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DISTURBANCES IN VISUOMOTOR BEHAVIOUR OF NONHUMAN PRIMATES AFTER CRANIAL IRRADIATION WITH HIGH-ENERGY PROTONS

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Abstract. *During long-duration space flights outside the Earth's magnetosphere, exposure to galactic cosmic rays and solar energetic particles may lead to early damage to human central nervous system, causing operator activity impairments in astronauts. A conditioned instrumental performance involving saccadic eye movements and manual reactions was studied in two nonhuman primates (Macaca mulatta). One monkey was exposed to a single cranial proton irradiation (170 MeV, 3 Gy). For the other control animal irradiation was simulated. Proton irradiation had no negative effects on instrumental performance during three months after irradiation. However, irradiated monkey showed an increase in saccadic and manual response latencies started at the one month after irradiation and observed next two months. No such effects were found in control monkey. Thus system processes, crucial for conditioned instrumental behaviour, turned out to be widely resistant to proton irradiation, although increase in saccadic and manual response latencies suggests its early negative effects on integrative and executive brain mechanisms.*

Keywords: *Operant conditioning, ionizing radiation, protons, monkeys, operator activity, saccades, manual reaction*

1. INTRODUCTION

During long-duration space flights outside magnetosphere of Earth fluxes of galactic cosmic rays (GCR) and solar energetic particles (SEP) are a source of radiation which may lead to disturbances of vital functions of the organism, and, particularly, of the central nervous system (CNS) [1], [2]. A substantial body of data exists on the effects different types of ionizing radiation have on behaviour and cognitive functions of rodents [3], [4]. This data, although very valuable, cannot provide information on how radiation affects human CNS and human behaviour, especially operator activity, which is of the highest importance in space missions. In order to tackle this subject, experimental work on nonhuman primates is needed, which is quite rare in comparison to the number of studies on animals with less developed brain. Modeling main components of operator activity on primates requires measurements of precise oculomotor and manual activity. Systems controlling these types of activity in primates are remarkably more complex than in rodents. In particular, hand movements in primates are controlled by highly developed extrapyramidal system.

Tasks involving complex cognitive processes such as perception, attention, working memory etc. are used to study the effects of irradiation on behaviour thus modeling possible behavioural impairments in deep space. Among these tasks, operant conditioning paradigms represent a universal yet simple way to assess such cognitive processes, easily applied to rodents. For example, delayed matching to sample (DMS) is widely used to estimate the stability of perception and memory after irradiation [5], [6], [7]. In the same time, such works are scarce and diverse in terms of types, methods and temporal structure of irradiation. The available data suggests that DMS performance decreases after a single dose gamma irradiation of the whole body at 5 Gy [5] as well as after multiple cranial gamma irradiation sessions (40 Gy in total, 8 sessions in course of 4 month) [7]. In the last case the severity of performance decrease depended on the complexity of the DMS task (from 2 to 6 alternatives), while overall performance was decreasing for the next 7 month after the last irradiation. V. Bogo [6] demonstrated 3 Gy of protons to be an adequate dose to model irradiation effects on cognitive functions in primates. This amount is also consistent with measurements of proton radiation outside Earth magnetosphere of SEP and GCR [8]. These two components provide a solar activity-dependent radiation hazard during long-term space missions. The galactic particle fluxes constitute permanent radiation, the intensity of which varies rather slowly in accordance with solar activity. The SEP occurrences are random and should be described by probabilistic models. Mean annual SEP event proton fluences are generally equal to the galactic cosmic ray fluences at

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proton energies of 170 MeV. Overbalance of one of the components highly depends upon the solar activity level [9].

Until recently, the tasks used in studies of effects of ionizing radiation on cognitive functions of primates did not provide an opportunity to measure quantitatively the key components of operator activity, such as temporal and spatial parameters of eye and hand movements. Changes to these parameters reflect dynamics of cognitive processes such as visual search and perception, attention and attention shift, as well as correctness and precision of hand manipulations [10]. Therefore, to predict risks of operator activity disturbances in humans during long-term space missions, instrumental tasks with precise measurements of these parameters are needed.

