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EFFECTS OF INHALATION OF LOW DOSES OF RADON IN THE KRUSHINSKY-MOLODKINA RAT STRAIN AND STUDY OF VARIOUS BEHAVIORAL CHARACTERISTICS

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რადონის დაბალი დოზების ინჰალაციის ეფექტი კრუშანსკი-მულოდკინას ვირთხებში და სხვადასხვა ქცევითი მახასიათებლის შესწავლა

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რეზიუმე

ბალნეოთერაპიაში რადონის ეფექტის შესასწავლად ექსპერიმენტულ ცხოველთა ჯგუფმა (მრავალჯერადი დაბალი დოზები გამოიყენეს KM და ეპილეფსიურ ვირთხებში) გაიარა რადონის ინჰალაციის პროცედურა წყალტუბოს მინერალური წყლის აუზში, დღეში ერთხელ, 20 წთ-ის განმავლობაში, 10 დღე. საკონტროლო ჯგუფების ცხოველებში რადონით ინჰალაცია არ იყო გამოყენებული.

წყალტუბოს მინერალური წყლით რადონის ინჰალაციის თერაპიის შემდეგ დაფიქსირდა ეპილეფსიური კრუნჩხვების არარსებობა ან შემცირება.

კვლევამ აჩვენა, რომ წყალტუბოს მინერალური წყლის რადონის ინჰალაცია იწვევს ჰორმეზს, მცირდება ან არ ფიქსირდება ეპილეფსიური კრუნჩხვები. შედეგი სტაბილური იყო 6-12 თვის განმავლობაში. სტაბილიზაციამ დადებითად იმოქმედა ოქსიდაციური სტრესის დონეზე, Na/K-ATF ეპილეფსიურ ვირთხებში. ჩვენი ექსპერიმენტის შედეგი გვაძლევს სტიმულს, გავაგრძელოთ მომავალი კვლევები, რათა ვიპოვოთ უფრო სპეციფიკური ნეიროქიმიური მექანიზმები რადონის ჰორმეზის პროცესებში.

INTRODUCTION: Recently, increased attention has been paid to hormesis in the treatment of age-related neurodegenerative diseases. The concept of hormesis refers to the biphasic phenomenon of dose-response, when low doses of the drug or stress have a protective beneficial effect, and high doses have harmful or toxic effects. It has been shown that neuro-hormesis, as an adaptive aspect of the hormonal response of neurons to a dose, slows down the onset of neurodegenerative diseases and reduces damage caused by aging, stroke, and traumatic brain injury. It was also observed that hormesis modulates anxiety, stress, pain, and severity of seizures. Neuro-hormesis can be considered a potentially innovative approach in the treatment of neurodegenerative and other neurological diseases. Studies have shown that hormesis mechanisms can prevent or improve neurodegenerative pathogenesis in animal models of epilepsy, Alzheimer's, and Parkinson's diseases. Moreover, hormesis activity at low doses of radiation and radon was evaluated in other neurological disorders, such as autism and Huntington's disease.

In this review, the neuro-hormetic concept of radon "dose-response" and possible mechanisms of neuroprotection for memory improvement in epileptic rats are discussed. The aim of this work was to study certain behavioral characteristics of the KM rat strain. Basic aspects of memory and learning mechanisms and to see the influence of hormetic effects of radon on some disorders of brain neurotransmitter systems. Specifically, the hormetic effect of radon on positive and negative behavioral stimuli 6 months after inhalation.

We also studied changes in oxidative stress markers (SH group), NA/K ATPase Activity, and animal behavior in response to both positive and negative stimuli before radon therapy and 6 months after radon inhalation. Activation of antioxidant functions during inhalation of radon plays a decisive role in alleviating inflammation and pain [4].

The uniqueness and novelty of the research lie in the study of the effect of radon inhalation on experimental models of epilepsy in the localization of epileptic foci in the hippocampus (audiogenic staining of rats according to Krushinsky-Molodkina (KM). We chose inhalation because it is a more direct method of radon application, although this is quite an advantage because we were able to reduce the dose of radon measurement, which was in the therapeutic range of 1 NC, 37 bq [2,3,6,11]. We decided to study the effects of radon inhalation on oxidative stress, namely changes in oxidative markers in both serum and brain. (SH group), NA/K ATPase Activity, and behavioral responses of animals to both positive and negative stimuli before radon therapy and 6 months after radon inhalation.

MATERIALS AND METHODS. Rats with a body mass of 200 - 250 g were placed under standard laboratory conditions with a "12 h light – 12 h dark" cycle, constant temperature of 22˚C ± 2˚C, and water and food ad libitum. Animal care and handling throughout the experimental procedures were in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC). Use of the animals in the experiments, animal care, and post-experimental euthanasia were performed in strict adherence to the officially adopted rules for animal use and care in biological laboratories [10]. Animals: for our experiment, we used 24-month Krushinsky-Molodkina (KM) male rats. They are predisposed to audiogenic epilepsy (seizures in response to a strong sound). Rapid (5-7 sec.) development of clonic-tonic seizures and the development of postictal catalepsy are characteristic of KM rats [12-14, 19].

Epileptic seizures: Genetically seizure-determined the Krushinsky-Molodkina (KM) rats were placed in an audiogenic stimulation chamber. The chamber represented a 60×60×60 cm plexiglass box, with a standard wall bell attached to the upper part. The animal was in the chamber and an audiogenic stimulus was delivered to it. A high pitch sound stimulus was presented to rats (bell intensity 110 dB, time 60 sec), in response to which they developed seizure reactions. Motor components of seizure activity were estimated by a slightly modified Jobe [8] scale: 0 - fear reaction; 1 - facial muscle clonus; 2 - head tremble, jaw myoclonus; 3 - wild run, forepaw myoclonus; 4 - myoclonus of fore- and hind paws, fall on a side; 5 - clonus of the four paws, skeletal muscle rigidity, ataxia, asphyxia. The mentioned KM rats fall into two sub-strains: a) animals, which, in response to a high pitch sound stimulus, develop fear reaction and facial muscle clonus - conventionally referred to as the first sub-strain, and b) animals developing fear and wild run to sound stimulus followed by clonic-tonic behavioral seizures - conventionally referred to as the second sub-strain. For the induction of epileptic seizures, we used an audiogenic signal before the study to which the experimental animal responded with cramps. In particular, the trigger caused the development of myoclonic seizures with "limbic" localization. Long-term (15 min) exposure of KM rats to the action of sound according to a special scheme with alternating 10 s periods of strong and weak sound causes cerebral circulation disorders in them, externally manifested in the form of paresis and paralysis of the limbs. On the 3- and 6-months assessment of epileptic seizure with the trigger - sound in BK rats was performed [10-19].

