# The orienting-exploratory activity of rats after oral intake of industrial dust of uranium ore

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► ***Original article***

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## ABSTRACT

***Background:*** The functional abnormalities of the nervous system induced by the injection of industrial dust of uranium ore (DUO) into rat's organism were studied. ***Materials and Methods:*** DUO was administered to nonlinear white male rats orally during a week in an amount of 5.8 g daily. The calculation of the individual absorbed and equivalent doses in the organs and tissues of rats allowed to include them to the range of small doses of ionizing radiation. To calculate the doses received by the animals during the experiment, the uranium content in biological samples was determined using an Agilent-7800 inductively coupled plasma mass spectrometer. The behavior of experimental animals was observed in the test in an open field condition for a month after incorporation into the body of long-lived radionuclides. ***Results:*** Analysis of behavioral acts in a group of males exposed to DUO at a dose of 50 maximum permissible concentration (MPC) indicated the predominance of passive-defensive forms of behavior, which was manifested by a decrease in motor and exploratory activity. The noted changes were evident throughout the study. At the same time, a slowdown in habituation processes was recorded, manifested in a small variability in the number of crossed squares and the number of vertical posts. ***Conclusion:*** The intake of DUO at a dose of 50 MPC changed the indices of locomotor and research activity of rats significantly along with an increased concentration of uranium in the organs and tissues of animals, including the brain.

## INTRODUCTION

The Republic of Kazakhstan is a unique uranium ore region, which contains about 30% of the world's uranium reserves. The country occupies a leading position in the uranium ore mining world (1-3). According to the Kazatomprom company, more than 20 thousand people in the Republic are directly involved in uranium industry enterprises (4).

Uranium industry workers are exposed to uranium isotopes at the radioactive workplace. Uranium isotopes emit alpha radiation, beta radiation, and gamma radiation due to uranium daughter products. Internal uranium exposure to human occurs primarily through inhalation and ingestion and has a potential health hazard for workers (5-7). The topicality of this problem is due to an increasing the number of persons, who exposed to occupational, technogenic radiation in small doses (8).

Scientists focus on the radiocerebral effects of small doses of ionizing radiation particularly, at which significant clinical effects do not develop (9). There is currently data of the uranium effect that significantly alter the psychoneurological condition of the nervous system. According to some authors, shifts in the nervous system have functional nature primarily (10,11). First of all, uranium exposure notifies the increased motor activity, sleep disturbances, memory loss and increased anxiety (12-14). It is known that the analysis of GNI in the experiment is impossible without determining an orientational research behavior, as it is the most important type of activity, that provides animals with the information about environment and it is an essential psychological mechanism for the adaptation of superior vertebrate animals and humans to changing living conditions

The aim of our research is to study the orientational research behavior of rats after prolonged intake of DUO in animals at a dose of 50


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MPC.

## MATERIALS AND METHODS

The experiments were performed on adult white outbred male rats. Experimental animals were obtained and kept in the own vivarium of the NpJSC “Medical University Astana”, Kazakhstan. The experiments were performed on 45 white mature male rats. To exclude the influence of individual reactivity factors on the results of the experiment, the studies were conducted on sexually mature animals weighing an average of 160–200 g. The rats were divided into 2 groups: I-control group, II-experimental. The first group consisted of intact animals that received portions of feed identical in composition and weight to the experimental animals, but without the addition uranium ore dust. The second experimental group of rats was exposed to uranium ore dust at a dose of 50 maximum permissible concentrations. The dust of uranium ore obtained at the factory of Stepnogorsk city of the Republic of Kazakhstan was used in the experiment. The Stepnogorsk mining and chemical plant is located on the territory of the North Kazakhstan uranium ore province and is a central industrial site near the city of Stepnogorsk, where workers come into contact with uranium ore dust [8].

