples with high LET heavy ions (Li, B, O, N, Ne to 50 MeV/n) in a wide range of absorbed doses →





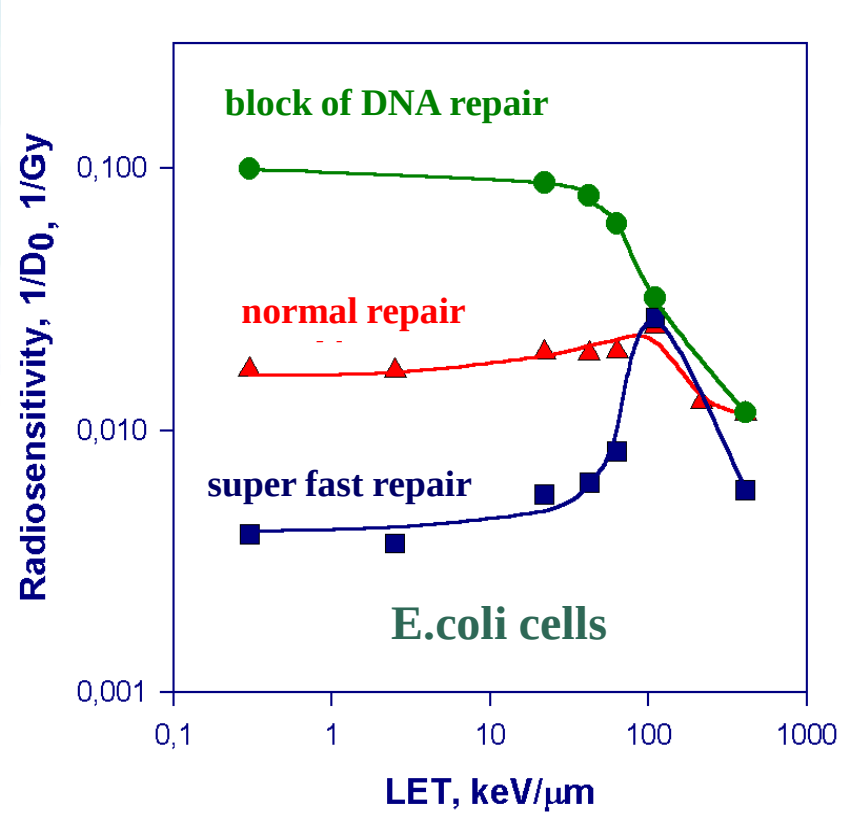
Irradiation of monkey's brain by 170 MeV protons at medical beam of DLNP phasotron

Irradiation of monkey's brain by 500 MeV/n ^{12}C ions and 2,56 GeV/n ^{78}Kr ions at Nuclotron



Precise conformal irradiation of rats by X-rays

Central Problem of Radiation Biology - biological effectiveness of radiation with different physical characteristics



RBE value is determined by two factors - the physical and biological.

The biological factor is dependent on the physical.

DNA damage caused by photon and hadron radiation is qualitatively different

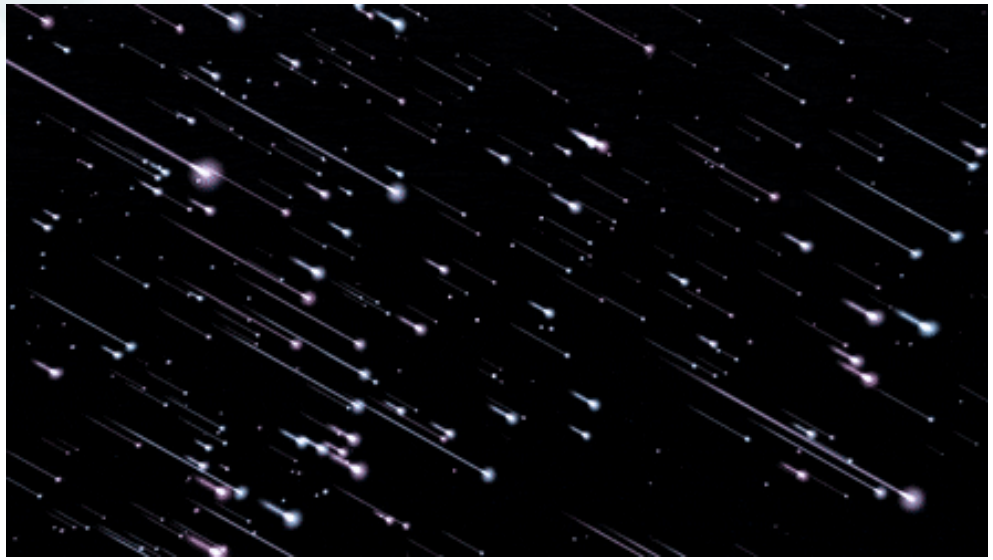
Красавин, 1984, 1989

Relative biological effectiveness of charged particles
Radiation-induced mutagenesis

JINR since 1959

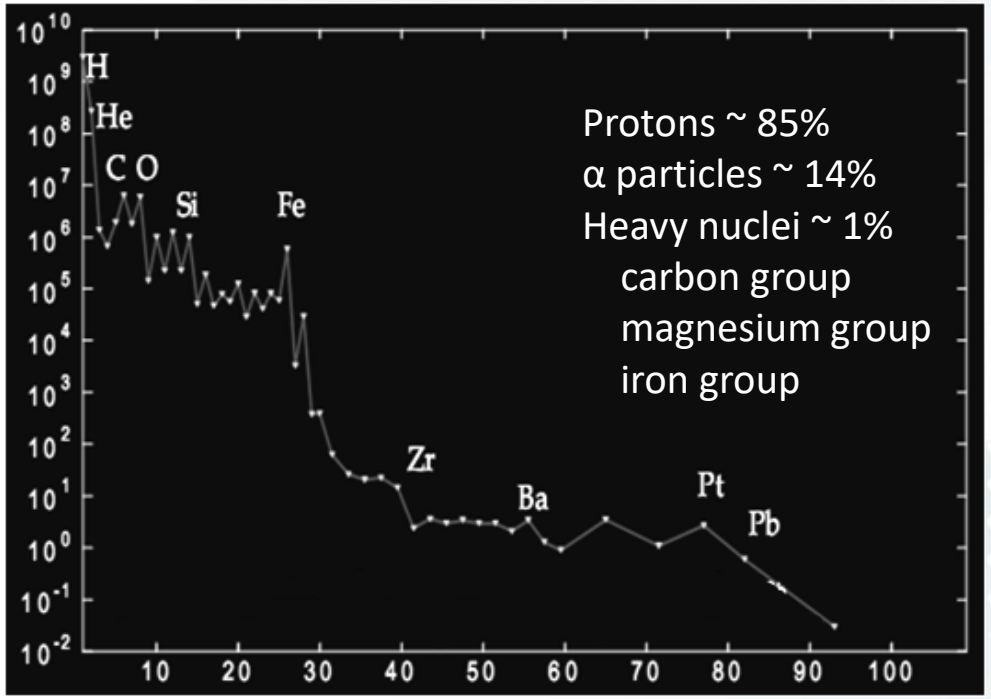
Focus: Space Radiobiology
Radiation risk in deep space

JINR since 1959



^{1}H to ^{56}Fe
100-10000 MeV/n
10^{6} cm^{-2} per year

Galactic Cosmic Rays



Charge spectrum of GCR particles

Cosmic radiation

- Solar energetic particles, galactic cosmic rays (protons - 92%, nucleus of helium atoms - 7%, heavy nuclei), stardust (exploding stars)

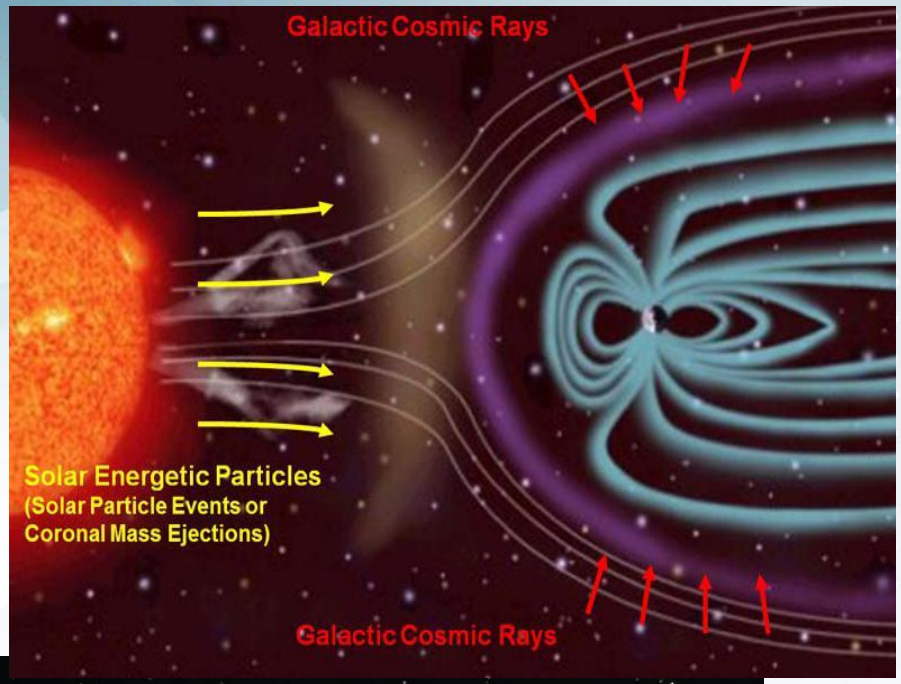
- Fully ionized nuclei + Secondary neutrons and charged particles - major sources of radiation exposure in an interplanetary spacecraft

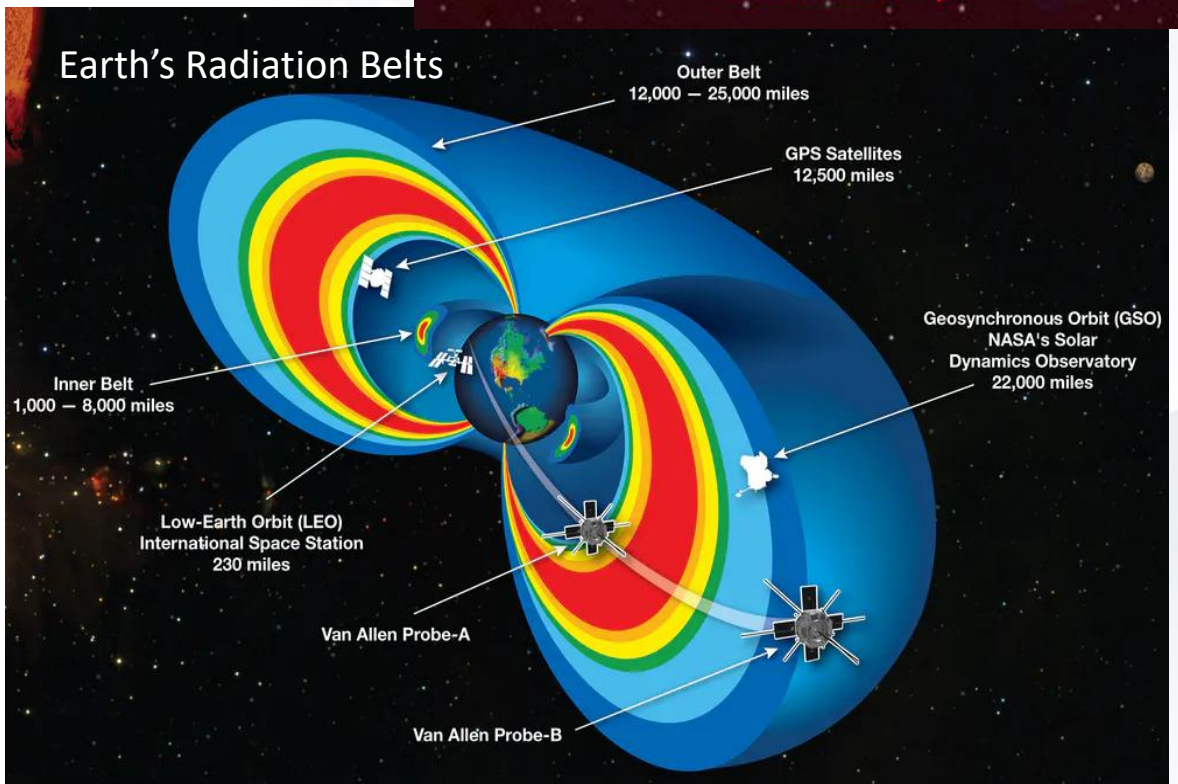
- solar particle events, solar storms

- Omnidirectional

- 10 ^ 8 - 10 ^ 21 eV

- Solar cycle modulation (11 years)





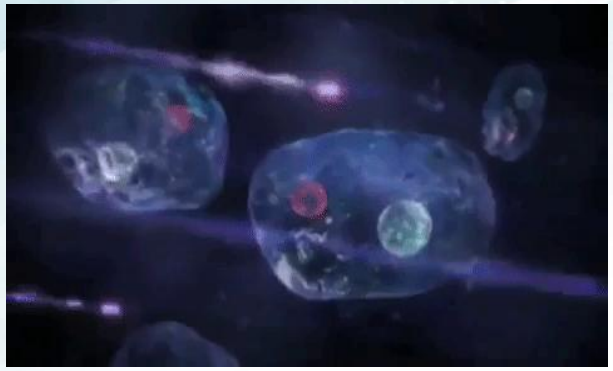
Radiation exposure of human organism

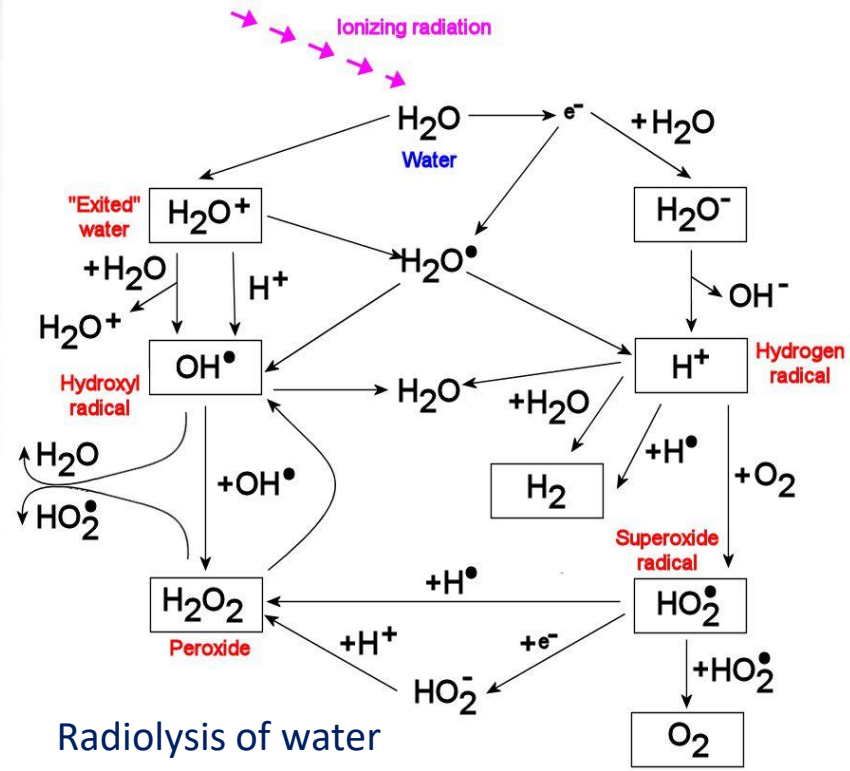
- **Earth: 0.1 µSv/h; 1 mSv/year**
- 0.1 mSv radiography chest, plane Brussels - Tokio
- 7 mSv CT chest
- 100 mSv 6 months at the ISS
- Astronauts on a mission 50 to 2,000 mSv
- Long flights: up to 10 µSv/h
- ISS: 20 µSv/h; 100 mSv/6 months
- Mars: 25 µSv/h; 300 mSv/500 days
- **Deep space: 75 µSv/h; 300 mSv/180 transit days**

- **200 mSv +1% cancer risk**
- **10000 mSv severe damage, death**

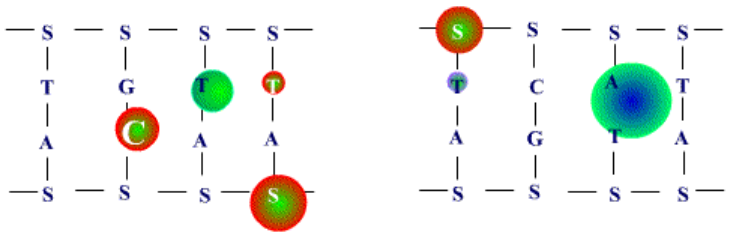
The molecular basis of cell death

Cell damage – free radicals formation – DNA damage, double-strand breaks – cell death/ mutations