Brain synaptic membrane fraction obtained from the adult albino rats of both sex is served as an investigation material. The synaptic membrane fraction is obtained by means of differential centrifugation, at 0.9-1.2 M concentration gradients of sucrose, according to De Robertis and Wittaker's recommendations. Na, K-ATPase activity is measured as a sensitive part of total ATPase activity. The total ATPase incubation medium contained 140 mM NaCl, 5 mM KCl, and 50 mM Tris-HCl buffer at pH 7.7. Control was carried out under the following conditions: 1 mM ouabain, 145 mM KCl, and 50 mM Tris-HCl buffer at pH 7.7. Na, K-ATPase activity is calculated by the difference between these two assays. Samples are incubated at 37° C for 15min. The ATPase activity is calculated according to the inorganic phosphorus (Pi) amount (per mg protein and per hour) resulting from the enzyme-induced ATP hydrolysis. Inorganic phosphorus is evaluated calorimetrically by using the modified Fiske-Subbarow and Kazanov-Maslova methods. Protein concentration is assessed by the Lowry method [4,18,28].

Sulf-groups. Cysteine thiols and their oxidized disulfide analogs are carefully balanced to maintain redox homeostasis in various cellular compartments [28]. In this review, we discuss the role of protein thiols as scavengers of hydrogen peroxide in antioxidant enzymes, using thiol peroxidases to illustrate how thiols of the protein, non-protein, thiol group contribute to the transmission of redox signals; we will provide an overview of a diverse set of small molecular weight thiols [23,9]. Determination using the ELISA kit [18,19,22,23].

Radon measurement: in the Tskaltubo spa center, where natural mineral water is used, we measured radon radioactivity in water. The radioactivity of radon was 37 Becquerel (Bq) in 1 m3 (37 Bq/m3) [9,15,20-24,25].

Radon inhalations procedure: we placed 10 experimental animals (KM rats) in Tskaltubo mineral water spa`s sauna (experimental group). Mineral water temperature was 36$^0$C, and humidity was 90%. A Control group of 10 KM rats was placed in another spa center`s sauna, where 36^{0}C mineral water (without radon) was delivered via inhalation. Humidity in this spa center`s experimental room was 90%. None (experimental and control group of rats) of the animals took a bath, they were just in two different saunas and living in the same conditions. Inhalation was administered through the nose, for 20 minutes, once a day, in conditions of high humidity (about 90%) for 10 days. After each procedure of inhalation, the rats were placed in a vivarium and given food and water [3,5,15,29].

Laboratory examination: we studied the physiological changes, caused by inhalation of Tskhaltubo water on an oxidative level, which prevents the development of brain disorders associated with peroxidation reactions. We measured the concentrations of free radicals (d-ROMs) - reactive oxygen metabolites in the blood plasma of rats, using a photometric test and measured the concentration of hydroperoxides (ROOH) in the brain tissue, which gives us a pro-oxidant status of the tissue. Hydro peroxides, also called Reactive Oxygen Metabolites (ROM), are formed during an oxidative attack when Reactive Oxygen Species (ROS) react with various organic substrates (e.g., carbohydrates, lipids, amino acids, proteins, nucleotides, etc.) [25,30,31]. To assess the antioxidant capacity of plasma, we used the PAT (Antioxidant Concentration Test) by measuring ferric reduction ability, and to evaluate the effectiveness of antioxidants, we determined the OSI (Oxidative Stress Index) and the OBRI (Oxidation Balance Status). All named measurements were provided by means of Photometric Analytical System FRAS 5 (H&D, Parma, Italy) [3,14].

Physiological profile: The area of locomotor activity includes such indicators as "number of squares passed", "speed of horizontal movements", "time spent on movements" and "total time of stops". The sphere of research activity is represented by the indicators: "the number of explored holes" and "the number of vertical racks". And the emotional sphere includes indicators: “leaving the center”, “number of acts of grooming”, “total duration of acts of grooming”, “duration of one act of grooming” and “average number of acts of defecation”. As for the movement of epileptic rats in the maze, conditioned-reflex behavior developed on a positive stimulus is learned in this trestle-type maze. It consisted of chambers 40-50 cm long, which were fixed on 20 cm high racks. That is how we used separate small racks to build a maze of a certain complexity by changing the position of the racks. We trained the rats to run on such bridges and climb a special ladder. The staircase ended with a box that represented the animal's den. Before the training, we placed a rat on a bridge near the stairs. After some time, and then, seeing the rats in his cage, it carefully began to descend the ladder into the den. After the time spent on the ladder stabilized, we placed the rat on the starting square and taught it to walk the entire maze (starting square, across bridges and stairs, and back to the den). The criteria for developed habits were considered to be 9-9 times without error in the shortest way, for about 1 minute, the time spent on passing the maze was recorded, which started counting from the moment the animal was placed on the starting square and continued until the rat returned to its den, and this time was 1 minute. The movement of the animal was recorded by recording these numbers, which allowed us to reconstruct the trajectory of the animal's movement after the end of the experiment.

Further research was conducted on behavior based on a negative emotion, namely by the method provided by Esman and Alpern [8]. In the chamber in which we conducted the experiment (consisted of two bright and dark compartments, which were connected to each other by a 5x6 cm hole. Under the condition of passive avoidance, the reaction was produced as follows. We placed the rat in the bright compartment 12x20 cm, which immediately entered the dark compartment, where it received electrical irritation of 30 V., intermittently 5 within a second. If he did not come out of the dark compartment during this time, in these experiments, the fear reaction was produced by a one-time irritation, and therefore, according to the classification given by I. Beritashvili [3], it belongs to the memory of a psycho-neurological nature. Preservation of the fear reaction, that is, under the condition of passive avoidance, the reflex was produced after 20 minutes, 2 hours, and on the 5th day. For this purpose, we placed the rat in the light compartment again, and if it did not move to the dark compartment within 1 minute, we considered the fear response preserved.