Modeling of prolonged effects of industrial uranium ore dust on rats was carried out by successively feeding the animals with uranium ore dust once a day for one week. The period of experimental uranium ore dust intake to animals was determined by the data of periodization of biologically similar stages of human’s and rat’s ontogenesis, according to which a week of a rat's life roughly corresponds to a year of a human's life [15]. In the dust composition of uranium ore, 0.4% was uranium trioxide.

Uranium ore dust was mixed with baby food (Nestle dairy-free porridge) until homogeneous mass. This mixture was the only source of nutrition for the experimental animals per day. Intact and experimental animals were kept in individual cages during the week-long experiment to control the consumption of food mixed with uranium ore dust. Every day after feeding the portion eaten by rats of the experimental and control groups was recorded. The volume of remains was less than 1% of the total volume of one portion of the feed mass.

Industrial uranium ore dust obtained for the research was studied in the radiochemistry and radiospectrometry testing laboratory of the Scientific Research Institute of Radiobiology and Radiation Protection. The total activity of α-emitting radionuclides in uranium ore dust was 98,8 Bq/g, and ß-emitting radionuclides was 30,4 Bq/g. The activity of uranium isotopes (238U, 235U, 234U) was subsequently determined to model the internal irradiation of animals. Then the values of uranium activity received by a worker in the uranium industry for 1 year of work and the annual effective dose from uranium α-radiation were calculated. As a result of the calculations, the value of the industrial radioactive dust mass for a single feeding of rats in the experimental group was 5.8 g.

Quadrupole mass spectrometer with inductively coupled plasma "Agilent 7800 ICP-MS" (Agilent Technologies, USA) was used for quantitative determination of uranium in samples of rat organs and tissues [16].

During sample preparation, microwave decomposition of samples of organs and tissues of experimental animals was performed using the decomposition program in Multiwave PRO 3000. The samples were decomposed for 50 minutes until completely transparent using nitric acid (at least 6 ml) and hydrochloric acid (1 ml) for each sample weighing 0.3 g. The concentration of uranium was determined in the blood, kidney tissue, liver, intestines and brain of rats.

To determine the degree of violation of the structure of orienting-research behavior and emotional response, the "open field" test was used. The experimental setup was an arena with a diameter of 1 m, bounded by a side with a height of 45 cm, the floor of which was divided into 16 squares with a side of 15 cm with 34 rounded holes with a diameter of 3 cm. During the experiments, the field was illuminated with a lamp of 500 W located at a height of 60 cm above the center of the field.

The testing of each rat was preceded by a three-minute dark adaptation. Then, the behavior of the animal in the “open field” was monitored during 10 minutes in each session of the experiment. The parameters of orienting research activity (ORA) were recorded in the first minute of the test: the first 5 minutes, the second 5 minutes. And their total number was determined for 10 minutes of the study. During the experiments, the following indicators were recorded: the latent period (LP) of exit from the center of the field; horizontal activity (HA) - the number of crossed lines, protected by the transition of the line with all four paws; vertical activity (VA) - the number of racks on the hind legs. In this case, the peripheral and central vertical activity of the rat were distinguished. In addition, the number of peeps (P) into the holes of the arena and the number of holes (H) themselves into which the rat looked were determined; the number of acts and the duration of grooming (the number of washing, shaking, scratching) in seconds (Gy), as well as the number of bowel movements (by the number of fecal boluses). To assess the nature of behavioral changes in animals at the beginning, the indices of ORA were determined - initial, and then on the 1st, 2nd, 3rd, 6th, 12th, 18th, 24th and 30th days after ingestion of dust of uranium ore.



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These experiments were performed during the first month after seeding animals with uranium.

***Statistical analysis***

Statistical processing of the research results was carried out using the IBM SPSS Statistics 20 software packages. Quantitative indicators were assessed for compliance with the normal distribution using the Kolmogorov-Smirnov criterion. To assess statistical significance between comparison groups, an unpaired Student's t-test was used. Differences were considered statistically significant at p<0.05.

## RESULTS

The results of the study summarized in table 1, showed that the highest concentrations of uranium are found in the tissue of the kidneys and small intestine in the early stages after internal exposure to uranium ore dust.

