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JOINT INSTITUTE FOR NUCLEAR RESEARCH

Laboratory of Radiation Biology

FINAL REPORT ON THE START PROGRAMME

Study of physiological parameters of a laboratory rat

Supervisor:

Mr Iurii Severiukhin

Student:

Zahra Aliyeva, Azerbaijan
Moscow State University Baku branch

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Abstract

This project is devoted to the study of behavioral reactions, hematological parameters and morphological changes in the tissues of small laboratory animals (SD rats). During the experiments, the behavioral reactions of rats in the Open Field test system, hematological parameters of mature individuals were studied, and the induction of apoptosis in animal tissues after irradiation was evaluated.

This kind of research is of great importance for modern neurobiology, physiology, and radiation biology.

Text

Laboratory rat as an object of research

The rat has extraordinary mental abilities. Currently, rats are bred in large quantities in special nurseries, used for staging various scientific experiments.

White rats are widely used for research in genetics, microbiology, virology, toxicology and radiobiology. An important advantage of rats as laboratory animals is that they are small in size, easy to care for and multiply quickly.

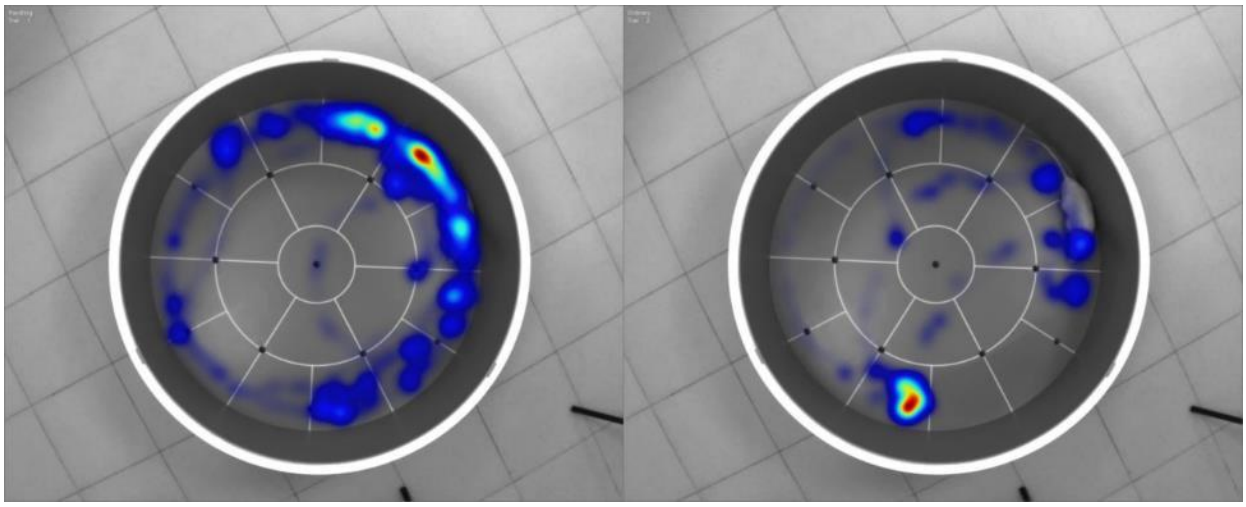
Experiments on rats have helped humans in the treatment of deadly diseases, in establishing the toxicity of drugs and poisons, in the study of nutrition and behavioral processes, as well as in space research.

Materials and methods.