In the study, we used adult males of the KM (N=13) and Wistar (N=17) rat strains at the age of 9 months and weighing 300-400 g. Rats were kept 4-5 individuals per cage with free access to food and water. All animals included in the experiment were intact. In order to assess zoo-social interaction, we chose a three-chamber test for social preference/social novelty. The experimental animal was placed in an experimental chamber divided into three compartments with the possibility of free movement between compartments. The outer sections contained an empty cage or a cage with a stimulus animal of the same sex and age (but not from the same cage, where the one had been before). In accordance with the choice of the tested rat (to approach a cage with an unfamiliar individual or move towards empty compartments), one can quantify the predisposition of the tested animal to zoo-social interaction or, conversely, its indifference to the presence of a stimulus animal or even the dominance of the avoidance reaction [7,18]. Contact meant that the head of a freely moving animal was located at a distance of less than 2 cm from the cage of the stimulus animal. The movement of the animal was studied with a video eye and also the following measurements were made: time spent in certain zones, path length, distance, number, and duration of fading episodes [14,16]. The ratio of the time spent by the free-moving rat in the compartments of the chamber was also recorded: contact time; vertical racks; short and full grooming; defecation and urination (if any) were counted. After each session, the surfaces of the devices were cleaned with a 50% alcohol solution. The rats that served as social stimuli were adapted to the cages used for 30-60 minutes prior to testing. Statistical processing of the results was performed in the STATISTICA 6.0 program using multivariate analysis of variance (ANOVA).

RESULTS AND DISCUSSION. Since we were studying different behaviors of epileptic rats, we decided to first determine their oxidative stress index, epileptic seizures, and Na/K ATP before and 6 months after radon inhalation, so we started our study by determining the oxidative stress index.

Reactive oxygen species (ROS), products of oxidative stress, contribute to the initiation and progression of the pathogenesis of various diseases. Although adequate levels of reactive oxygen species (ROS) act as signaling molecules required for cell growth and proliferation, increased ROS production can cause oxidative damage to cells [1,2]. Since mitochondria are one of the main intracellular ROS production organelles and are the most vulnerable targets of ROS, inadequate accumulation of ROS due to oxidative stress has been recognized as one of the mechanisms leading to apoptosis after DNA damage associated with mitochondrial dysfunction [14,22,27]. An increase in the level of ROS is associated with a change in the intracellular redox balance of cells, and this may be facilitated by the inability of antioxidant mechanisms to eliminate the production of ROS [20,27,31]. Moreover, accumulation of ROS beyond the antioxidant function of cells can decrease the mitochondrial membrane potential (MMP), a measure of the efficiency of the electron transport chain, leading to impaired production of adenosine triphosphate (ATP) [4,7.9]. Subsequently, apoptogenic factors such as cytochrome c are released into the cytoplasm from the mitochondrial intermembrane space due to the loss of MMPs and the caspase cascade is activated, which can eventually induce apoptosis. Ultimately, intracellular ATP levels can also be used as an important indicator to assess the homeostasis of mitochondrial energy metabolism associated with oxidative stress [8,9]. From the data presented in Table 1, we can see the following. A study of dROM in genetically epileptic MK rats before exposure to radon showed that 3 and 6 months after inhalation dROM, PAT, OBRI, and OSI were within the normal range. However, it should be noted that after 3 months the above data decreased, and after 6 months even more and turned out to be exactly within the normal range, which affected the behavior of the rats, and in 80-90% of the rats there were no tonic-clonic convulsions (Tab. 1, Fig. 1).

Table 1. Oxidative stress in epileptic rats

Epileptic rats	D-ROMs FAST Ucarr.	PAT	OBRI	OSI REDOX
Control	525±3.67 Free radicals, very high	2765±5.85 Antioxidants There is a deficit	1.953±0.3 Oxidative status is at a dangerous level in relation to cholesterol	45±2.3 Oxidative status index is on the critical edge
Steam control	301±2.27 Normal range	2844±5.75 Slight deficiency	1.25±0.2 Normal	42±2.2 Normal
3 months after inhalation	259±1.13 Normal range	2324±2.82 Normal value	0.8±0.001 Normal	33,2±2.1 Normal
6 months after inhalation	255±1.12 Normal range	2000±2.81 Normal value	0.8±0.001 Normal	34,2±2.01 Normal

Fig. 1. Oxidative stress in epileptic rats

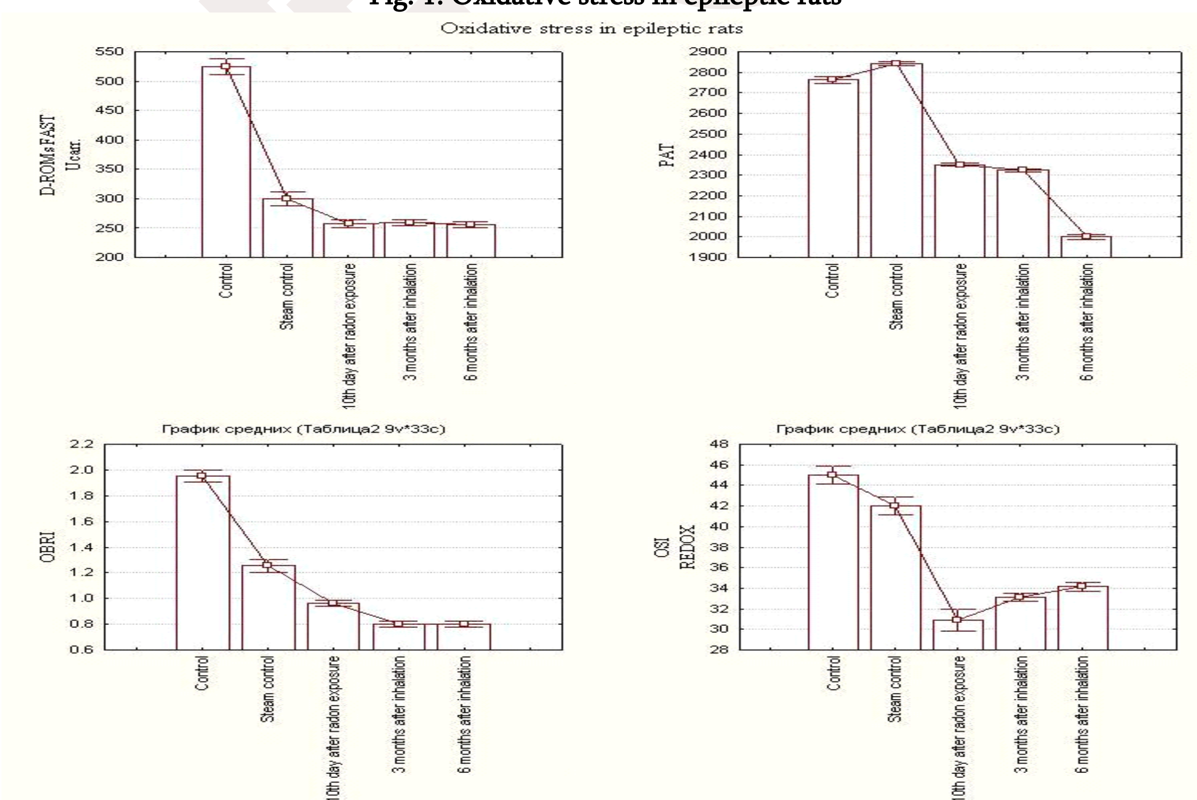
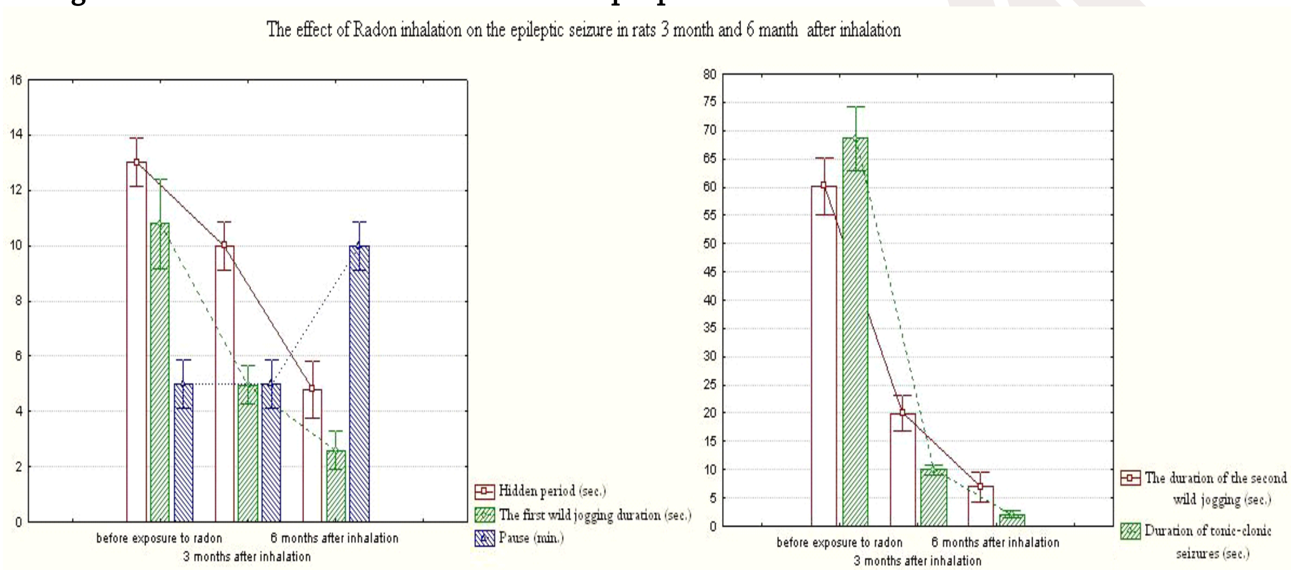


