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Effects of exposure to ^{12}C and ^{4}He particles on cognitive performance of intact and ovariectomized female rats

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ABSTRACT

Exposure to the types of radiation encountered outside the magnetic field of the earth can disrupt cognitive performance. Exploratory class missions to other planets will include both male and female astronauts. Because estrogen can function as a neuroprotectant, it is possible that female astronauts may be less affected by exposure to space radiation than male astronauts. To evaluate the effectiveness of estrogen to protect against the disruption of cognitive performance by exposure to space radiation intact and ovariectomized female rats with estradiol or vehicle implants were tested on novel object performance and operant responding on an ascending fixed-ratio reinforcement schedule following exposure to ^{12}C (290 MeV/n) or ^{4}He (300 MeV/n) particles. The results indicated that exposure to carbon or helium particles did not disrupt cognitive performance in the intact rats. Estradiol implants in the ovariectomized subjects exacerbated the disruptive effects of space radiation on operant performance. Although estrogen does not appear to function as a neuroprotectant following exposure to space radiation, the present data suggest that intact females may be less responsive to the deleterious effects of exposure to space radiation on cognitive performance, possibly due to the effects of estrogen on cognitive performance.

1. Introduction

On exploratory class missions outside the magnetic field of the earth, such as a mission to Mars, astronauts will be exposed to types and doses of radiation that are not experienced in low earth orbit (Cucinotta et al., 2014; Schimmerling et al., 2003) where the International Space Station operates. The radiation environment in space (cosmic rays) is composed of alpha particles (^{4}He), protons and particles of high energy and charge (HZE particles) such as ^{56}Fe, ^{48}Ti and ^{12}C. On a projected three-year mission to Mars, astronauts may be exposed to a total dose of 20–30 cGy of protons and heavy particles (Zeitlin et al., 2013).

Exposure to the radiation environment in space can affect the capacity of an organism to perform a variety of cognitive tasks. Exposing male mice and rats to HZE particles using a ground-based model for exposure to cosmic rays, produces deficits in spatial learning and memory (Shukitt-Hale et al., 2000, 2003; Britten et al., 2017); in general (non-spatial) learning and memory (Rabin et al., 2005a, 2015; Parihar et al., 2016); in the ability of the organism to respond to changes in environmental contingencies (Rabin et al., 2009, 2015); and in fear conditioning (Raber et al., 2013).

Despite the fact that the crew on exploratory class missions will probably be composed of both male and female astronauts, little research on the effects of exposure to the types of radiation encountered on exploratory class missions has involved the use of female models. The limited data available suggests that females may not respond to exposure to HZE particles similarly to males. Raber and colleagues (Villasana et al., 2010, 2013) have shown differences in the effects of exposure to space radiation on the cognitive performance of mice as a function of sex and ApoE isoform. Working with mice exposed to ^{40}Ca ions, Raber et al. (2016) have shown impaired contextual freezing in irradiated female mice compared to irradiated male mice. Comparing the performance of males and females on a variety of cognitive tasks following exposure to a simulation of the galactic cosmic ray spectrum, Krukowski et al. (2018a) reported deficits in novel object recognition performance and in social recognition memory in male mice but not in female mice. The behavioral changes were paralleled by microglial changes in male but not female mice. In an initial experiment, Rabin et al. (2013) began an evaluation of the possible role of estrogen in mediating the observed sex differences in the cognitive effects of exposure to HZE particles. They reported that the cognitive effects of

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exposures to ^{56}Fe particles in female rats vary as a function of the hormonal status of the subject (estradiol or vehicle) at the time of irradiation and the specific task (novel object recognition or operant responding).

Exposure to space radiation causes oxidative stress (Bounanno et al., 2011; Li et al., 2014; Poulose et al., 2011), neuroinflammation (Jenrow et al., 2013; Rola et al., 2005; Krukowski et al., 2018b) and changes in neuronal function (Davis et al., 2014; Poulose et al., 2011). These effects of exposure to space radiation are similar to those observed with neurodegenerative diseases (Giles and McArthur, 2010; Nilson, 2008; Pal et al., 2016; Stephenson et al., 2018). Because estrogen can function as a neuroprotectant (Dluzen, 1997, 2000; Engler-Chiurazzi et al., 2017), it may influence how exposure to the types of radiation encountered in space could affect cognitive performance in females. While many of the neurophysiological/neurochemical markers that characterize the disruption of cognitive performance by exposure to HZE particles in male subjects are absent in females (Krukowski et al., 2018a), the underlying cause for the sex differences remains to be established. It is possible that sex differences in the sensitivity of an organism to the disruptive effects of exposure to space radiation are related to sex differences in brain organization (Galea et al., 2017; Choleris et al., 2018) and in the generation of oxidative stress (Tenkorang et al., 2018).

Given that both male and female astronauts will participate in exploratory class missions, it is essential that the effects of exposure to space radiation of the cognitive performance of female subjects be evaluated in addition to effects of irradiation on male subjects. The present experiment was designed to evaluate the effects of exposure to low linear energy transfer (LET) ^{12}C and ^{4}He particles on two measures of cognitive performance in female rats as a function of hormonal status at the time of irradiation. The specific tasks utilized were novel object recognition, which is a measure of non-spatial learning and memory, and operant responding which is a measure of an organism's motivation to work for reinforcement and its ability to respond to changes in environmental contingencies. Within this context, the possible role of estrogen mediating the effects of exposure to ^{4}He particles on cognitive performance may be of particular importance because a significant proportion of the total dose within the spacecraft will be provided by ^{4}He particles (Norbury and Slaba, 2014) and because exposure to very low doses of ^{4}He particles disrupt cognitive performance (Rabin et al., 2019).