In our study, we implemented such a task and measured temporal characteristics of visually guided saccadic movements and manual responses of non-human primates before and after cranial irradiation with high-energy protons that make up to 92% of GCR.

2. Materials and methods

Two male Rhesus monkeys (*Macacca mulatta*) were used in this study. One monkey (I-, 13 y.o.) was subjected to sham irradiation while the other monkey (I+, 16 y.o.) underwent cranial irradiation with high-energy protons. All manipulations with the animal were performed in accordance with the requirements of the Directive of the European Parliament and the Council of the European Union Council of the European Community (2010/63 / EU) on the use of animals for experimental research.

The monkeys were trained to fix gaze on central stimulus and then perform a visually guided saccade to a peripheral stimulus. Stimuli were presented at 34 locations with respect to the central stimulus of the monitor (see Figure 1).

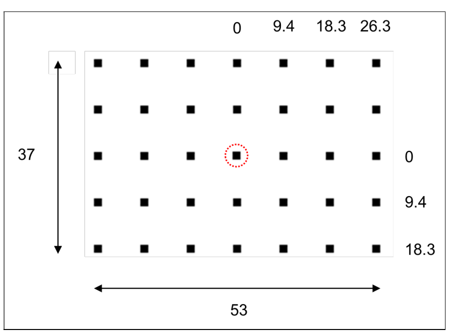

Figure 1. Locations of stimuli in the visual field of monkeys. The numbers indicate angular distance

Having fixated on peripheral stimulus, the monkeys were to wait for its dimming and respond manually by pulling the ipsilateral lever with the respective hand, after which they received juice rewards. For stimuli on central axis any lever could be selected to response. Basing on temporal characteristics, manual responses were divided into four groups: preemptive (before the peripheral stimulus started to dim), late (after the peripheral stimulus was turned off), refusals (no response took place) and correct responses. Only the latter were rewarded. The monitor (Mitsubishi Diamond Pro 2070SB, 22”) was located within their visual field, in 51 cm in front of the animal. Central stimuli were presented for 500-800 ms, peripheral – for 800-1000 ms, interstimuli interval was set between 300 and 500 ms. The intervals were chosen pseudorandomly with 100 ms step. Lever position was recorded at frequency of 100 Hz. Monkey head was immobilized with a 3D-printed plastic holder designed using a CT scan to be anatomically fit and comfortable for it. All experimental procedures were performed on a unique computer system. Habituation and training procedures are described in [11].

Movements of left eye were recorded using 200 Hz camera (Fast-Video-250, NPO ‘ASTEK’) under IR-illumination provided by 28 LED-source (L-53SF6C, λ_{peak} = 860 nm). Gaze position was determined by the original software [12]. Saccades were extracted using original software on Matlab. Assessed parameters were averaged over every experimental session for further analysis.

The cranial irradiation with protons took place at the Laboratory of Nuclear Research of the Joint Institute for Nuclear Research (Dubna, Moscow Region). The monkey I+ were irradiated for 5 minutes with a 8x8 cm beam of protons with the energy of 170 MeV. Total radiation dose was 3 Gy. The accuracy of the dose is ensured by placement of the ionization chamber in the path of the beam at the entrance to the treatment box. Before start of the irradiation session, the indications of this chamber are calibrated by a clinical dosimeter installed instead of the irradiated animal. The accuracy of this dose measurement is 3%, which is verified by other dosimetric methods. The sham-irradiated animal underwent all the procedures except for irradiation itself.

Minitab 17 software package was used for statistical analysis. We utilised Fischer z-test of frequency comparison for manual reactions rates, Kruskal-Wallis test - to determine influence of ‘day of experiments’ factor on performance in instrumental task. Linear correlations of saccade and manual response latencies were counted using Pearson coefficient (r). Comparison of latencies was performed using Student t-test.