**Table 1.** Results of calculation of doses received by organs and tissues of experimental animals after uranium intoxication.

| 50 Maximum permissible concentration (MPC) | | |
|---|---|---|
| **Blood** | **per year** | **per day** |
| **D** | 0,49 µGy | 1,3 nGy |
| **H** | 98 µSv | 26 nSv |
| **Kidneys** | **per year** | **per day** |
| **D** | 0,14 mGy | 0,38 µGy |
| **H** | 2,7 mSv | 7,4 µSv |
| **Intestines** | **per year** | **per day** |
| **D** | 0,017 mGy | 0,047 µGy |
| **H** | 0,34 mSv | 0,9 µSv |

**Note:** D – absorbed dose; H – equivalent dose.

Based on the data on the uranium content in the organs and tissues of rats, we calculated the activity of uranium in biological samples, absorbed dose rate, and directly absorbed, equivalent received by each organ of the animal after seven days of uranium incorporation. For the calculation, the data presented by A.A. Moiseev were used. in the section "Internal exposure" from alpha and beta emitting radionuclides [17]. The results are presented in table 1. The calculation of a dose load allowed to identify the received doses are small by experimental animals.

The concentration of uranium in the brain of group II rats increased significantly and exceeded the data of intact animals by more than 3.5 times and amounted to 155.8±97.3 ng/l.

The study of behavioral acts began with an analysis of the total indicators reflecting the orientational research activity (ORA) of experimental animals in 10 minutes of finding them in the arena of the "open field". Also, for a more detailed and dynamic recording of rat behavioral indicators, we analyzed the ORA of males at the 1st minute of observation of them in the “open field” test, and we compared their activity at different stages of the study: the first and second 5 minutes of finding them in the arena of the installation.

In the group of intact sexually mature male rats, the dynamics of ORA was characterized by low variability and a tendency to get used to the “open field” installation, which was characterized by a gradual decrease in the motor activity of animals. The study of ORA at different stages of the study made it possible to find out that individuals are more active in the first 5 minutes of examining a new territory for them. However, the values of the number of mink peeks and grooming recorded in the open field test were higher in the second 5 minutes of the experiment.

An analysis of the dynamics of behavioral reactions revealed the following features of ORA in male rats exposed to DUO at a dose of 50 MPC. The latent period of the animals in the center of the site throughout the entire research period did not differ from that before uranium incorporation. Significant differences were revealed only on the 30$^{th}$ day of the experiments, when the latent period was almost 2 times lower than the initial values and amounted to 18.25–5.95 s (p <0.05) (figure 1).

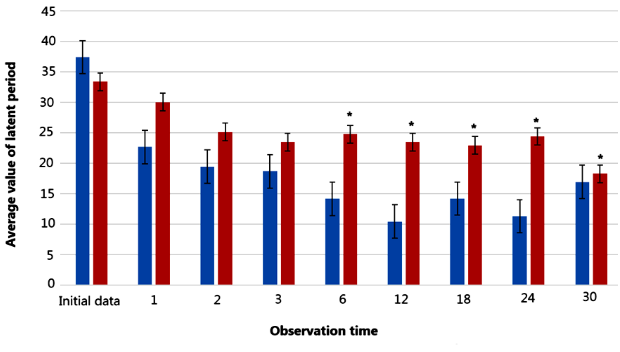


**Figure 1.** Effect of uranium ore dust at a dose of 50 maximum permissible concentrations on the latent period of exit from the center of the “open field”.

However, it is worth noting that in this group there were animals that did not leave the center of the “open field” for 10 minutes of testing, so on the second day of the experiment, 2 rats remained almost motionless during testing, another four days of research on one rat behaved similar way.

The total horizontal activity of animals significantly decreased during all days of the study after the receipt of uranium ore in the animal organism. Moreover, during the first landing of animals on the arena of the “open field”, the studied parameter immediately decreased by 36.6% from the initial level and amounted to 39.31-19.99 (p <0.01) against 62.0-20.07 shoots. In the following days of the experiment, the extinction rate of the general horizontal activity continued. By the 12th day of the study, the total number of locomotions was minimal - 24.5–9.79 (table 2).


