Life Sciences in Space Research 27 (2020) 56–63

Contents lists available at ScienceDirect

Life Sciences in Space Research

journal homepage: www.elsevier.com/locate/lssr

Effects of partial- or whole-body exposures to ^{56}Fe particles on brain function and cognitive performance in rats

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ABSTRACT

On exploratory class missions, such as a mission to Mars, astronauts will be exposed to particles of high energy and charge (HZE particles). Exposure to HZE particles produces changes in neuronal function and can disrupt cognitive performance. Cells throughout the entire body, not just the brain, will be impacted by these particles. To determine the possible effects that irradiation of the body might have on neuronal function and cognitive performance, rats were given head-only, body-only or whole-body exposures to ^{56}Fe particles. Cognitive performance (novel object recognition, operant responding) was tested in one set of animals; changes in brain function (oxidative stress, neuroinflammation) was tested in a second set of rats. The results indicated that there were no consistent differences in either behavioral or neurochemical endpoints as a function of the location of the irradiation. These results suggest that radiation to the body can impact the brain, therefore it may be necessary to re-evaluate the estimates of the risk of HZE particle-induced changes in neuronal function and cognitive performance.

1. Introduction

On exploratory class missions, astronauts will be exposed to cosmic rays, which are composed of alpha particles, protons and particles of high energy and charge (HZE particles). Exposure to low doses of HZE particles (20-40 cGy) can disrupt cognitive performance (Britten et al., 2016, 2017; Davis et al., 2014; Raber et al., 2013; Rabin et al., 2011, 2015) which has the potential to compromise successful completion of mission requirements. In contrast to simulations using a particle accelerator, only a small fraction of neurons in the central nervous system will be hit by an HZE particle during a 3-year Mars mission (Curtis and Letaw, 1989; Curtis et al., 1998; Cucinotta et al., 1998, 2014). Also in contrast to simulations using whole body exposures to HZE particles (Britten et al., 2016, 2017; Davis et al., 2014; Parihar et al., 2018; Raber et al., 2013; Rola et al., 2005; Sweet et al., 2016; Vilisana et al., 2011; Vlkolinsky et al., 2007), which provide a uniform pattern of exposure across the entire body, the distribution of "hits" to cells will be asymmetrically located across the entire body: particles may be expected to randomly hit cells located throughout the body as well as neurons in the central nervous system. Because the area of the body is greater than that of the head, the body will suffer a greater exposure to HZE particles than the head. Previous research using ^{16}O or ^{4}He particles (Rabin et al., 2014; 2019) indicated that the effects of partial- or whole-body exposures on cognitive performance could vary as a function of the specific task.

The present experiment was designed to answer a series of questions about the possible mechanisms by which exposure to HZE particles alters cognitive performance. First, does the disruption of cognitive performance result only from the direct effects of HZE particles on brain, or is the disruption of performance also an indirect effect of exposure to HZE particles which does not require direct hits on the brain? Second, to what extent does head- and/or body-only exposure to HZE particles contribute to the disruption of cognitive performance following whole-body exposures? And third, do partial-body exposures produce changes in neurochemical functioning, specifically oxidative stress and neuroinflammation, in the brain which may affect cognitive performance?

2. Methods

2.1. Subjects

The subjects were 112 male Sprague-Dawley rats weighing 225-250 g at the time of exposure. The rats were maintained on a 12:12 hr light:dark cycle. Food and water were continually available except as required by the experimental protocol. The research protocols were approved by the Institutional Animal Care and Use Committees of Brookhaven National Laboratory (BNL), the University of Maryland, Baltimore County (UMBC), and the Human Nutrition Research Center on Aging (HNRCA). The facilities at BNL and HNRCA are AAALAC

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https://doi.org/10.1016/j.lssr.2020.07.006
Received 6 May 2020; Received in revised form 1 July 2020; Accepted 22 July 2020

accredited; the animal facilities at UMBC are supervised by Veterinary Medicine Resources of the University of Maryland School of Medicine.

Following irradiation at BNL, 36 rats (n = 5/treatment/dose + 6 control) were shipped to HNRCA and were euthanized 60 days following exposure for neurochemical testing. The remaining 76 rats (n = 11/treatment/dose + 10 non-irradiated control) were shipped to UMBC for behavioral testing.

2.2. Radiation

The subjects were exposed to 25 or 50 cGy of ^{56}Fe particles (600 MeV/n) at the NASA Space Radiation Laboratory (NSRL) at BNL. During irradiation the rats were restrained in well-ventilated tubes. Dosimetry was performed using parallel plate ionization chambers (La Tessa et al., 2016). Tungsten bricks were used to shield either the head or body, as appropriate. The dose to the subject behind the bricks was < 4% of the total delivered dose. The bricks were removed for whole-body exposures. Control rats (0 cGy) were taken to the NSRL, placed in the restraining tubes and walked about the facility, but were not irradiated.

2.3. Behavior

Two behavioral tests were utilized: (1) novel object recognition, which measures perirhinal cortex/hippocampal-dependent learning and memory (Rabin et al. 2009); and (2) operant responding on an ascending fixed-ratio reinforcement schedule, which is dependent upon the integrity of the striatum, and which measures the motivation of an organism to respond to changes in reinforcement and its ability to respond to changes in environmental contingencies, including changes in reinforcement contingencies (Salamone and Correa, 2002). Each test was run twice: the first series occurred 1-2 months following exposure and the second test was administered ~10 months following irradiation.

For the novel object recognition task, the subjects were placed in an open field (93cm x 93cm). On the conditioning day there were two identical (familiar) stimulus objects placed in the field. The rat was allowed to explore the stimuli until it accumulated 25-30 sec total object exploration time (i.e., exploration of either object) or until 15 min had passed. On the test day, 24 hr later, the rat was placed back in the field with one familiar and one novel object and allowed to explore both stimuli until it had accumulated 25-30 sec of object exploration on either object or until 15 min passed. Subjects that did not meet the criterion of 25-30 sec exploration time were eliminated from the specific test in order to minimize the effects of different amounts of exploration time on subsequent performance. The number of subjects achieving criterion did not differ as a function of radiation location or dose (data not shown).