Table 2. The effect of Radon inhalation on the epileptic seizure in rats 3 and 6 month after inhalation

	Hidden period (sec.)	The first wild jogging duration (sec.)	Pause (min.)	The duration of the second wild jogging (sec.)	Duration of tonic-clonic seizures (sec.)
Before exposure to radon (p<0.05)	13±1.1	11±1.1	5±0.2	60±1.8	69±1.9
3 months after inhalation (p<0.05)	10±1.4	5,1±0.1	6.0±1.4	20±1.1	10±0.1
6 months after inhalation (p<0.05)	4±1.4	3±0.1	10±1.1	7±1.1	2.±0.1

Fig. 2. The effect of Radon inhalation on the epileptic seizure in the 3 and 6 months after radiation



By inhalation of low doses (37 Bq / m3) (Table 2 and Fig. 2), the latent period before attacks, and the pauses between attacks significantly increased (p<0.05) in the group of radon irradiation, compared to the control. The latency period before inhalation of radon in rats with epilepsy was (13±1.1). After 3 months, the inhalation (10±1.4) and after 6 months of inhalation (4.0±0.1) respectively. The duration of the first and second jumps after the trigger decreased in the group of rats that received radon inhalation, after 3 months after inhalation - (6.0±1.4) (p<0.05) and 6 after months inhalation (10±1.1). In all groups, the second wild run started later and continued in the control group (60±1.8), 3 months after inhalation of radon it was only (20±1.1), and after 6 months (7±1.1). As for the tonic-clonic episodes in the control, they lasted - (69±1.9), on the 3rd month after inhalation of radon, (10±0.1) seizures disappeared, and after 6 months after inhalation of radon, 95% of seizures disappeared [23].

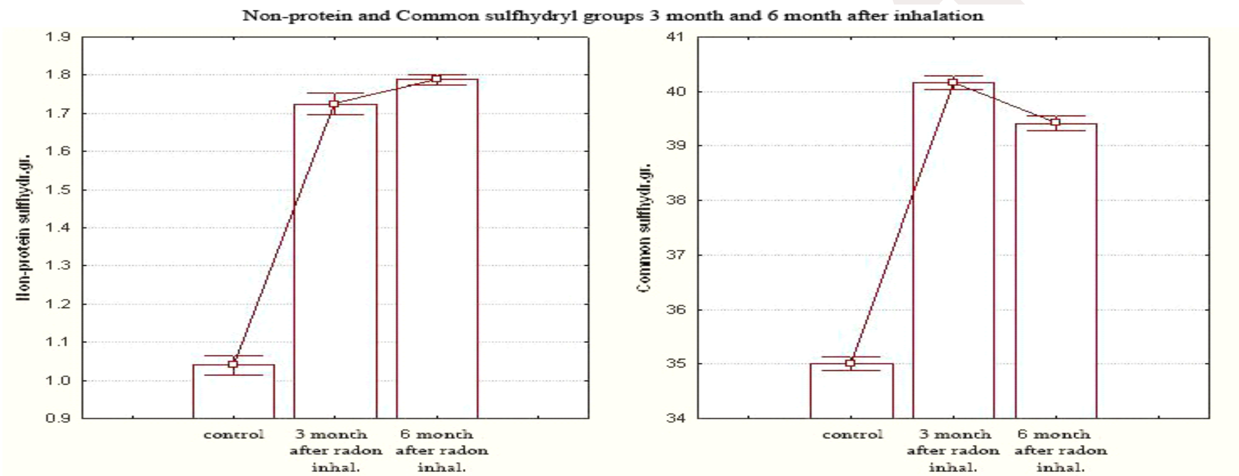
Definition of sulfhydryl groups: It is known from the literature that protein cysteine thiols respond to the cellular redox state. They can oxidize and inhibit thiol-proteins and enzymes and therefore have antioxidant action. In particular, when oxidants increase in the cell, thiol-disulfide is involved in redox regulation. These redox-sensitive mechanisms are involved in redox various changes including cell hypoxia. Under hypoxic conditions, the concentration of thiols decreases. This is due to the association of metabolites produced during the recovery of hypoxia with glutathione (GSH), a cellular nonprotein thiol (NPSH). That is, the metabolites react with GSH instead of oxygen. When cellular thiols are depleted, peroxide is produced [12] and excessive oxidative stress leads to cell death. Within the frames of our study, we examined the quantitative variation of non-protein and total Sulfhydryl groups [24,25]. On the 3 and 6 months after radon inhalations, we determined the concentration of non-protein and total SH groups in the rat brain (Tab3. Fig3).

