The Scientific Conference of Young Scientists and Specialists
"Alushta-2024"

Kinetics of DNA double-strand breaks formation in mature neurons of primary rat hippocampal cell culture under the action of ionizing radiation with different physical characteristics

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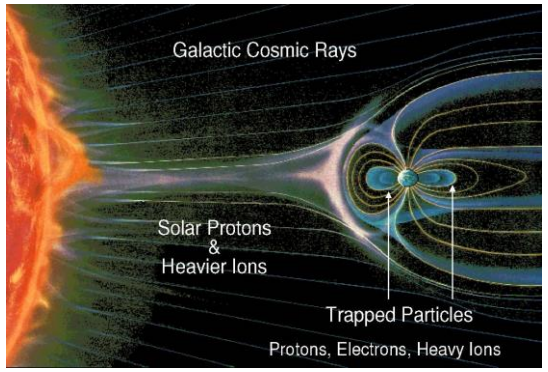
Introduction

The effects of radiation on the central nervous system are of major interest because of several issues:

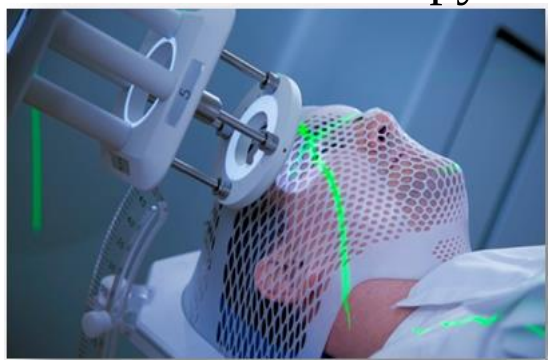
- the manned deep-space missions

Cosmic radiation

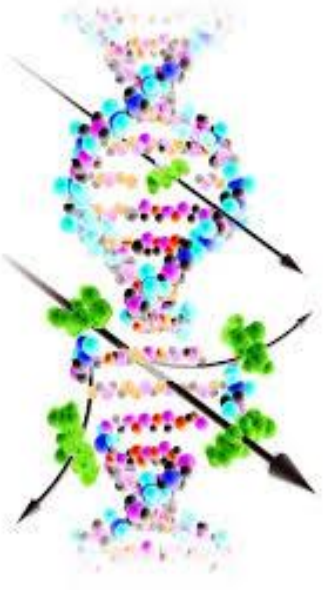


- the widespread use of radiation in medical treatment

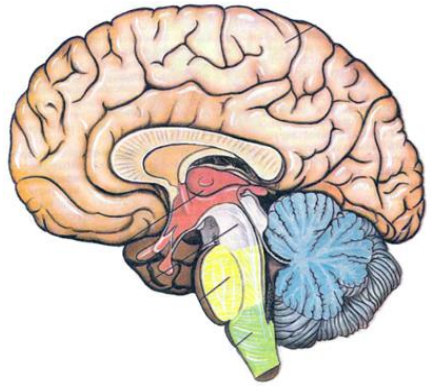
Radiation therapy



DNA damage



Cognitive impairment

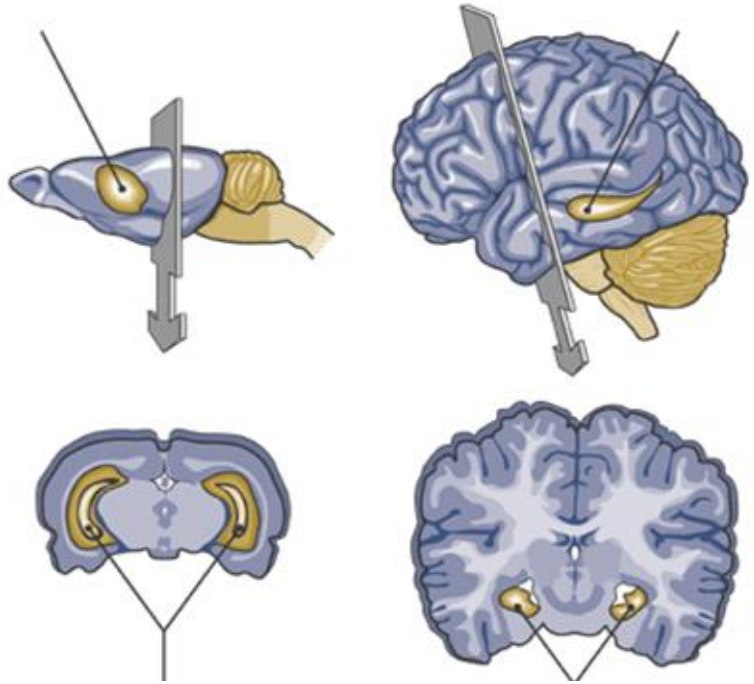


Double-strand break is one of the most critical types of DNA damage

Hippocampal functions

Rodent hippocampus

Human hippocampus



Rodent hippocampus

Human hippocampus

Functions:

- Spatial orientation
- Memory
- Learning

Ionizing radiation exposure:

- Inhibits the adult hippocampal neurogenesis
- Reduces the dendritic arborization
- Impairs nerve impulse conduction
- Changes proteins expression and forms mutant proteins
- Causes cognitive impairment
- Violates animal spatial memory

The hippocampus is considered as one of the most radiosensitive brain structures due to the adult neurogenesis processes occurring in it.

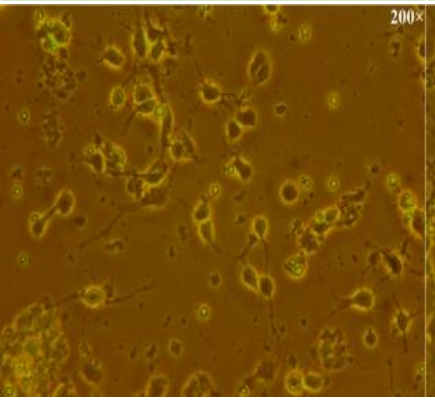
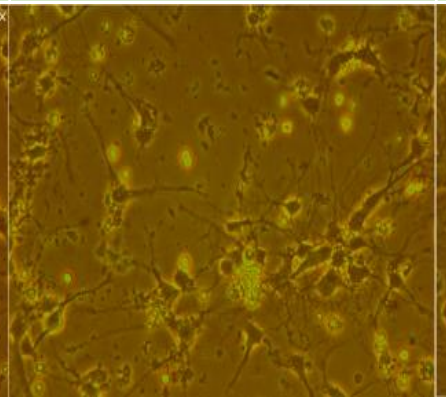
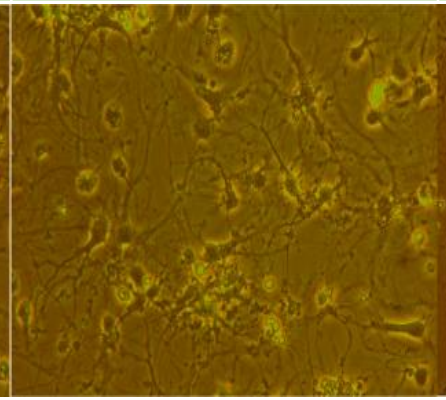
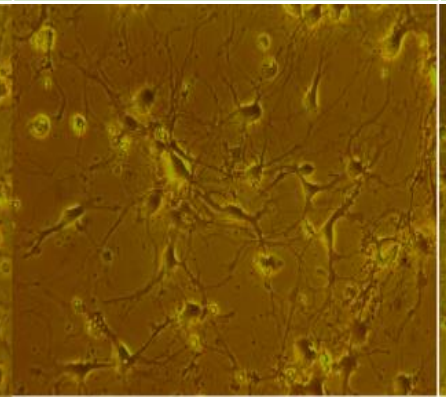
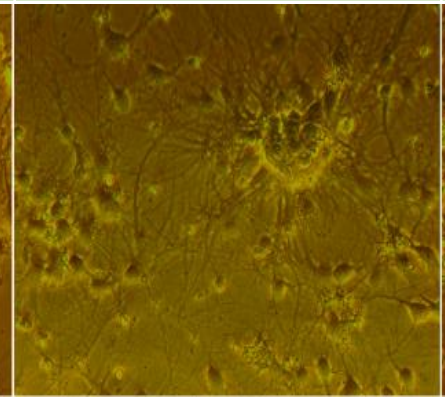
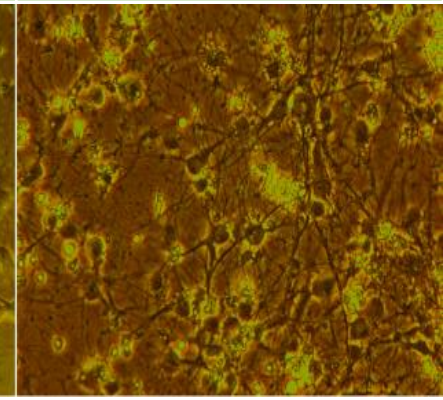
Research aim