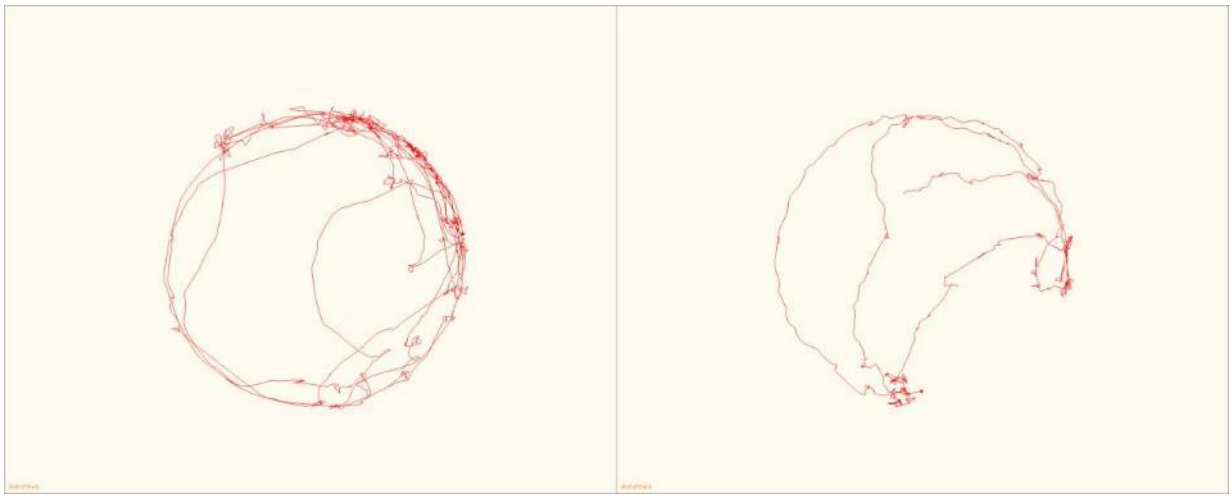
In this paper, we consider a number of experiments involving 2 sexually mature individuals of male rats of the Sprague-Dowley(SD) line aged 20 weeks with an average weight of 800 g. The animals came from the laboratory nursery "Pushchino" and were kept in a vivarium with free access to feed and water. The lighting mode included 12 hours of light, 12 hours of darkness. The conditions of keeping animals and conducting the experiment corresponded to the rules of bioethics.

The photos above show a vivarium (left) and containers with rats inside the vivarium itself (right).

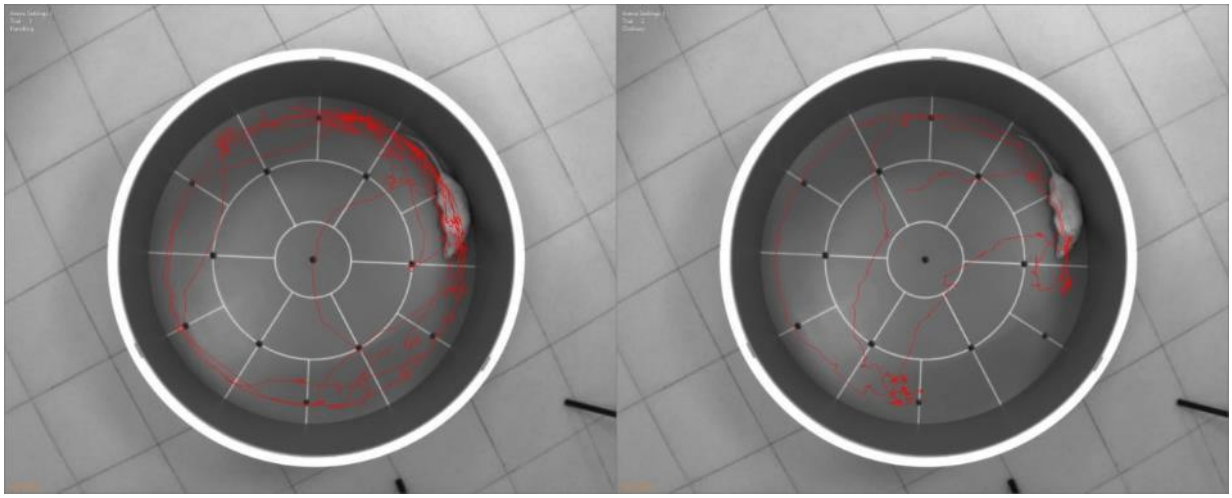
Test system open field:



Heat map of the movement of rats



Tracking a rat without marking the boundaries of the available field of movement



Tracking rats by marking the boundaries of the available field of movement

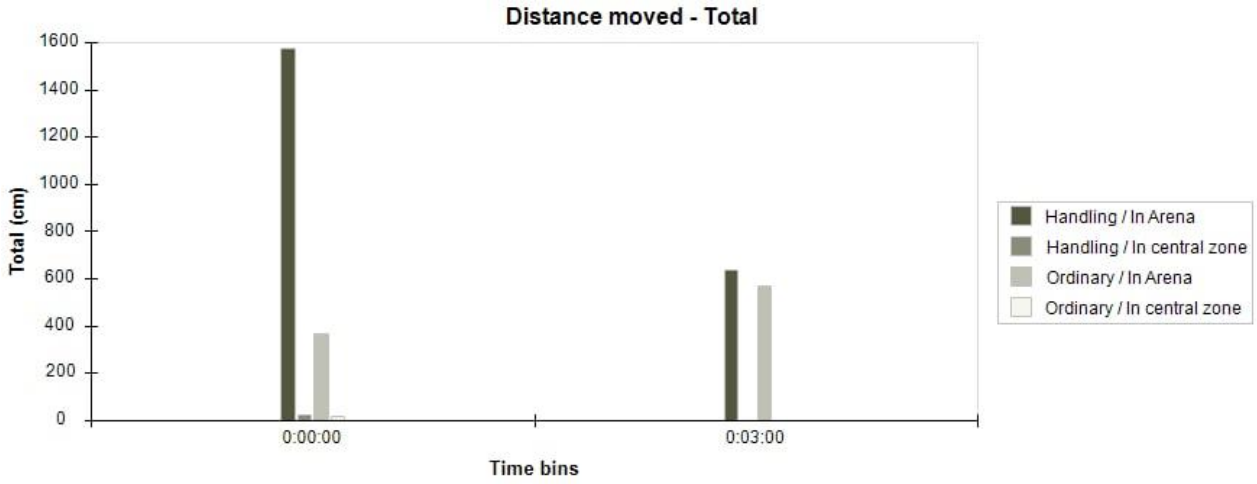
Noldus EthoVision XT Test System:

EthoVision XT is the most widely used video tracking software that tracks and analyzes the behavior, movements and activity of any animal.

The results calculated by the program are shown below in the table:

				Distance moved	Velocity
				center-point	center-point
				Total	Mean
				cm	cm/s
Handling	Trial 1	Start-0:03:00	In Arena	1576.51	8.76032
Handling	Trial 1	Start-0:03:00	In central zone	22.1641	23.0876
Handling	Trial 1	0:03:00-0:06:00	In Arena	638.275	3.55071
Handling	Trial 1	0:03:00-0:06:00	In central zone	-	-
Ordinary	Trial 2	Start-0:03:00	In Arena	369.246	2.05182
Ordinary	Trial 2	Start-0:03:00	In central zone	15.4661	19.3327
Ordinary	Trial 2	0:03:00-0:06:00	In Arena	570.165	3.16759
Ordinary	Trial 2	0:03:00-0:06:00	In central zone	-	-

The table shows the results of the speed and distance traveled in the center and in the periphery of the study area at different time intervals of two rats: those who underwent handling and those who did not undergo handling.



A bar chart with the values of both subjects in two time intervals(00:00:00-00:03:00 and 00:03:00-00:06:00) in the central zone and in the periphery.

Behavior:

In the course of conducted studies of behavioral, motor activity reactions, research behavior and anxiety. An increase in motor activity and research behavior was found in the group of animals that underwent handling, at the same time, prolonged grooming during the first testing period and a large

number of acts of urination and defecation indicate an anxious state of animals that did not undergo handling.

Clinical urine analysis:

Urine collection was carried out with the natural urination of the animal during fixation. The urine was collected in a clean glass tube. The analysis was performed using URITU CVET 13 Plus test strips on the ZOOMED UC-32 Vet analyzer.

Hemanalyzer (general clinical blood test):

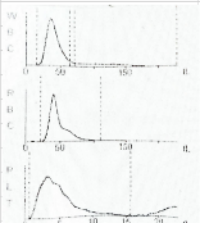
For analysis, blood was collected from the tail vein into IMPROMINI tubes with EDTA-K3. The analysis was carried out on the BC-2800Vet analyzer.

The count of the hematological analyzer for the first rat.

	Отсчет гемалог. анализатора					
Хоз: Захра А.		Имя жив: handled_rat_1_FATMAN		Тип животного: Крыса		
Код: 1	Время: 05-08-2022 16:15	Пол: M	Возр: 18 мес	Режим: Цельн. кровь		
Параметр	Рез.	Номин. Диапаз.	Параметр	Рез.	Номин. Диапаз.	
WBC	9.7*10^9/L	2.9-15.3	HCT	46.00%	36.0-46.0	
Lymph#	6.7*10^9/L	2.6-13.5	MCT	53.0 fL	53.0-68.8	
Mon#	0.4*10^9/L	0.0-0.5	MCH	19.2 pg	16.0-23.1	
Gran#	2.6*10^9/L	0.4-3.2	MCHC	H 363 g/l	300-341	
Lymph%	69.20%	63.7-90.1	RDW	12.70%	11.0-15.5	
Mon%	3.80%	1.5-4.5	PLT	778*10^9/L	100-1610	
Gran%	27.00%	7.3-30.1	MPV	5.6 fL	3.8-6.2	
RBC	H 8.68*10^(12)/L	5.60-7.89	PDW	16.4		
HGB	H 167 g/L	120-150	PCT	0.44%		