Table 3. Non-protein and Common sulfhydryl groups 3 months and 6 months after inhalation.

	Control	3 months after radon inhalation	6 months after radon inhalation
Non-protein sulfhydryl groups	1,04±0,121	1,72±0,107**	1.79±0,109**
Common sulfhydryl groups	35,01±1,23	40,16±1,44**	39,46±1,43**

Note: **P<0.05. n=28 (14 in each group)

Fig. 3. Sulfhydryl amount (mcg / g in the brain), (p<0.05) in the control and experimental rat group on the 3 and 6 months after inhalation

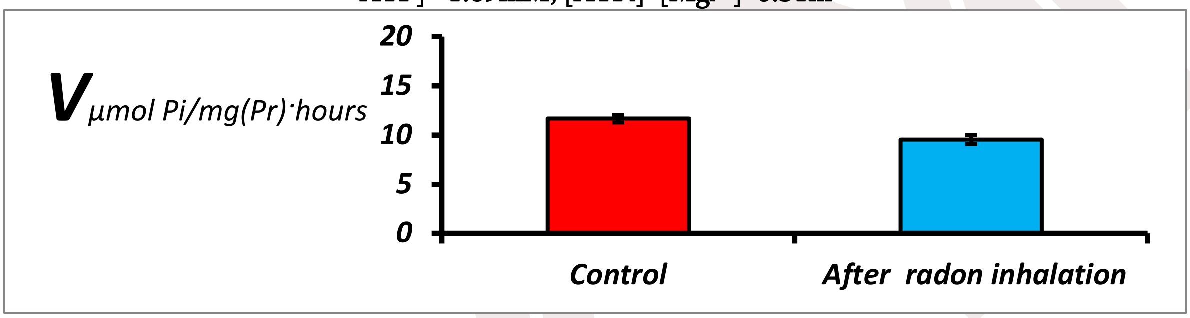


In a study of non-protein SH-groups, we found that total sulfhydryl groups 3-6rd month after radon inhalation, it increased statistically and became (40.16±1.44**) and (control 35.01±1.23), respectively, also had higher concentrations compared to the control group (39.46±1.43) and (35.01±1.23), respectively, which indicates an increase in protein concentration and the number of sulfhydryl groups, on the 3-6rd month after radon inhalation, compared to the control group performing inhalations with ordinary mineral water. We see the effect of radon inhalation on physiological processes, which acts as an activator or inhibitor of certain neurotransmitters [6,13,20]. In view of the foregoing, it can be said that exposure to radon regulates oxidative stress, the clinical manifestation of which may be a decrease in epileptic seizures, which is confirmed by studies. Considering that audio-genic epileptic seizures begin immediately after the bell and last several minutes before the Tskaltubo water inhalation in experimental rats, as shown in Fig. 2, the duration of epileptic seizures does not exceed 2 seconds after inhalation with Tskaltubo water; the 6 months after inhalation, no audiogenic convulsions were also manifested. Na-K ATPase, which is active in animals, is known to consume large amounts of ATP. At present, there is no doubt that the energy and transmitter processes in brain tissues are interconnected. Therefore, ATP (adenosine triphosphate) is a powerful source of energy, along with the fact that it interacts with the glutamine system, the links of which, in turn, are glutamate (excitatory transmitter) and GABA (inhibitory). Naturally, the recovery processes are disrupted in terms of consumption. In accordance with the previously discovered concepts of the occurrence of paroxysmal shift depolarization (PDS), disturbances in neurophysiological events are associated with disturbances in ionic, transient energy processes. Thus, the concept of the emergence of a neuron in the membrane allows for a primary violation in the membrane or the possibility that, as a result, is associated with insufficiency of the potassium-sodium pump, increased membrane permeability, and increased expansion to depolarization, and, consequently, excessive excitability of the neuron. Development of changes in the environment, neurons, increase in MPD, dysregulation of the concentration of electrolytes or transmitters, or both. Therefore, we decided to study the activity of Na/K-ATP 6 months after radon inhalation. Changes in glucose metabolism deficiency under the action of Na+/K+-ATPase are associated with neuronal hyperactivity (Tab. 4, Fig. 4). This is one of the leading mechanisms for reducing the concentration of extracellular K+ accumulated after seizure activity. Low activity of Na+/K+-ATPase is associated with the development of epileptic seizures. In addition, the activity of Na+/K+-ATPase decreased within a few minutes after transient focal ischemia in the cerebral cortex and hippocampus of rats, as well as in an experimental model of brain injury. Altered ion homeostasis may also partly explain the interaction between seizure activity and hypoglycemia.

Table 4. The effect of radon on the 6-month-old rat brain synaptic membrane Na/K-ATPase activity

Experimental rats	Na/K-ATPase activity	%
Control	11.66±0.39	100%
After radon inhalation	9.53±0.45	82.59%

Fig. 4. 6-month-old rat brain synaptic membrane Na/K-ATPase activity. The reaction medium was [Mg-ATP] =1.69mM; [ATP_f]=[Mg_f^{2+}]=0.31m