2. Methods

2.1. Subjects

The subjects for the present experiment were intact and ovariectomized (OVX) female Sprague–Dawley rats approximately 7 weeks of age obtained from Taconic Farms. The rats were ovariectomized by the supplier and shipped to Brookhaven National Laboratory (BNL) together with the intact rats. At BNL half the OVX rats were implanted with silastic tubing containing either estradiol (Eβ) in sesame oil or vehicle (sesame oil) only. Implantation of the tube containing estradiol or vehicle was performed at BNL using the procedure described by Strom et al. (2008). Briefly, 30 mm segments of silastic tubing (Inner/outer diameter: 1.575/3.175 mm) were filled with a solution of 180 pg Eβ/mL in sesame oil or sesame oil alone (vehicle). The ends of the tubing were sealed with 5 mm pieces of wooden applicator sticks. Before use, the capsules were stored overnight in a vial containing sesame oil with the same concentration of Eβ or vehicle as inside the capsules. To implant the capsules the rats were anesthetized with i.p. injections of ketamine (100 mg/kg) and xylazine (10 mg/kg). A 5 mm incision was made in the loose skin of the rat's neck, and a pocket bluntly dissected caudally in which the silastic capsule was gently installed using forceps; the incision was then closed with sutures. Half of the OVX rats were implanted with silastic tubing containing Eβ and half were implanted with tubing containing vehicle.

Following surgery the rats were monitored to make certain that they recovered from the anesthesia. Strom et al. (2008) have used radioimmunoassay procedures to evaluate the levels of estradiol in serum following implantation of silastic capsules containing estradiol as detailed above. They reported that this procedure results in a relatively stable level of estradiol in serum equivalent to that in female rats during estrous. The level of estradiol reaches an equilibrium level within 48 h of implantation and lasts for 4–5 weeks following implantation.

At Brookhaven National Laboratory (BNL), the rats were maintained in the AAALAC-accredited animal facility. For behavioral testing, the rats were shipped to the University of Maryland, Baltimore County (UMBC). The animal facilities at UMBC are supervised by Veterinary Medicine Resources of the University Of Maryland School Of Medicine. At both facilities, the rats were maintained on a 12:12 h light:dark cycle with food and water continuously available except as required by the experimental protocol. All procedures were approved by the IACUCs of BNL, UMBC, and Human Nutrition Research Center on Aging (HNRCA).

2.2. Radiation

The rats were irradiated at the NASA Space Radiation Laboratory (NSRL) at BNL 65–72 h following the implantation of the silastic tubing. The rats were given head-only exposures to 5, 10, or 25 cGy of ^{12}C particles (290 meV/n; LET $\approx$ 13 keV/μm) or 0.025 or 0.050 cGy ^{4}He particles (300 MeV/n; LET $\approx$ 1.4 keV/μm) (n = 10/dose) at a nominal dose rate of 0.01 cGy/min. The doses selected for this experiment are based upon prior research (Rabin et al., 2011, 2019) with male rats which established effective doses for the disruption of cognitive performance. This research indicated that the relationship between particle LET and the effectiveness of a specific particle in disrupting cognitive performance is such that as LET decreases the dose needed to disrupt the threshold dose also decreases. This research has also indicated that the mechanisms for the disruption of cognitive performance are not particle specific. Dosimetry was provided by the staff of the NSRL using parallel plate ionization chambers (La Tessa et al., 2016). The non-irradiated rats (0 cGy, n = 10/treatment condition) were taken to the NSRL, placed in restraining tubes, but were not irradiated.

For irradiation, the rats were placed in well-ventilated plastic tubes which were placed perpendicular to the beam. The animal was positioned with the center of its head in the beam. The body of the rat was shielded with tungsten bricks. As such, some of the neck of the animal was also exposed. Head-only exposures were utilized to make the current research compatible with our previous research using ^{12}C and ^{4}He particles. Previous research (Rabin et al., 2011, 2014, 2019) has shown that there are no differences in the cognitive effects of whole body or head only exposure to ^{4}He particles.

2.3. Behavioral testing

Following irradiation the rats were shipped to UMBC for behavioral testing. The first sequence of tests (recognition memory and operant responding) was begun 4 months following exposure to carbon particles and 2 months following exposure to helium particles. The second sequence started 10 (carbon) and 11 (helium) months following exposure.

The specific tasks were novel object recognition and operant responding on an ascending fixed-ratio (FR) reinforcement schedule. The novel object recognition task is a measure of general learning and memory and is a standard task used to evaluate the effects of ovariectomy and estrogen replacement on cognitive performance (Luine, 2015; Tuscher et al., 2015). The operant task is a measure of an organism's motivation to work for reinforcement and its ability to respond to changes in environmental contingencies. Performance on this task is dependent on the dopaminergic system (Lindner et al., 1997) and may be related to the activational aspects of motivation and the effort put into obtaining reinforcement and is dependent upon the integrity of the

striatum (Salamone, 1994; Salamone and Correa, 2002; Salamone et al., 2018).

2.3.1. Object recognition memory

Recognition memory (novel object performance) was tested by placing the subjects in a dimly lit open field (93 cm x 93 cm). The stimulus objects (which are no smaller than the size of the rat and no larger than two and a half times its size) vary in shape and color. After habituation to the apparatus, two identical (familiar) stimulus objects are placed in symmetrical locations in the open field. The rat is allowed to explore the stimuli until it accumulates 25–30 s total object exploration time (i.e., exploration of either object) or until 15 min have passed. After 24 h delay, the rat is placed back in the field with one familiar and one novel object and allowed to explore both stimuli until it has accumulated 30 s of object exploration on either object or until 15 min have passed. Subjects that did not achieve the criterion of 25–30 s exploration time on either the conditioning or test day were excluded from the analysis. Typically, 7–10 subjects (out of 10) met criterion. When tested a second time different sets of stimulus objects are used.