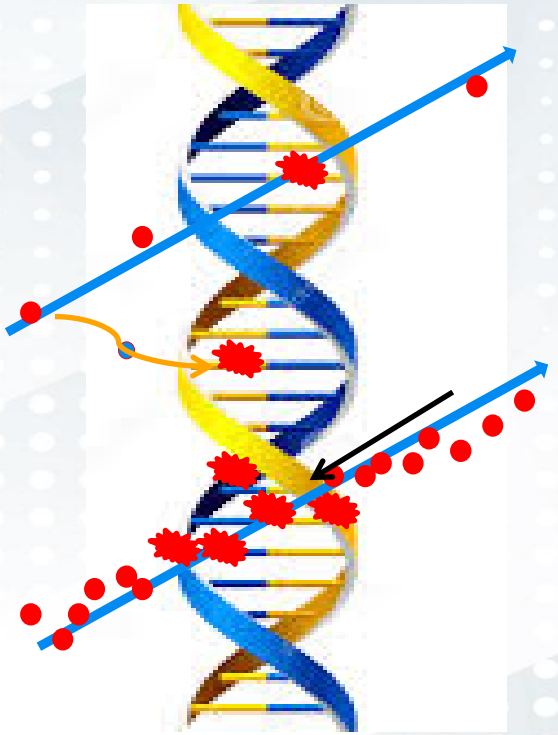
Radiolysis of water



Clustered double-strand break

low - LET

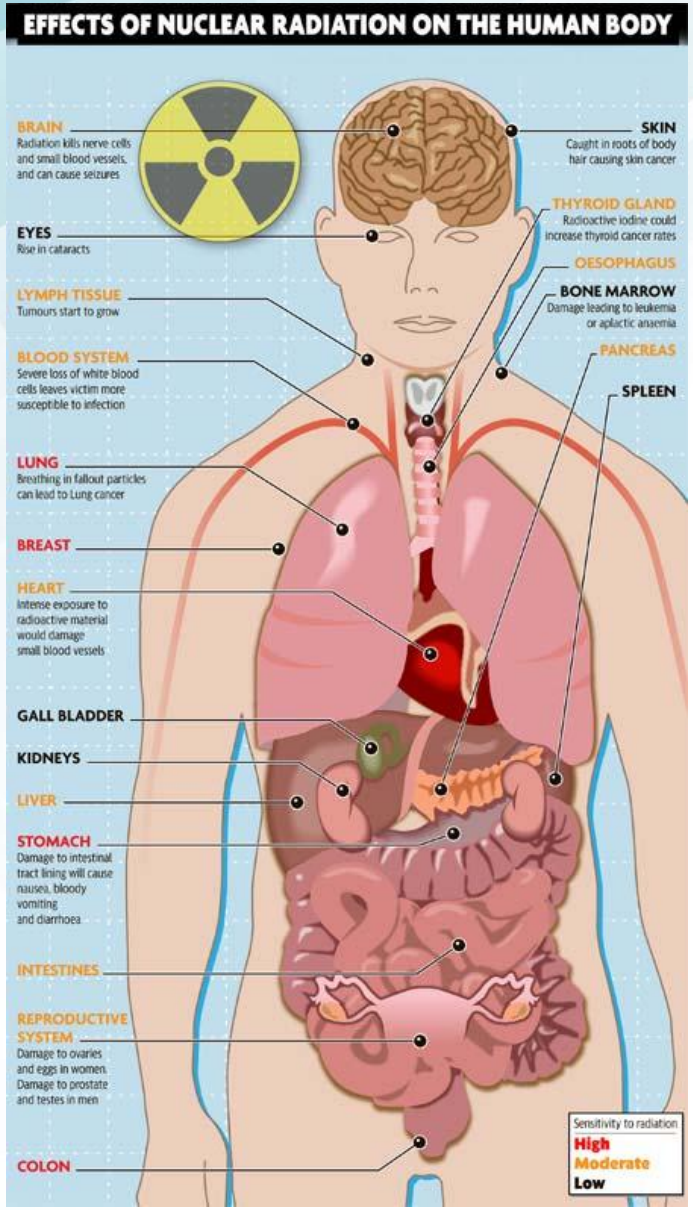
high - LET



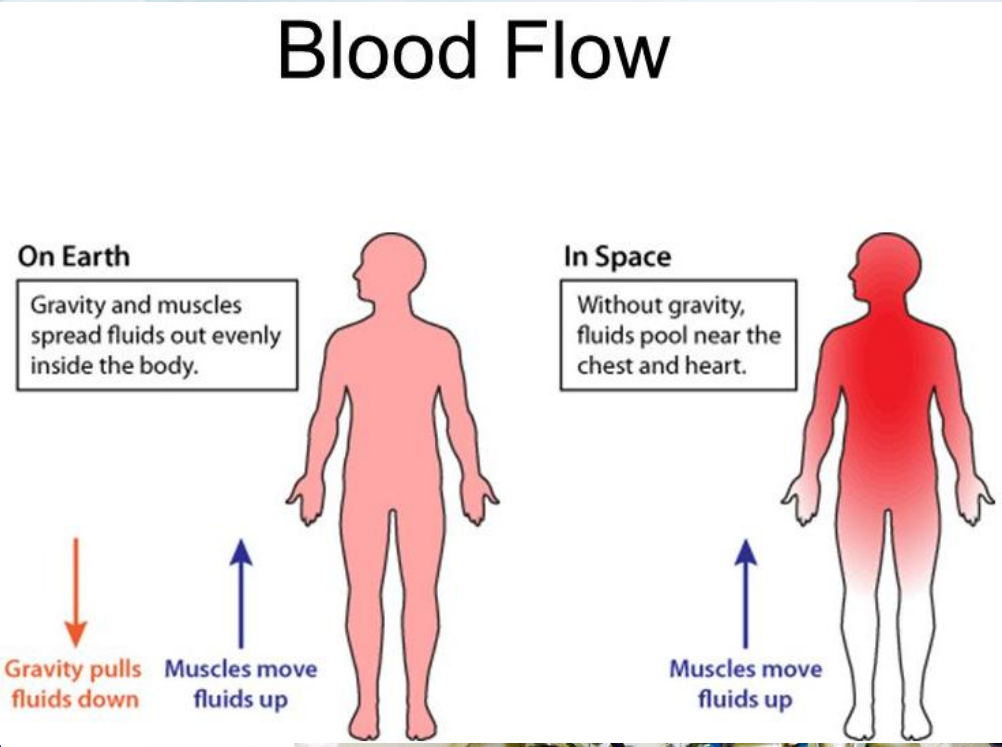
ionization

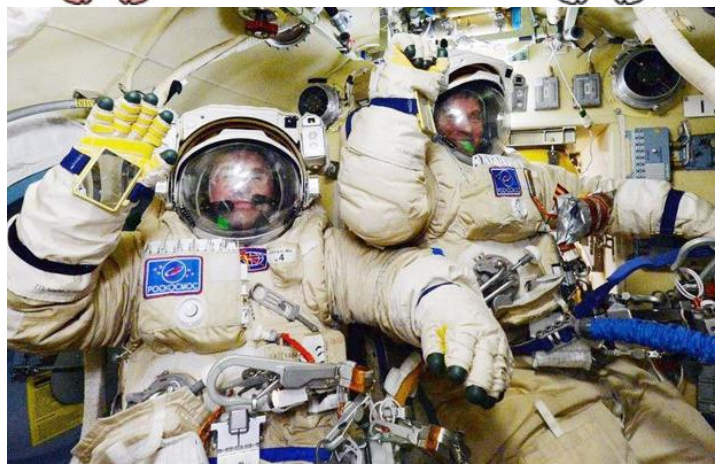
DNA lesion

Radiation effects on organs and CNS
During the cosmic flight

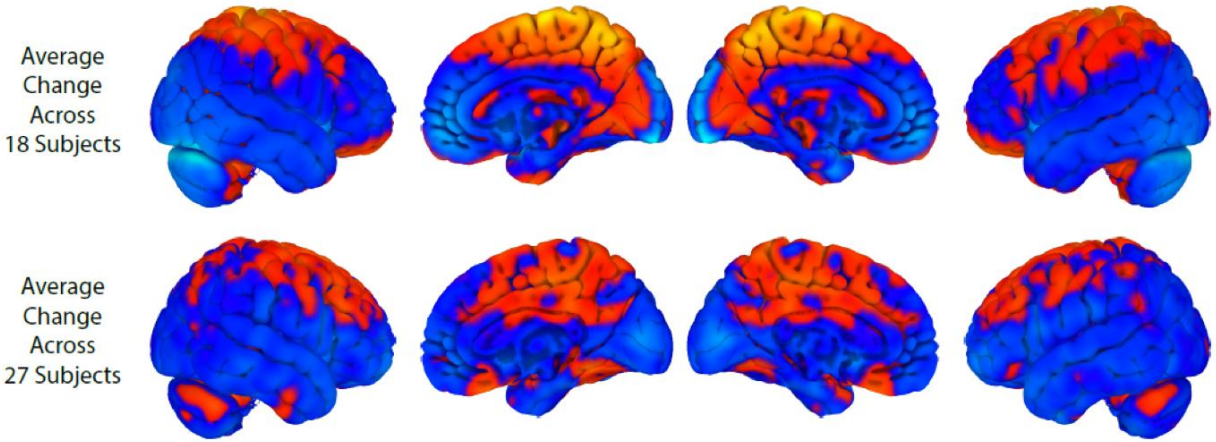


- **stress and psychological changes**
- space adaptation syndrome
- muscle atrophy
- bone loss
- **cardio-vascular problems**
- immunity changes
- skin problems
- changed sleep patterns
- changes in microflora
- digestive problems
- motion sickness
- water retention, kidney function lowers
- **cancer + cataract risk**
- **changes in brain activity, decreased neurogenesis, altered neuron morphology reduced neuronal connectivity, oxidative stress, onset of neurodegenerative diseases…**



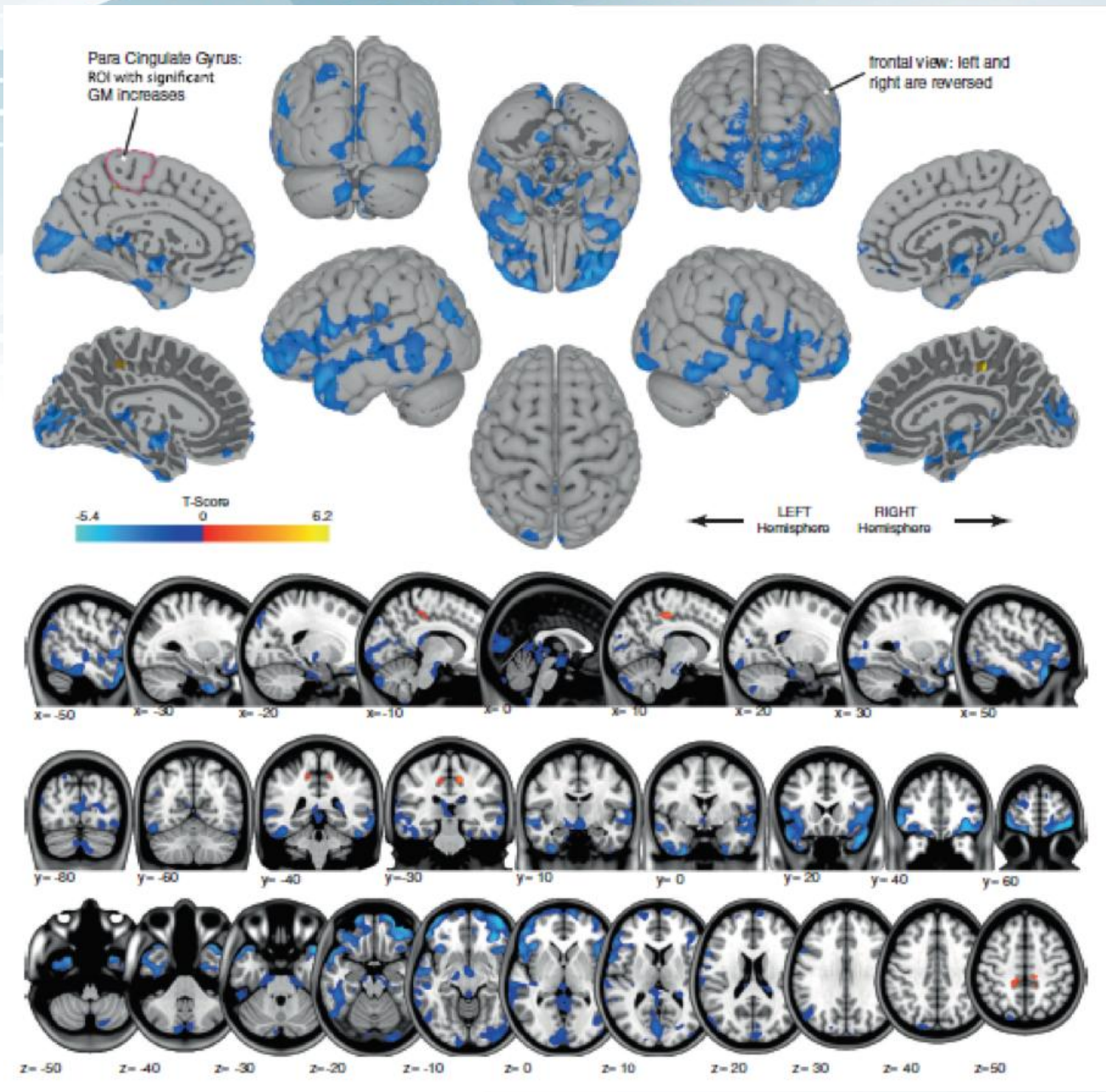


The structural brain changes in astronauts



MRI scans from 27 astronauts,
NASA Lifetime Surveillance of Astronaut Health

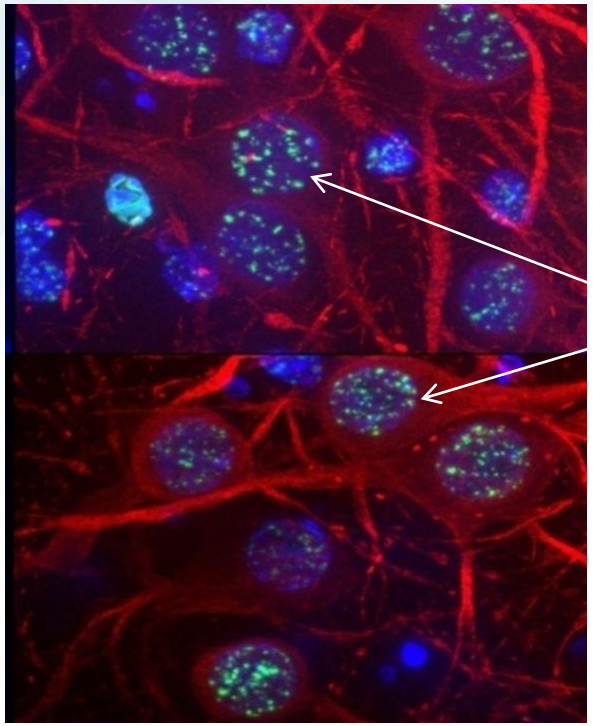
Koppelmans et al., 2016

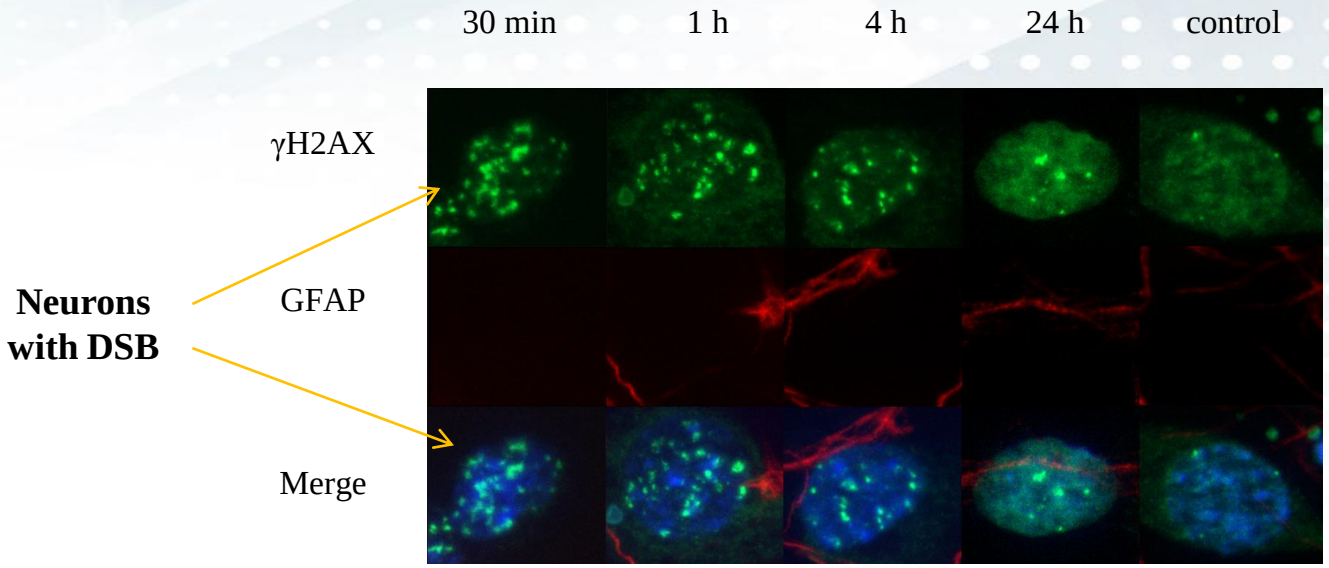


Molecular Radiobiology

DNA damage formation in a brain tissue culture after irradiation

Visualization of damaged sites in DNA



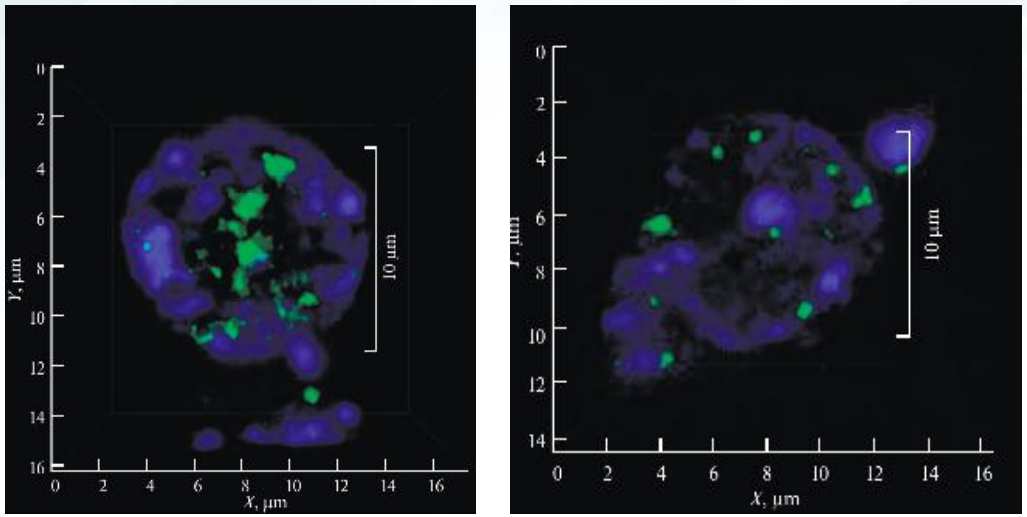


DNA double-strand break (DSB) repair kinetics in hippocampal neuron culture after exposure to 3 Gy of γ-rays.