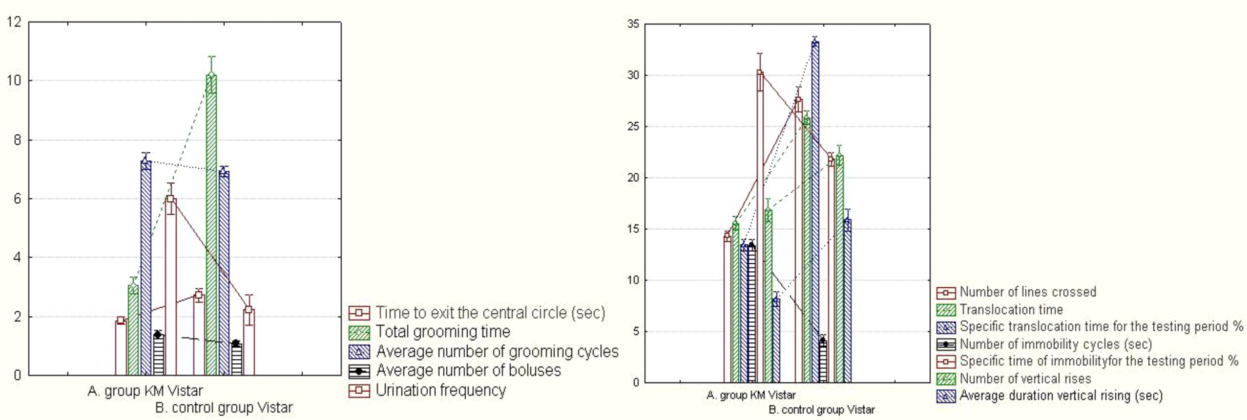
Based on the obtained results, we began to study the behavior of epileptic rats to achieve our goal. The main part of this article was to study the behavior of epileptic rats to both positive and negative stimuli, to determine the quality of memory of epileptic rats during various behavioral tests, and to see what effect radon inhalation has on memory performance using various behavioral tests. Therefore, we began to study the behavior of epileptic rats using the "open field" method in order to determine their emotional condition. The comparison of behavioral responses in the "open field" test showed that the locomotor activity of the studied rat strains differs only in the outcome (Table 5). Crossing an equal number of squares, the males of the KM line show a higher speed of horizontal movements in the “open field” than Wistar, but spend more time on stops. As they spend more time perceiving the environment in which they move. As for the locomotor activity in the open field, we saw that the control rats had more crossed cells (14.53±0.06) than the epileptic rats (27.8±0.07). As for the time spent on crossed cells, in control rats, it is (15.6±0.06) and in epileptic rats (25.9±0.06), there is a significant difference in both the time spent in translocation and the percentage of time spent in translocation in relation to the total time spent in the experiment. Therefore, we can say that the control rats move more slowly and crossed fewer cells, which can be explained by grooming, by standing in the center and at the walls. They tried to perceive the environment they are in and make the right decision for their family members, which is the right social position for them. It points to the perception of the environment, which epileptic rats do not have, they are more emotional and move quickly, which is confirmed not by the amount of grooming (7.3±0.01), but also by the time spent on grooming (3.1±0.01), Wistar rats (6.9±0.01) and time (10.2±0.01), as well as the number of standing up to the wall in epileptic rats (8.2±0.02) and time (16±0.03), which is also confirmed by the time of exit from the central circle in epileptic rats (1.86±0,07) and Wistar rats (2.78±0.06). An increase in the number of upright positions and a significant increase in the time spent on orientation while in one or another compartment means that the rats are exploring the compartment in which they are placed, i.e., they are engaged in "open field exploration". A comparison of the stereotypic activity of epileptic and Wistar rats in the open field showed that, although the number of grooming cycles in the animals differed slightly, it can be said that Wistar rats spent time standing up both in the vertical and in the number of center movements during the grooming time. Wistar rats have more than epileptic rats, an indicator of their excessive emotion and anxiety.

Indicators of exploratory activity of Wistar rats are stable. While in KM rats they significantly change (Table 5). KM rats paid less attention to looking down than Wistar rats, at the same time it should be noted that, it should be noted that not all live lines of KM showed interest in holes located on the arena. Some animals did not react at all to them, while Wistar rats explored every hole in their path [6]. In the study of the emotional sphere of behavior, special attention is usually paid to the analysis of the level of emotional reactivity, as well as to the assessment of the observed manifestations of grooming. As you can see, the evaluation of the values of the «number of acts of defecation», which is traditionally associated with the level of emotional reactivity in small rodents [4], did not reveal any reliable changes in the investigated lines. Moreover, the KM rat strains did not differ from Wistar throughout the experiment (Tab. 2). The data presented in table 2 indicate that the value of the indicator «number of acts of grooming» in epileptic rats of the experiment was at a significantly lower level. The indicator "total duration of all acts of grooming" turned out to be stable, and at the KM strain, it was always at a lower level [5]. Another stable indicator of grooming in Wistar can be called «duration of one act of grooming». It was always at a higher level compared to the one at KM, which was shorter. As a rule, they are interpreted as a manifestation of tension and/or conflict of research motivation and fear [6]. Prolonged acts of grooming, on the contrary, are evaluated as elements of comfortable behavior [2]. In connection with this, the indicator of the average duration of one act of grooming comes to the fore in the assessment of the emotional component of behavioral reactions, which, with the sufficient quantitative manifestation of this reaction, can be a direct characteristic of the animal's emotional state. The shorter one act of grooming - the higher the probability that there is a misplaced activity. The behavior of the animal does not correspond to the actual motivation and situation. And the longer it lasts, the less emotionally the animal reacts to the novelty of the situation artificially created in the "open field" test. In the experiment, the rats of the KM rats strain turned out to be more emotional than the Wistar rats' strain. Perhaps the fact that epileptic rats, throughout the entire experiment, leave the center of the arena early should be connected with this. This feature is preserved throughout all fifteen days of research. The increased emotionality/sensibility is not only related to the nature of grooming but, most likely, also to the nature of their locomotor activity. The more emotional KM move around the arena with "short runs", while the less emotional Wistar rats maintain a relatively low speed of movement. In the emotional sphere, there is a gradual increase in the share of "displaced activity" in the general structure of behavior, which further distances the KM rat strains from Wistar (Tab. 5, Fig. 5).

Table 5. Locomotor activity, orienting-exploratory reactions, and emotional sphere of the behavior of rats of the Krushinsky-Molodkina strain

	A. group KM	B. Control gr, Wistar	P_{A-B}
Time to exit the central circle (sec)	1,86±0,8	2,78±0,06	<0.05
Number of lines crossed	14,53±0,06	27,8±0,07	<0.05
У translocation time	15,6±0,06	25,9±0.06	<0.001
specific translocation time for the testing period %	13.31±0,05	33.5±0,08	<0.001
Number of immobility cycles (second)	13,2±0,2	4,1±,0,5	<0.001
Specific time of immobility for the testing period %	30,3±4,7	21,9±4,2	<0.001
Number of vertical rises	17,0±1,01	22,1±0,5	<0.001
Average duration, vertical rising. sec	8,2±0,2	16.0±0,3	<0.005
Total grooming time sec	3,1±0,01	10,2±0,01	<0.05
Average number of grooming cycles	7,3±0.021	6,9±0.01	<0.05
Average number of boluses	1,4±0,1	1,1±1,0	>0.05
Urination frequency	6,0±0,2	2,3±0,2	<0.05