3. Results

3.1. Instrumental conditioned performance

Throughout the experimental period the animals demonstrated an individually stable level of performance. The rate of correct responses varied in range of 80-90% for I- and of 80-95% for I+ (see Figure 2).

Performance of I- slightly increased in course of 3 month after sham irradiation (see Figure 2), which can be explained by further improvement of conditioned response during a prolonged training period. Performance of I+ was not affected by irradiation (see Figure 2), as proportion of correct responses demonstrated similar dynamics to I-. Two-way ANOVA did not reveal any main effects of independent variables (factors) 'day' (F1 (77.6)=1.730, p=0.253) and 'subject' (I-/I+) (F1(1.6)=1.760, p=0.233) on percent of correct responses. The obtained results suggest that performance in instrumental task is largely unaffected by irradiation.

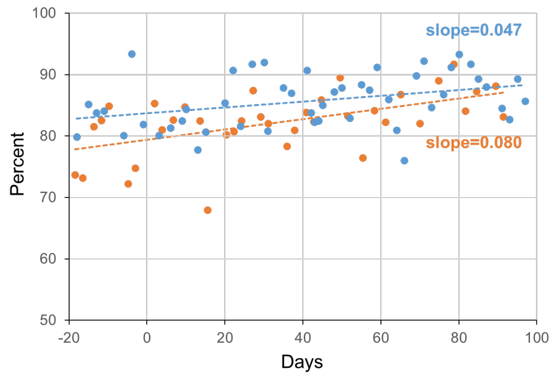

Figure 2. Percentage of correct operant responses of I- (blue) and I+ (orange). Zero point represents the day of sham (I-) or actual (I+) irradiation

The monkeys had substantial individual characteristics with respect to performance due to different levels of motivation. In particular, the mean proportion of correct responses by I- per session was 475±38, while for I+ this value was 1391±58, which is a highly statistically significant difference (t=−14.21, df=40, p<0.0001). Throughout the experimental period the raw number of correct responses decreased in both animals with Pearson correlation (r) between session number and correct responses being −0.156 (p=0.329) for I- and −0.462 (p=0.018) for I+. High significance of r in I+ may reflect decreasing level of motivation caused by consequences of proton irradiation, while motivation of I- remained largely unaffected.

3.2. Saccadic and manual response latencies

Saccadic latencies in I+ were not stable throughout the experimental period (see Figure 3).

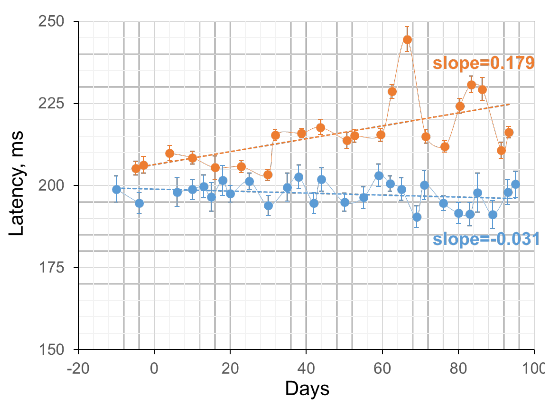

Figure 3. Saccadic latencies of I- (blue) and I+ (orange)

Nonetheless, at the 32th day after the irradiation (a.I.) we recorded a small but significant increase by 4%. Later, from 63 to 87 day a.I., a substantial increase took place in comparison to the period before irradiation (b.I.). This increase developed through two phases, during the first of which the increase was in average 35 ms (the 67th day, +17%), while during the second phase - in average 22 ms (84th day, 11%). After that, the latencies returned to the level of the 32th day a.I. In the control animal (I-) there was no increase and, in fact, saccadic latencies decreased marginally significantly (see Figure 3), once again showing an improvement due to continuing training. We also should notice that until irradiation effects took place there was a small difference of about 5% between average latencies of I+ and I- (207 and 197 ms). These results point to the presence of irradiation effects on saccade latencies, which underwent a small, then a profound increase and subsequent decrease to the initial elevated level.