The count of the hematological analyzer for the second rat

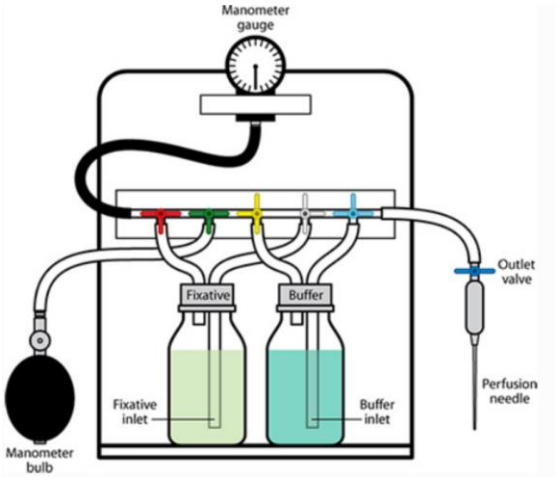
	Отсчет гемалог. анализатора						
Хоз: Захра А.			Имя жив: handled_rat_2_BABY		Тип животного: Крыса		
Код: 1	Время: 05-08-2022 16:15		Пол: M	Возр: 18 мес	Режим: Цельн. кровь		
Параметр	Рез.		Номин. Диапаз.	Параметр	Рез.	Номин. Диапаз.	
WBC		8.3*10^9/L	2.9-15.3	HCT	H 48.1%	36.0-46.0	
Lymph#		6.4*10^9/L	2.6-13.5	MCT	L 52.8 fL	53.0-68.8	
Mon#		0.2*10^9/L	0.0-0.5	MCH	19.6 pg	16.0-23.1	
Gran#		1.7*10^9/L	0.4-3.2	MCHC	H 372 g/l	300-341	
Lymph%		77.00%	63.7-90.1	RDW	11.30%	11.0-15.5	
Mon%		2.50%	1.5-4.5	PLT	793*10^9/L	100-1610	
Gran%		20.50%	7.3-30.1	MPV	5.5 fL	3.8-6.2	
RBC	H	9.12*10^(12)/L	5.60-7.89	PDW	16.2		
HGB	H	179 g/L	120-150	PCT	0.44%		



The result of the analysis shows that all indicators are within the age norm.

1)Perfusion:

Replacement of blood in the bloodstream with a 10% sucrose solution (without a fixative) was performed on an animal in a state of deep anesthesia using the Sacrifice Perfusion System, Leica.



Perfusion scheme:

Before perfusion is performed, anesthesia is administered to the subject. After the introduction of anesthesia, a check is carried out for deep pain sensitivity, if the animal does not react, then we proceed to the autopsy, and then to perfusion.

Operation stages:

The first stage:

a) A lateral incision is made through the skin and abdominal wall.

b) Then parallel incisions are made on both sides from the ribs to the collarbone.

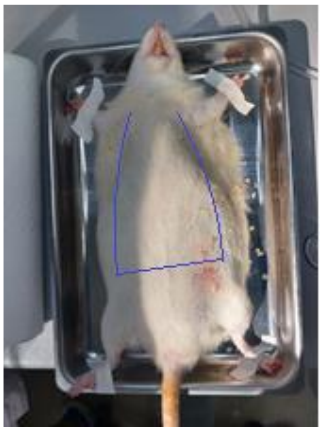
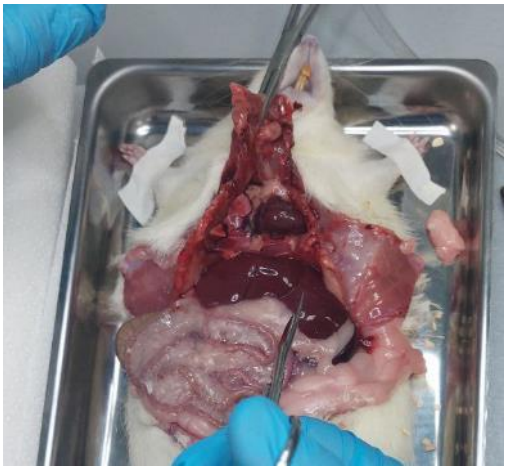