2.3.2. Operant responding

For the operant task, the rats were placed on a mild food deprivation schedule and maintained at 90% base weight. During deprivation the rats were weighed daily and food obtained in the operant chamber was supplemented with experimenter-provided food. The rats were trained to press a lever for 45 mg food pellets in a continuous reinforcement schedule using an autoshaping procedure in which they are placed in an operant chamber for 8–14 h. Most rats learn the response within a single session. The rats were then trained to respond on a fixed-ratio (FR) schedule by placing them in the chamber for 30 min and rewarding them on FR-1, FR-5, FR-10, and FR-20 reinforcement schedules. For testing they are then given 30-min sessions in which they are rewarded on FR-1, FR-5, FR-10, FR-15, FR-20, FR-25, FR-30 and FR-35 reinforcement schedules on consecutive days. The same pattern was utilized for the second run.

2.4. Statistics

The initial analysis of novel object recognition performance was a one-way ANOVA comparing the percentage of time spent interacting with the novel object across all treatment conditions. However, because rats will normally spend significantly more time with the novel object, statistical analysis of novel object performance involved individual two-tailed *t*-tests to determine whether or not the performance of the treatment groups spent significantly more time with the novel object (i.e., greater than 50% of their time exploring the novel object).

The initial analysis of operant performance utilized a 3-way ANOVA to determine whether or not there were significant differences in performance between the different treatment groups (intact, OVX + Eβ, OVX + V). Where the main effect for treatment was significant ($p < 0.05$), independent 2-way ANOVAs were run for each treatment condition. The statistical package (NCSS[11]) provides a critical value for the planned comparisons following an ANOVA. The program calculates the critical Bonferroni value from the ANOVA. It then automatically runs all comparisons using the ANOVA error term. Where the difference between treatment conditions or dose exceeds that value, the difference is considered to be significant at the 0.05 level. The output gives us the results of the comparisons indicating where the differences between doses/treatments are equal to or greater than the critical value.

3. Results

The effects of exposure to ^{12}C particles on cognitive performance are shown in Figs. 1–3. As shown in Fig. 1, there were no clear effects of ovariectomy and estrogen replacement at the time of exposure on novel object recognition performance following irradiation. A one-way ANOVA indicated that four months following irradiation there was no significant difference in performance as a function of hormonal status or dose ($F[11,89] = 0.96$, $p > 0.10$). Ten months following irradiation the overall ANOVA was significant ($F[11,77] = 2.26$, $p < 0.05$), although individual comparisons using the Bonferroni test did not indicate any significant differences in performance as a function of hormonal status or irradiation.

Performance on the operant task four months following irradiation (Fig. 2) differed as a function of hormonal status (intact, OVX + Eβ, OVX + V) at the time of irradiation ($F[2,776] = 91.18$, $p < 0.01$), and as a function of dose ($F[3,776] = 6.75$, $p < 0.01$). The treatment by dose interaction was also significant ($F[6,776] = 19.08$, $p < 0.01$). Individual comparisons using the Bonferroni test indicated that the performance of the intact (non-OVX) animals was significantly different than that of both groups of OVX subjects, showing an increased responsiveness to changes in reinforcement contingencies following exposure to 5 or 25 cGy of ^{12}C particles. Analysis of performance using two-way ANOVAs of the separate treatment conditions indicated a significant main effect of dose for all treatment conditions (intact $F[3,280] = 24.80$, $p < 0.01$; OVX + Eβ $F[7,256] = 4.17$ $p < 0.01$; OVX + V $F[3,240] = 5.78$ $p < 0.01$). Further analysis indicated that the performance of the intact rats exposed to 5 or 25 cGy of ^{12}C particles was significantly better than that of the non-irradiated control subjects. In contrast, the performance of the non-irradiated (0 cGy) OVX rats that had been given estradiol at the time of exposure was significantly better than that of the subjects exposed to 10 or 25 cGy and the performance of the non-irradiated rats given vehicle implants at the time of exposure was significantly better than that of the rats exposed to 25 cGy of ^{12}C particles.

When retested 10 months following exposure (Fig. 3), the main effect for treatment condition ($F[2,270] = 161.41$, $p < 0.01$) and the treatment by dose interaction ($F[3,720] = 6.87$, $p < 0.010$) were significant, although the main effect for dose was not significant ($F[3,720] = 1.08$ $p > 0.10$). As at the earlier time point the performance of the intact subjects was significantly different from the performance of the two OVX conditions. The performance of the non-irradiated intact rats was significantly poorer than that of the rats exposed to 10 cGy of ^{12}C particles. The performance of the non-irradiated OVX rats with Eβ implants was significantly better than that of the rats exposed to 5 and 10 cGy whereas there were no differences in performance as a function of dose for the OVX rats with vehicle implants at the time of exposure.

The effects of exposure to ^{4}He particles on novel object recognition in intact and ovariectomized female rats given implants of estradiol or vehicle at the time of irradiation and tested two and eleven months following exposure are shown in Fig. 4. At neither time point was there a significant difference in performance of the non-irradiated controls as a function of hormonal status (intact, OVX + Eβ, OVX + *V*) at the time of irradiation ($F[2,23] = 1.34$, $p > 0.10$; $F[2,18] = 0.16$, $p > 0.10$; 2 and 11 months, respectively).