Investigation of the influence of radiation with different physical characteristics on DNA DSBs formation in mature neurons of rat primary hippocampal cell culture

Materials and methods

Primary rat hippocampal cell culture

DIV-days in vitro

DIV1	DIV3	DIV4	DIV5	DIV6	DIV10
					

Brain tissue isolation of *Sprague Dawley* rat pups (P0-P1)

Plating of cell suspension

Cell cultivation

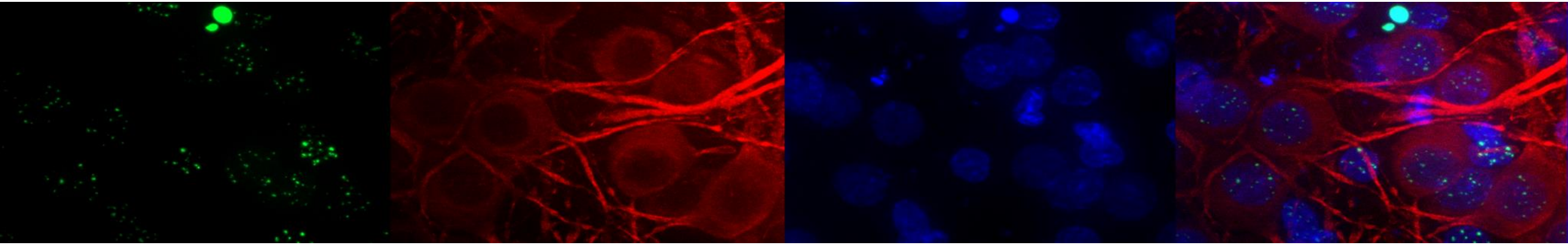
Irradiation DIV10

Materials and methods

- Irradiation
- Fixation 4% PFA
- Immunocytochemical staining for DNA DSBs markers and cell type markers
- Fluorescent microscopy
- Quantitative analysis

Radiation source	E	LET, keV/μm	Angle, º	Dose, Gy	Facility
^{60}Co γ-rays	1.25 MeV	0.3	90	3	«Rocus-M» MTC LNR JINR
Protons	150 MeV	-	10	3	Synchrocyclotron MTC LNR JINR
^{15}N	14 MeV/nucleon	175	10	1.25	U400-M, LNR JINR