Fig. 1



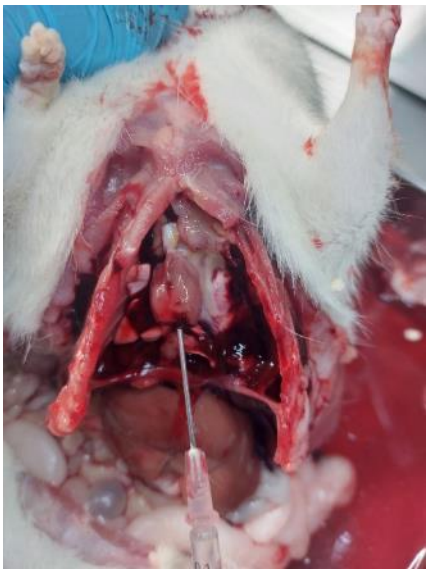
c) The bottom of the sternum is fixed so that free access to the heart is open. (fig. 2)

Fig. 2

The second stage:

a) A perfusion needle is passed through the incised ventricle into the ascending aorta and fixed.

b) With the help of rainbow scissors, a small incision is made at the posterior end of the left ventricle.



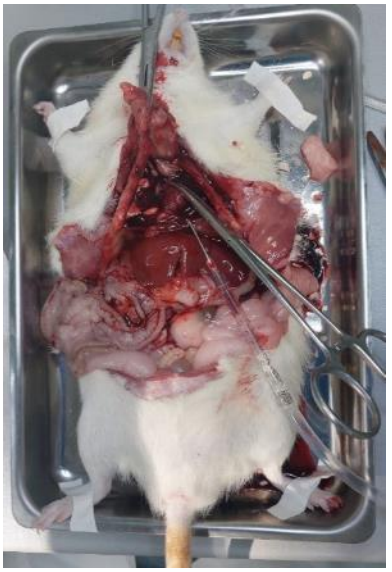


Fig. 3

To create pressure in the pipeline, a rubber bulb of the pressure gauge is used. It is very important to make sure that no air bubbles form. The pressure gauge cylinder is inflated to a pressure of 100-150 mm Hg. and then this pressure is maintained throughout the entire period of buffer infusion. (see Fig. 4)

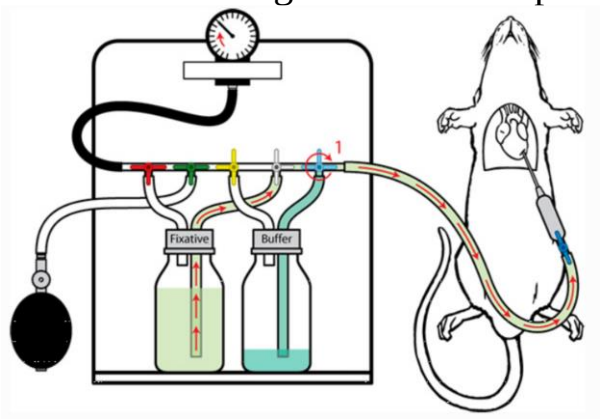


Fig. 4

Decapitation: at this stage, the brain is extracted from the skull.

The head is cut off from the body and the skull is opened. A spatula is used to cut off the olfactory bulbs and nerve connections in the very anterior location of the brain. After the extracted brain is placed in a bottle with a retainer

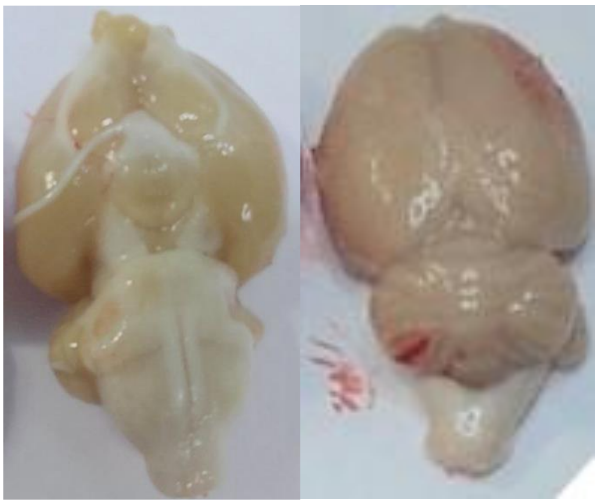
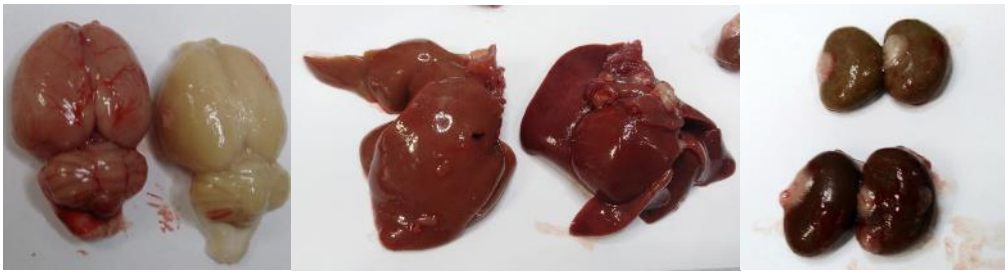