Two months following exposure only the OVX rats exposed to 0.025 cGy of ^{4}He particles showed a disruption of recognition memory by spending equal amounts of time with both the familiar and novel objects (Fig. 4(A)). All of the subjects exposed to 0.050 cGy spent significantly more time with the novel than the familiar object. These results were reversed when retested 11 months after exposure: the OVX females with either Eβ or vehicle implants at the time of irradiation and exposed to 0.025 cGy were the only subjects to spend significantly more time with the novel object (Fig. 4(B)).

The initial test of operant responding (Fig. 5) was performed 3 months following exposure to ^{4}He particles. The overall 3-way ANOVA indicated that the main effects for treatment ($F[2,623] = 68.80$, $p < 0.01$) and dose ($F[2,623] = 12.23$, $p < 0.01$) were both significant, as was the dose by treatment interaction ($F[4,623] = 11.87$, $p < 0.01$). The Bonferroni comparison across the three treatment conditions indicated that the baseline responding of the intact subjects

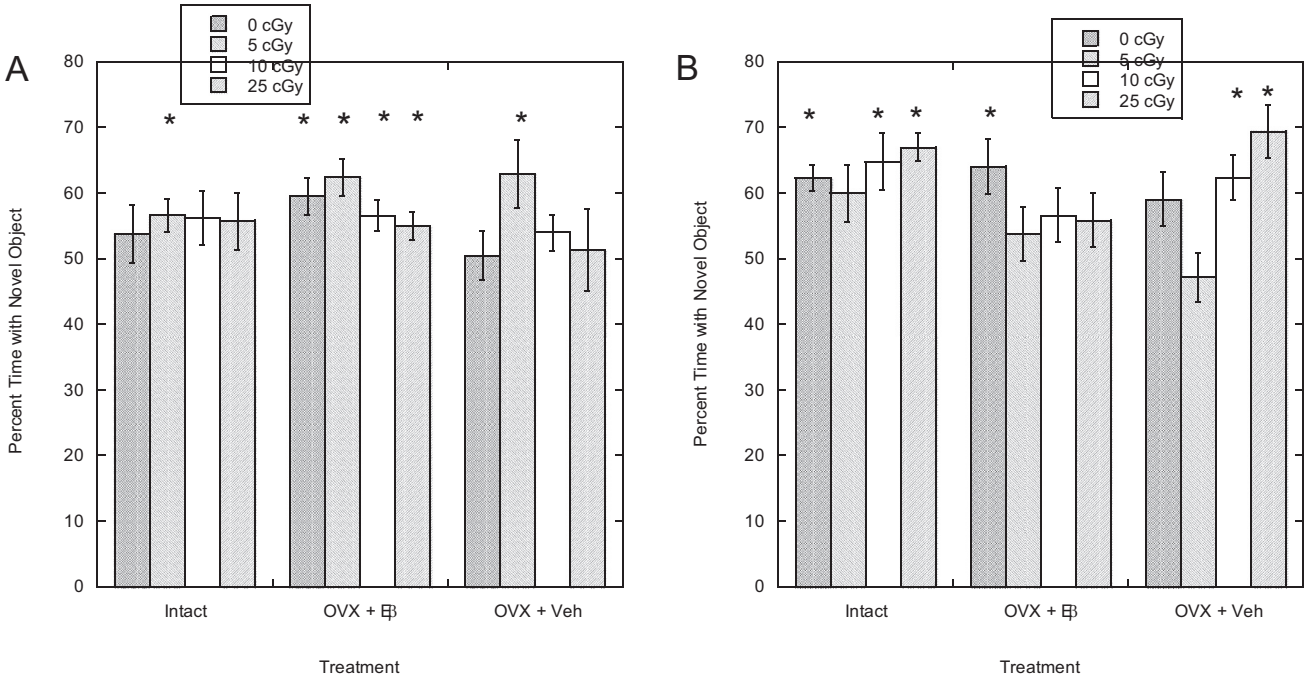

Fig. 1. Effects of exposure to ^{12}C particles on novel object recognition. (A) 4 months following irradiation; (B) 10 months following irradiation. *Significantly different than 50% time with novel object.

was significantly higher than that of the two OVX groups. Also, the schedule by treatment interaction was significant ($F[14,623] = 3.24$, $p < 0.01$) indicating that the responsiveness of the subjects to changes in reinforcement contingencies was different for the three treatment contingencies, with the intact subjects being the most responsive.

For all three treatment conditions (intact, OVX + Eβ, OVX + Veh) the main effect for dose was significant ($F[2,168] = 10.52$, $p < 0.01$; $F[2,184] = 5.13$, $p < 0.01$; $F[2,200] = 19.81$, $p < 0.01$; respectively). However, the pattern of radiation effects was different. Both the intact and the OVX + Veh subjects showed significantly higher rate of responding following exposure to 0.05 cGy of ^{4}He particles compared to the non-irradiated subjects. In contrast, the OVX + Eβ rats showed a significant performance decrement. For all treatment conditions, the performance of the subjects exposed to 0.025 cGy of ^{4}He particles did not differ significantly from the performance of the control subjects (0 cGy).

When retested 11 months following exposure (Fig. 6), the overall ANOVA indicated that the main effects for treatment ($F[2,592] = 192.43$, $p < 0.01$) and for dose ($F[2,592] = 6.59$, $p < 0.01$) were significant, although the dose by treatment interaction was not significant ($F4,592] = 1.44$, $p > 0.10$). As was observed 3 months after exposure, the baseline rate of responding was significantly greater in the intact subjects than in the OVX groups and the intact subjects continued to be the most responsive to changes in reinforcement contingencies.