RIF (γH2AX, γH2AX/53BP1) – radiation induced foci

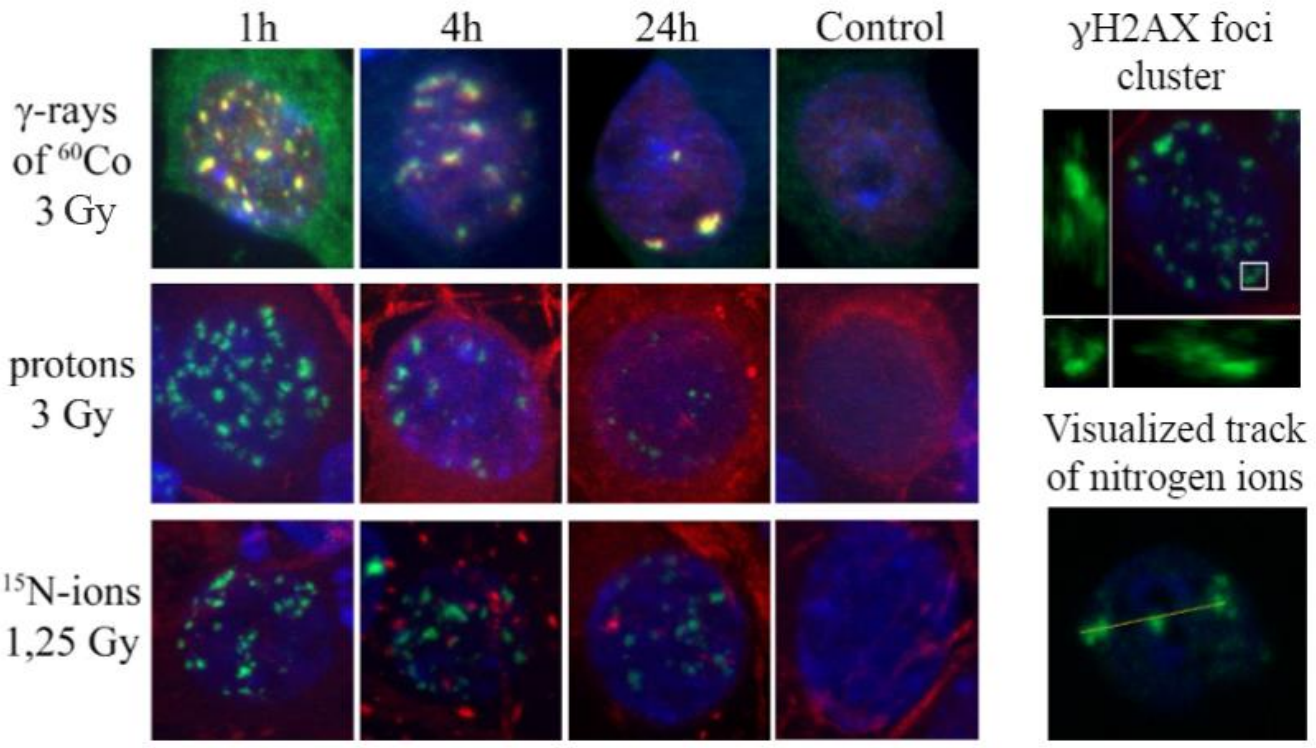


γH2AX - DNA DSBs MAP2 – soma, axon and dendrites DAPI - cell nuclei Merge

The kinetics of RIF formation in mature neurons of primary rat hippocampal cell culture

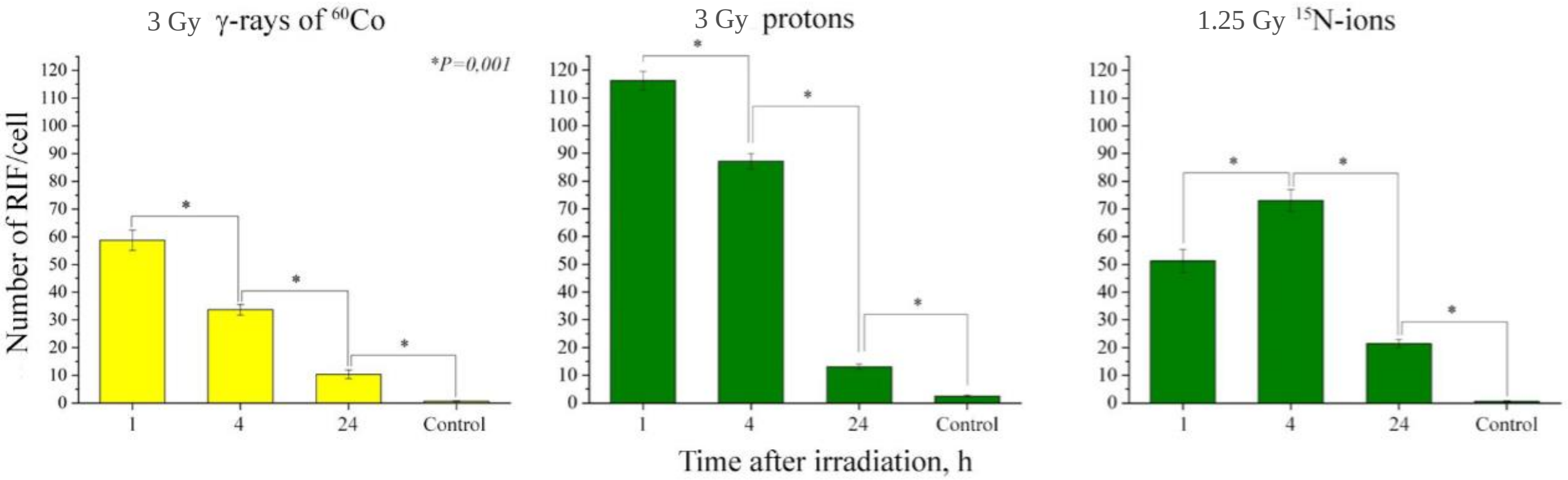
Measured parameters:

- The number and complexity of colocalized γH2AX/53BP1 foci in neuronal cells after γ-irradiation
- The number and complexity of γH2AX foci in mature neurons (MAP2+) after proton irradiation
- The number and complexity of γH2AX foci in mature neurons (MAP2+) after ^{15}N ions irradiation
- The number and foci complexity shaped by nitrogen ions tracks