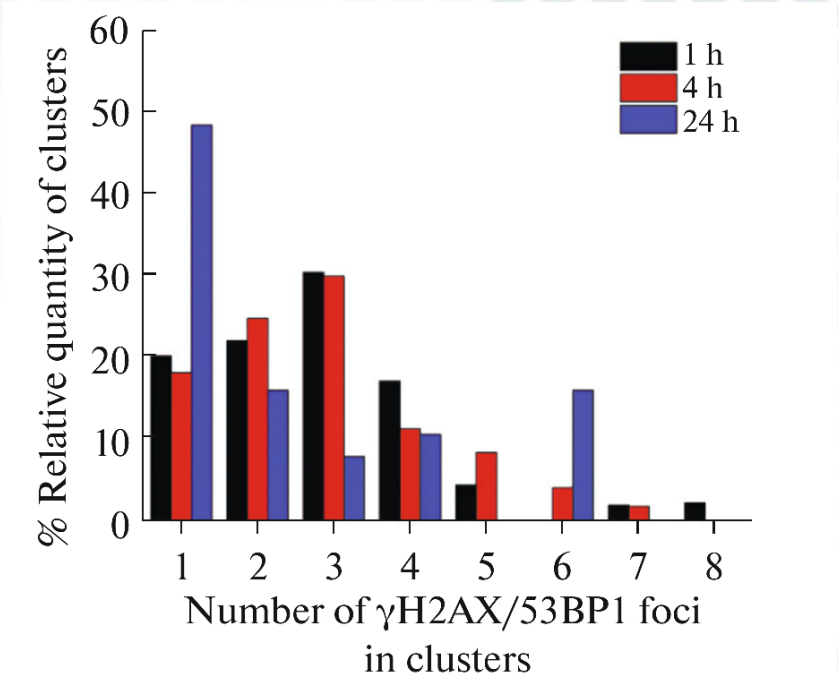
Formation of DNA Double-Strand Breaks in Rat Brain Neurons after Irradiation of rats with Krypton Ions

krypton ions

protons



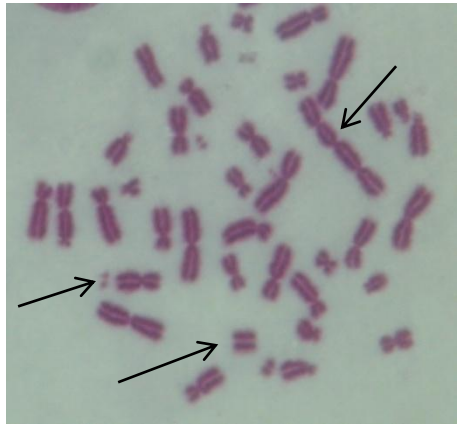
DNA damage visualization in a dentate gyrus cell



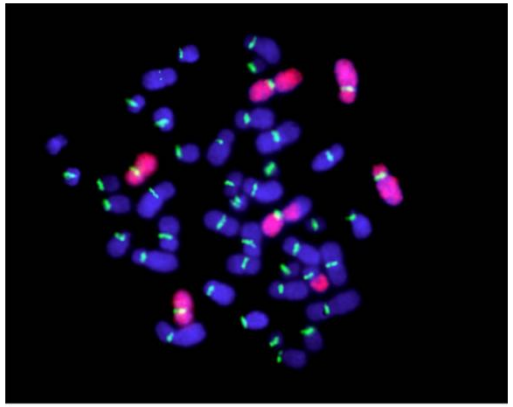
Boreyko, Bulanova, et al, 2019

Radiation Cytogenetics

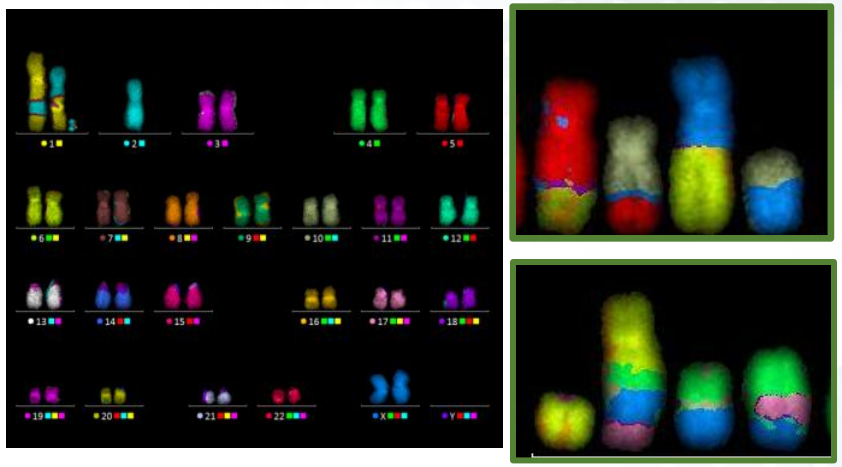
- Evaluation of biological efficiency of particle beams at JINR facilities by cytogenetics methods
- The effects of low-dose radiation exposure
- **Long-term consequences of radiation exposure**
- **Evaluation of complex chromosome aberrations induction**



visualization of chromosome aberrations by metaphase method

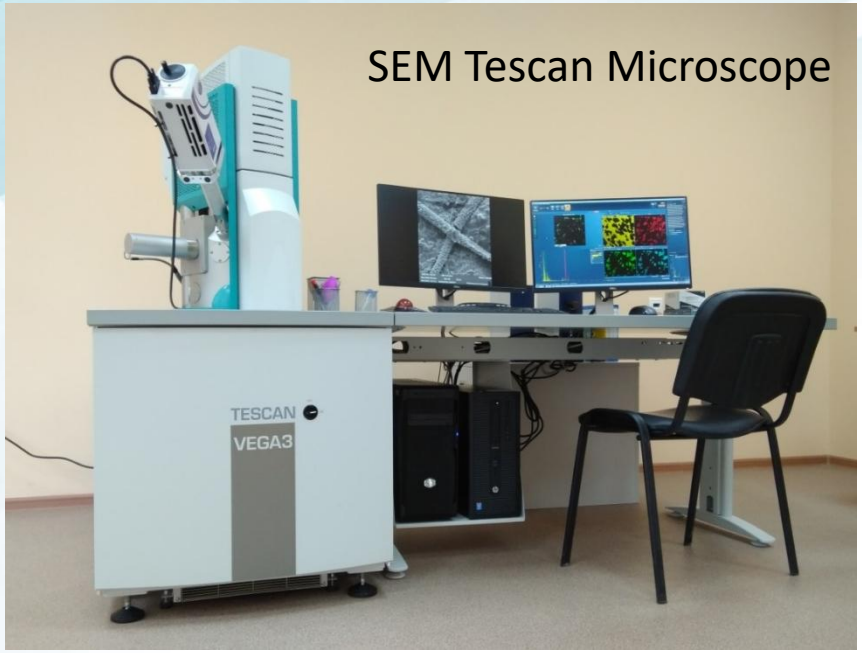


visualization of chromosome aberrations by FISH method



visualization of complex chromosome aberrations by mFISH method

Astrobiology

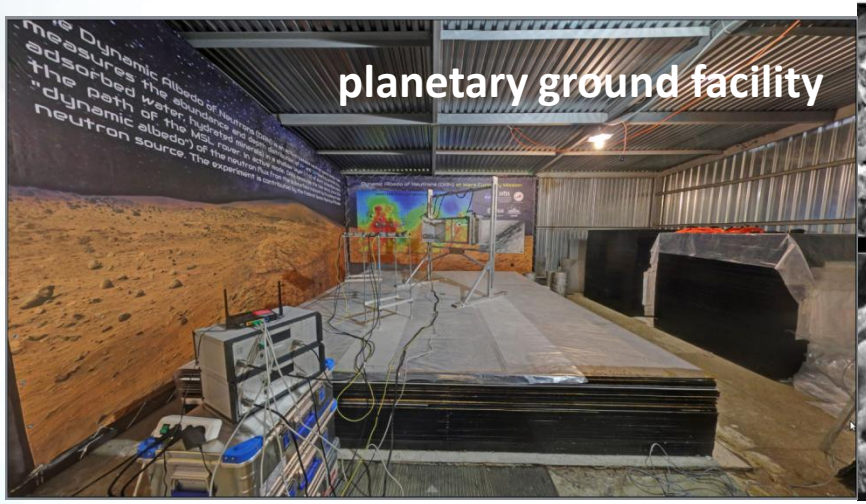


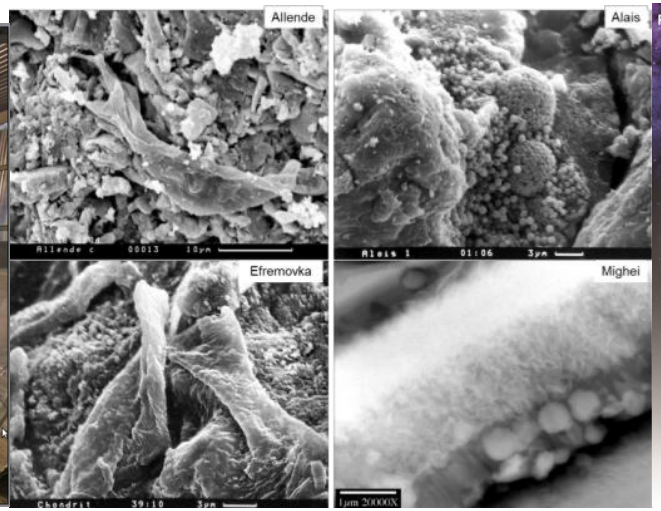
- Biogeochemical studies of cosmic matter and cosmic dust
- Studies of biofossils and organic compounds in meteorites
- **Synthesis of prebiotic compounds** from “formamide + meteorite matter” under particle exposure

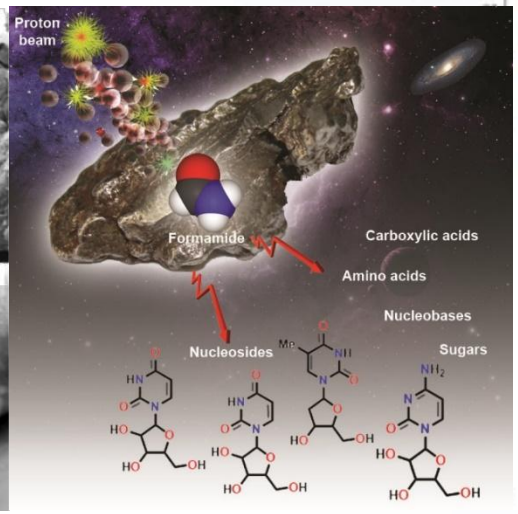
<u>Tasks:</u> Life formation in space

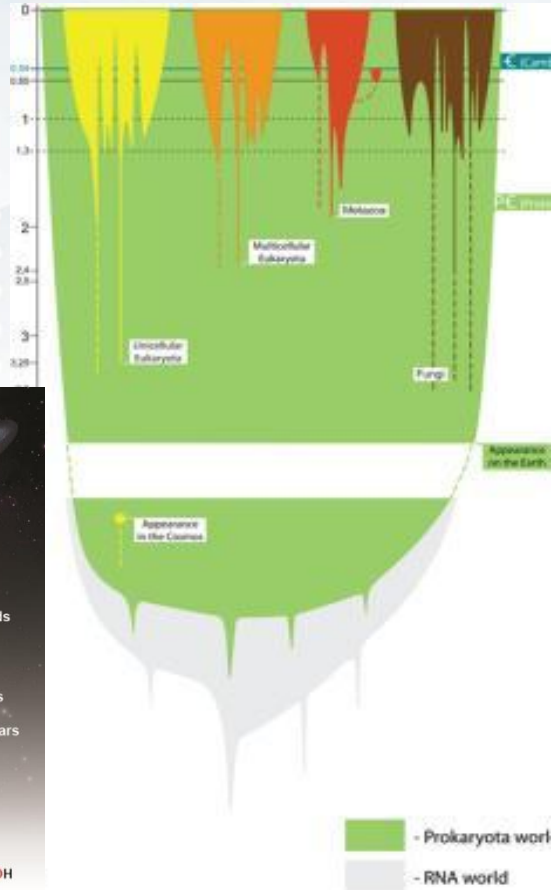
Synthesis of biomolecules: nucleosides, nucleotides, oligo- and polymer molecules

- **search for biofossils in meteorites**

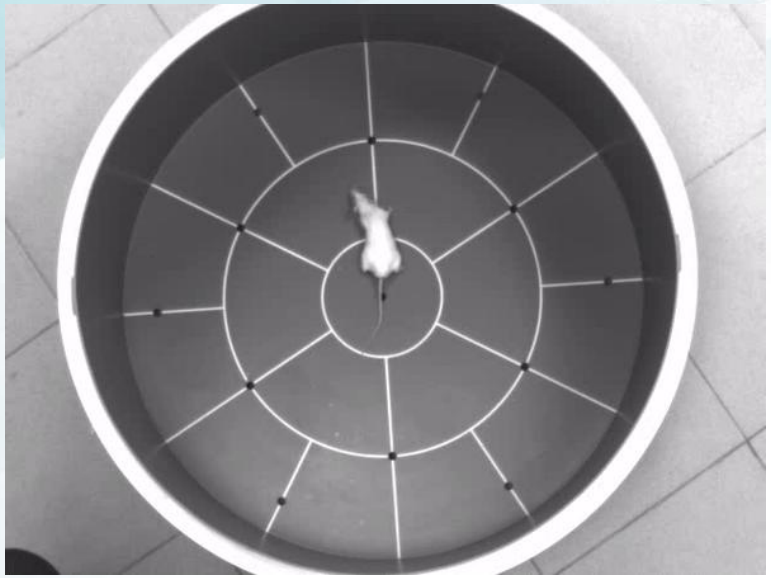




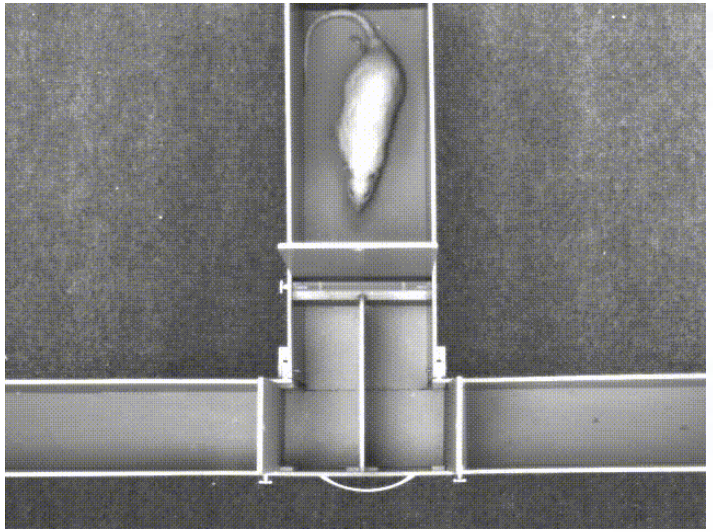


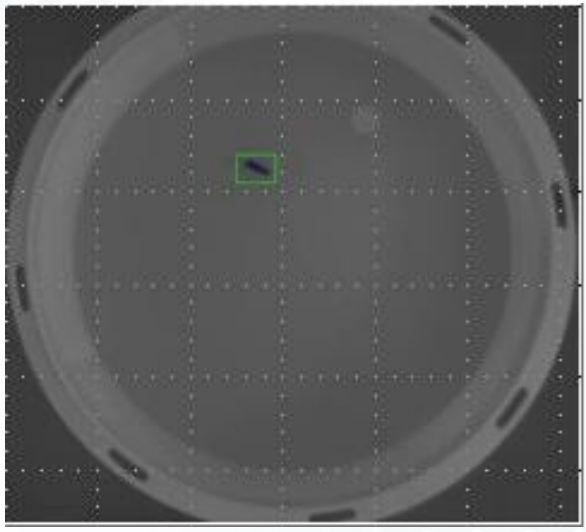


Sector of Radiation Physiology



- study of behavioral disorders in irradiated animals
- pathomorphological changes in various structures of the brain and spinal cord, and other organs
- hematological tests
- study of radioprotective and radiosensitizing properties of pharmacological drugs