In contrast to their performance three months after exposure, the performance of neither the intact ($F[2,200] = 2.47$, $p > 0.05$) nor the OVX + Veh ($F[2,208] = 0.40$, $p > 0.10$) subjects exposed to either dose of ^{4}He particles differed significantly from that of their respective controls. Only the subjects in the OVX + Eβ treatment condition exposed to both 0.025 and 0.050 cGy of ^{4}He particles showed a significant decrease in performance ($F[2,207] = 6.01$, $p < 0.05$).

4. Discussion

There are two aspects that must be considered in evaluating the

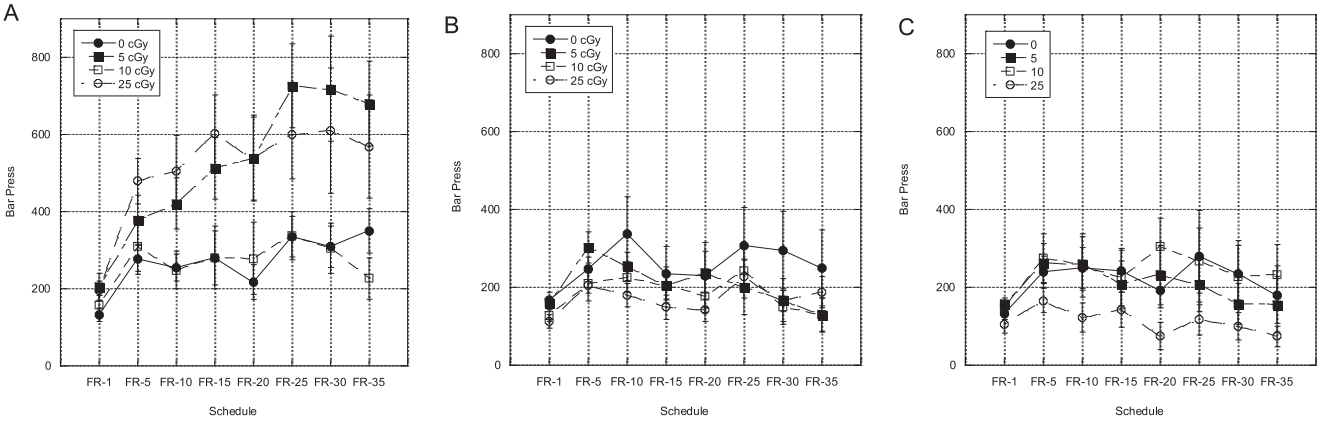

Fig. 2. Effects of exposure to ^{12}C particles on operant responding on an ascending FR reinforcement schedule 4 months following irradiation. (A) Intact (non-ovariectomized subjects); (B) OVX subjects with silastic implants of estradiol at the time of irradiation; (C) OVX subjects with silastic implants of vehicle at the time of irradiation.

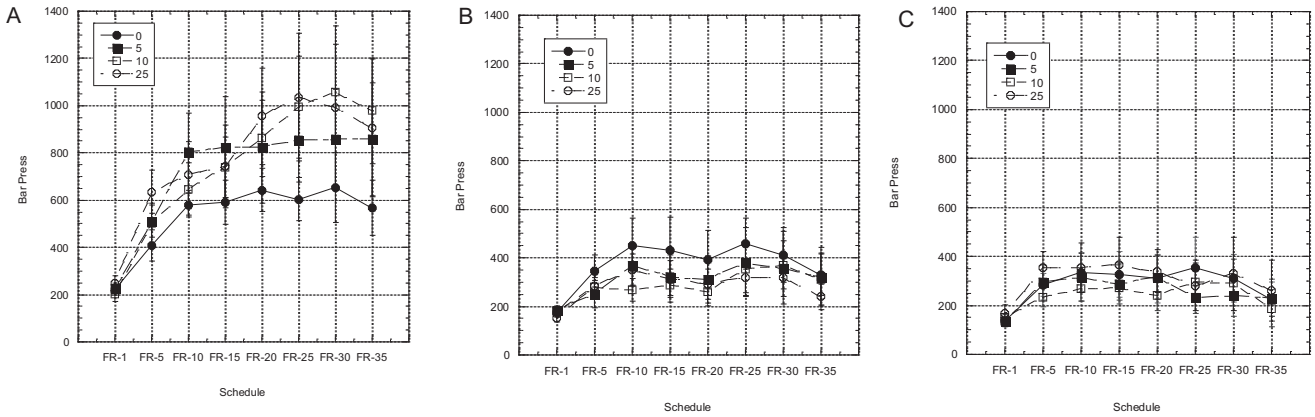

Fig. 3. Effects of exposure to ^{12}C particles on operant responding on an ascending FR reinforcement schedule 10 months following irradiation. (A) Intact (non-ovariectomized subjects); (B) OVX subjects with silastic implants of estradiol at the time of irradiation; (C) OVX subjects with silastic implants of vehicle at the time of irradiation.

present results. First, the effects of OVX and estradiol replacement on the two measures of cognitive performance: novel object recognition and operant responding on an ascending FR schedule. Second, the effects of exposure to ^{12}C and ^{4}He particles on these endpoints and the possible role of estrogen as a modifier of the responsiveness of the organism to exposure to the types of radiation encountered on exploratory class missions.