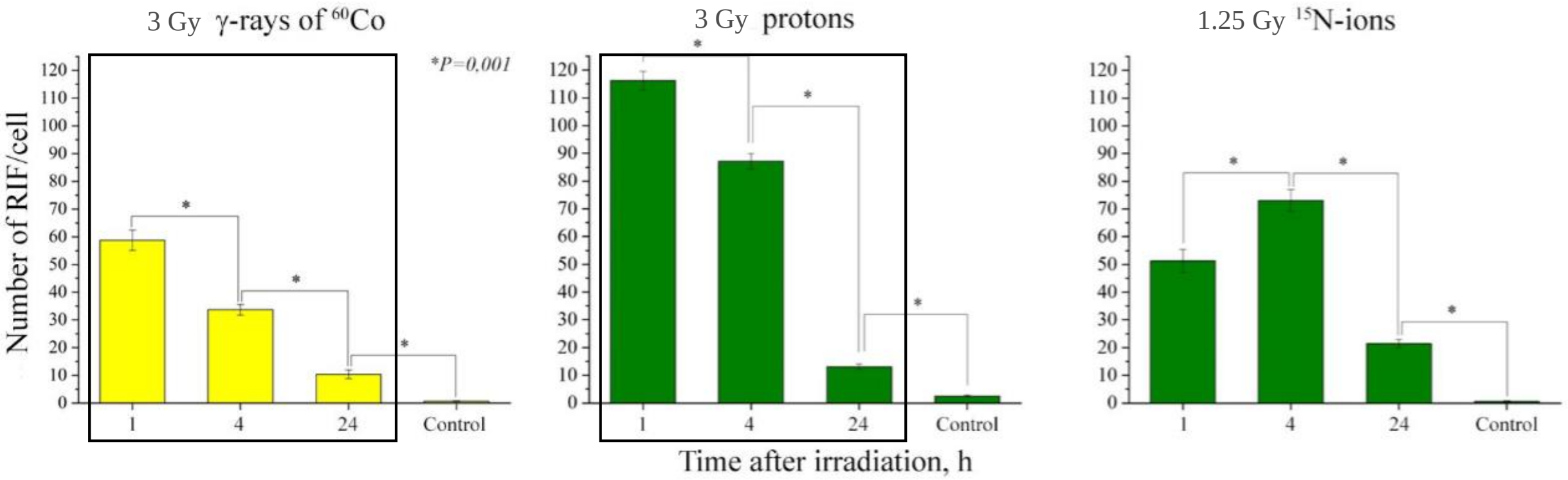
The kinetics of γH2AX and 53BP1 foci formation in mature neurons of primary rat hippocampal cell culture after exposure to radiation with different physical characteristics (x1000 magnification).

The kinetics of RIF formation in mature neurons of primary rat hippocampal cell culture



The statistically significant difference between groups was shown with the Mann-Whitney test (*P=0,001)

The kinetics of RIF formation in mature neurons of primary rat hippocampal cell culture



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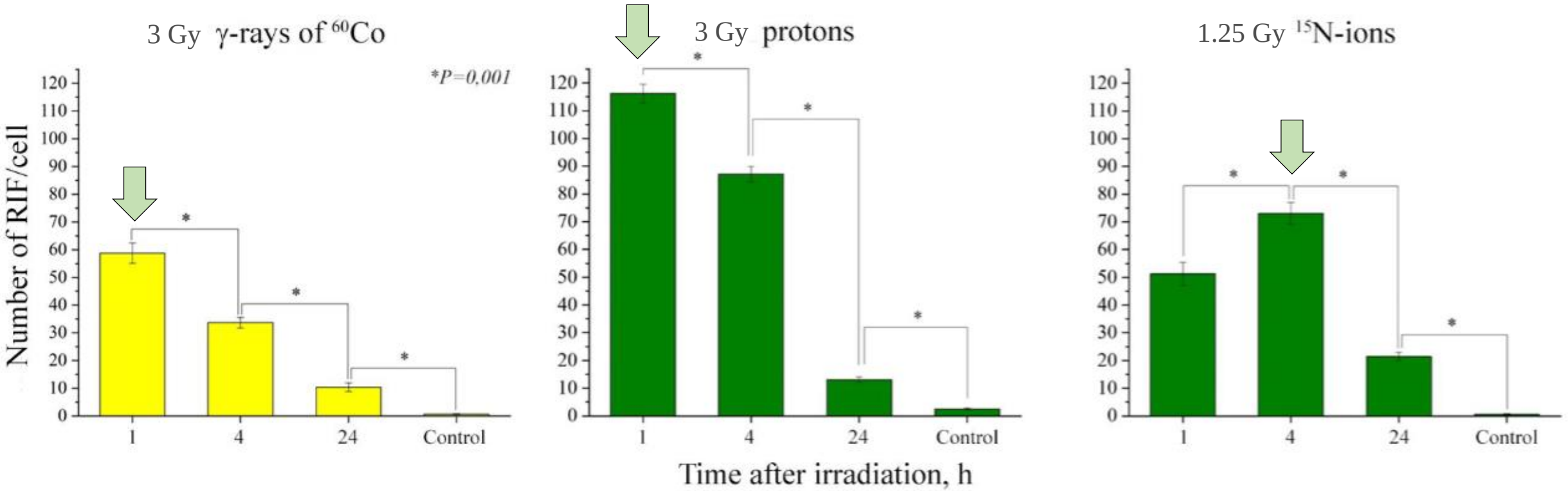