The operant response involved pressing a lever in order to obtain a 45 mg food pellet. The rats were placed on a mild food deprivation schedule to maintain their body weight at approximately 90% of their weight prior to the start of the protocol. Throughout the training and testing periods, the rats were weighed daily, and the amount of food obtained in the operant chamber was supplemented by experimenter-provided food needed to maintain this body weight. For the initial acquisition of the response, an autoshaping procedure was utilized, which involved placing the rats in the operant chamber for 7-12 hrs on a continuous reinforcement schedule in which the rat was given a pellet for every lever press. Once the rats learned the response, they were trained to respond on a fixed-ratio (FR) reinforcement schedule. With a FR schedule, the rat was rewarded with a food pellet after a specific number of lever-presses. On an FR-1 schedule, every response was rewarded; whereas on an FR-20 schedule, 20 lever presses were required in order to obtain a single pellet; and on an FR-35 schedule, the rat had to press the lever 35 times in order to obtain a single pellet. For testing, an ascending fixed-ratio reinforcement schedule was used from FR-1 to FR-35 with each session lasting 30 min.

2.4. Neurochemistry

Western blotting procedures were performed on the hippocampus and frontal cortex of animals exposed to 50 cGy of ^{56}Fe particles according to previously published methods (Poulose et al. 2014). In brief, sixty days following irradiation, the rats were euthanized by decapitation. Brains were removed from the skull and bisected, and from one half of the horizontally cut brain, the frontal cortex and hippocampus were dissected out. Brain regions were snap frozen in liquid nitrogen and stored at -80°C before processing. The specific tissues were selected because of their potential role in mediating cognitive performance. The neurochemical endpoints included measures of oxidative stress (NOX2, NADPH-oxidoreductase-2); neuroinflammation (NF-κB, Nuclear Factor kappa B); protein damage (P62, ubiquitin-binding sequestering protein p62/SQSTM1); endogenous antioxidants SOD (Cu–Zn-superoxide dismutase), and GST (glutathione S-transferase); protective signaling (CREB, cAMP-response element-binding protein); and transcription factors Nrf2 (nuclear factor erythroid 2-related factor 2), and Keap1 (Kelch-like ECH-associated protein 1). β-actin was used as a loading control marker.

Upon thawing, each region was homogenized using a handheld homogenizer, in 300–600 μl homogenization buffer containing 50 mM Tris (pH 7.4), 150 mM sodium chloride, 2 mM ethylene diamene tetraacetic acid (EDTA), 2 mM ethylene glycol tetraacetic acid (EGTA) and 0.1% Triton X-100, with fresh addition of 1:100 mammalian protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN), 50 mM sodium fluoride, 1 mM sodium orthovanadate, 1 mM sodium pyrophosphate and 10 μg/ml phenylmethylsulfonyl fluoride (PMSF). The homogenate was centrifuged at 1000g for 5 min at 4°C, and protein concentration of the supernatant (cleared homogenate) was measured using the Bio-Rad Dc Protein Assay kit (Hercules, CA). The cleared homogenate of each brain region was subjected to Western blot analysis by loading 20 μg of protein on 12.5% SDS gels and electrophoretically transferring proteins to PVDF membranes. Blots were blocked with RapidBlock Solution (Amresco LLC, Solon, OH) and incubated overnight with primary antibody diluted in RapidBlock. Primary antibodies were obtained commercially (Abcam, Cambridge, MA).

Following primary antibody incubation, the blots were washed with Tris-buffered saline/0.5% Tween-20 (TBST) 3×10 minutes and incubated with the appropriate HRP-conjugated secondary antibody diluted in RapidBlock for 1 hour. After washing in TBST, 4×10 minutes, specific immunoreactivity was assessed using IgG-horse radish peroxidase (HRP)-conjugated secondary antibodies (EMD Millipore, Billerica, MA), and developed using Clarity Western ECL Substrate (Bio-Rad, Hercules, CA). The immunoreactive bands were visualized with a digital CCD camera attached to a BioImaging System (EC^3 Darkroom, UVP, Upland CA), and the optical densities were quantified with the LabWorks Imaging Acquisition and Analysis software (version 4.5, UVP).

2.5. Statistics

The initial analysis of novel object performance involved a one-way analysis of variance (ANOVA) to determine whether there were significant differences in performance as a function of the location of the exposure (head- or body-only or whole-body) followed by comparisons between the individual treatments using the Tukey-Kramer comparison when the initial ANOVA was significant. Subsequently, because rats will normally spend greater than 50% of their time interacting with a novel object, t-tests were used to compare the performance of control and radiated subjects to the 50% chance level. The initial analysis of operant performance was done using a 3-way ANOVA with 2 independent (treatment condition/dose) and one repeated factor (reinforcement schedule). Secondary analyses were performed with a