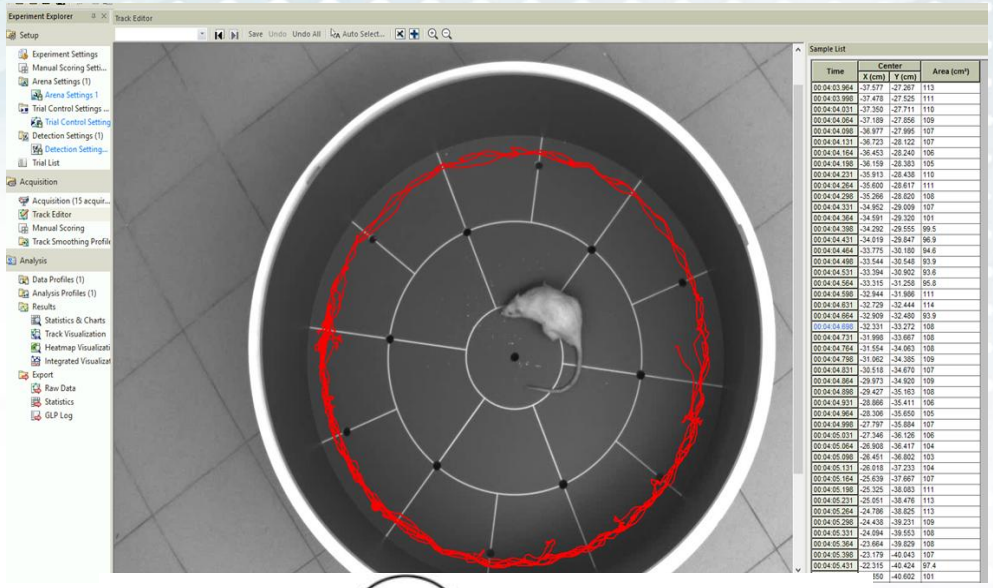




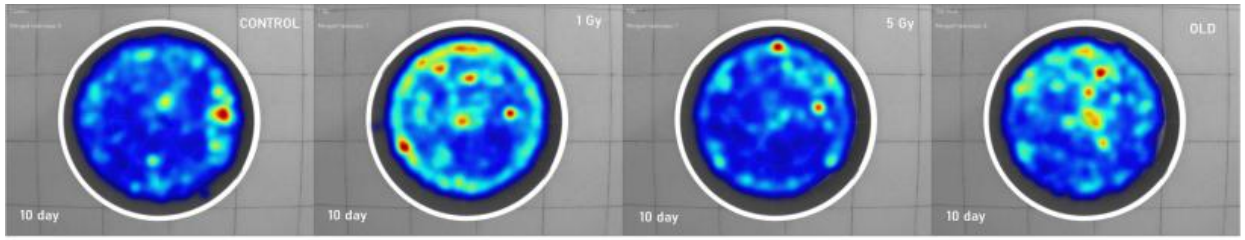
Radiation Physiology

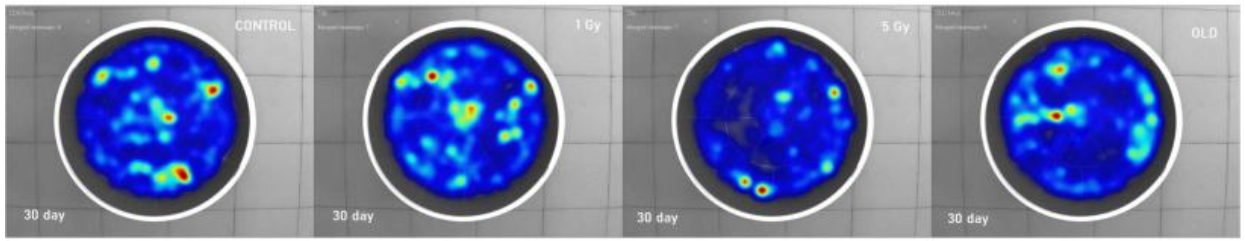
Study of behavioral reactions and memory changes after IR

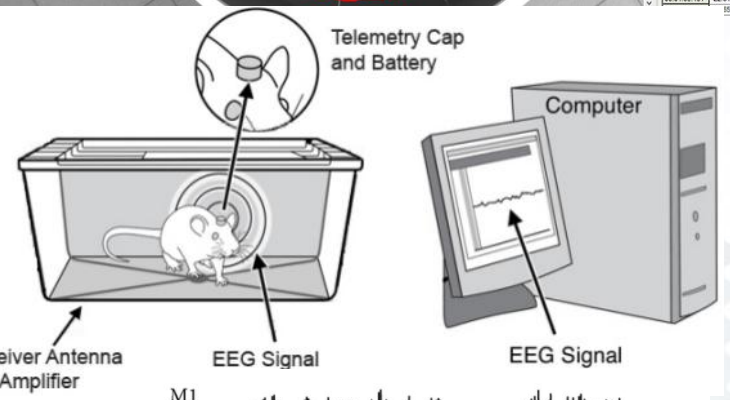
- Open field
- T - maze
- Morris water maze
- Barnes maze



Video-tracking information system







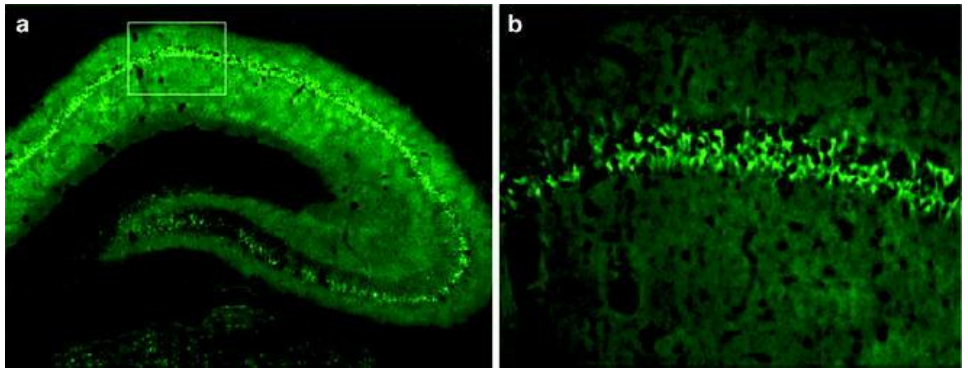
wireless EEG system

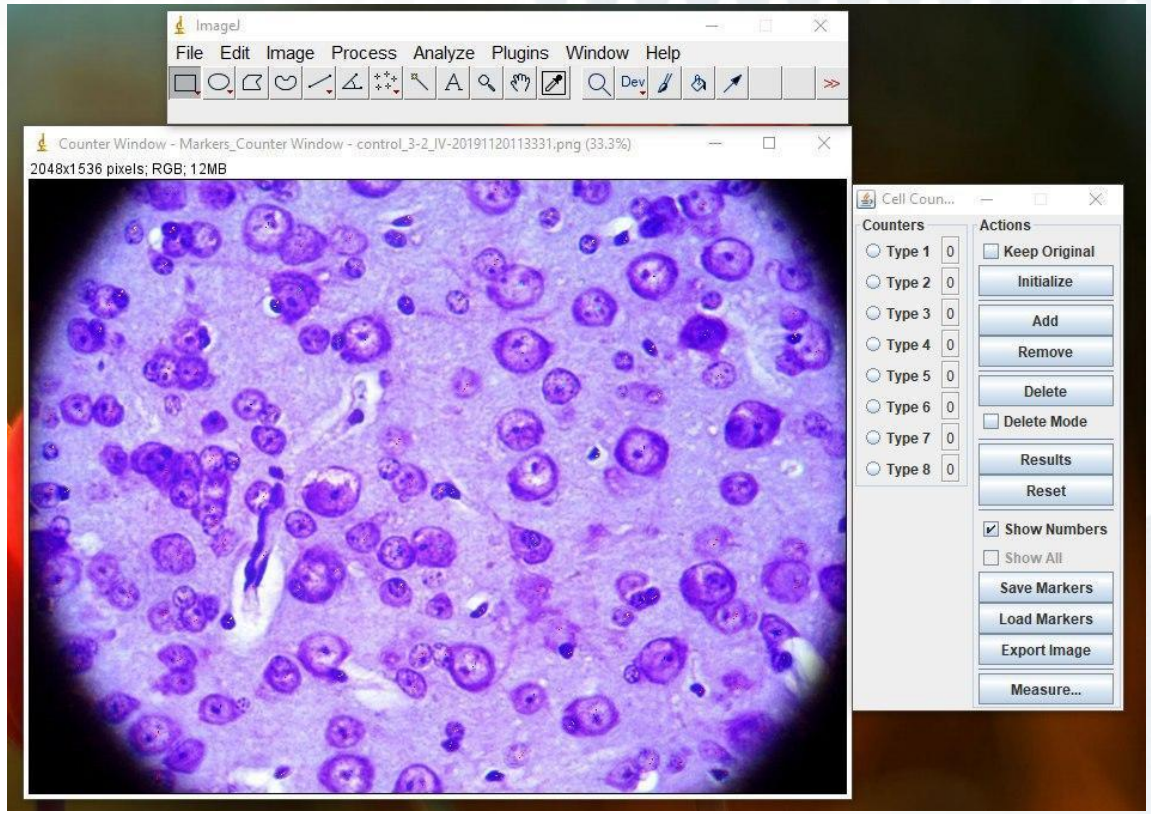
Histological analyses

- methods of anesthesia, electrophysiological and hematological analysis, perfusion of internal organs of laboratory animals
- study of pathomorphological changes in tissues
- histological and immunohistochemical methods on light and fluorescent microscopic equipment

Nissl staining

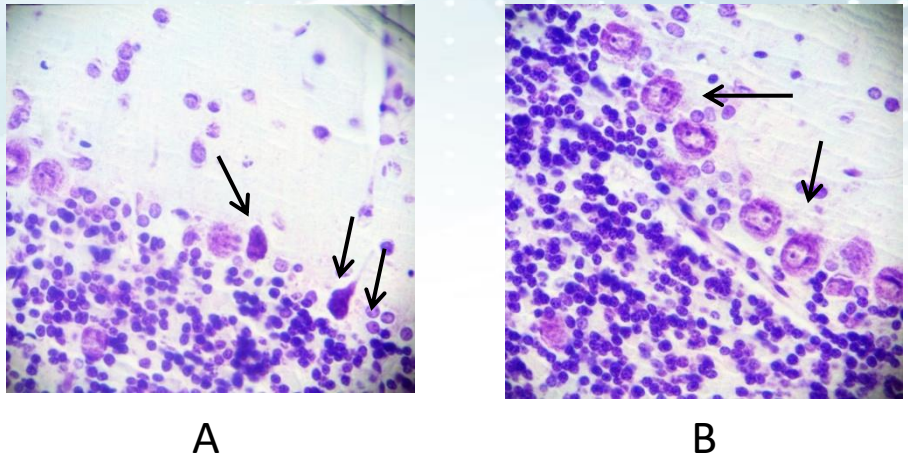
Fluoro Jade B





ImageJ

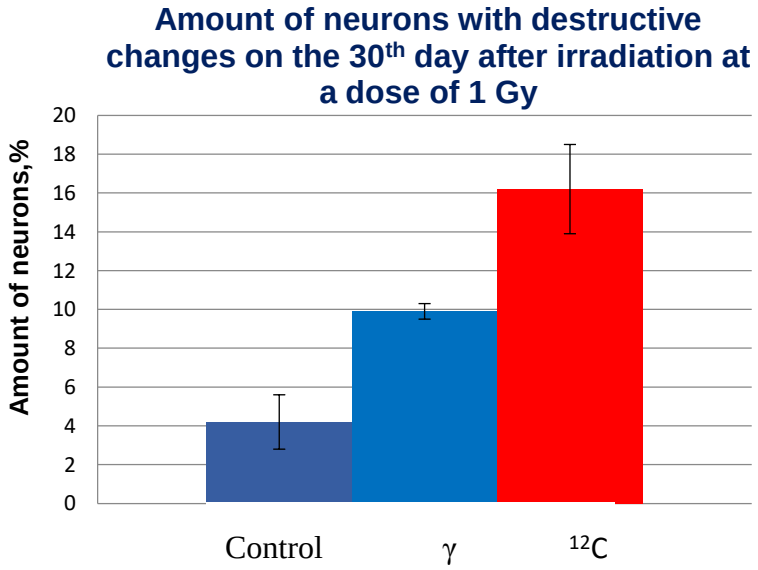
Morphological changes in rat cerebellar neurons after exposure to ^{12}C ions

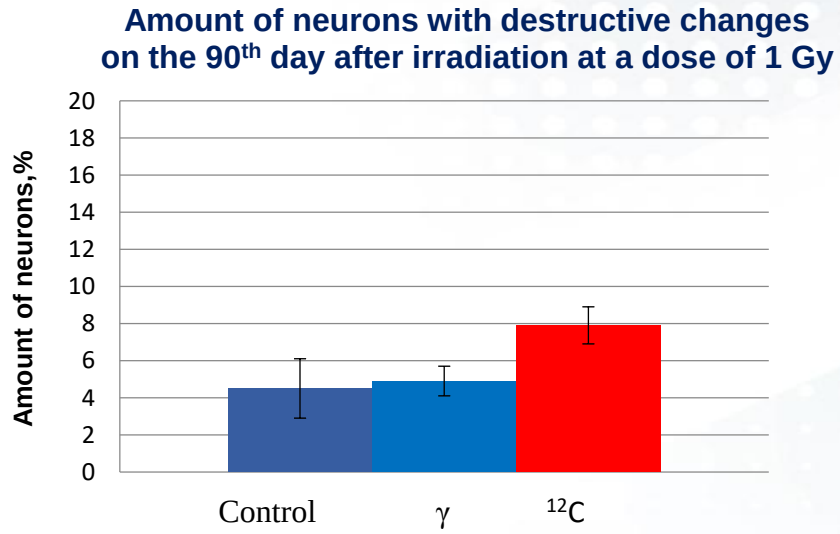


A B

(A) Destructive changes in Purkinje cells in rat cerebellar cortex exposed to carbon ions. Nissl staining, 4000-fold magnification.

(B) A ganglionic layer of the rat cerebellum without visible damage (control animals). Nissl staining, 4000-fold magnification.





The effect of 1 Gy ^{12}C particle radiation exposure on rats
Behavior and emotional status

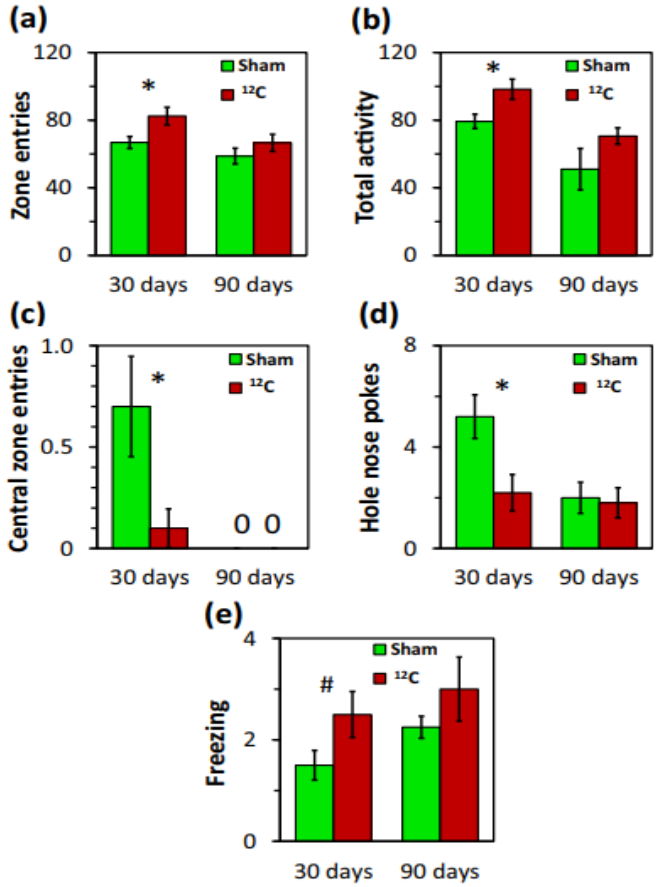


Fig. 1 The open field test results - including zone entries (a), total activity (b), central zone entries (c), hole nose pokes (d), and freezing frequency (e). (/) $p < 0.05$ and (#) $p < 0.1$ between the exposed and sham-irradiated rats at the same periods of time after exposure

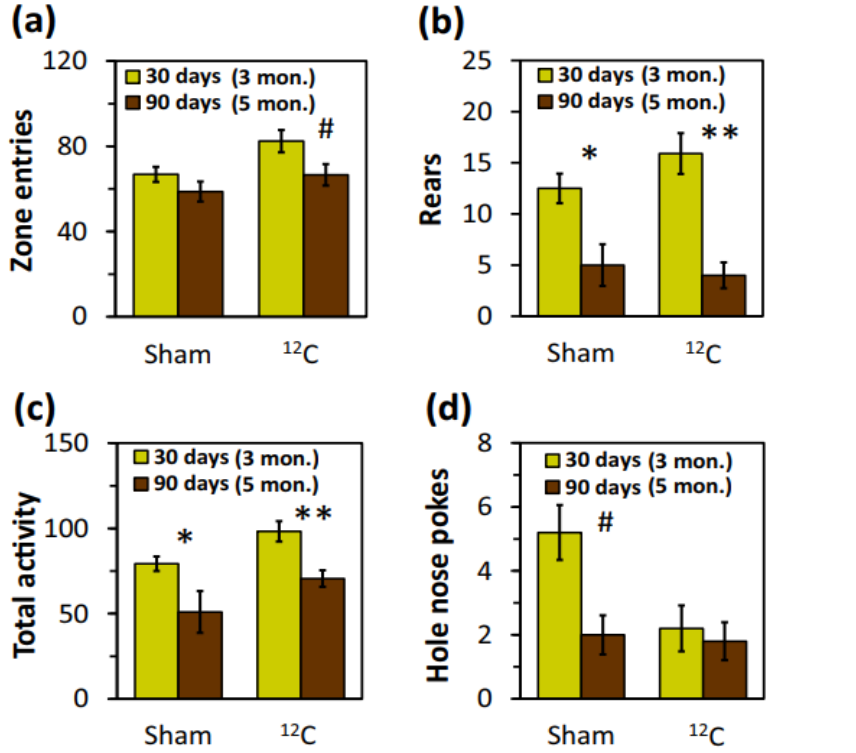
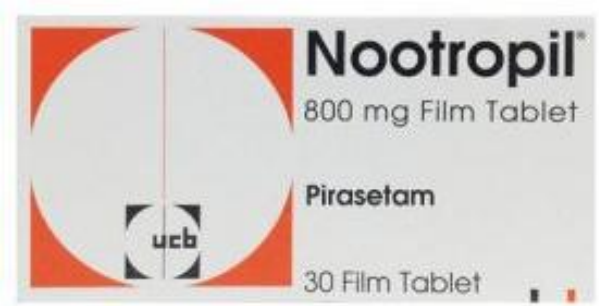


Fig. 2. The temporal dynamics of the open field test - including zone entries (a), rearings (b), total activity (c), and hole nose pokes (d). (/) $p < 0.05$, (//) $p < 0.01$, and (#) $p < 0.1$ between 3- and 5-month-old rats in the exposed and sham-irradiated groups. The corresponding periods after exposure were 30 and 90 days, respectively.