The novel object recognition task is routinely used to study the effects of hormones on memory (Luine, 2015). The general finding is that ovariectomy disrupts performance on this task and estrogen replacement results in improved performance (Aubele et al., 2008; Tuscher et al., 2015; Inagaki et al., 2010). The effects of irradiation on novel object recognition performance varied as a function of the specific ion, dose, hormonal status at the time of irradiation and time since exposure. While the overall ANOVA indicated that there were no significant differences as a function of treatment condition, a disruption of recognition memory was observed under some conditions. The factors that might have influenced the present results are not clear, except it has been noted that the effects of estrogen on learning and memory are complex, and can result in either an enhancement or impairment of performance depending upon the specific task and the brain regions that mediate the performance (Inagaki et al., 2010; Pratap et al., 2016; Walf et al., 2006; Gervais et al., 2016).

Three and four months following exposure, the operant performance of the intact subjects exposed to either ^{12}C or ^{4}He particles was not decreased compared to that of the controls. Rather the subjects exposed to several doses of either particle showed an increase in responding compared to the non-irradiated controls as the reinforcement ratio increased from FR-1 to FR-35. The observation that exposing intact female rats to HZE particles did not decrease performance on a cognitive task is consistent with the results reported by other investigators using exposure to ^{56}Fe particles (Villasana et al., 2010, 2013; Raber et al., 2016; Rabin et al., 2013) and by Krukowski et al. (2018a) using a simulated galactic cosmic ray pattern. These results with female rats differ from those obtained using intact male subjects exposed to various components of space radiation which

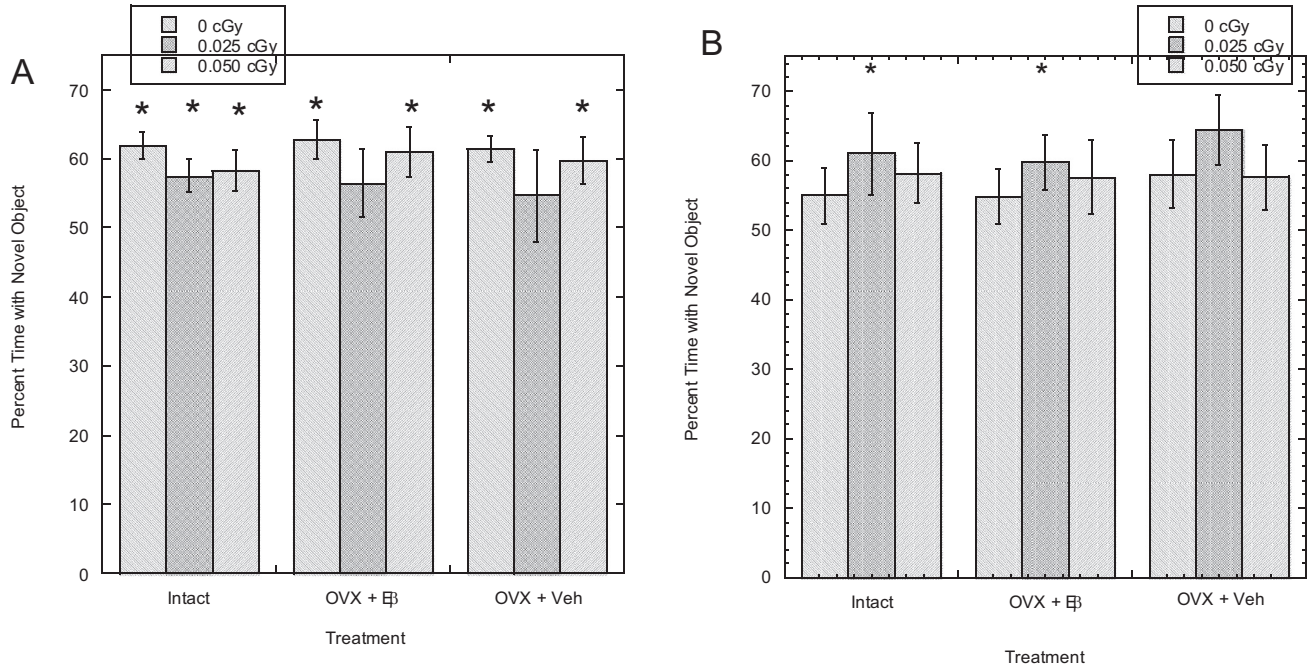

Fig. 4. Effects of exposure to ^{4}He particles on novel object recognition. (A) 2 months following irradiation; (B) 11 months following irradiation. *Significantly different than 50% time with novel object.