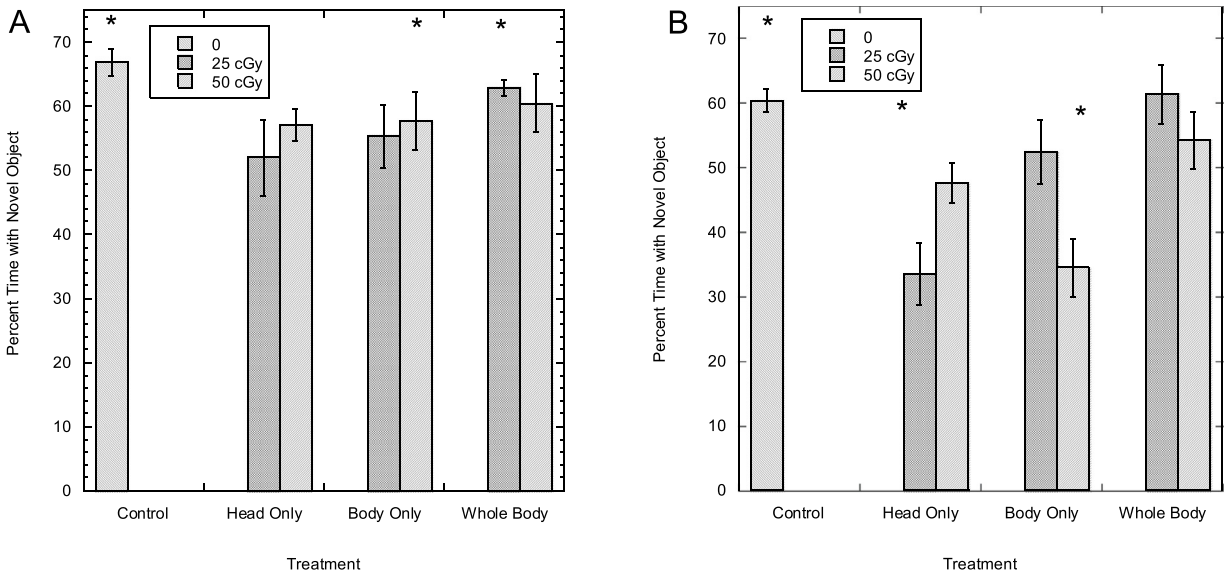

Fig. 1. Effects of partial/whole-body exposures to 25 or 50cGy ^{56}Fe particles on Novel Object Recognition Performance. **A**: 3 months post-irradiation; **B**: 12 months post irradiation. *Significantly different ($p < 0.05$) than 50%, indicating that the subjects remembered the previous stimulus object.

separate 2-way ANOVA for each treatment condition. Comparisons within individual treatment conditions were done using the Tukey-Kramer statistic. The statistical package (NCSS) provided the critical value and which comparisons were significantly different at the $p \leq 0.05$ level.

For the neurochemical data, statistical analyses were performed using SYSTAT software. The densitometric data, based on the intensity of the protein bands, were analyzed by one-way ANOVAs to determine if there were differences between the treatment groups. Post-hoc comparisons to determine differences among radiation groups were performed using Fisher's least significant difference (LSD) analysis. Results were considered statistically significant if the observed significance level was $p \leq 0.05$.

3. Results

3.1. Behavior

The effects of partial- and whole-body exposure to ^{56}Fe particles on novel object performance are shown in Fig. 1. The overall ANOVA indicated that two months following irradiation (Fig. 1A) there were no overall significant differences between the three treatment groups (head-, body-only, whole-body; $F_{6,46} = 2.08$, $p > 0.05$). However, examining performance as a function of location and dose indicated that there were differences in novel object performance. The head-only subjects exposed to either 25 or 50 cGy failed to spend significantly more time with the novel object. In contrast, the rats given 50 cGy exposures to the body-only or 25 cGy whole body exposures, as well as the control (0 cGy) subjects, spent significantly more than 50% of the time interacting with the novel object.

When retested 12 months following irradiation (Fig. 1B), the overall ANOVA comparing the three treatment groups was significant ($F_{6,5} = 7.09$, $p < 0.01$). However, only the subjects given head-only exposures of 25 cGy or body-only exposures of 50 cGy spent significantly less than 50 % of the time with the novel object. The performance of the other rats given partial body or whole-body exposures did not differ significantly from 50%.

For the operant task, the overall 3-way ANOVA (dose, treatment, reinforcement schedule) indicated that there were no significant differences in performance three months following irradiation between the subjects given head-only, body-only or whole-body exposures ($F_{2,504} = 2.36$, $p > 0.05$). While the main effect for dose was significant ($F_{2,504} = 16.92$, $p < 0.01$) the treatment by dose interaction was not significant ($F_{4,504} = 1.17$, $p > 0.10$) indicating that all groups of rats responded to exposure to ^{56}Fe particles similarly. Two-way ANOVAs evaluating each of the treatment conditions independently indicated that main effect for dose was significant for all treatment conditions (head-only, $F_{2,152} = 4,21$, $p < 0.05$; body-only, $F_{2,184} = 3.26$, $p < 0.05$; whole-body, $F_{2,191} = 13.06$, $p < 0.01$), suggesting a consistent disruption of operant performance regardless of location of irradiation. While dose was a significant factor for all treatment conditions, multiple comparisons using the Tukey-Kramer statistic indicated there were differences in the effectiveness of the irradiation (defined as the threshold dose) as a function of the irradiated tissue. For the body-only exposures, none of the comparisons between radiated (25 or 50 cGy) and control (0 cGy) subjects were significant; for the head-only treatment group, only the comparison between the subjects exposed to 50 cGy and the 0 cGy control subjects was significant; and for the whole-body treatment condition, the performance of the subjects exposed to either 25 and 50 cGy was significantly poorer than that of control (0 cGy) subjects.

When retested 11 months following exposure, the main effects for treatment ($F_{2,496} = 3.60$, $p < 0.05$) and dose ($F_{2,496} = 18.03$ $p < 0.01$) were both significant. The treatment by dose interaction was also significant ($F_{4,496} = 11.76$, $p < 0.01$) indicating that the different treatment conditions responded differently to exposure to ^{56}Fe particles as a function of dose. Two-way ANOVAs for each of the three treatment conditions indicated that although the main effect for dose was significant for each treatment, there were differences in the dose needed to alter cognitive performance. For the head-only treatment group, there was a significant enhancement of performance compared to the control subjects following exposure to 50 cGy whereas the performance of the rats exposed to 25 cGy did not differ from that of the control (0 cGy) subjects. In contrast, both the body-only and whole-body exposed subjects showed a significant enhancement in performance following exposure to 25 cGy but not following exposure to 50 cGy.

