A nonhuman primate model of early effects of irradiation with high-energy protons on operator activity

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Abstract. During long-term space missions irradiation with Galactic Cosmic Rays may result in acute damage to human central nervous system (CNS) and, possibly, lead to disturbances of operator activity. Three monkeys (Macacamulatta) were trained to perform a visuomotor task consisting of saccade and manual responses to visual stimuli, thus constituting a model of operator activity. Upon training completion two animals (M1 and M2) were exposed to cranial irradiation with high-energy protons (170 MeV, 3 Gy), while the third (M3) underwent selective irradiation of parietal lobe in Bragg peak (155 MeV, 3 Gy). We noticed no change in instrumental task performance in course of three months after irradiation (a.i.). Both cranially irradiated animals demonstrated a highly correlated increase in both saccade and manual response latencies one month a.i. M3 experienced earlier and stronger increase in these parameters starting on the 20th day a.i. Our results suggest that systemic behavioural mechanisms are resistant to proton irradiation while visual motor integration and executive control systems might be affected, resulting in reaction time increase.

1. Introduction

During long-term space flights outside the Earth magnetosphere one of the limiting factors is irradiation with Galactic Cosmic Rays which may result in disturbances of vital functions including central nervous system activity (CNS) [1, 2].

Investigations of the effects of different types of ionizing radiation on behaviour and cognitive abilities of rodents are abundant [3, 4], but despite their unquestionable value they do not predict effects of radiation on humans accurately. Therefore a physiologically closer model animal is needed. Lower primates represent an almost perfect candidate as key structural elements of their visual and motor systems highly resemble those of humans. In order to properly model operator activity, an experimental task should require execution of precise visually-evoked manual and oculomotor movements. To our knowledge, such experiments have not been reported yet.

Belyaeva et al. 2017 [5] have employed a task in which monkeys were required to follow a moving target with a cursor manually guided with a joystick. General visuomotor performance parameters such as the number of animal-initiated trials and the percentage of correctly completed trials were recorded. The authors found no effect of single irradiation with high-energy protons (170 MeV, 3 Gy) on performance parameters in course of 40 days a.i. the task, although mimicking certain elements of operator activity, unfortunately lacked quantitative analysis of precise parameters of manual activity such as trajectory, spatial and temporal tracking parameter.
Currently it is possible to present animals with tasks requiring complex manual and oculomotor activity and to record precise parameters of their performance at the same time. Therefore, slight effects of irradiation on operator activity of model animals can be assessed using such research design.

2. Objectives
Intending to replicate radiation conditions in deep space, for this study we implemented irradiation with high-energy protons as they represent around 92% of ionizing particles in space [6]. Proton energies were chosen to be in compliance with those obtained outside the Earth magnetosphere.

Human operator activity requires coordinated eye and hand movements. As a consequence, appropriate model animals should possess physiologically similar systems controlling these types of activity. As neural circuits of lower primates meet this criterion, monkeys (Macacamulatta) were chosen to participate in the present study. We utilised instrumental visuomotor task consisting of visually evoked saccades and manual responses to mimic the key components of human operator activity. High-frequency eye and hand movement recording allowed us to measure the parameters of animal motor activity with high precision.

Therefore, the goal of this study is to investigate early effects of cranial or selective irradiation with high-energy protons on performance and temporal parameters of the responses in a visuomotor instrumental task in model animals.

3. Materials and methods

3.1. Instrumental task
Three male monkeys (Macacamulatta, M1, M2, M3) participated in the study. The animals were kept under moderate water deprivation (Protocol of Ethics Committee of the Biological Faculty of Lomonosov Moscow State University N.34 effective as of 26.10.2012).

Monkeys were trained to execute saccades to visual stimuli presented in one of equally distributed 34 positions in the 39x26 degree visual field on the monitor placed in 50 cm in front of the eyes of the animal. Monitor screen colour was dark green; its luminosity was 0.25 lux at eye level. Experiments were conducted two or three times a week.

An experimental session consisted of array of consecutive trials. In course of a single trial central stimulus (CS) was presented for 500-800 ms. Then without delay a peripheral stimulus (PS) appeared and lasted for 800-1000 ms, after which it dimmed for 1200 ms allowing the animal to execute manual response. Inter-trial interval was 300-500 ms with all stimuli turned off. Both PS and CS were white-coloured and square-shaped with 0.38 degrees side. The degree of contrast with the background was 0.96 for bright stimuli and 0.9 for dimmed stimuli. The monkeys were trained to fix gaze on CS and execute a saccade to PS upon its appearance, then fix gaze on it until it dims and respond to dimming by pulling lever. Correct responses within 1200 ms after dimming start were rewarded with 0.2 ml of fruit juice.