Fig. 5. Locomotor activity, orienting-exploratory reactions, and emotional sphere of the behavior of rats of the Krushinsky-Molodkina line

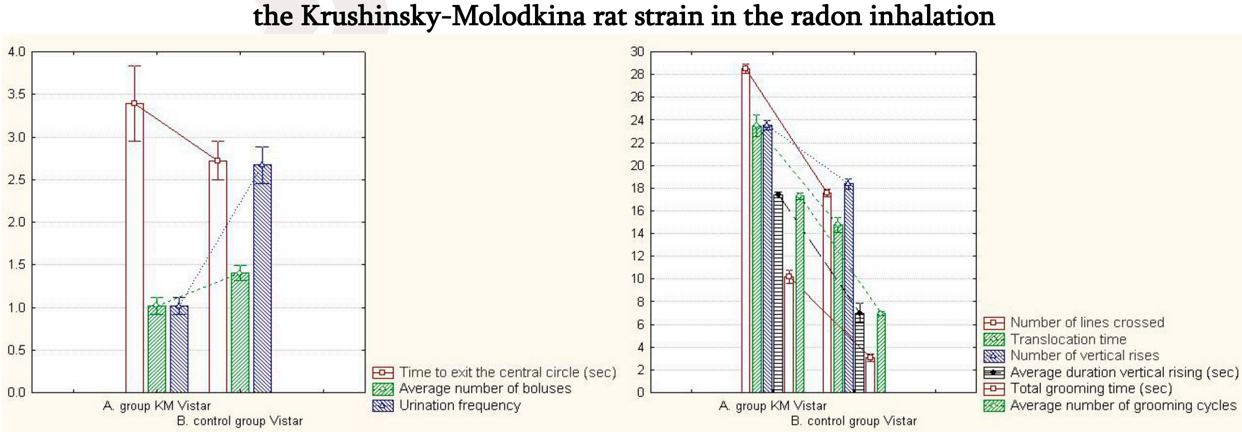


As a result of exposure to radon, it was found that epileptic rats lose fear and anxiety, which is manifested by an increase in the grooming phase, as well as increased time spent on looking down, which expresses one of the orientation behaviors of rats, as it is known from the literature, it helps epileptic rats to perceive the environment. Moreover, they increased the number of vertical standings and decreased the number of wall stands, an indicator of their reduced anxiety. The number of getting up in the center and staying in the center for a long time is of great importance, which the epileptic rats did little of during the entire experiment and only increased after inhalation of radon, which gives us another reason to say that the behavior of the epileptic rats stabilized and the fear and anxiety were really removed, after radon inhalation (Tab. 6, Fig. 6).

Table 6. locomotor activity, orienting-exploratory reactions and emotional sphere of behavior of rats of the Krushinsky-Molodkina line in the radon inhalation

	A. group	B. Control gr, Vistar	P_{A-B}
Time to exit the central circle (sec)	3.40,8	2,780,06	<0.05
Number of lines crossed	28,420,06	17,60,07	<0.05
У translocation time	23,70,06	14,80.06	<0.001
Number of vertical rises	23,41,01	18,50,6	<0.001
Average duration, vertical rising. sec	17,40,3	7.00,1	<0.005
Total grooming time sec	10,20,01	3,20,01	<0.05
Average number of grooming cycles	17,30.021	6,90.01	<0.05
Average number of boluses	1,00,1	1,31,0	>0.05
Urination frequency	1,00,2	2,70,2	<0.05

Fig. 6. Locomotor activity, orienting-exploratory reactions, and emotional sphere of the behavior of rats of the Krushinsky-Molodkina rat strain in the radon inhalation



As for the movement of epileptic rats in the maze, as we mentioned above, the maze is a conditioned-reflex behavior developed on a positive stimulus, which we studied in a trestle-type maze. The movement of the animal was recorded by recording the numbers, which allowed us to reconstruct the movement of the animal in a given maze with its trajectory. As a result, it was shown that 10 epileptic rats completed this maze in 1 minute without making a mistake after 7 days, which is an indicator of their relatively good memory, while Wistar control rats gave a positive response to this behavior already after 9 days. We can note a difference of 2 days between the epileptic rats and the control non-epileptic rats, which indicates a relatively good memory of the epileptic rats. Further research was conducted on negative emotion-based behavior, specifically using the method provided by Essman and Alpern. The conducted tests showed that 8 out of 10 epileptic rats did not move to the dark compartment. Tests conducted on animals of control and experimental groups showed us that they maintained an adequate reaction. They remember the electrical stimulation and do not go from the light to the dark compartment, again indicating their good memory. Based on the obtained tests, we can say that the psycho-neurological memory of epileptic rats does not decrease.

Moreover, we studied the zoo-social relationships of the Krushinsky-Molodkina rat strains. The presence of a social stimulus (unfamiliar male Wistar, "social preference" test) resulted in increased anxiety and reduced exploratory response in KM: dramatically reduced the time and number of contacts with other animals, as well as reduced free movement to the box and increased the number of interrupted grooming sessions, indicative of anxiety. There was catatonia, thus in KM rats, there was a reduction in intraspecific motivation for intraspecific interactions and increased anxiety/fear responses.

Since the animals were in catatonia for a long time and had very few stand-ups ($p<0.001$) and many interrupted grooming and sometimes inverted grooming or tail-starting grooming ($p<0.001$), it indicates a high anxiety/fear directed towards the presence of a new social stimulus. All this suggests that epileptic rats have a deficit in social relations or motivation, which is clearly seen in the test we studied (Fig. 7, Fig. 8).

In the cross maze, we see their long stay in the closed arm ($p<0.001$), limited locomotor activity, and high anxiety interrupted grooming. and a short stay in the open arm KM ($p<0.01$). [10,21,26].