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**Table 2.** The total indicators of ORA in male rats after the intake of DUO at a dose of 50 MPC.

| Index | Observation time | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Initial data | 1 | 2 | 3 | 6 | 12 | 18 | 24 | 30 |
| LP | 33,3 | 30,0 | 25,11 | 23,45 | 24,7* | 23,46** | 22,91** | 24,37** | 18,25** |
| HA | 62,0 | 39,31* | 28,36** | 30,27** | 37,83** | 24,5** | 26,58** | 34,67** | 24,67** |
| VA | 4,4 | 2,75 | 2,42 | 1,67* | 1,91 | 1,25* | 1,75* | 2,0* | 2,0* |
| P | 28,80 | 15,83 | 9,58* | 10,08* | 11,0 | 18,33 | 13,83 | 13,25 | 13,08 |
| Gy | 25,96 | 22,28 | 22,75 | 24,84 | 26,59 | 28,80 | 26,30 | 30,81 | 28,36 |
| fecal boluses | 1,90 | 2,67 | 1,33 | 2,08** | 1,50 | 1,70 | 2,10 | 1,93* | 1,50** |

Note: *p<0,05, **p<0,01

The decrease in total motor activity on all days of the experiment occurred due to a decrease in movements in the second 5 minutes of the experiment, which was manifested in a significant decrease in horizontal activity. When studying the temporal representation of the number of movements in different periods, significant changes were revealed during the first minute of testing. Thus, a significant increase in locomotion was recorded from the 3rd day of the experiment (13.54-6.4 versus 6.7-4.7, $p < 0.05$) until the 24th day of the experiment (11.08–6.1 versus 6.7±4.7, $p < 0.05$).

Analysis of the vertical activity of male rats exposed to uranium revealed several changes: during the first landing, the studied parameter decreased by 38% of the initial data and amounted to 2.75–1.76 ($p < 0.05$). The following experimental sessions were characterized by even lower indicators of vertical activity, which did not recover by the end of the experimental study. Minimum indicators of vertical activity were recorded on the 12th day of the study and amounted to 1.25–0.97 ($p < 0.01$). It should be emphasized that the dynamics of the decrease in vertical struts completely coincided with changes in the horizontal activity of rats in the arena of the "open field". When studying the indicators that make up the research matrix of animal behavior in the "open field", significant differences were revealed compared to background values throughout the study.

The males showed a pattern of extinction of research activity, as evidenced by the data on the number of peeps into the arena holes, which on the first day after feeding the uranium ore dust decreased by almost 50% compared with the initial data. In the subsequent days of the study, this indicator was low and did not approach the background values. The minimum number of peeks in minks was observed on the 2nd day of the experiment -9.58±4.17 versus 28.8±8.99. An identical picture was observed when using the method of dividing the observation process into two test periods. It should be emphasized that rats examined the "minks" more actively in the first five minutes of the experiment.

The total duration of grooming acts remained at the same level throughout all 8 testing sessions in the installation. Registration of this parameter at various time intervals during one experiment showed its greater representation in the first five minutes on the 2nd, 3rd, 6th day of the study.

Comparative analysis of the orienting-exploratory activity of II group male rats with control animals revealed a number of significant differences.

The latent period of exit from the center of the "open field" site during the entire observation period was longer in individuals who underwent uranium intoxication. In this case, significant differences were detected from the 6th day to the end of the experiment. These days, the latent period exceeded on average 2 times the data recorded in the group of intact individuals.