3.2. Neurochemistry

The effects of partial or whole-body exposures to 50 cGy ^{56}Fe particles on measures of oxidative stress and neuroinflammation are summarized in Fig. 4. F or both frontal cortex ($F_{3,16} = 1.49$, $p > 0.10$) and hippocampus ($F_{3,16} = 1.56$, $p > 0.10$), the overall ANOVAs indicated that there were no significant changes in NOX2 levels following

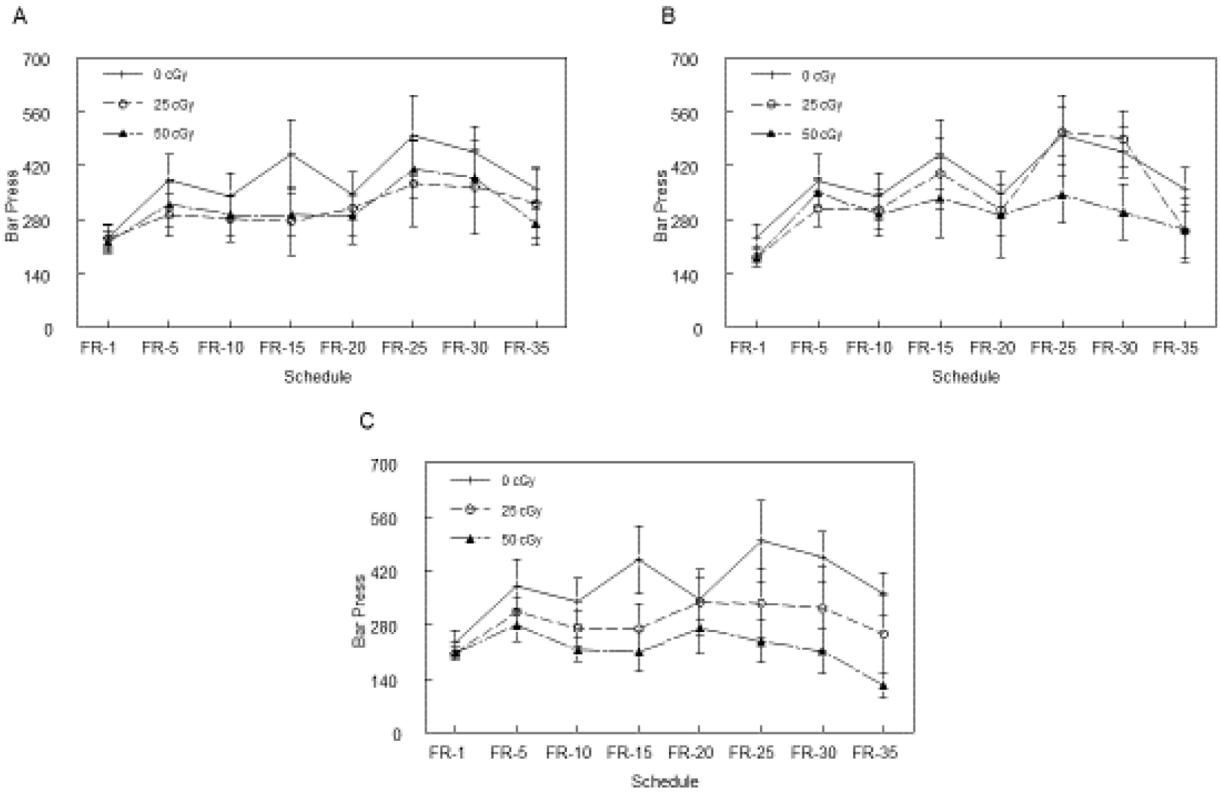

Fig. 2. Effects of partial/whole-body exposures to 25 or 50cGy ^{56}Fe particles on operant responding on an ascending fixed-ratio schedule 3 months following irradiation. **A**. Body-only exposures; **B**. Head-only exposures; **C**. Whole-body exposures.