Medicinal agents in experiments

Piracetam-like Nootropics
2-oxo-1-pyrrolidine acetamide

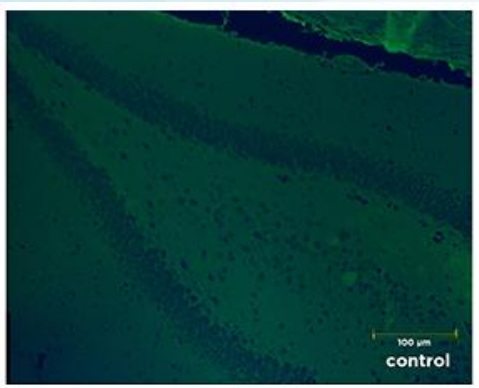


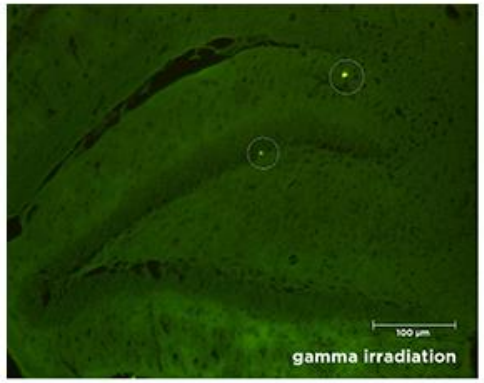
Ginko Biloba based drugs

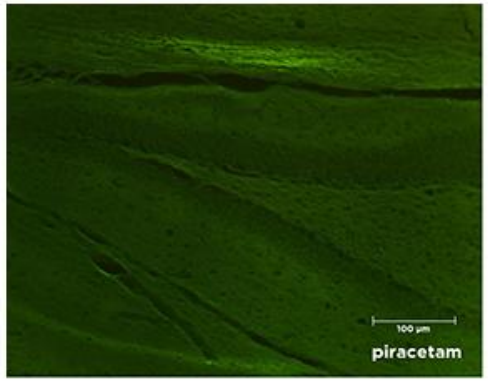
Semax – 0.1% neuropeptid, Met-Glu-His-Phe-Pro-Gly-Pro

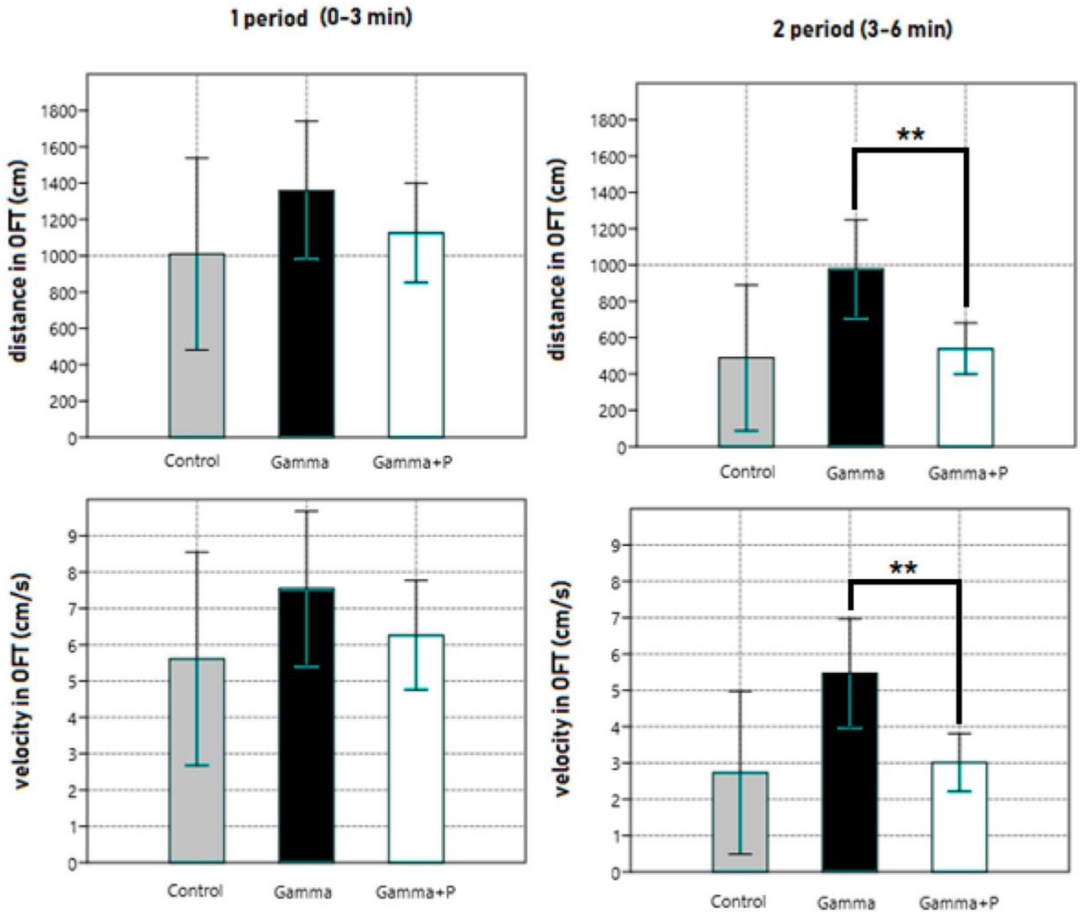
Cerebrolysin

Effect of Piracetam after fractionated gamma irradiation

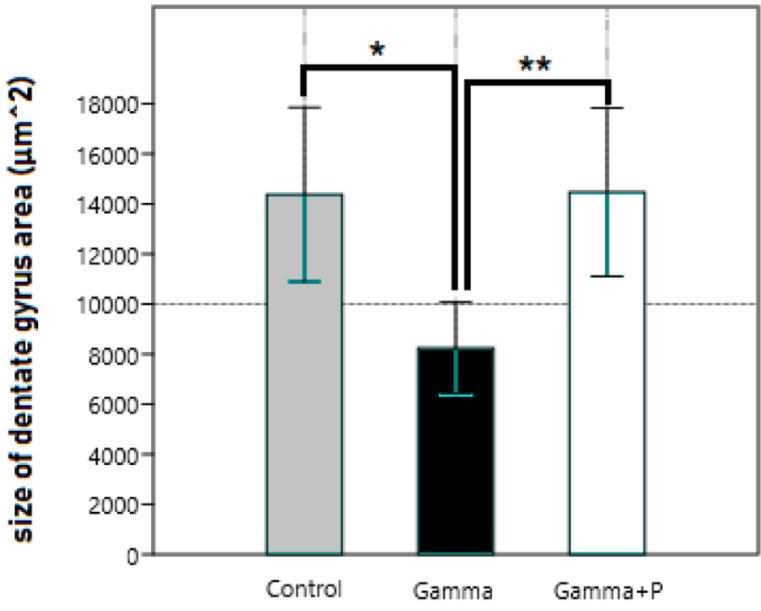








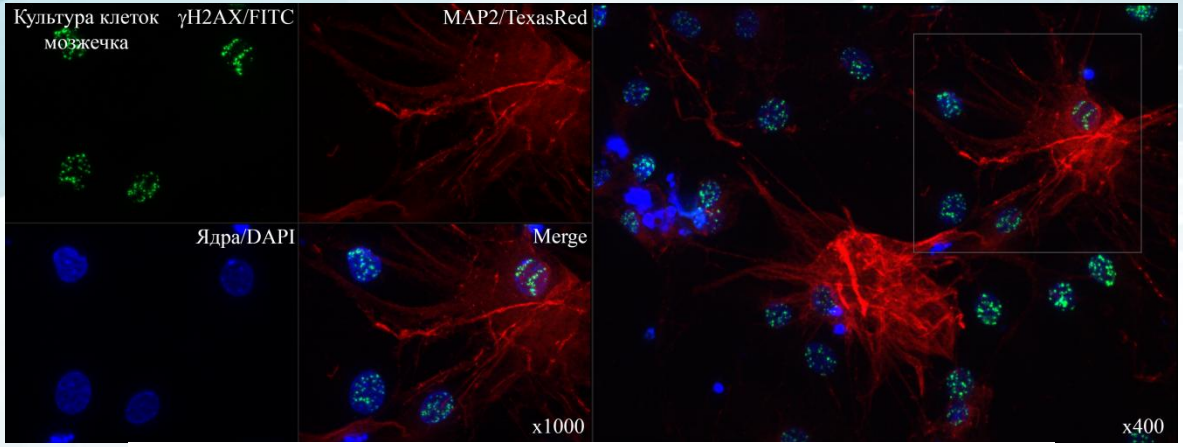
Flouro Jade B and Open field test results



The effect of 2 week - piracetam injection after irradiation on size of DG area of hippocampus

Severyukhin et al., 2021

Information system for radiation biology
(joint project of LIT and LRB)



ALGORITHMIC APPROACH TO THE RECOGNITION OF CELLS IN THE SENSORIMOTOR CORTEX FROM MICROPHOTOGRAPHS

I.A. Kolesnikova[1,2], N.N. Budennaya[1,2], Yu.S. Severyukhin[1,2], M.G. Lalkovicova[1,3]

[1] *Laboratory of Radiation Biology, Joint Institute for Nuclear Research, Dubna, Russia*

[2] *Federal State-Funded Educational Institution of Higher Education of Moscow Region "Dubna University", Dubna, Russia*

[3] *Slovak Academy of Sciences, Institute of Experimental Physics, Kosice, Slovakia*

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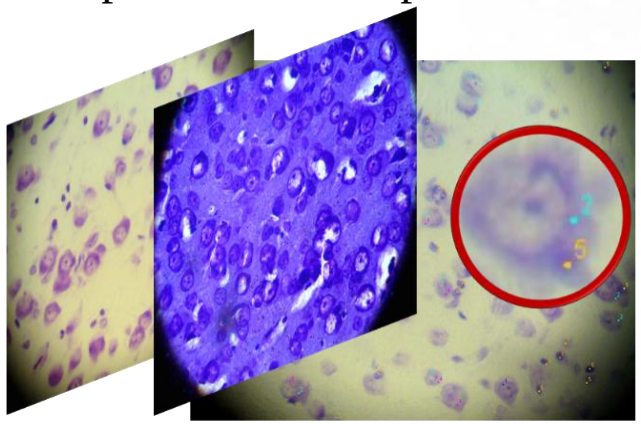
The aim of the work is to study the effect of ionizing radiation on the central nervous system of laboratory animals. The Sector of Radiation Physiology of LRB JINR studies the effects of radiation on the cellular and organismal levels. Experimental animals are irradiated with protons or gamma rays in the JINR Medico-Technical Complex. The collection of biological material, its fixation, slicing, staining, microscopy and the creation of photographic images of the samples take two weeks or more, and the morphological analysis of the histological preparations takes approximately six months. Achievements in the application of artificial neural networks in the biomedical field indicate the possibility of automating the stage of analysis of histological preparations. The purpose of this work is to show the effectiveness of modern computer vision algorithms and machine learning methods for automating the stages of the morphological analysis of micropreparations of the brain of experimental animals after exposure to ionizing radiation. Thereby the speed of obtaining qualitative results increases, and the subjectivity of the approach to processing experimental data decreases.

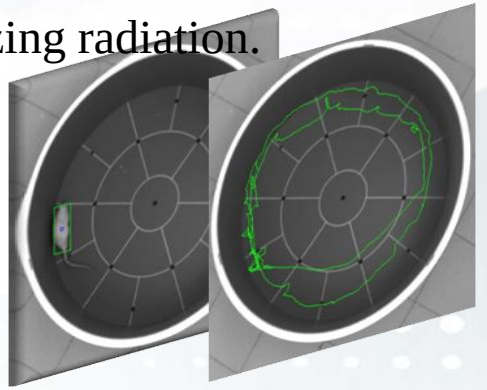
The information system is based on:

- computer vision algorithms based on **machine and deep learning technologies**;
- modern IT solutions for storing, processing and visualizing data

The information system will allow:

- to **speed up and simplify the work** with experimental data for various groups of researchers
- to simplify and **accelerate the diagnosis of pathologies** of the central nervous system, and in a particular case, the development of effective methods of prevention and protection from ionizing radiation.





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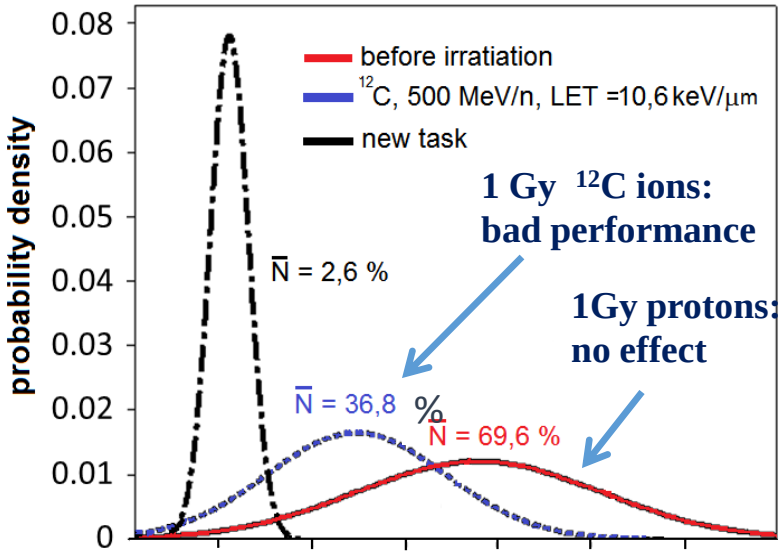
Radiation Physiology on primates

- **First experiments with primates exposure to protons and carbon ions**
- **Manned deep space flight simulation at accelerator of heavy ions**
- **Mechanisms of radiation-induced cognitive disorders**



Automated computer system for the simulation of operator activity during the flight

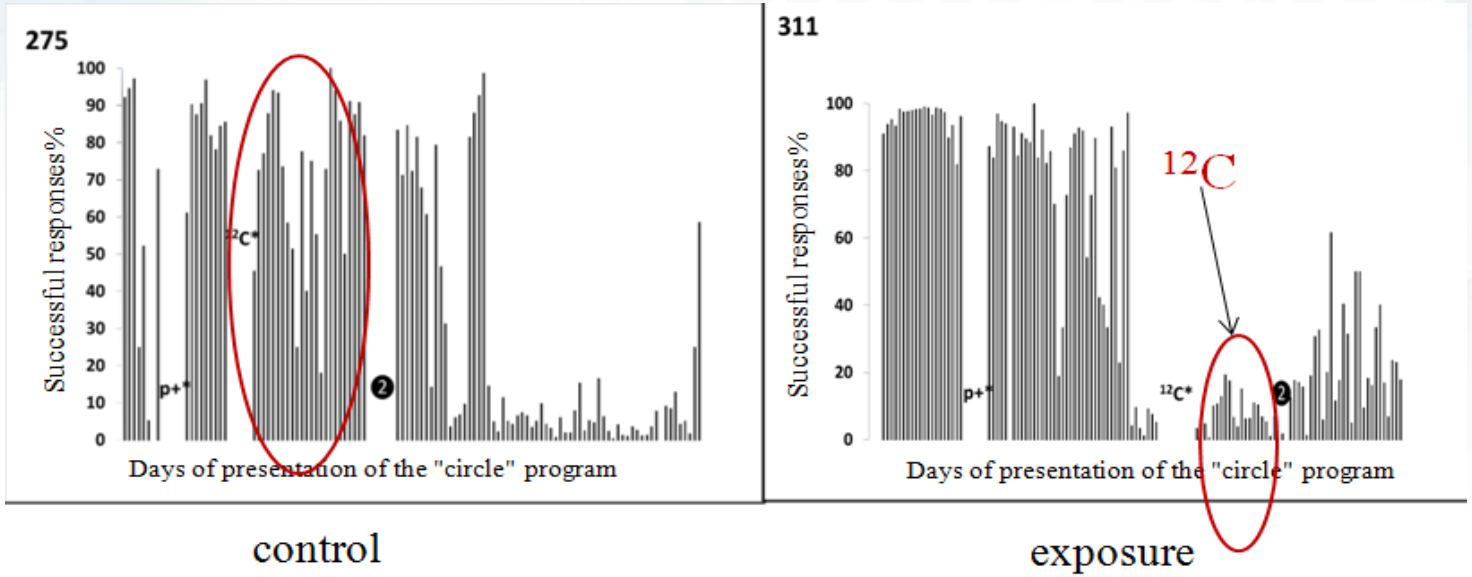
Collaboration:

RAS Institute of Biomedical Problems,
RAS Institute of Medical Primatology,
RAS Institute of Higher Nervous Activity and Neurophysiology,
Moscow State University

Macaca mulatta irradiation (^{12}C ions, 500 MeV/nucleon)

Psychological Test System — a series of 18 computer gaming tasks of increasing difficulty to simulate the basic elements of the operator's activity

Indicators of cognitive functions in the irradiated and control monkey groups



control exposure

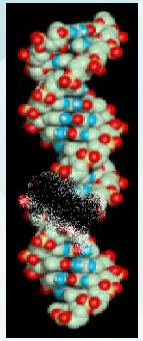
Dose: 1 Gy

p + — proton irradiation day; ^{12}C — carbon ion irradiation day; 2 — a new level of the game program difficulty.