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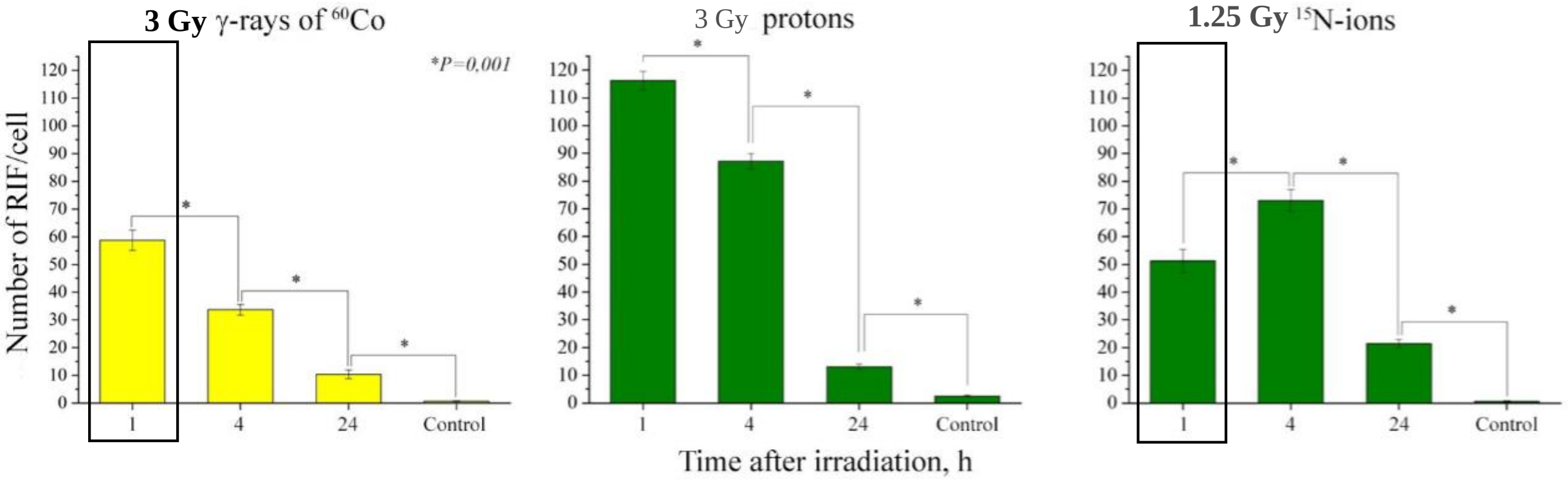

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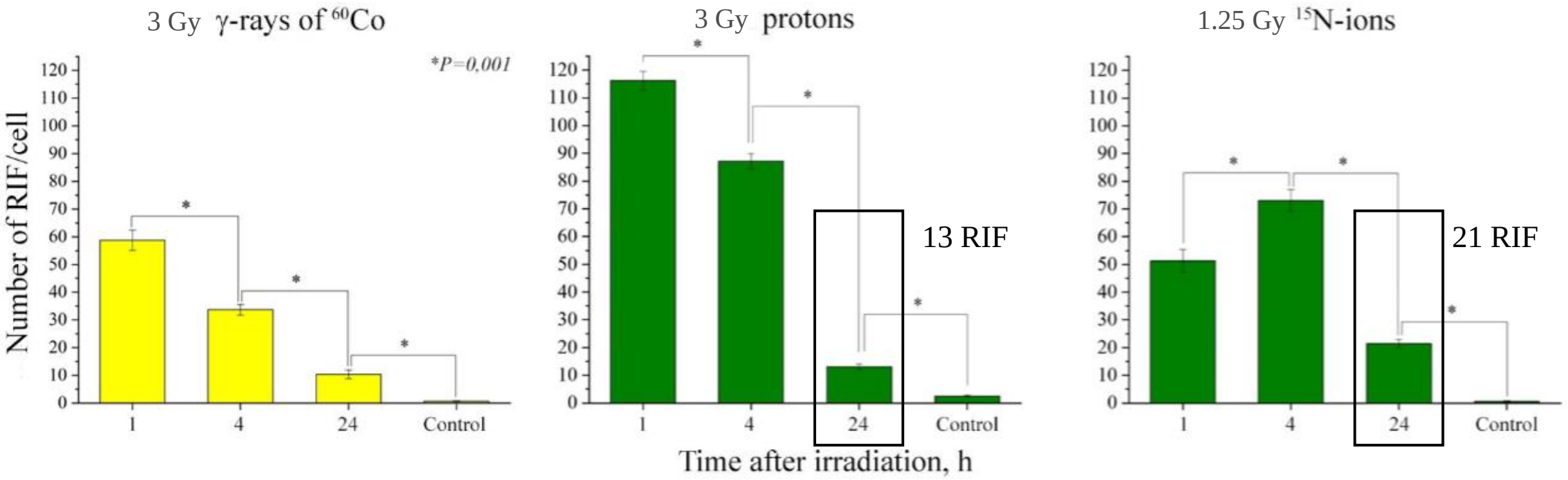
The kinetics of RIF formation in mature neurons of primary rat hippocampal cell culture