When comparing the total horizontal activity, it was found that on all days of the study, the indicator in group III was on average 2 times lower compared to the control group. Significant differences were determined on the 2nd, 3rd, 12th, 18th and 30th days of the experiment. The maximum differences were recorded on the 2nd day of the study after uranium intoxication when the number of paces of rats of group II exceeded more than 2 times the horizontal activity of males in the control group: 28.36±18.45 versus 60.71±19.92 ($p < 0, 01$). In males who received uranium ore dust at a dose of 50 MPC, the total number of locomotion significantly decreased both due to testing in the first and second 5 minutes. There were no significant differences in the amount of horizontal activity in the 1st minute of testing.

An intergroup difference in the total indicators of vertical activity was detected only on the 3rd day of the experiment when the studied trait in the experimental group was significantly lower than the vertical activity of intact males (1.67±1.07 versus 4.78±1.76, $p < 0, 05$). It should be noted that the total vertical activity on all days of the study was below the control values. A study of the number of "minks" in the study groups revealed that the number of peeps in holes was less in rats that underwent DUO intoxication during the study up to 24 days. Significant differences were recorded on the 2nd and 3rd day of the experiment when the number of males peeping into the arenas of the arena of males who underwent DUO exposure was 1.5 times lower than in the control group of animals. However, the 30th day of the experiment was marked by an increase in the research activity of animals of group II and the indicators of these groups were practically equalized. Differences between animals in the number of bowel movements were observed on the 3rd, 24th and 30th days of the study: this indicator was higher in males



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who underwent exposure to PUR. The maximum difference in the number of fecal boluses was recorded on the 30th day: 1.5±0.94 against the absence of bowel movements in relatively healthy rats.

## DISCUSSION

In general, an analysis of behavioral acts in a group of males exposed to uranium ore dust at a dose of 50 MPC (II) indicated the predominance of passive-defensive behaviors, which was manifested by a decrease in motor and research activity. Marked changes were evident throughout the study. At the same time, a slowdown in habituation processes was recorded, which manifests itself in a small variability in the number of crossed squares and the number of vertical posts. It is known that in the systematic organization of behavior, the main role belongs to orienting and research activity, which is aimed at assessing the significance of an active stimulus for organizing targeted adaptation. ORA is based on a complex set of reflexes reflecting the functional state of the cerebral cortex [18].

Observation of the orientation research activities of male rats (group I) revealed the following: from the moment healthy animals were placed on the open field arena, the total indicators of horizontal sprouts, vertical racks, and the number of peeps into minks gradually decreased and were characterized by low variability, and in general this testified to the addiction of intact animals to the installation of "open field".

The emotional state of animals in the open field test is evidenced by the number of fecal boluses, which tended to decrease and be absent by the 30th day of the study. The number of bowel movements, according to some authors, reflects the emotional stress of animals [19]. In general, such behavior of intact rats in the "open field" is logical and indicates the selection of an adequate strategy for the behavior of animals.

The behavior of rats of group II was uniform and depleted, males were inactive, research activity was negligible. As a result of the impact of the DUO, there was a sharp decrease in horizontal movements and vertical racks. The decrease in motor activity was due to the fact that these animals were hiding under the walls of the chamber, because this part is covered less intensely, that is, at the initial stages of observing animals, a passive-defensive behavior strategy prevailed. In addition, we observed a decrease in the number of "minks," which is the main expression of a broken search strategy in an "open field". It should be emphasized that on all days of testing there was a long latent period of exit from the center of the "open field", which is another confirmation of the increased anxiety of rats exposed to DUO at a dose of 50 MPC, since biological motivation aimed at meeting the needs for the conservation and development of the species, which determines purposeful behavior, was implemented by making a decision to cross the border that separated the central platform from the rest of the field [20]. To make this decision, rats of group II took longer than intact animals

Under conditions of combined toxic and radiation exposure of uranium, such behavioral changes can be explained by intoxication syndrome developed in animals. The timing of studies of the ORA structure from the moment of DUO administration was 30 days, taking into account the toxicokinetics of uranium compounds in the body, changes in animal behavior can be clinical manifestations of intoxication. In this case, general inhibition of animals was observed, which affected the level of horizontal and vertical movements [20]. The behavioral activity of animals was characterized by a violation of its components with a predominance of inhibition processes, which was expressed by sitting, fading, and the absence of other patterns. Violations of the structure of orientational research activities and the high anxiety of rats subjected to the incorporation of SDI can be explained from several perspectives. Firstly, the inhibition of inhibition, which occurs in almost all forms of pathology of the nervous system. Most likely, this is since inhibitory mechanisms are phylogenetically younger mechanisms, and therefore more vulnerable when exposed to various damaging factors. In case of a violation of the inhibitory component, the excitatory component is disinhibited and pathologically expressed [21-23].