Fig. 7. Movement of epileptic rats in the cross maze

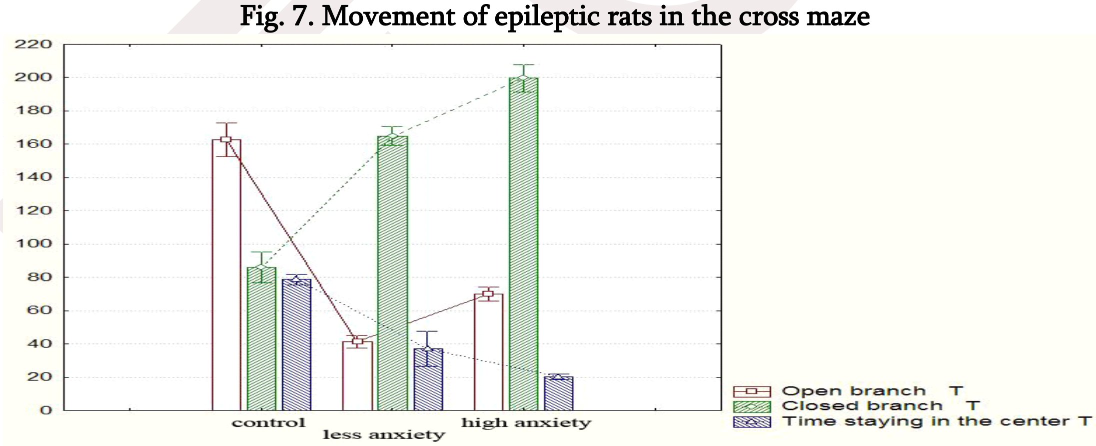
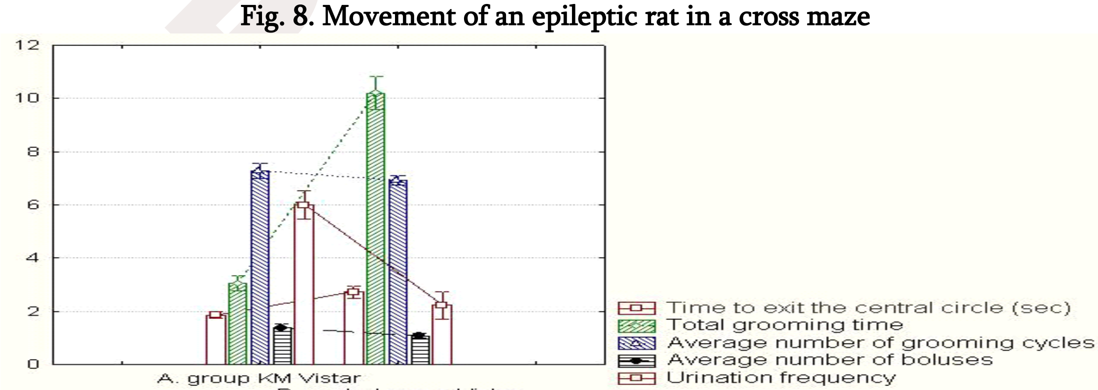


Fig. 8. Movement of an epileptic rat in a cross maze



Based on the experiments, it can be concluded that the radon hormetic effect has a great impact on the performance of different behavioral actions. In particular, the restoration of the integrity of the interrupted grooming of rats in the open field, the number of crossed cells, the reduction of the number of wall stands, and the increase of the number of stand-ups in the center, as well as the increase of being in the open arm of the cross-maze and the establishment of social relations with familiar and unfamiliar rats, all of these indicate the induction of anxiety in epileptic rats and, accordingly, the reduction of oxidative stress.

CONCLUSIONS. Six months after radon inhalation it was found that zoo-social interaction in KM rats achieved quite good results, because of the radon inhalation, epileptic rats socialized with unfamiliar rats within 2-3 minutes, and their behavior was not restricted to the middle of the arena.

They stayed longer in the center of the cross-maze than in the closed arm. This further confirms that the anxiety and fear of the epileptic rats, which were characterized by various behavioral tests, approached the behavior of the control rats. Predictors of oxidative stress were studied, PAT, D-ROM (reactive oxygen metabolite index), OBRI (oxidative stress balance risk index), and OSI (oxidative stress index) were evaluated to assess the antioxidant capacity of plasma. Based on the obtained data we can conclude that: inhalation of Tskaltubo water develops the effect of hormesis, which causes positive changes in all of the above markers of oxidative stress in the brain, which has a definite effect on behavior and on memory rats.

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EFFECTS OF INHALATION OF LOW DOSES OF RADON IN THE KRUSHINSKY-MOLODKINA RAT STRAIN AND STUDY OF VARIOUS BEHAVIORAL CHARACTERISTICS

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SUMMARY

To study the effect of radon in balneotherapy, a group of experimental animals (multiple low doses in KM and epileptic Wistar rats were used) went through the procedure of inhalation of radon by the Tskaltubo mineral water pool, once daily, during 20 min, 10 days. In animals of the control groups, inhalation with radon was not used. After radon inhalation therapy with Tskaltubo mineral water, it was analyzed the absence or reduction of epileptic seizures.

This research showed that Tskaltubo mineral water radon inhalation caused hormesis, absence or reduction of epileptic seizures. The result was stable during 6-12 months. Stabilization positively influenced oxidative stress levels, Na/K-ATF in epileptic rats. The result of our experiment gives us a stimulus to continue future research to find more specific neurochemical mechanisms participating in radon hormesis processes.

Oxidative stress resulting from excessive free-radical release is likely implicated in the initiation and progression of epilepsy. An increase in active forms of oxygen or free radicals of oxygen is believed to play an important role in epileptic rats. To investigate the relationship between oxidative stress and memory impairment in epileptic mice, we determined the level of activity of antioxidant enzymes in blood plasma. The results showed that the level of lipid peroxidation in the plasma was significantly higher in epileptic rats than in control Wistar rats. Therefore, our study aimed to investigate the effects of radon inhalation on different forms of behavior in epileptic rats, both to positive and negative stimuli. As known from the literature, antioxidant therapies aimed at reducing oxidative stress have received considerable attention in epilepsy treatment. This article discusses various forms of behavior in KM rats as revealed by experiments. The KM rat strain differs from the Wistar rat strain in the nature of the locomotor activity, a higher level of exploratory activity, and increased emotionality. For rats of the KM rat strain, instability of behavioral reactions is characteristic, which manifests itself in all areas of their activity in the “open field” test. As for memory in epileptic rats, the behavior based on negative stimuli is not impaired in their psycho-neurological memory. Conditioned-reflex behavior developed on a positive stimulus, which we studied in a trestle-type maze, the obtained results showed that 10 epileptic rats complete the exit of this maze in 1 minute, already after 7 days without error. We have studied three sections of social environment awareness. It was determined that the time ($p<0.001$) and the number of contacts ($p<0.001$) were statistically significantly reduced among KM male rats. Decrease in indicators of social interaction: Group KM reliably spent less time in the compartment with animals ($p<0.01$) and spent more time near its cell. As a result of movement in the cross-maze, it was determined that KM rats spent more time in the closed arm, had more interrupted and rotated (tail-to-front) grooming than in the open arm, and also had a higher number of stand-ups than in the open arm, which is an indicator of its high anxiety. Inhalation of radon reduced oxidative stress in epileptic rats, and alleviated anxiety, together with definite memory improvement.

Keywords: behavior, Na / K-ATPase, Oxidative Processes, radon