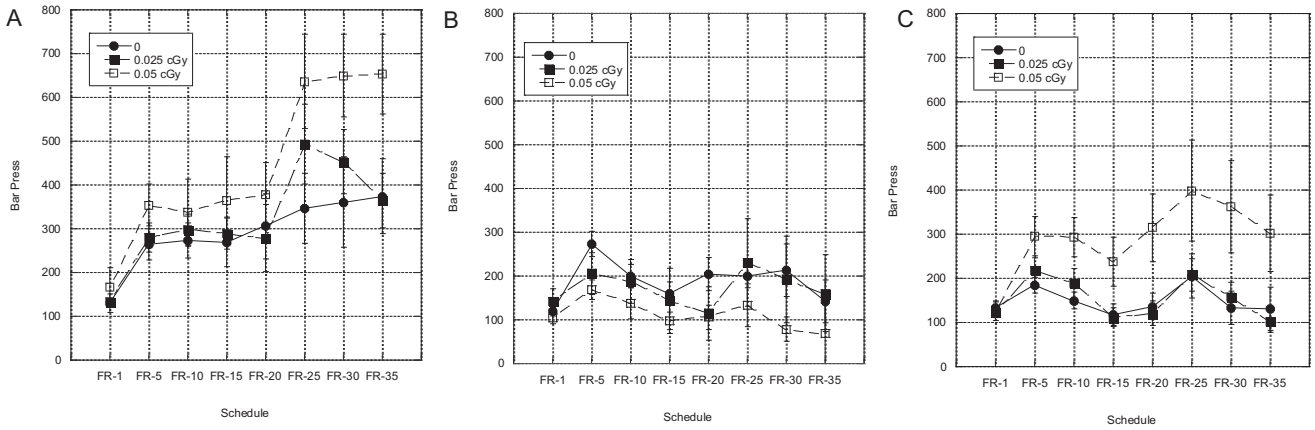

Fig. 5. Effects of exposure to ^{4}He particles on operant responding on an ascending FR reinforcement schedule 3 months following irradiation. (A) Intact (non-ovariectomized subjects); (B) OVX subjects with silastic implants of estradiol at the time of irradiation; (C) OVX subjects with silastic implants of vehicle at the time of irradiation.

show a disruption of cognitive performance (Shukitt-Hale et al., al.,2000, 2003; Britten et al., 2017; Rabin et al., 2005a, 2015; Parihar et al., 2016; Rabin et al., 2009, 2015; Raber et al., 2013). While improved performance, shown by an increased responsiveness to changes in reinforcement contingencies, is not typical of the effects of exposure to HZE particles, a similar increase in the responsiveness of the organism has been reported following irradiation of aged male rats (Rabin et al., 2018), indicating that an increase in the rate of responding following irradiation can be observed with other subjects.

As with recognition memory, the effects of exposure to ^{12}C or ^{4}He particles on operant responding in OVX subjects were not straightforward, varying as a function of the specific ion, dose, hormonal status at the time of irradiation and time since exposure. For both particles the differences in performance between the OVX+ Eβ and OVX+Veh subjects could not be due to differences in hormonal status at the time of testing because all behavioral tests, which were begun 3–4 months following irradiation, were conducted at a time during which there were no differences in estradiol levels (Strom et al., 2008). There were differences in the pattern of responding 3–4 months following exposure as a function of particle and hormonal status at the time of irradiation. OVX rats with either Eβ or Veh implants exposed to ^{12}C showed decreased operant performance following irradiation. Following exposure to 0.05 cGy of ^{4}He particles the OVX subjects given Eβ implants at the time of irradiation showed a disruption of performance; the subjects given Veh implants showed enhanced responsiveness to change in reinforcement contingencies following exposure to 0.05 cGy. The underlying mechanisms for the different effects of irradiation for the two particles are not immediately obvious. It is possible that the different effects may reflect the differences in the doses needed to affect cognitive performance: 5 cGy for ^{12}C particles compared to 0.05 cGy for ^{4}He particles.

When operant performance was retested 11–12 months following irradiation the performance of the intact (non-OVX) rats was not disrupted compared to that of the non-irradiated controls. However, there continued to be differences in the effects of irradiation on cognitive performance between the subjects exposed to ^{12}C particles and those exposed to ^{4}He particles such that the performance of the intact subjects exposed to ^{4}He particles did not differ significantly from the non-irradiated controls, whereas exposure to ^{12}C particles resulted in enhanced responsiveness. The effects of irradiation to either particle in the OVX subjects were similar, with the irradiated rats given Eβ showing decreased performance compared to the non-irradiated controls (0 cGy), and the irradiated rats given Veh implants showing no differences compared to the control subjects. While these results are consistent with previous research showing an interaction between exposure to HZE particles and age in male subjects (Rabin et al., 2005b, 2018), the mechanisms underlying the relationship between age, the specific particle and hormonal status at the time of irradiation remains to be clarified.

The failure of exposure to HZE particles to disrupt operant

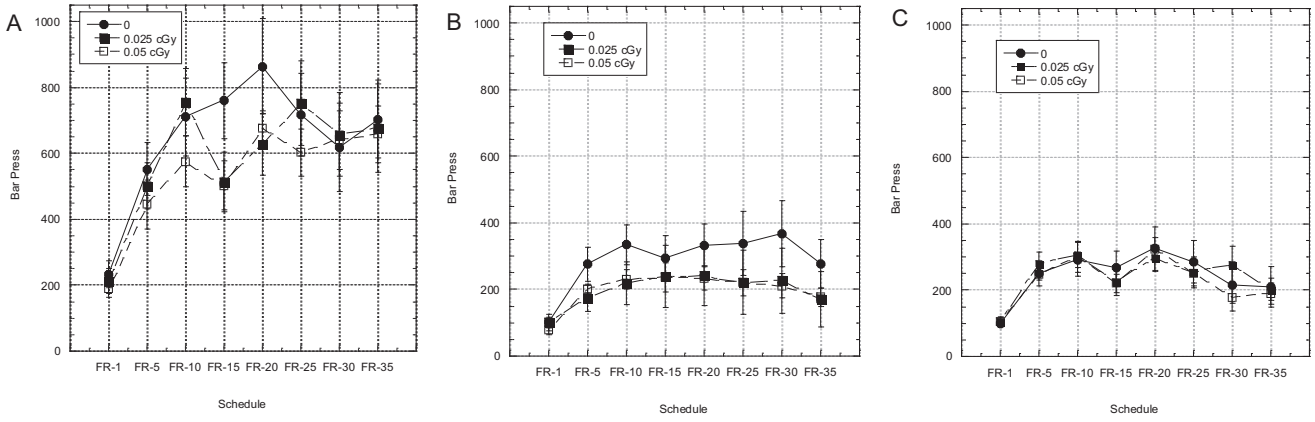

Fig. 6. Effects of exposure to ^{4}He particles on operant responding on an ascending FR reinforcement schedule 11 months following irradiation. (A) Intact (non-ovariectomized subjects); (B) OVX subjects with silastic implants of estradiol at the time of irradiation; (C) OVX subjects with silastic implants of vehicle at the time of irradiation.