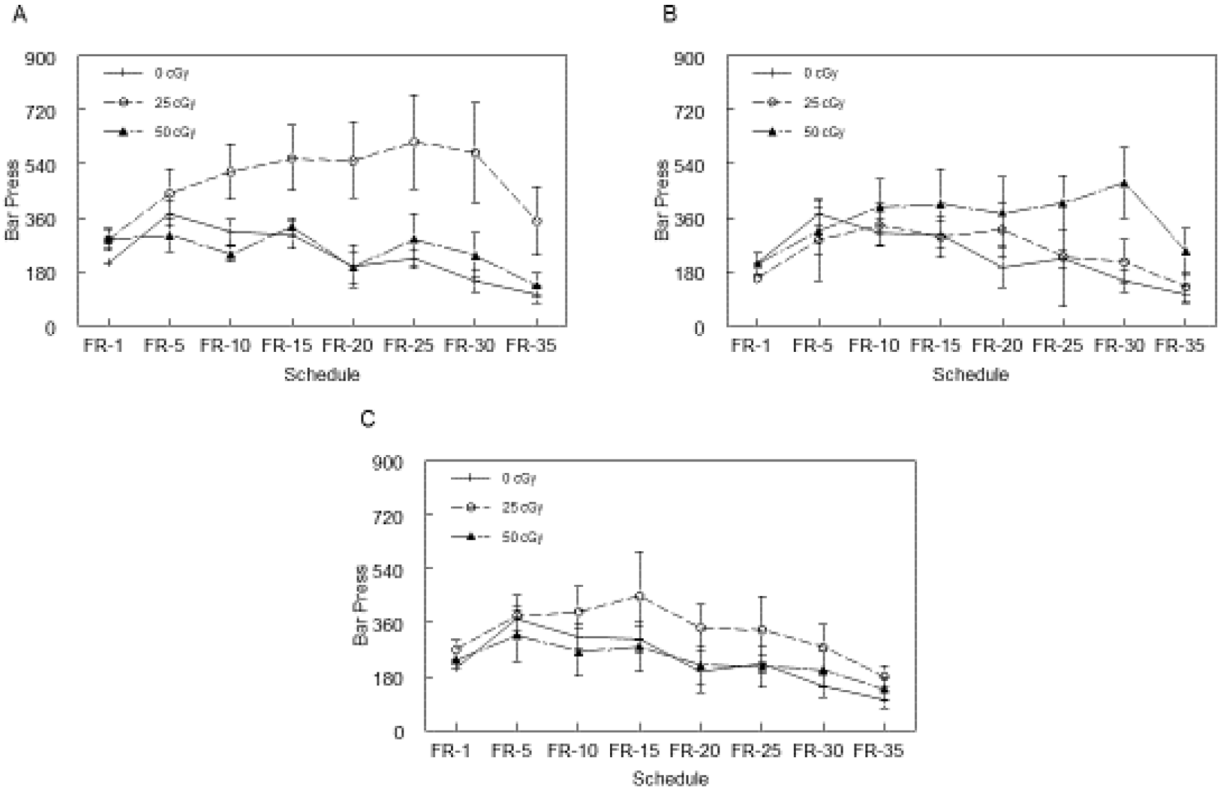

Fig. 3. Effects of partial/whole-body exposures to 25 or 50cGy ^{56}Fe particles on operant responding on an ascending fixed-ratio schedule 11 months following irradiation. **A**. Body-only exposures; **B**. Head-only exposures; **C**. Whole-body exposures.

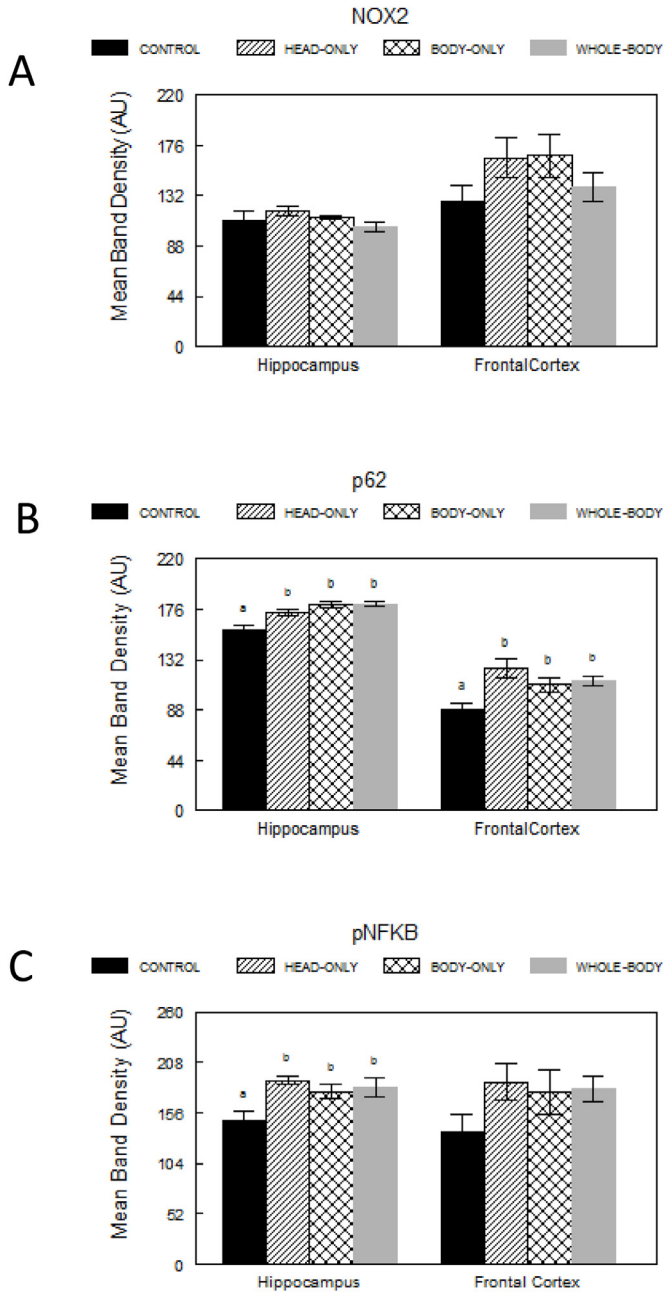

Fig. 4. Effects of partial/whole-body exposures to 50cGy ^{56}Fe particles on the oxidative stress marker NADPH-oxidoreductase-2 (NOX2) and the inflammatory markers ubiquitin-binding sequestering protein p62/SQSTM1 (p62) and Nuclear Factor kappa B (NFKB), in the hippocampus and frontal cortex.

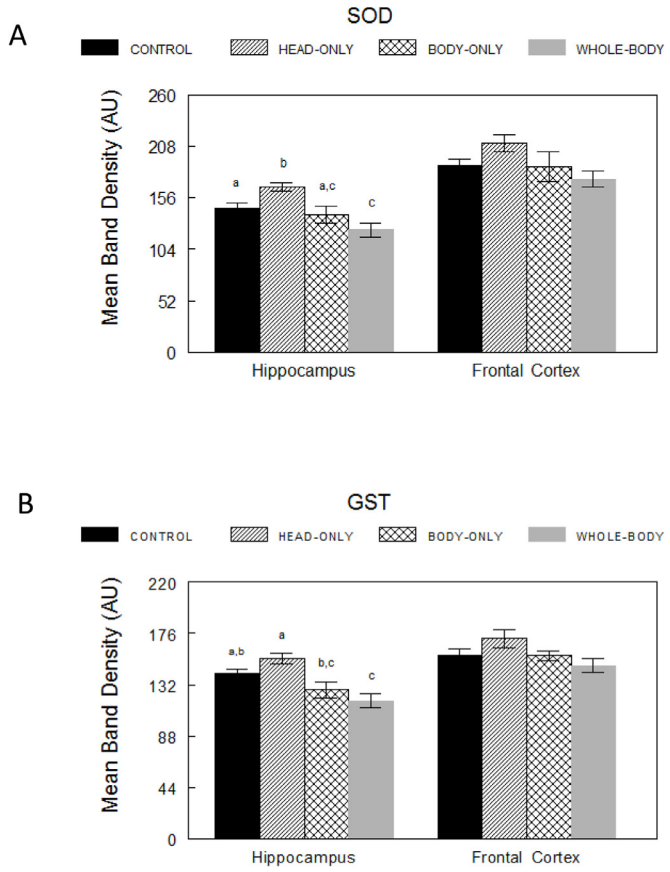

Fig. 5. Effects of partial/whole-body exposures to 50cGy ^{56}Fe particles on the endogenous antioxidants Cu–Zn-superoxide dismutase (SOD) and glutathione S-transferase (GST), in the hippocampus and frontal cortex.