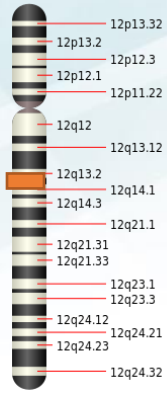
Mathematical modeling

Bugay et al, 2019

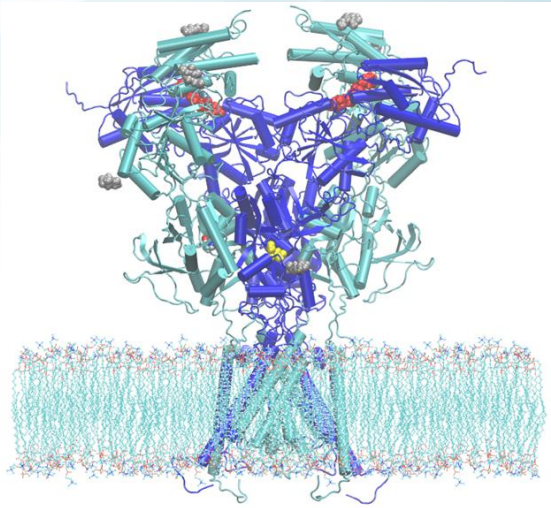
Simulation of genetic and molecular mechanisms of neurodegenerative diseases



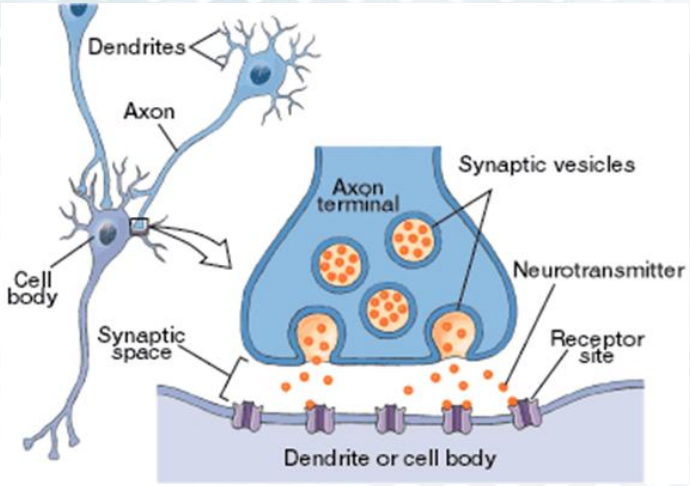
DNA damage



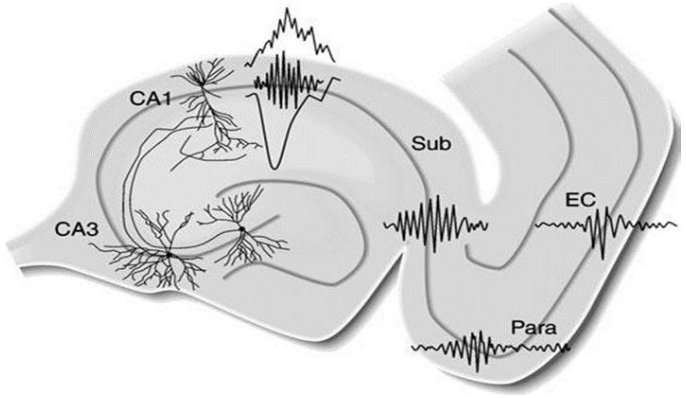
Chromosome 12
GRIN2B gene



NMDA synaptic receptor



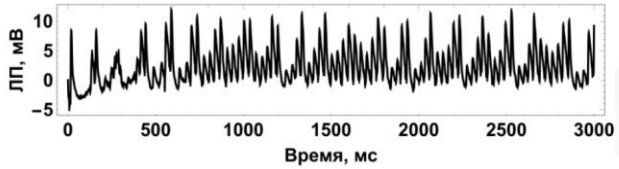
Interconnections between neurons



Brain neural networks

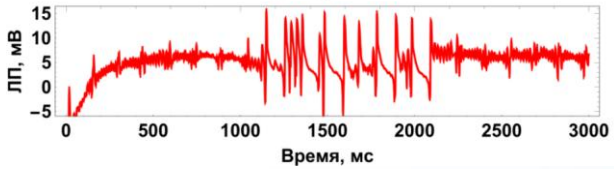
Mutation	Phenotype
p.Asn615Leu p.Phe671_Gln672del	West syndrome (epilepsy) Mild ID, Autism

Native

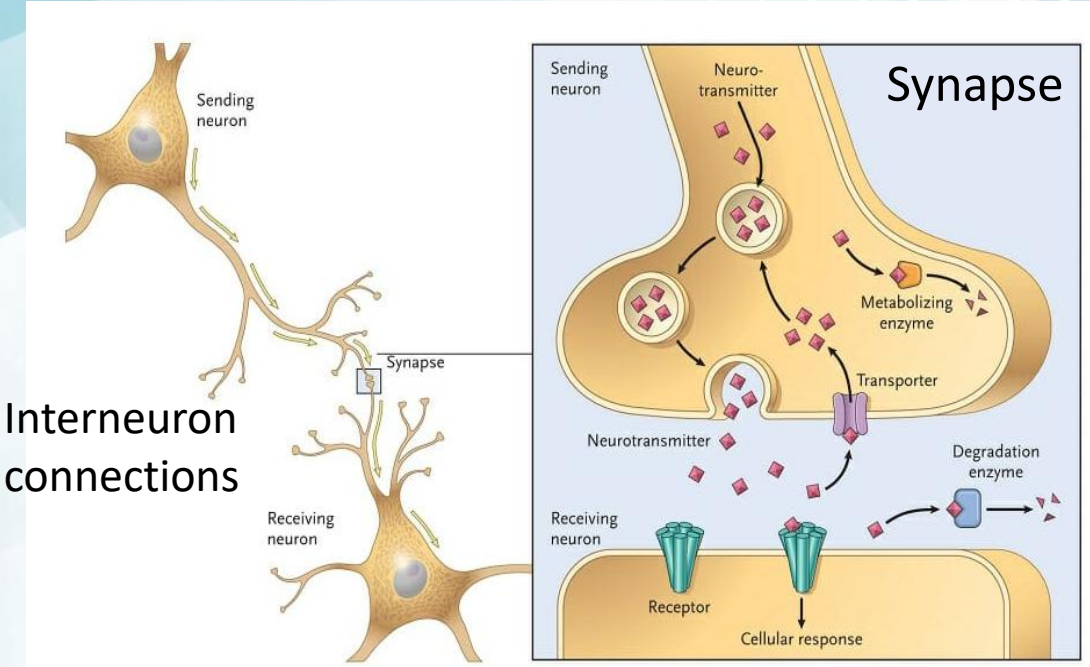


p.ASN615LEU

Epileptic seizure

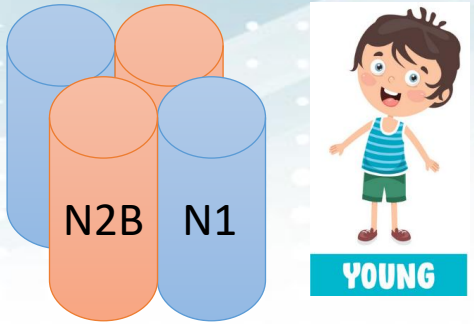


Simulation of molecular mechanisms of brain aging

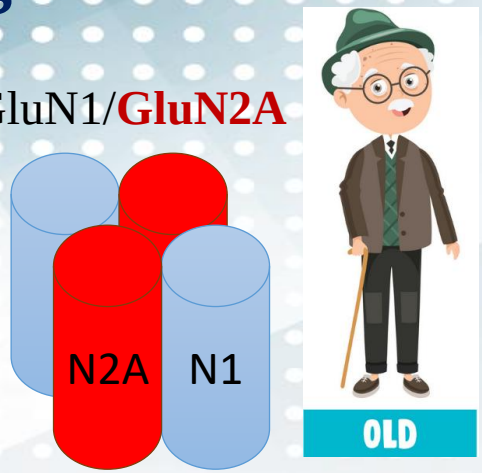


Interneuron connections

GluN1/**GluN2B**



GluN1/**GluN2A**



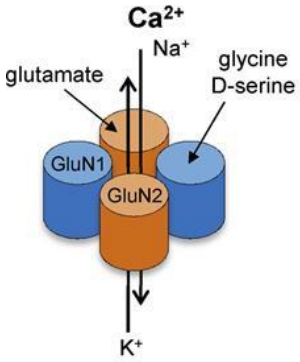
Higher receptor conductance	Lower receptor conductance	(-12%)
Slower receptor deactivation	Faster receptor deactivation	(+250%)
Increased theta-rhythm	Decreased theta-rhythm	(-30%)

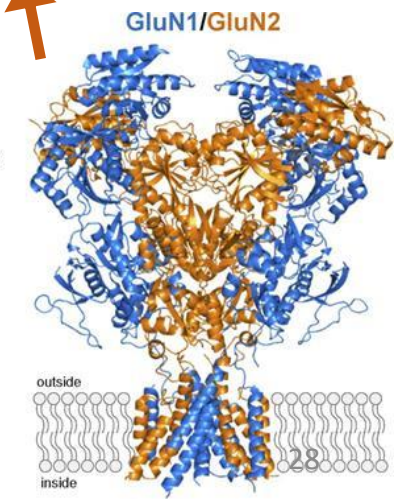
BETTER LEARNING AND MEMORY

NMDA receptor:

GluN1 & GluN2 subunits

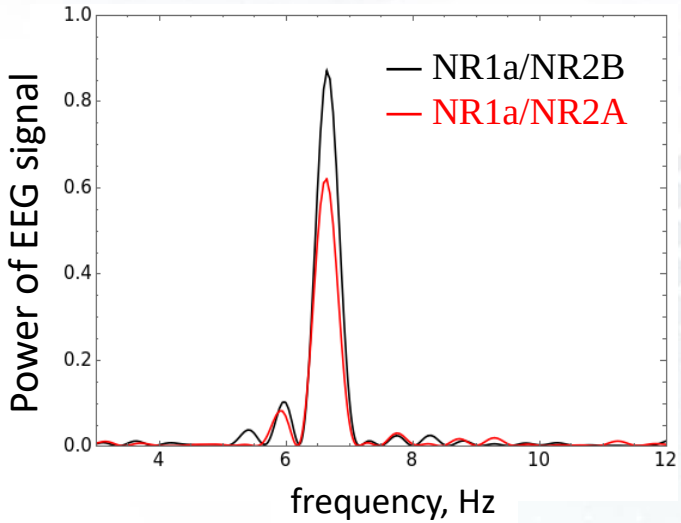
Subunit expression changes with age





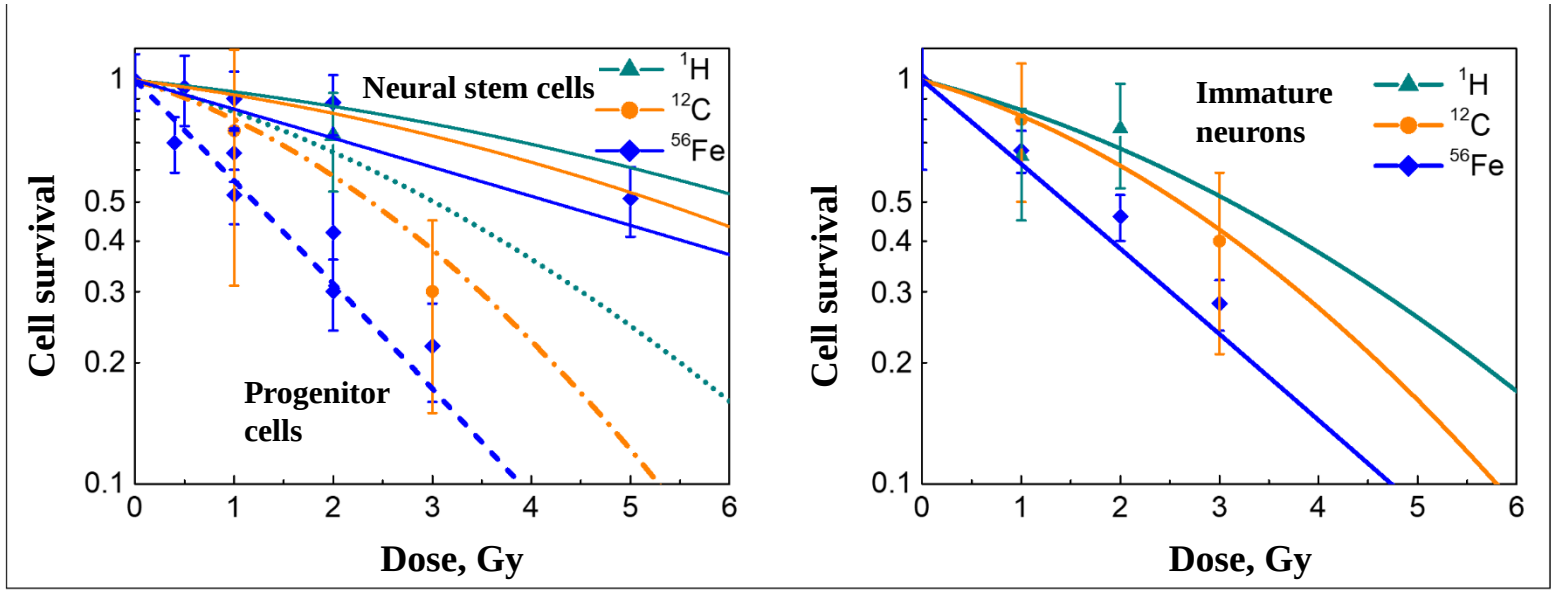
Multiscale simulation approach

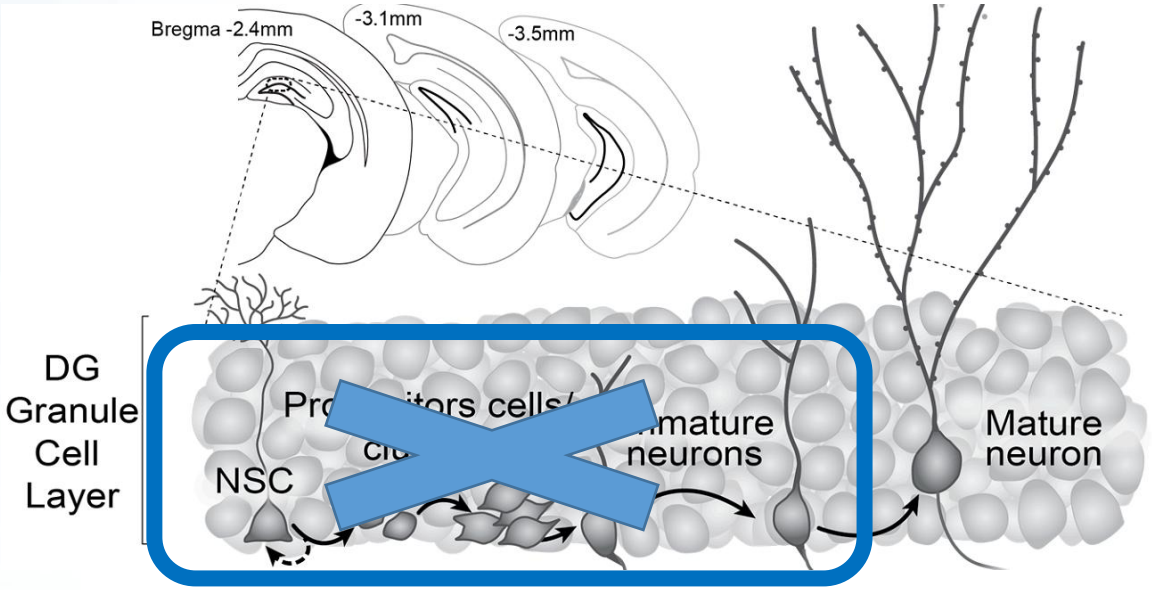
- Molecular dynamics
- Biochemical kinetics
- Neural networks