Dynamics of manual reactions latencies is remarkably similar. From the 46th to the 87th day a.I. I+ demonstrated a statistically significant increase in latency (see Figure 4). This process developed through 3 phases. The first phase was characterised by 40 ms increase (day 51th a.I., +13%), the second – by 70 ms increase (day 67th a.I., 23%) and the third – by 63 ms (day 81th a.I., 20%) with a subsequent decrease to the level of the 42th day a.I. The control animal (I-) showed no such dynamics (see Figure 4). In the same time, average manual response latencies during the last month b.I. varied substantially in both animals due to individual characteristics. It should be noted that saccadic and manual reactions latencies were highly correlated in I+ (r=0.837, p<0.001). The obtained results contribute to the hypothesis about a transient effect of proton irradiation on saccadic and manual reactions latencies of the animal.

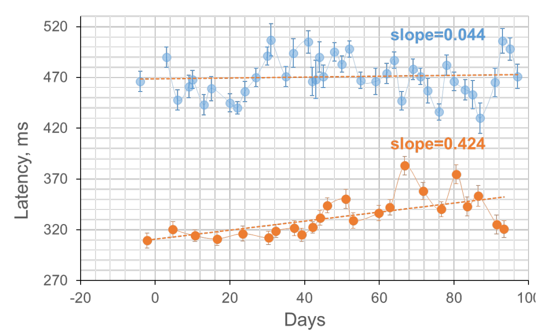

Figure 4. Manual reaction latencies of I- (blue) and I+ (orange)

4. Discussion

We already know that different types of ionizing radiation cause deterioration of performance in cognitive tasks such as DMS [5], [6], [7]. In the same time, in tasks with less cognitive demands, for example, eye-hand tracking with joystick, 3 Gy high-energy proton irradiation did not result in any disturbances [13]. Our results are in line with those of [13] as well as with available data on operant conditioning in irradiated rats [14], [15].

In particular, we found no negative effects of irradiation on performance of monkeys in operant conditioning task (see Figure 2). However, we did show a monotonous decrease of the number of correctly performed trials, reflecting possible disturbances of motivational system, especially its dopamine-dependent part. This is consistent with results of [16], reporting the decrease in dopamine transporter and dopamine receptors concentration after proton irradiation at similar doses.

Assessing quantitative temporal parameters of eye and hand movements is of especially high significance, as they mimic basic components of operator activity performed by humans and, to our knowledge, have not been investigated in primates exposed to irradiation earlier. We found a small but significant (up to 4%) increase of saccade latencies in the irradiated monkey one month after irradiation, after which a substantial increase (up to 11-17%) followed for about 3 weeks with a subsequent return to the slightly elevated level (see Figure 3) [17]. Similar dynamics was recorded for manual response latencies, having begun one week later than the first changes of saccadic latencies (see Figure 4). These changes bear high resemblance with the effects of MPTP-induced hemiparkinsonism in monkeys, also pointing to dopaminergic system involvement [18], [19].

These results suggest a possible transient decrease in efficiency of integrative and executive mechanisms performance because of damage by high-energy protons, which lead to increase in manual and saccadic latencies while performing an operant conditioning task. Possible mechanism for this effect is dysfunction of striate dopamine-dependent mechanisms of goal-directed behaviour [16], which can also be found in monkeys with MPTP-induced hemi parkinsonian syndrome [19].

The obtained results should be extrapolated to irradiation of the entire body (not only the cranium) for practical purposes, which is likely to enhance the demonstrated changes. The results will be refined due to the ongoing involvement of new animals into the experiment.

5. Conclusion

The obtained results suggest systemic processes involved in execution of operant conditioning tasks to be largely impervious to irradiation with high-energy protons. In the same time, early negative effects of radiation can be seen in changes of temporal characteristics of oculomotor and manual responses. These effects might have a substantial significance for human operator activity. Our preliminary results contribute to one of the aspects of the problem of early damaging effects of ionizing radiation on complex and diverse activities of humans during long-term space missions in the nearest future.

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