exposure to ^{56}Fe particles or between the irradiated groups. In both the hippocampus ($F_{3,16}=15.58$, $p<0.001$) and frontal cortex ($F_{3,16}=6.18$, $p<0.01$), exposure to ^{56}Fe particles caused significant increases in p62 levels compared to the non-irradiated control subjects. In both brain regions, post-hoc testing indicated greater p62 expression in head-only, body-only and whole-body exposures compared to controls ($p<0.05$), but no differences by area of exposure. Exposure to ^{56}Fe particles caused a significant increase in the neuroinflammatory marker pNF-κB in the hippocampus ($F_{3,16} = 4.87$, $p < 0.05$), but not in the frontal cortex ($F_{3,16} = 1.55$, $p > 0.10$). Post hoc analyses indicated that there were significant increases in NF-κB levels in the hippocampus of all irradiated groups compared to the non-irradiated controls ($p < 0.05$), but no significant differences in pNF-κB levels as a function of exposure location.

Two endogenous antioxidants were measured, SOD and GST (Fig. 5). The overall ANOVAs indicated that there were no significant changes in SOD ($F_{3,16} = 2.13$, $p > 0.10$) or GST ($F_{3,16} = 2.65$, $p > 0.05$) in the frontal cortex following exposure to ^{56}Fe particles. In contrast, the overall ANOVAs were significant for ^{56}Fe particle-induced changes in SOD ($F_{3,16} = 7.68$, $p < 0.01$) and GST ($F_{3,16} = 8.75$, $p < 0.01$) in the hippocampus. Post hoc comparisons indicated that compared to control subjects, there was a significant increase in SOD in the hippocampus of rats given head-only exposures and a significant decrease in SOD in the rats given whole-body exposure to ^{56}Fe particles. The SOD levels of the rats given body-only exposures did not differ significantly from those of the control subjects. Additionally, head-only exposures generated significantly higher levels of SOD in the hippocampus than body-only and whole-body exposures ($p<0.05$). There was a significant decrease in GST levels in the subjects given whole-body exposures compared to the control subjects. The GST levels of the rats given either head- or body-only exposures did not differ significantly from controls, but GST expression was significantly increased ($p<0.05$) for head-only compared to body-only and whole-body exposures.

The effects of partial- or whole-body exposure to ^{56}Fe particles on the Nrf2/Keap1 pathway, a major regulator of cytoprotective responses to oxidative stress (Kansanen et al., 2013), are summarized in Fig. 6. There was no significant effect of irradiation on Nrf2 levels in either the frontal cortex ($F_{3,16} = 1.81$, $p > 0.1$) or hippocampus ($F_{3,16} = 2.30$, $p > 0.10$). The overall ANOVA indicated that exposure to ^{56}Fe particles produced a significant decrease in Keap1 levels in the hippocampus ($F_{3,16} = 9.86$, $p < 0.01$), but not in the frontal cortex ($F_{3,16} = 0.22$, $p > 0.10$). Although all irradiated groups showed significantly lower

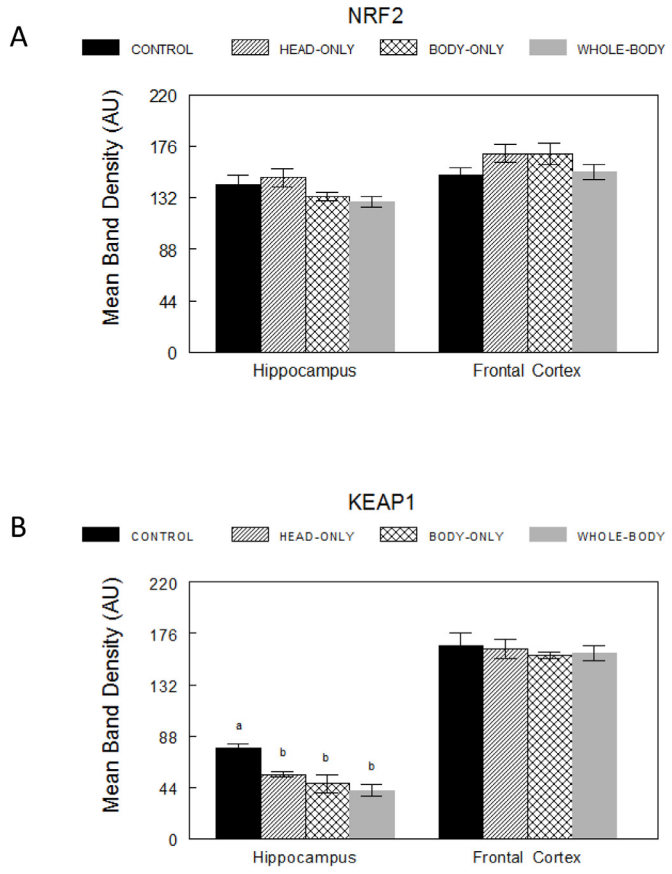

Fig. 6. Effects of partial/whole-body exposures to 50cGy ^{56}Fe particles on nuclear factor erythroid 2-related factor 2 (Nrf2) and Kelch-like ECH-associated protein 1 (Keap1), in the hippocampus and frontal cortex.

levels of Keap1 than control subjects, post hoc comparisons indicated that the decrease in Keap1 levels in the hippocampus did not differ as a function of the location of irradiated tissue.