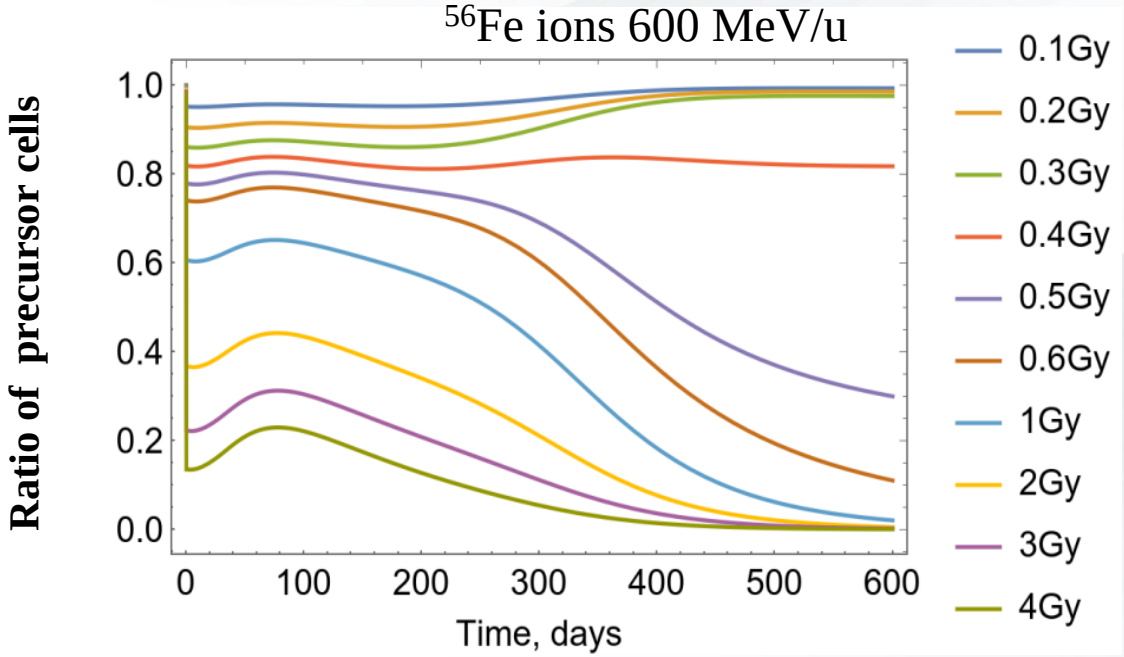


Mathematical modeling of radiation-induced neurogenesis impairment

Calculated survival of radiosensitive cells (neural stem cells, neural progenitor cells, immature neurons) after action of 1000 MeV protons, 290 MeV/u carbon ions, 600 MeV/u iron ions as compared with experimental data [Rola 2004, 2005, Tseng 2014].

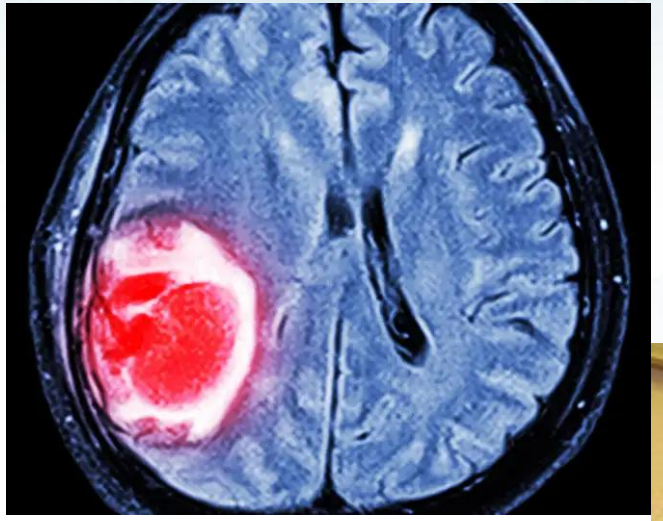


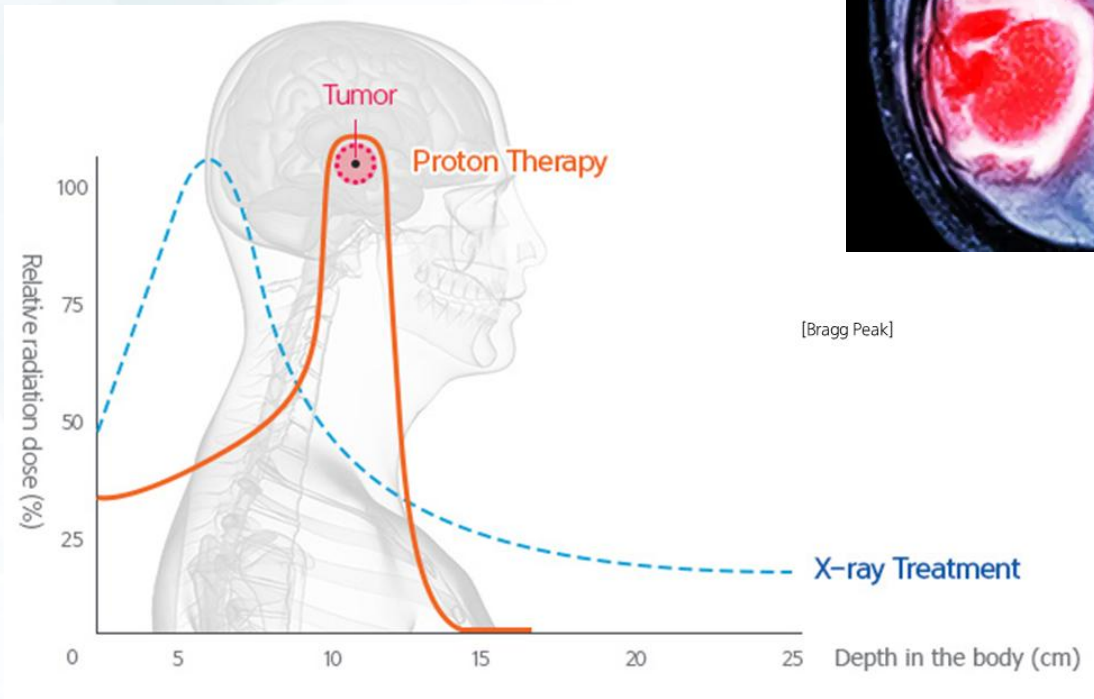




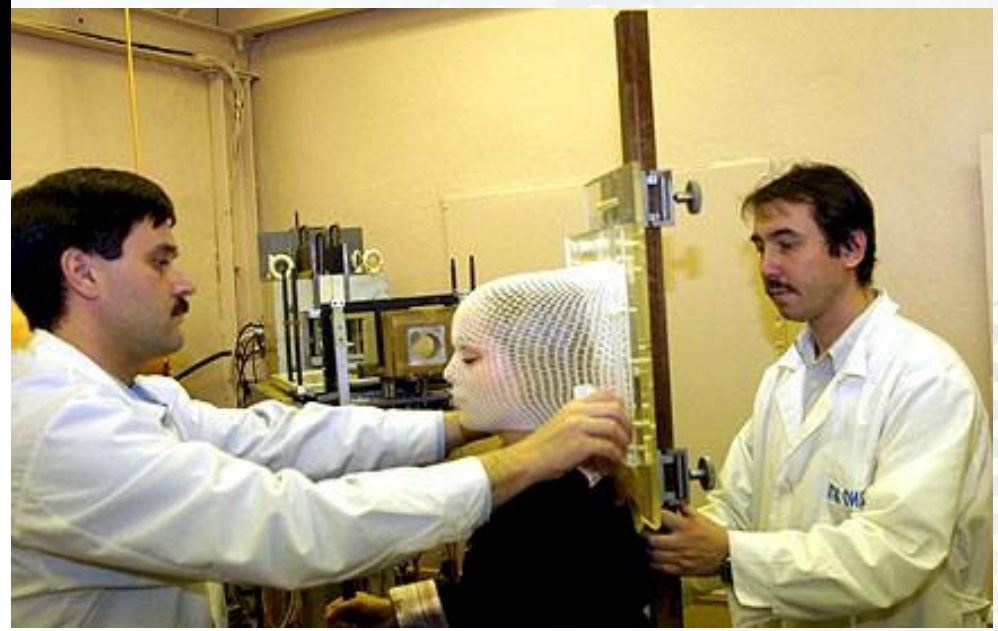
Focus: Biomedical radiation research
Radiation cancer therapy

JINR since 1967





[Bragg Peak]

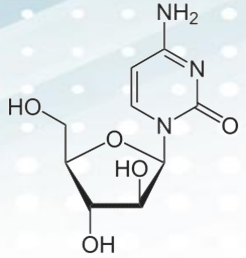


Medical and Technical Complex of JINR

Clinical Radiobiology

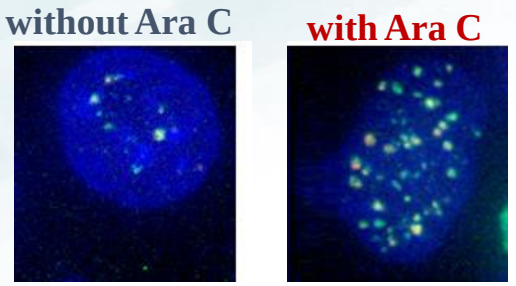
A new promising method for cancer therapy:
increasing the biological effectiveness of γ- and proton radiation by special drugs

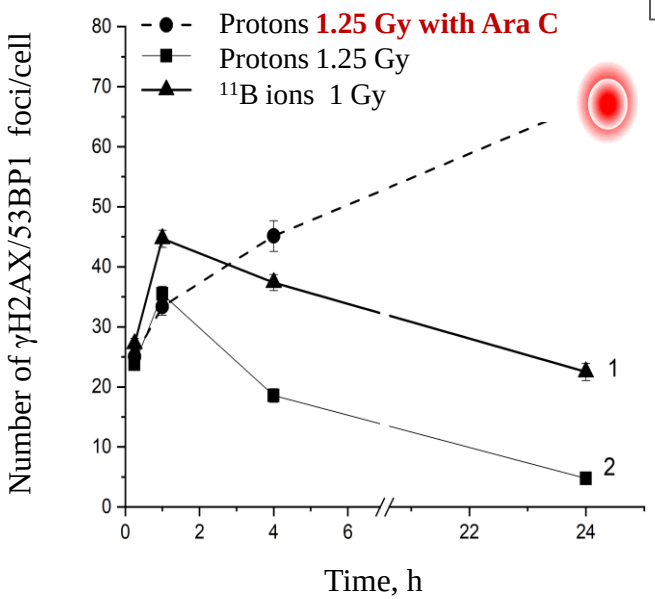
- Induction of **DNA damage** in **human cells** (*in vitro* experiments)



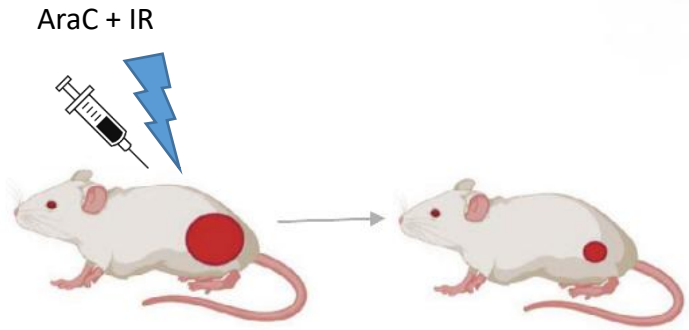
DNA synthesis inhibitor

Ara C, cytarabine



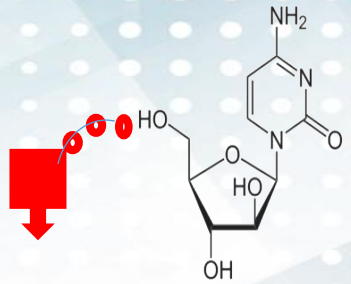


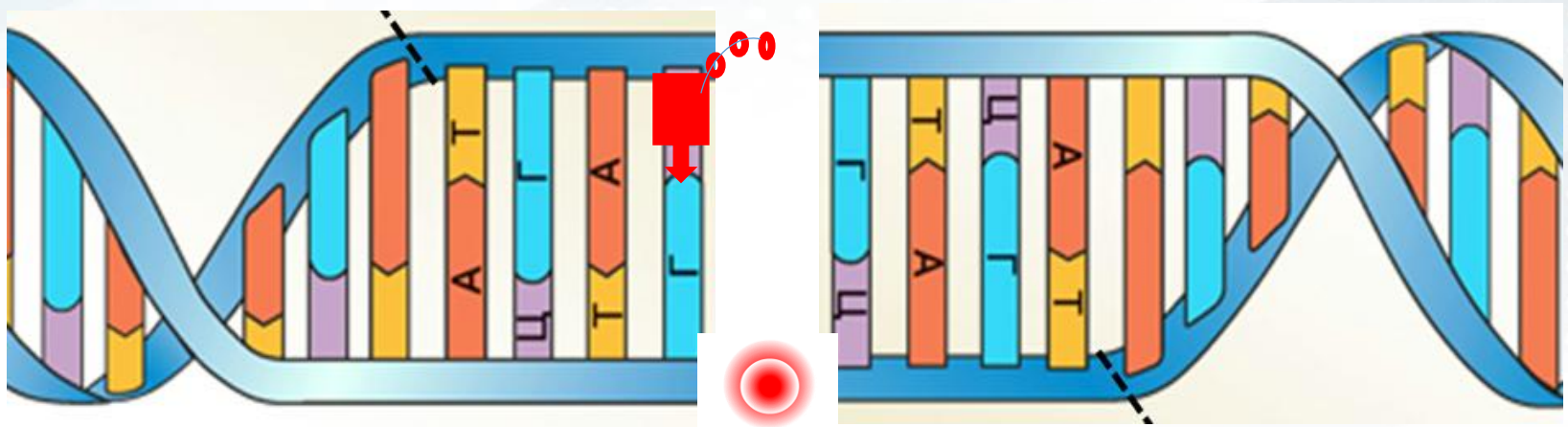
15-fold increase of proton beam efficiency by DNA synthesis inhibitors



The mechanism of AraC effect

AraC- "The Trojan Horse"