The monkeys were in training until their performance stabilised. Then one month-long control sessions was collected and the animals underwent an irradiation. In order to investigate its early effects the experiments were conducted for three months constituting an experimental sessions.

We utilised high-frequency videooculography (200 Hz for M1 and 500 Hz for M2 and M3; FastVideo-250 camera to record eye movements. Lever pulling was recorded with high-frequency high-precision accelerometer (100 Hz). Eye movement and manual response properties were assessed using original software.

3.2. Irradiation
The irradiation was carried out at the phasotron of the Joint Institute for Nuclear Research (Dubna, Moscow region). M1 and M2 underwent a cranial irradiation with square proton beam 8x8 cm. In M3 parietal lobe was selectively irradiated in Bragg peak using custom collimator.
Proton energy for M1 and M2 was 170 MeV while for M3 it was 155 MeV. Total dose for all animals was 3 Gy delivered in course of 5 minutes [7].

4. Results

Animals were found to have individual training capabilities: M1 responded in 1364±66 (M±SEM) trials per session while M2 – only in 722±31 and M3 – in 572±26 trials per session. We believe this to be due to individual traits, in particular, to temper, as it was reported earlier [5]. Therefore, performance baseline was established individually on the basis of values obtained during control sessions.

4.1. Conditioned task performance dynamics after irradiation

Despite the irradiation all animals demonstrated a slight improvement in performance in conditioned task (Pearson’s correlation coefficient for performance between consecutive days - 0.546 (p<0.0001) in M1, 0.282 (p=0.043) in M2 and 0.420 (p<0.001) in M3. Such positive statistically significant dynamics points to high resistance of conditioned reflex to irradiation in course of 3 months a.i. and most likely represents reflex settling due to prolonged task presentation.

![Figure 1a](image.png) Mean saccadic latencies (±SEM) dynamics over time in M1, cranial irradiation being day 0. Linear regression shown in line (p<0.0001)

![Figure 1b](image.png) Mean saccadic latencies (±SEM) dynamics over time in M2, cranial irradiation being day 0. Linear regression shown in line (p<0.023)

4.2. Saccadic and manual latencies dynamics after cranial irradiation

We found a positive dynamics of mean saccadic latencies after cranial proton irradiation in M1 and M2 (figure 1a and 1b respectively). Pearson’s correlation coefficients for this parameter between consecutive days were 0.755 (p<0.0001) and 0.364 (p=0.023) in M1 and M2 respectively.

After irradiation saccadic latencies in M1 increased by 15 ms on average in comparison to control values. We also found two extremes between 63th and 87th days pointing to the fact that negative effects of irradiation were not equally distributed. Peak latencies of these extremes exceeded control values by 25 and 40 ms. M2 demonstrated a temporal increase in saccadic latencies between 35th and 50th days a.i. and several extremes later. Due to data irregularities saccadic latencies a.i. on average exceeded control average by 11 ms although peak values exceeded control average by 30 ms. Saccadic latencies increase rate (measured as slope value with respect to experiment number) was 0.188 (p<0.0001) and 0.098 (p=0.023) in M1 and M2 respectively.
Manual response latencies demonstrated similar elevating dynamics as did saccadic latencies (figure 2a and 2b in M1 and M2 respectively). Pearson’s correlation coefficients for manual latencies between consecutive days were 0.642 (p<0.001), 0.364 (p=0.023) in M1 and M2, respectively. Manual latencies in M1 increased stepwise by 20 ms between 40th and 95th days a.i. M2 also demonstrated similar stepwise increase (but by 100 ms on average) initiating on the 5th day and gradually decreasing from the 26th to the 99th day a.i. During the latter period manual latencies exceeded control values by 70 ms on average.

Saccadic latencies increase rate (measured as described above) was 0.347 (p<0.0001) and 0.334 (p=0.060) in M1 and M2 respectively. Both animals demonstrated a highly statistically significant correlation between saccadic and manual latencies: 0.756 (p<0.0001) in M1 and 0.614 (p=0.015) in M2.

4.3. Saccadic and manual latencies dynamics after parietal lobe irradiation in Bragg peak
A positive dynamics of saccadic latencies a.i. with Pearson’s correlation coefficient of 0.669 (p<0.0001) was revealed in M3 (figure 3). We found an acute increase by 20 ms in this parameter starting from the 20th day a.i. in M3. On average they exceeded control values by 25 ms in the following days. We also determined several extremes of saccadic latencies exceeding control average by 55 and 70 ms.