4. Discussion

For both novel object recognition and operant responding, the overall ANOVAs indicated that there were no significant differences in performance as a function of treatment condition. Although there were differences in the threshold dose needed to disrupt performance for both cognitive tasks as a function of the location of the irradiation, exposure of the head-only or whole-body was not consistently more effective (did not disrupt performance at a lower dose) than body-only exposures, which did not directly affect the brain. These results would support the hypothesis that irradiation of the brain by exposure to HZE particles is not a necessary condition for the disruption of cognitive performance.

While the mechanisms by which body-only exposure can influence the brain and cognitive performance are not certain, it is possible that these effects result from irradiation-induced oxidative stress and neuroinflammation, which, in turn, affect neuronal function. While neuronal function was not directly measured in the present experiment, behavior is dependent upon the activity of neurons and changes in cognitive performance reflect changes in neuronal activity. Previous experiments have shown that exposure to HZE particles produces changes in the electrophysiological and neuroanatomical characteristics of the central nervous system (CNS) (Allen et al., 2015; Alp and Cucinotta, 2017; Carr et al., 2018; Vlkolinsky et al., 2007; 2010). Similarly, producing oxidative stress or neuroinflammation by treatment with a variety of toxic stimuli results in the death of neurons or changes in neuronal excitability (Berezhnaya et al. 2018; Patel et al., 2011; Ransohoff, 2016; Taylor and Crack, 2004; Vezzani and Viviani, 2016). Experiments using whole-body or head-only exposures to HZE particles have shown oxidative stress and neuroinflammation in various brain structures, including the hippocampus, striatum and cortex (Kumar et al., 2015; Li et al., 2014; Poulose et al., 2011; Poulose et al., 2014). Body-only exposure may produce oxidative stress and neuroinflammation in the brain by affecting the vagus nerve (Marquette et al. 2003; Fung et al., 2012) or by causing the release of cytokines into the bloodstream (Maier and Watkins, 1998; Marinelli et al., 2019; Vezzani and Viviani, 2016). More recently, several investigators have indicated that there is an interaction between the gut microbiome and the brain such that dysregulation of the gut can lead to the development of neurodegenerative processes in the brain (Ma et al., 2019; Zhao et al., 2018). Radiation exposure may produce changes in the gut microbiome (Casero et al., 2017), resulting in dysregulation of the gut-brain axis and neurodegenerative processes including inflammation and cognitive deficits. (Jones et al., 2020). Regardless of the mechanism, the present results show the occurrence of oxidative stress and neuroinflammation in the brain and a related disruption of cognitive performance following body-only exposure.

Despite the fact that the individual comparisons between the radiated subjects (25 or 50 cGy) and the non-irradiated controls (0 cGy) for operant performance using the Tukey-Kramer statistic did not always achieve significance, the overall ANOVA indicated that dose was a significant factor affecting performance for all treatment conditions. Differences in the disruption of operant performance were observed as differences in the overall pattern of responding as the reinforcement ratio increased from FR-1 to FR-35. The observation that body-only exposure was effective in disrupting cognitive performance suggests that the direct effects of HZE particles on neurons are not a critical factor for HZE particle disruption of neuronal function and cognitive performance. However, as reported in another study using ^{16}O particles (Rabin et al., 2014), whole-body exposure was more effective in disrupting cognitive performance than head-only exposure in that whole-body exposure affected performance at a lower dose than head-only exposure (25 compared to 50 cGy) in the initial test of performance 3 months following exposure.

When tested 11 months following exposure, the differences between the control and irradiated subjects in all treatment conditions were observed as enhanced performance such that the irradiated subjects were more responsive to the changes in reinforcement contingencies. These results are consistent with previous research indicating an interaction between exposure to HZE particles and age in the alteration of cognitive performance (Rabin et al., 2018). The observation of enhanced responsiveness to changes in reinforcement contingencies provides additional support for the hypothesis that irradiation of the head is not a necessary for the HZE particle-induced alteration of cognitive performance.

Changes in cognitive performance following exposure to HZE particles have been linked to changes in neuronal function. Exposure to HZE particles produces oxidative stress and neuroinflammation which, in turn, leads to inhibition of autophagy and altered hippocampal proteins and neurogenesis (Britten et al., 2017; Poulose et al., 2011; Parihar et al., 2018). Prevention of oxidative stress and neuroinflammation following head-only exposure to HZE particles by the use of antioxidant-rich diets (Rabin et al., 2005, 2009; Poulose et al., 2014; Shukitt-Hale et al., 2007) or by inhibiting microglia following whole-body exposure (Jenrow et al., 2013; Krukowski et al., 2018) attenuates the disruption of cognitive performance. Although additional testing is needed, the present results which show that body-only exposure to ^{56}Fe particles causes oxidative stress and neuroinflammation in specific brain structures is consistent with the previous research suggesting that the changes in CNS activity and cognitive performance are likely to be indirect effects resulting from radiation-induced oxidative stress and neuroinflammation and not a direct effect of HZE particles on neuronal

function.

As noted with behavior, exposure of the head, either by itself or as a component of whole-body irradiation, was not consistently more effective in producing oxidative stress and neuroinflammation in either the hippocampus or frontal cortex. As such, the present results suggest that the direct effects of HZE particles on neurons are not necessary to produce changes in neuronal function. However, in contrast to the behavioral data, interpretation of the neurochemical data is more complex. Not only were there occasional differences in neurochemical endpoints as a function of location of exposure, but there were also some differences as a function of the specific tissue analyzed, hippocampus or frontal cortex. In general, ^{56}Fe particle irradiation produced more changes in neuronal endpoints in the hippocampus than in the frontal cortex. Differential sensitivity of specific brain structures to the inflammatory effects of whole-brain exposure to gamma rays has been reported previously by Lee et al. (2010). The factors that influence the sensitivity of specific tissues to irradiation are not certain. Although it is tempting to ascribe differences in the irradiation sensitivity of different structures to specific neurotransmitter systems, there is no evidence to support this hypothesis.