Secondly, the revealed changes in the behavior of animals can be directly associated with a change in the level of neurotransmitters, which play a significant role in the organization of higher nervous activity in normal and pathological conditions [24,25]. Analyzing the available data, it can be assumed that the neurotoxic effects of uranium can be caused, among other things, by a disturbance in the balance of nervous system mediators such as serotonin, norepinephrine, and gamma-aminobutyric acid and underlie the formation of anxiety syndrome. A decrease in the concentration of serotonin in various areas of the rat brain upon receipt of PUR leads to an indifferent attitude to potentially threatening situations, and an increased secretion of serotonin in the structures of the central nervous system causes the occurrence of pathological anxiety disorder. An increasing of concentration of norepinephrine in the structures of central nervous system is associated with astheno-depressive and anxious conditions [26].

In addition, the stimulation of certain structures of the limbic system, temporal cortical zones, certain zones of the brain bridge, which contains most of the cells of noradrenergic neurons are in attendance with fear reactions in experimental animals, and their destruction is accompanied by a decrease in the level


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of fear and aggression. Besides that, blockade of benzodiazepine receptors that regulate gamma-aminobutyric acid metabolism can cause acute anxiety syndrome in animal experiments (27, 28). Uranium intoxication, like poisoning with other heavy metals, is a stress that is accompanied by changes in neuroimmunoendocrine processes. In this case, the stress-realizing system of the hypothalamic-pituitary-adrenal system is activated. In turn, the level of biologically active substances that increase anxiety may increase in the blood.

Judging by the concentration of uranium in the brains of experimental animals and expressed locomotor deviations, we can speak with a high degree of confidence about the direct effect of uranium ore dust on the nervous system (29). However, in an irradiated organism, along with direct radiation damage to some tissues, organs and systems, reactive indirect changes occur in other systems. Such changes are also called remote changes, since in many cases the recorded effects appear outside the irradiated tissue. The above mechanisms may also occur in our study, along with the direct damaging effect of DUO. Taken together, the results of the study indicated that intoxication with uranium ore dust has a negative effect on the behavior and emotional status of animals. Extrapolating the results obtained to workers, it can be assumed that deviations of the nervous system can reduce the efficiency and reliability of the professional activities of workers involved in the mining and processing of uranium ore. Further experimental and epidemiological studies may provide the basis for the development of effective preventive and rehabilitation measures to ensure radiation safety and social adaptation of uranium industry workers.

## CONCLUSIONS

Thus, on the basis of the presented data, it can be concluded that the prolonged enteral intake of uranium ore dust into the body caused a change in the orientation and research activity of experimental animals. After receiving dust of uranium ore at a dose of 50 MAC, the males were in a state of behavioral depression, which was characterized by a sharp inhibition of motor activity and an increase in negative emotionality. The results of the experiment indicate the feasibility of further research and systematization of data in a general biological concept, which is of interest in connection with the increasing scale of the uranium industry in Kazakhstan and throughout the world.

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***Authors' contributions***: Conducting the experimental study, idea and design of the article prepared by ES, DT and Zh A., preparation of research materials, collection and analysis of data prepared by ES, Ye K and Zh A. Conceptualization was done by PK, AM and DT, methodology prepared by ES, Ye K, ST, KK, formal analysis was performed by Ye K, TT. The first draft of the article was prepared by AM, ES. Reviewing and editing the article prepared by PK, MA and DT. All authors reviewed the manuscript and agreed to the final version.

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