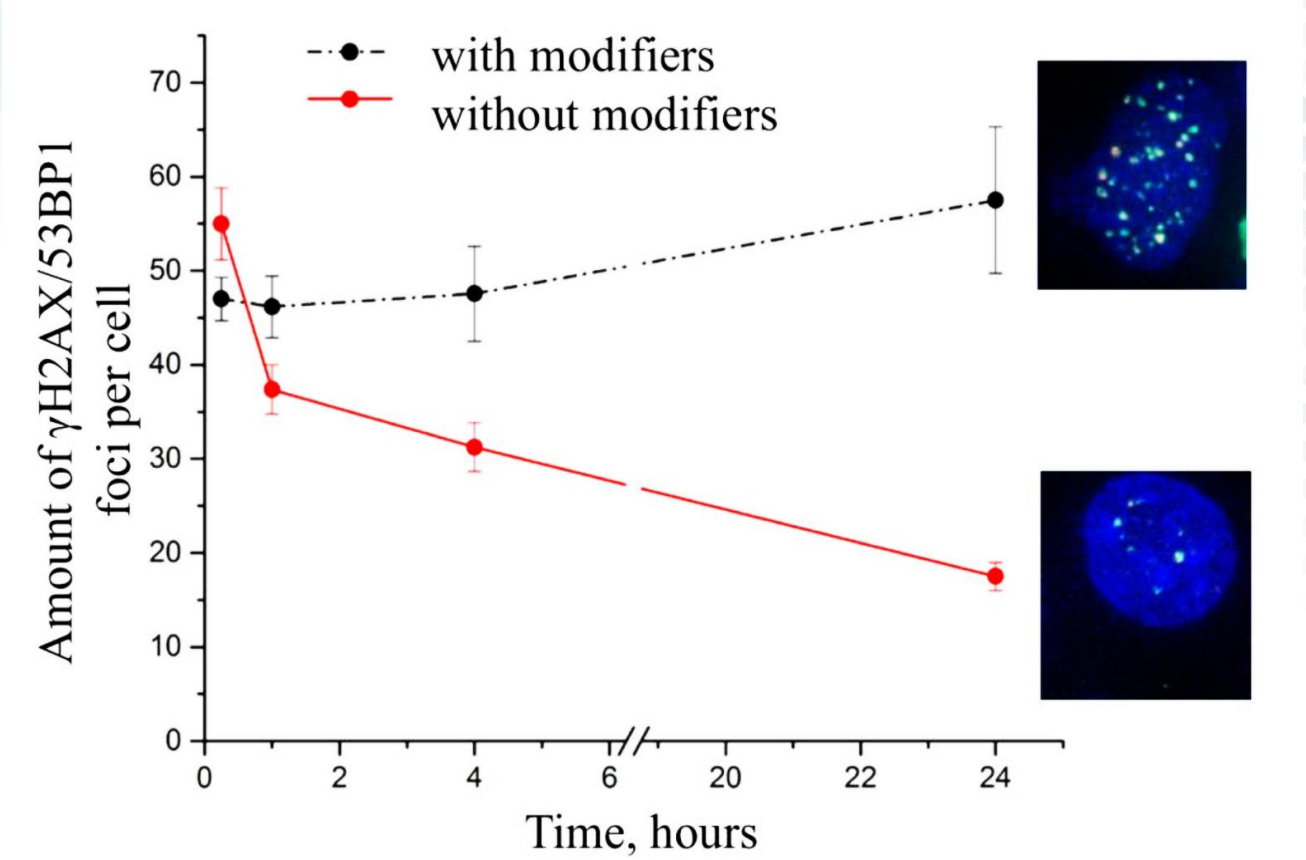


S1 endonuclease

Stopping DNA synthesis and forming a double-strand break

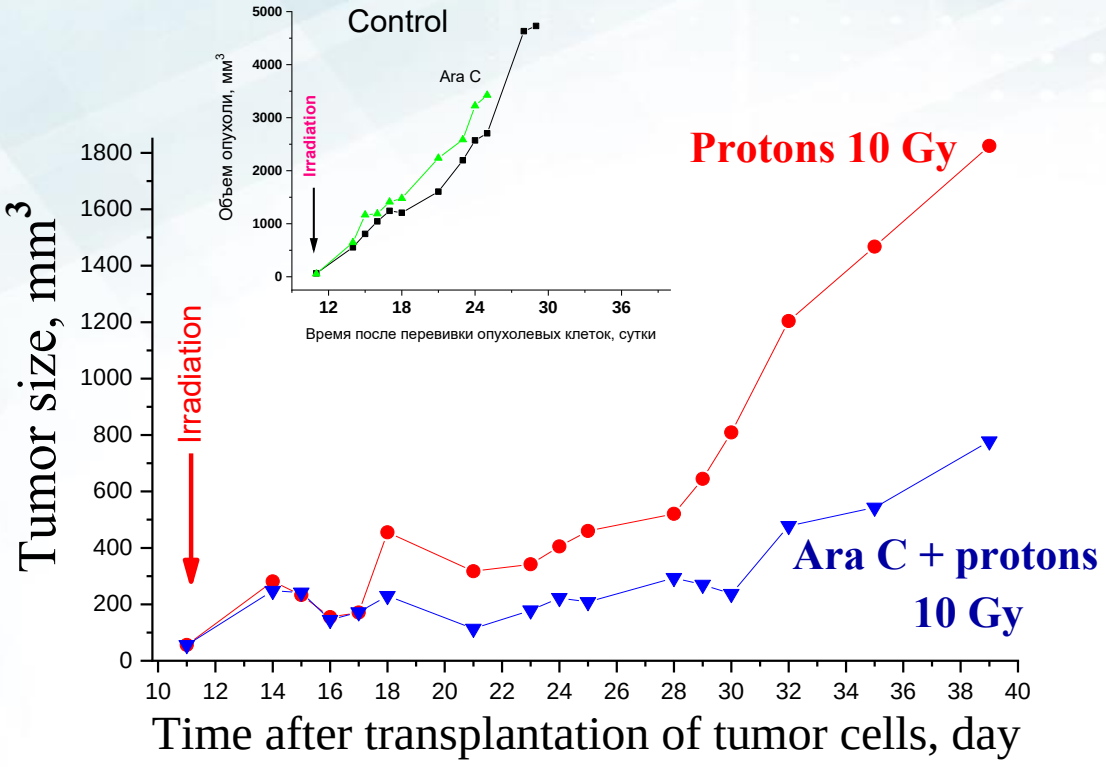
Human glioblastoma (U87)

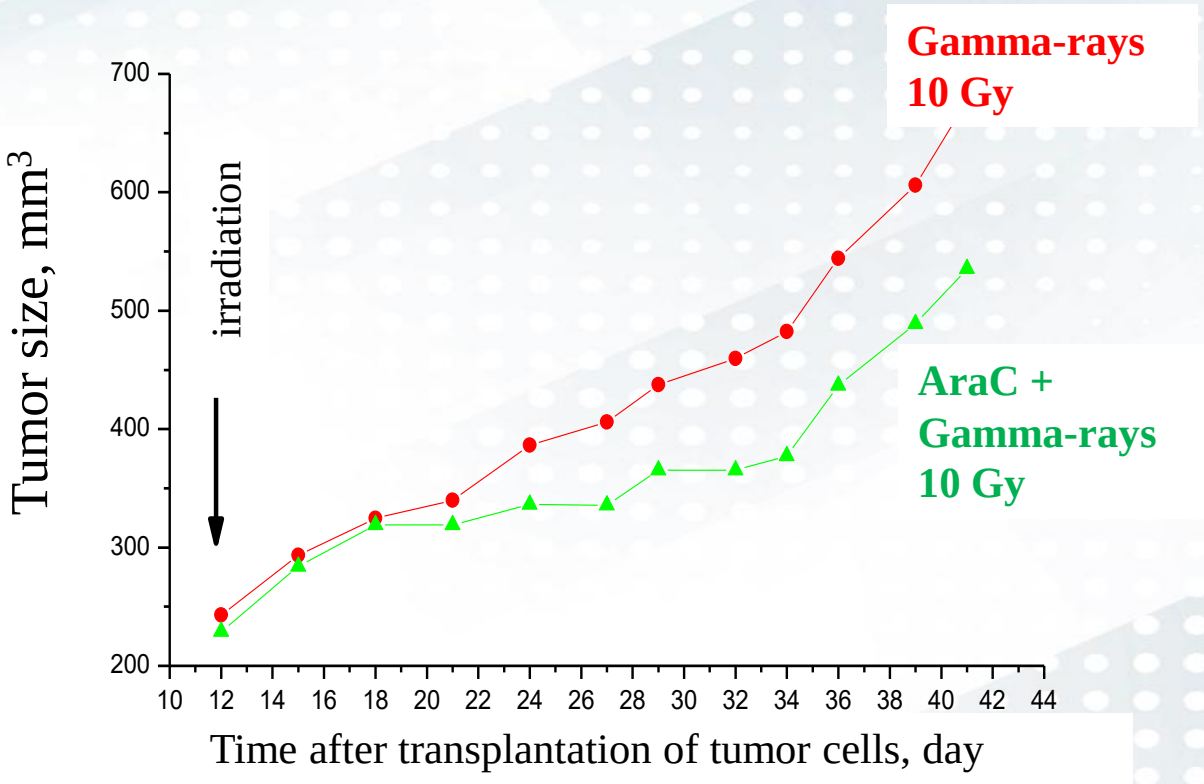
Irradiation with protons (dose 1.25 Gy) in the extended Bragg peak



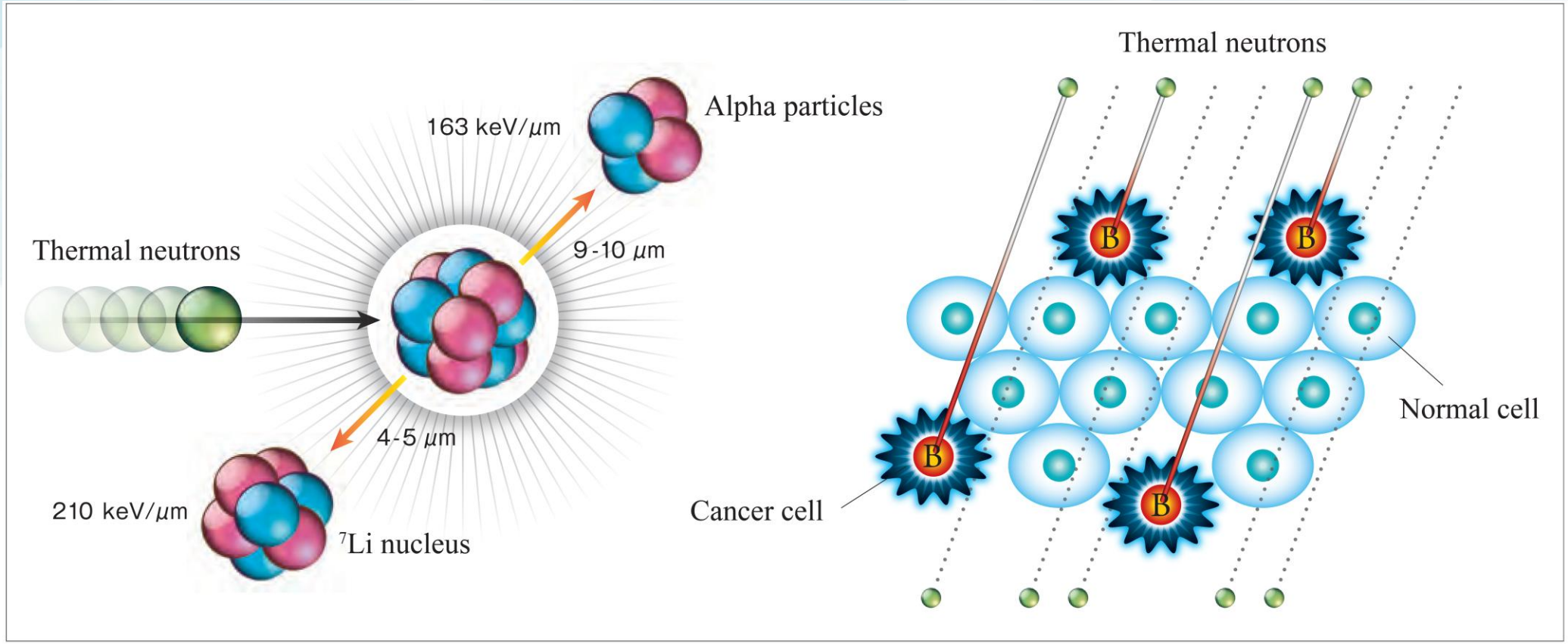
Preclinical studies on laboratory animals with transplanted melanoma tumor

Krasavin, Zamulaeva, Kaprin, 2019,2020





Approaches to increase the efficiency of particle therapy
Boron Neutron Capture Therapy



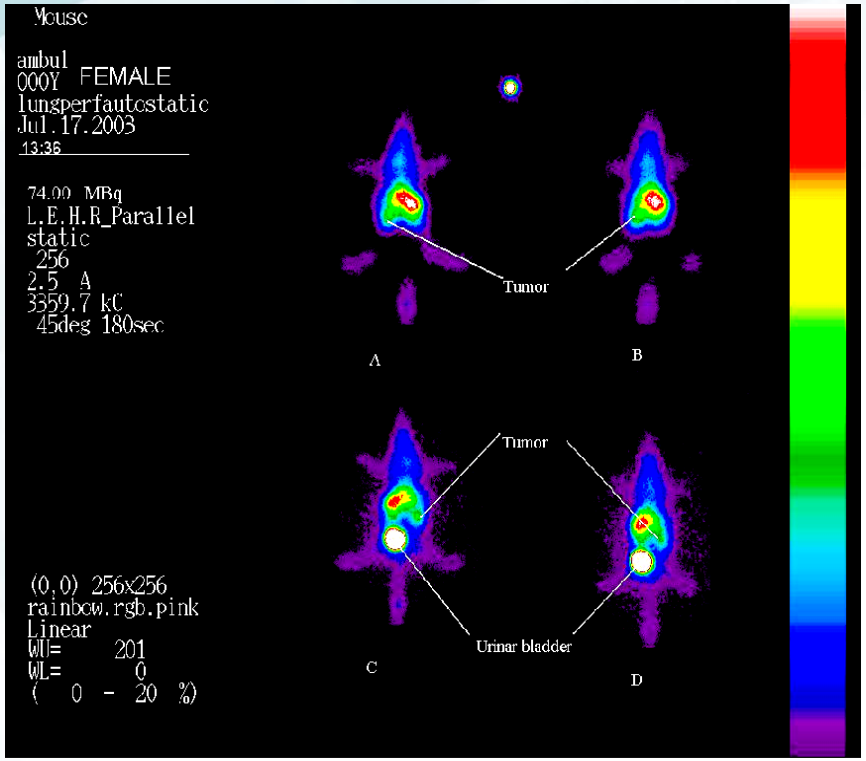
- Selective accumulation of boron-containing drug in tumor cells, for example, using "vector" technologies;
- Formation of a field of thermal neutrons with a high flux density in the tumor zone;
- the destruction of the tumor cell but neighboring cells are not affected

Nuclear medicine: targeted delivery of radionuclides

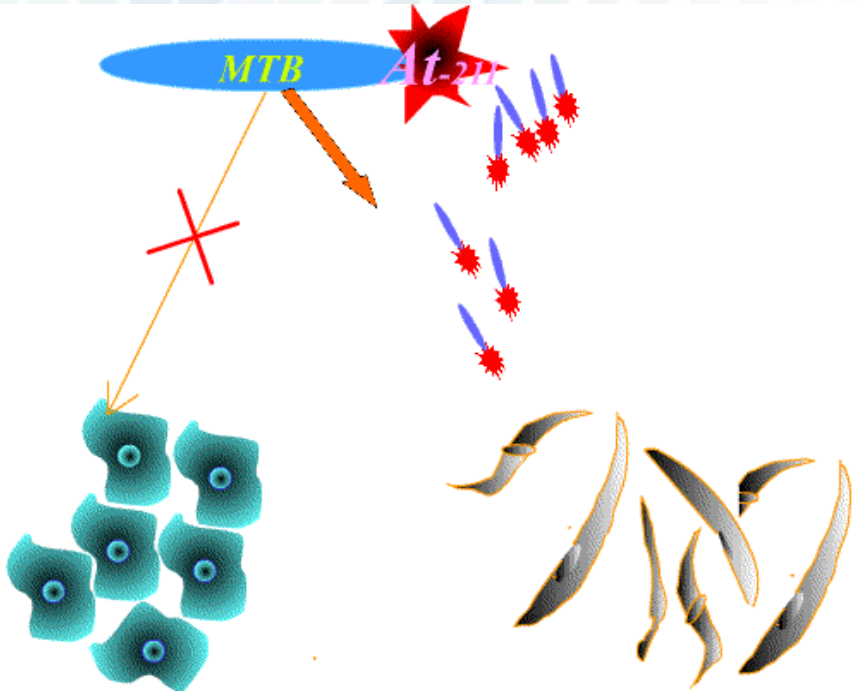
JOINT INSTITUTE FOR NUCLEAR RESEARCH

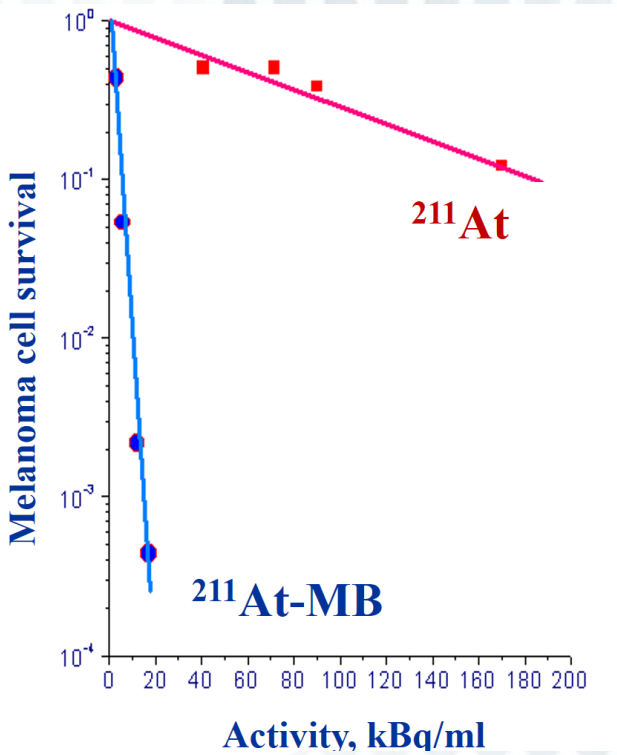
Methylene blue (MTB)

Shmakova, Norseev, Krasavin, Kodina et al, 2002



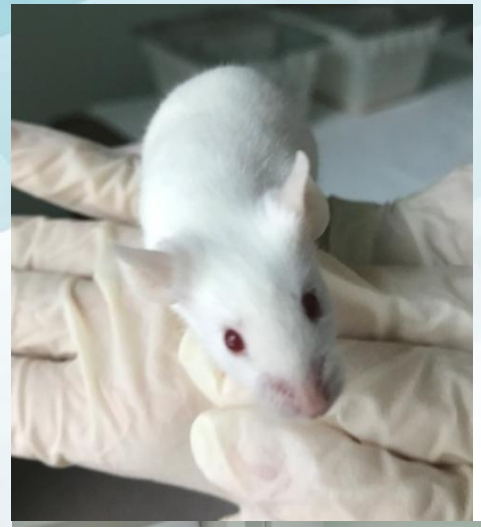
^{131}I-MTB accumulation in animals with inoculated melanoma





Melanoma tumor cell survival *in vitro*

Education



LRB education programs and trainings at JINR University Centre

- *Radiation protection and dosimetry*
- *Geant4-based computer modelling*
- *Study of DNA repair using immunocytochemistry*
- *Cytogenetics using mFISH*
- *Genetic instability*
- *Animal behavioral experiments*

Dubna State University, Department of Biophysics

- *bachelors*
- *masters*
- *postgraduates*

Thank you for your attention!

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