Fig. 5

2) Conducting an autopsy: The second method considered in this paper is autopsy. This is done in order to show the difference between the organs that the perfused test subject had. The body of the initially decapitated test subject is not filtered from the blood as the body of the perfused:



Stereotaxis:

Stereotactic surgery is a minimally invasive form of surgical intervention that uses a three-dimensional coordinate system to determine the location of small objects inside the body and perform certain actions with them, such as removal, biopsy, injury, injection, stimulation, implantation, radiosurgery (SRS), etc.

In neurosurgery, the use of stereotaxis makes it possible to detect and treat affected areas of the brain with great accuracy, which are often inaccessible when using other techniques.

Histological technique:

HISTOLOGICAL TECHNIQUE is a set of methodological techniques used in histology and pathology in the manufacture of preparations of cells, tissues and organs for their subsequent microscopy.

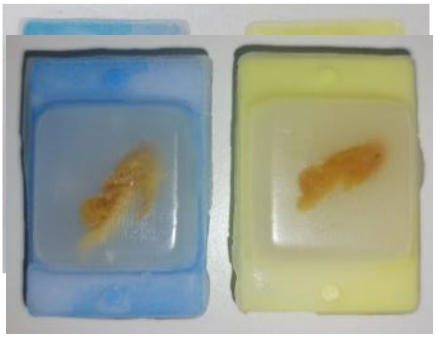
Preparation of permanent histological preparations in the form of thin sections consists of the following main stages: fixation, washing, dehydration and filling of pieces, preparation of sections, staining, dehydration and conclusion.

Fixation is the preservation of the structure and chemical composition of cells and tissues by rapid exposure to chemical or physical reagents that prevent the development of postmortem changes. Optimal for fixation is a formalin solution buffered at a pH of 6.8-7.0. For the laboratory preparation of buffered formalin, it is recommended to use the R.D.Lillie19 prescription. The final fixation of tissue samples should be complete and adequate. Buffered formalin is the optimal fixing liquid, and excess exposure in a high-quality and uncontaminated solution for hours or even a day does not have a negative effect on tissues.

All manipulations with samples – from taking the material to transportation to the laboratory, must be carried out extremely carefully to prevent their mechanical, thermal and chemical damage.

The material is poured into paraffin wax with molten paraffin (BioVitrum, Russia) using a dispenser (Medax 43900, Germany) and special filling molds and cassettes (BioVitrum, Russia). The molds are left to solidify, after which the cassette is removed from the filling mold and ready for use on the microtome.

When pouring into paraffin, the fabric washed from the fixator is dehydrated (through alcohols of increasing strength), passed through an intermediate solvent (xylene or chloroform) and impregnated with paraffin at t 55-56 ° C, then, quickly cooling the pieces, paraffin blocks are obtained. To obtain sections from paraffin and celloidine blocks, a sledge microtome is used. By staining the sections, contrast is achieved between various structures of cells and tissues that perceive certain dyes differently.



The sections are stained after removal of paraffin (xylene) from them, wiring them through alcohols of decreasing concentration and washing in water. Methods of staining drugs are diverse. Of the main dyes, hematoxylin, carmine, safranin, methyl green, gallocyanin are more often used. For the preparation of nerve tissue preparations, supravital staining with methylene blue and various methods of impregnation are used — the restoration of metals, mainly silver, by nerve structures. After coloring, well-washed preparations are dehydrated in alcohols of ascending strength (up to 100%),

enlightened in a mixture of carbolic acid and xylene (1:3), kept in 2 portions of xylene and then enclosed in an environment that ensures the preservation of the object's structures, its color and transparency, using Canadian or fir balsam, polystyrene, etc.

Tunnel staining(technique)

TUNEL Assay Kit - HRP-DAB (ab206386) allows the recognition of apoptotic nuclei in paraffin-embedded tissue sections, frozen tissue sections, or in preparations of single cell suspensions fixed on slides. In this assay, Terminal deoxynucleotidyl Transferase (TdT) binds to exposed 3'-OH ends of DNA fragments, which are generated in response to apoptotic signals, and catalyzes the addition of biotinlabeled deoxynucleotides. Biotinylated nucleotides are detected using a streptavidin-horseradish peroxidase (HRP) conjugate. Diaminobenzidine (DAB) reacts with the HRP-labeled sample to generate an insoluble brown substrate at the site of DNA fragmentation. Counterstaining with methyl green aids in the morphological evaluation and characterization of normal and apoptotic cells.