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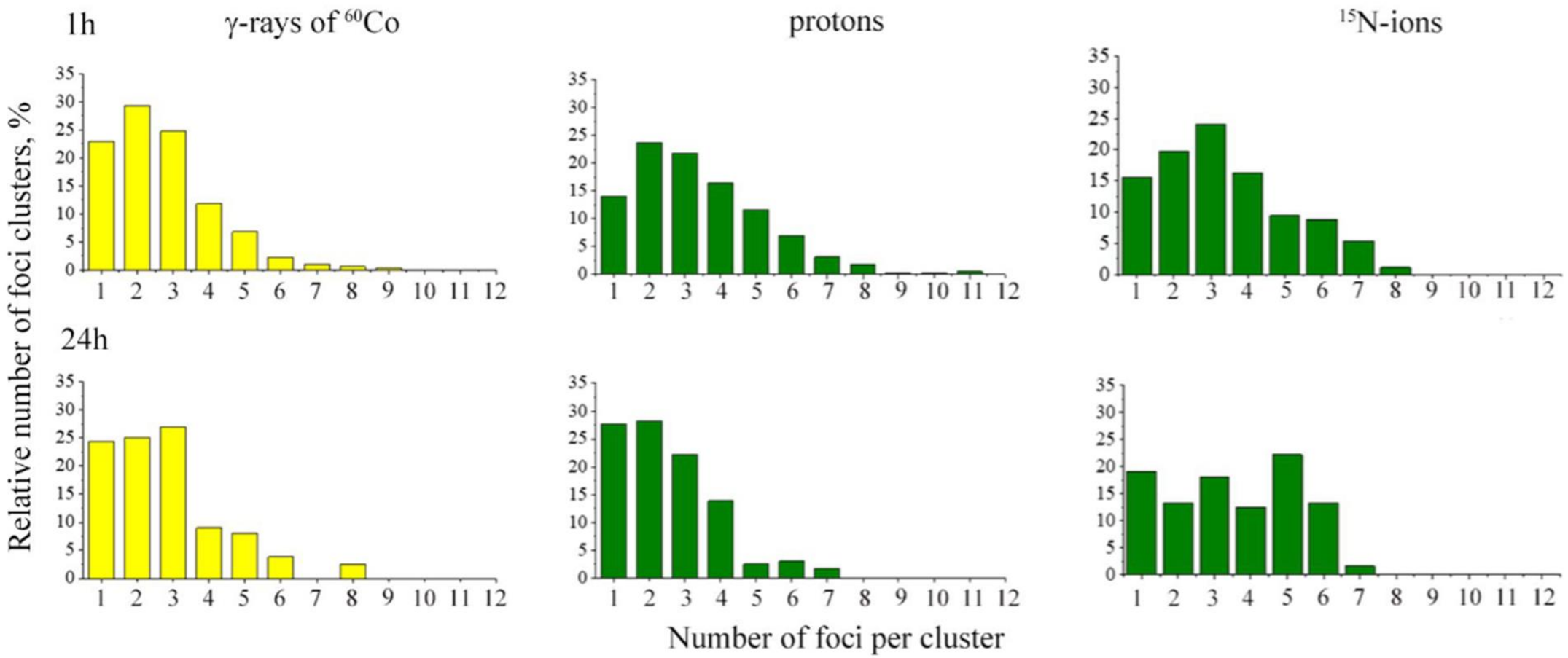
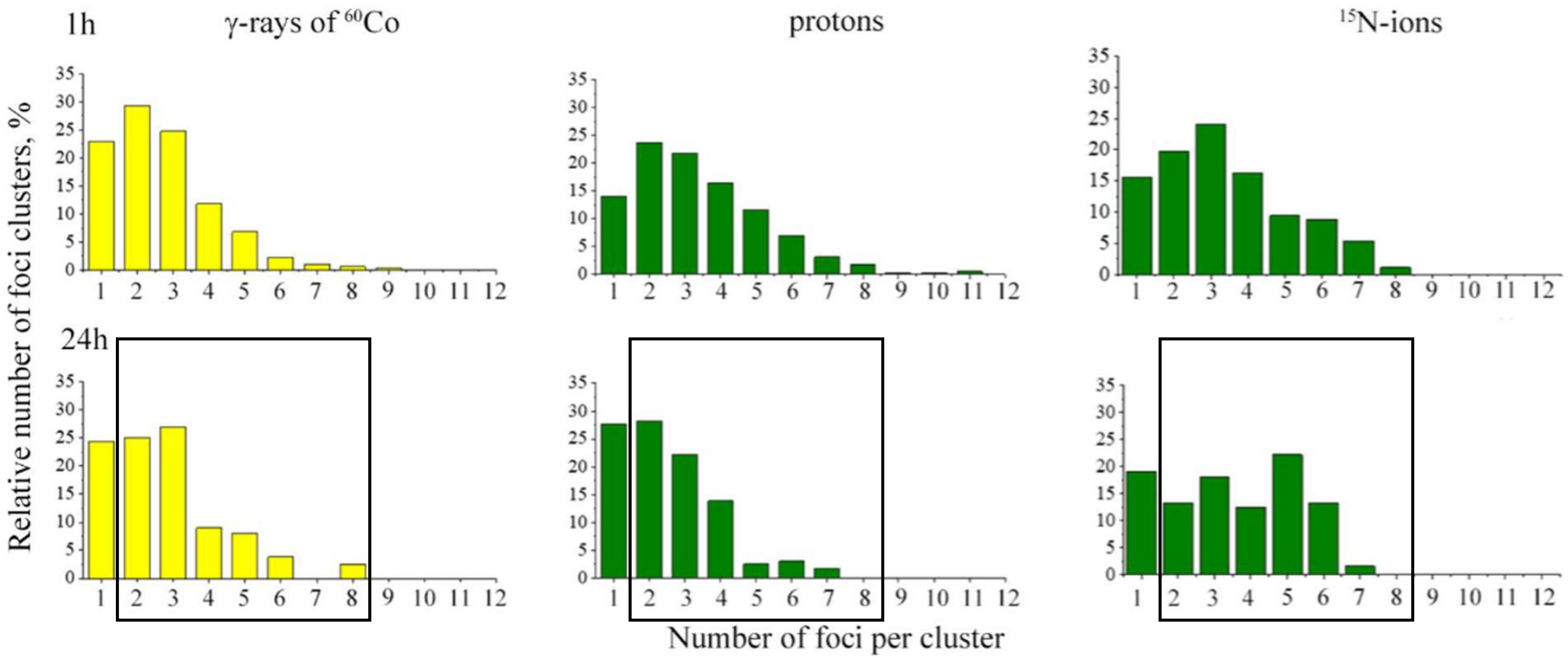
The structure of complex RIF clusters in mature neurons of primary rat hippocampal cell culture



The X-axis is a number of individual foci in a complex cluster, the Y-axis is a number of foci in clusters with defined complexity calculated relatively to the total number of analyzed RIF clusters in three dimensional space of nuclei.

The structure of complex RIF clusters in mature neurons of primary rat hippocampal cell culture



The X-axis is a number of individual foci in a complex cluster, the Y-axis is a number of foci in clusters with defined complexity calculated relatively to the total number of analyzed RIF clusters in three dimensional space of nuclei.

Conclusions

- The number of RIF is significantly higher after proton-irradiation in comparison with γ-irradiation that could be connected with the features of the proton energy transmission
- The peak of RIF formation shifts as LET increases to the later post-irradiation time
- The number of RIF is 2.4 times higher 1h after nitrogen irradiation compared to gamma irradiation
- 24h h after nitrogen irradiation the RIF number remained preserved in contrast to proton irradiation: 21 γH2AX foci - ^{15}N-ions, 13 γH2AX foci – protons
- The foci clusters complexity increases as LET grows that is clearly demonstrated by the dominance of foci clusters with the higher number of individual foci

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Thank you for your attention!