Saccadic latencies increase rate (measured as described above) turned out to be higher after irradiation in Bragg peak than after cranial irradiation – 0.330 (p<0.0001). Apart from higher slope values we found a statistically relevant increase of this parameter dispersion (standard deviation) after irradiation in comparison to control dispersion values (58 vs 35 ms, Mann-Whitney test, p=0.023).
effect was not found in the animals who underwent cranial irradiation (31 vs 33ms ($p=0.817$) in M1, 39 vs 33 ms ($p=0.566$) in M2).

Manual response latencies in M3 increased stepwise by 35 ms on the 15th day a.i. and decreased for three following weeks almost to control values (figure 4). After that they returned to elevating dynamics till the end of experimental session, being by 40-60 ms greater than the control average. Pearson’s correlation coefficient for manual latencies between consecutive days was 0.512 ($p<0.0001$). Increase rate for this parameter was higher than in cranially irradiated animals and was estimated to be 0.473 ($p<0.0001$). Manual and saccadic latencies in M3 turned out to be also highly correlated with correlation coefficient being 0.594 ($p=0.006$).

Such dynamics of saccadic and manual latencies in M3 suggests greater effect of parietal lobe irradiation in Bragg peak in comparison to non-collimated cranial irradiation.

5. Discussion
Primate studies of the effects of irradiation are of exceptional importance to the understanding of the risks for human health and performance after radiation exposure. It is well known that monkey performance in cognitive visual tasks such as delayed-match-to-sample decreases after exposure to ionizing radiation [8,9]. Concerning manual activity, there was no performance decrease in tasks with lesser cognitive demands such as eye-hand tracking using joystick after 3 Gy irradiation with high-energy protons [5]. We expected similar results and, in fact, did find no decrease in conditioned task performance after irradiation with similar dose, which proves simple behavioural patterns to be resistant to such exposure.

As to the studies investigating precise parameters of eye and hand movements in visuomotor task simulating operator activity, there seems to be very little of them.

In our work the animals were presented with such a task and then exposed to high-energy proton irradiation. The animals demonstrated an increase in visually evoked saccadic and manual response latencies starting one month after exposure and persisting for two following months. Concerning the individual differences, in M1 the increase of manual latencies started 1 week later than saccadic latencies, while in M2 these processes occurred almost simultaneously. This difference might be due to individual characteristics of the animals.

Negative effects of proton irradiation on saccade and manual latencies also resembled those in monkeys during MPTP-induced Parkinsonian syndrome at the stage before the clinical symptoms arise [10]. It is possible that protons and MPTP both disturb functions of basal ganglia which are involved in eye and hand movement control. In particular, proton irradiation and MPTP might both be causing dysfunction of dopamine dependent mechanisms of voluntary movements [11].

As parietal cortex is strongly involved in spatial perception necessary for completion of visuomotor task, we expected its irradiation in Bragg peak to result in more profound changes to saccadic latencies. Even greater negative effects were to follow from increased linear energy transfer in the brain region of interest (parietal cortex in M3) due to its placement in Bragg peak of the proton beam. Saccadic dynamics in M3 were in complete accordance with our predictions. Saccadic latencies increase rate was substantially higher than that of cranially irradiated animals (0.330 in M3 vs 0.188 and 0.098 in M1 and M2 respectively) (figure(1)). Parietal lobe irradiation in Bragg peak also resulted in greater dispersion of saccadic latencies most likely reflecting instabilities in visuospatial information processing. The present results demonstrate key role of parietal cortex in spatial perception and visuomotor behaviour in general as well as dangers of its radiation-induced injury.

Therefore, our findings suggest that proton irradiation might temporarily decrease the efficiency of integrative and executive brain mechanisms resulting in increased latencies of saccadic and manual responses in a conditioned task.

6. Conclusion
Our results demonstrate high resistance of monkey conditioned behaviour to high-energy protons irradiation. However, we found acute negative effects on temporal characteristics of visually guided
Saccades and manual reactions. The magnitude of these effects varied between cranially irradiated animals and animal that underwent parietal lobe irradiation in Bragg peak.

Such effects might be of concern regarding temporal parameters of human reactions while executing operator activity. Our results contribute to the general problem of early damaging effects of ionizing radiation on complex human activity during long-term space missions in deep space.

7. References

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