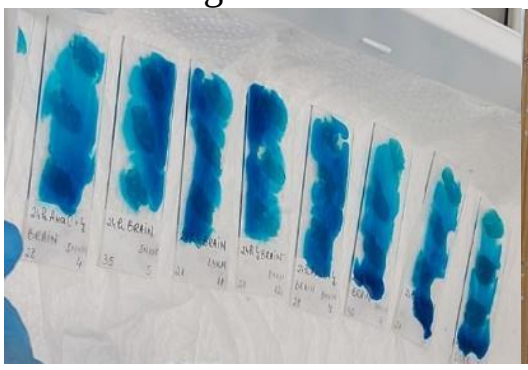
Apoptosis is the result of a cascade of molecular and biochemical events involving endogenous endonucleases that cleave DNA into the prototypical 'ladder of DNA fragments' that may be visualized in agarose gels. This apoptosis detection assay exploits the fact that, when the apoptotic endonucleases produce the classical DNA ladder, they generate free 3'-OH groups at the ends of these DNA fragments. These free 3'-OH groups are end-labelled by TUNEL Assay Kit - HRPDAB, allowing for the detection of apoptotic cells using a molecular

biology-based end labelling technique.

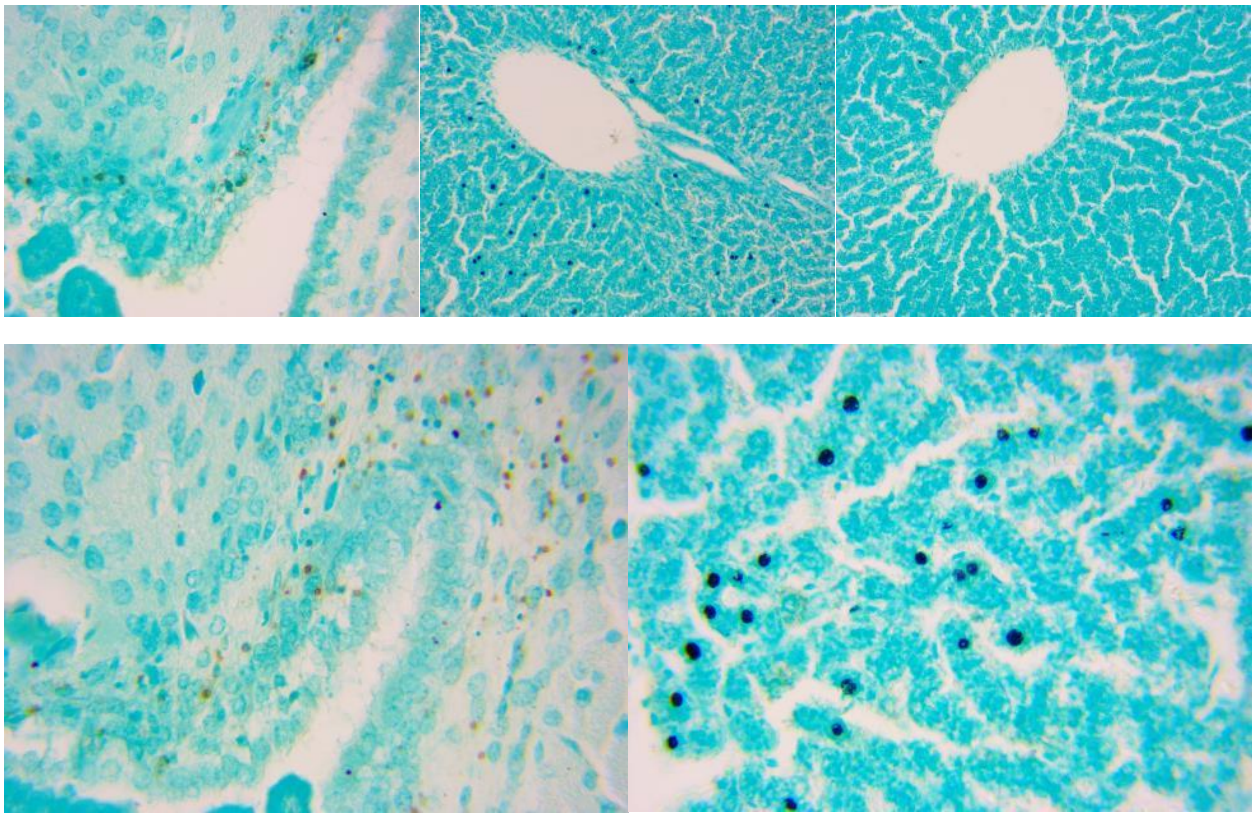
Preparations used in tunnel staining.