The results of the present experiment show that irradiation of the head is not necessary for the HZE particle-induced development of oxidative stress and neuroinflammation in the brain and the related disruption of cognitive performance. Although some differences as a function of location were noted, for the most part there were no differences in any measure of neurochemical activity or cognitive performance following body- or head-only or whole-body exposures. In addition, in contrast to previous research (Rabin et al., 2014), irradiation of the entire organism did not enhance nor diminish the effectiveness of exposure on any endpoint. These results suggest that HZE particle-induced oxidative stress and/or neuroinflammation was the underlying cause of the disruption of neuronal function and cognitive performance and that these effects of exposure to HZE particles is not dependent upon the direct effect on neurons.

Despite the fact that there were no significant changes in NOX2 measured 60 days following exposure to ^{56}Fe particles, there were changes in several neurochemical endpoints which are related to the occurrence of oxidative stress. However, the pattern of these changes was not consistent with the occurrence of enhanced oxidative stress. In the hippocampus, but not in the frontal cortex, there was a significant increase in the endogenous antioxidant SOD following head-only exposures and decreased levels of both SOD and GST following whole-body exposures. Similarly, there were tissue specific changes in the Nrf2-Keap1 pathway, which functions to counteract the effects of oxidative stress to protect against neurodegenerative diseases (Kansanen et al., 2013; Deshmukh et al., 2017), following irradiation. These observations suggest that there are long-term consequences on performance resulting from the occurrence of oxidative stress at the time of exposure.

The role of HZE particle-induced oxidative stress and neuroinflammation in the disruption of cognitive performance is not certain. The present results show a long-term disruption of cognitive performance ($\approx$ 10 months after exposure) whereas the markers of oxidative stress (NOX2, SOD, GST) did not indicate a consistent elevation of oxidative stress in the hippocampus and frontal cortex two months after exposure to ^{56}Fe particles. The neurochemical results reported here are similar to those reported by Poulose et al. (2014), showing that the levels of NOX2, SOD and GST in the hippocampus were significantly increased 36 hr following exposure to ^{56}Fe particles but had returned to nearly non-irradiated control levels by 30 days following irradiation. Similarly, short-term increases in oxidative stress have been reported by other investigators (Li et al., 2014; Limoli et al., 2007). Given the generally short-acting time course of oxidative stress, it is difficult to determine how increases in the levels of reactive oxygen species can affect cognitive performance many months following exposure. However, Britten et al. (2017) have reported changes in the hippocampal proteome indicative of oxidative stress 90 days following exposure to ^{56}Fe particles. It is possible that the short-term occurrence of radiation-induced oxidative stress initiates a cascade of changes resulting in long-term neuroinflammation and related changes in cellular function (Buonoanno et al., 2011; Sawal et al., 2017).

In contrast to the measures of oxidative stress, both partial- and whole-body exposures to ^{56}Fe particles produced a significant increase in the neuroinflammatory marker NF-κB in the hippocampus measured 60 days following exposure. Long-term changes in neuroinflammatory markers following whole-body irradiation have been reported by other investigators (Parihar et al., 2018; Poulose et al., 2011; Rola et al., 2005). The present experiment indicates that neuroinflammatory responses do not require exposure of the head. The increase in NF-κB following body-only exposures may be mediated by vagal afferents (Marquette et al., 2003) or by the release of peripheral cytokines which affect neuronal microglia (Schaue et al., 2012). Inhibiting the increase in microglial activation prevents the disruption of cognitive performance by exposure to heavy particles (Jenrow et al, 2013; Krukowski et al., 2018). It is also possible that an irradiation-induced dysregulation of the gut microbiome can lead to the development of neurodegenerative processes in the brain (Jones et al., 2020; Ma et al., 2019; Zhao et al., 2018).

Historically, the assumption has been that exposure to HZE particles affects neuronal function and cognitive performance because of direct effects on neurons as the particles pass through the brain (e.g., Cucinotta et al, 1998, 2014; Curtis et al., 1989, 1998). The present results suggest that HZE particle-induced oxidative stress and neuroinflammation and the disruption of cognitive performance are not dependent upon a direct action of HZE particles on neurons. Rather, there are no consistent differences in neurochemical and behavioral endpoints as a function of location of the irradiation (head-only, body-only or whole-body). Although the conclusions are somewhat limited by the fact that neuronal function was not directly assessed and that oxidative stress and neuroinflammation in the striatum were not measured, previous research using HZE particles has established that exposure alters the structure of neurons and neuronal activity in the CNS (Allen et al., 2015; Alp and Cucinotta, 2017; Carr et al., 2018; Vlkolinsky et al., 2007; 2010) and that there is increased oxidative stress (Poulose et al., 2014; Rabin et al., 2015) and changes in dopamine signaling in the striatum (Joseph et al., 1992, 1993). Additional research will be needed to fully establish the brain structures involved and mechanisms by which exposure restricted to the body is capable of disrupting cognitive performance.

Nonetheless, the present results show that ^{56}Fe particle exposure limited to the body is capable of affecting cognitive performance. As such, these results raise questions about the contribution of direct effects of HZE particles on neurons in producing changes in neuronal function and behavior. In addition, because there were no significant differences in neurochemical or behavioral endpoints as a function of location, these results suggest that “hits” from HZE particles anywhere on the body must be taken into account when attempting to assess the risk to the central nervous system of astronauts during long duration missions outside the protection of the magnetic field of the earth.

Declaration of Competing Interest

None.

Acknowledgments

This research was supported by NASA Grant NNX16AE06G.

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