Staining results:

Irradiated organs were examined (24 hours after exposure).

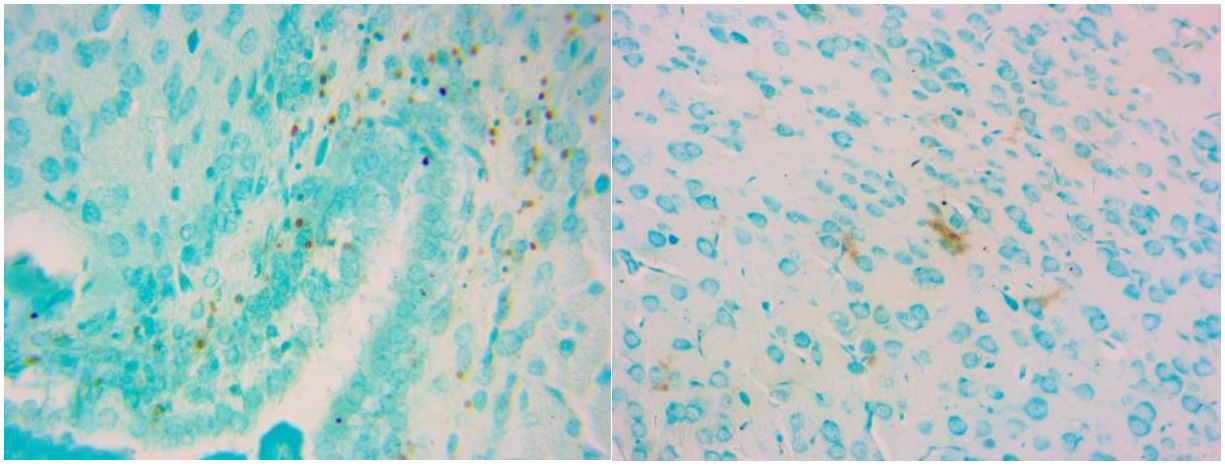


Liver:



The black dots on the images are apoptotic nuclei of hepatocytes of the liver.

Brain:



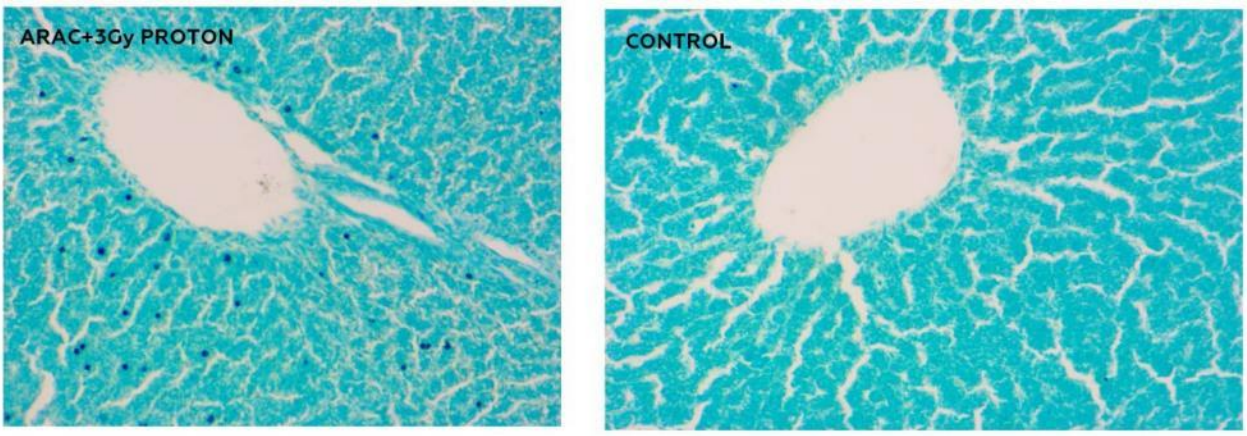
Few apoptotic cells (brown and black) were found in the brain. Apoptosis in the ventricular areas of the brain.

Results:

Methods of radiation physiology, ethology, histology have been mastered.

Differences in behavioral reactions were found between animals that underwent handling and did not undergo handling.

Hematological changes in age-related rats at the age of 18 months were identified and analyzed, tunnel staining was performed to detect apoptosis.



The optimal method of transcardial perfusion was determined.

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