responding in intact female rats is not due to presence of estrogen: the effect of estrogen at the time of exposure to either particle was to produce a decrease in the responsiveness of the subject to changes in reinforcement contingencies. However, the absence of estrogen at the time of irradiation may not necessarily be protective in as much as the OVX rats with vehicle implants continued to show poorer performance following exposure to ^{12}C particles in contrast to the subjects exposed to ^{4}He particles who showed increased responsiveness to changes in reinforcement contingencies.

The possible role of estrogen as a mediator of these differences is not clear. Estrogen is a neuroprotectant, delaying the onset and progression of neurodegenerative diseases (Engler-Chiurazzi et al., 2017), providing protection against the effects of traumatic brain injury (Brotfain et al., 2016; Chakrabarti et al., 2016) and protecting against dopaminergic system dysfunction following the administration of a variety of neurotoxins, including kainic acid and 6-hydroxydopamine (Dluzen, 1997, 2000). The mechanisms underlying the neuroprotective effects of estrogen may involve the activation of free radical scavenging systems to reduce oxidative stress and neuroinflammatory processes (Nilson, 2008; Vegeto et al., 2008) and changes in mitochondrial function (Simpkins and Dykens, 2008).

Although estrogen is a neuroprotectant, the results of the present experiment indicate that the presence of estrogen at the time of irradiation is not the mechanism for the preservation of cognitive performance in female rats. All of the OVX rats given Eβ at the time of irradiation showed decreased operant performance. An alternative possibility for the maintained and improved operant performance of intact female rats following exposure to both ^{12}C and ^{4}He particles may be the presence of estrogen at the time of testing. Operant responding on an ascending FR schedule, which measures an organism's motivation to work for reinforcement and its ability to respond to changes in environmental contingencies, is dependent upon the integrity of the nigrostriatal dopamine system (Lindner et al., 1997; Salmone, 1994; Salmone et al., 2018). Estrogen promotes dopamine release which is reduced by ovariectomy (reviewed in McEwen and Milner, 2017). A consequence of the lack of estradiol which affected dopamine levels in the nigrostriatal system at the time of testing may be responsible for the reduced performance on this task by the OVX subjects. In this regard, it may be noted that exposure to HZE particles also disrupts dopaminergic functioning (Joseph et al., 1992; Davis et al., 2014).

Although the present experiment has not clarified the role of estrogen as a potential mediator of differences in the responsiveness of male and female models to exposure to the types of radiation that will be encountered by astronauts on exploratory class missions, the results are consistent with previous research showing that the cognitive performance of females may be less affected by exposure to space radiation than males.

Conflict of interest

There is no conflict of interest.

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References

Aubele, T., Kaufman, R., Montalmant, F., Kritzer, M.F., 2008. Effects of gonadectomy and hormone replacement on a spontaneous novel object recognition task. Horm. Behav. 54, 244–252.

Britten, R.A., Jewell, J., Miller, V.D., Davis, L.K., Hadley, M.M., Wyrobek, A.J., 2017. Impaired spatial memory performance in adult Wistar rats exposed to low (5-20 cGy) doses of 1 GeV/n 56Fe particles. Radiat. Res. 187, 287–297.

Brotfain, E., Gruenbaum, S.E., Boyko, M., Kutz, R., Zlotnik, A., Klein, M., 2016. Neuroprotection by estrogen and progesterone in traumatic brain injury and spinal cord injury. Curr. Neuropharmacol. 14, 641–653.

Buonanno, M., de Toledo, S.M., Pain, D., Azzam, E.I., 2011. Long-term consequences of radiation-induced bystander effects depend on radiation quality and dose and correlate with oxidative stress. Radiat. Res. 175, 405–415.

Chakrabarti, M., Das, A., Samantaray, S., Smith, J.A., Banik, N.L., Haque, A., Ray, S.K., 2016. Molecular mechanisms of estrogen for neuroprotection in spinal cord injury and traumatic brain injury. Rev. Neurosci. 27, 271–281.

Choleris, E., Galea, L.A.M., Sohrabji, F., Fricki, K.M., 2018. Sex differneces in the brain: implikcations for behavioral and biomedical research. Neurosci. Biobehav, Rev. 85, 125–145.

Cucinotta, F.A., Alp, M., Sulzman, F.M., Wang, M., 2014. Space radiation risks to the central nervous system. Life Sci. Space Res. 2, 54–69.

Davis, C.M., DeCicco-Skinner, K.L., Roma, P.G., Hienz, R.D., 2014. Individual differences in attentional deficits and dopaminergic protein levels following exposure to proton radiation. Radiat. Res. 181, 258–271.

Dluzen, D.E., 1997. Estrogen decreases corpus striatal neurotoxicity in response to 6-hydroxydopamine. Brain Res. 767, 340–344.

Dluzen, D.E., 2000. Neuroprotective effects of estrogen upon the nigrostratal dopaminergic system. J. Neurocytol. 29, 387–399.

Engler-Chiurazzi, E.B., Brown, C.M., Povroznik, J.M., Simpkins, J.W., 2017. Estrogens as neuroprotectants: estrogenic actions in the context of cognitive aging and brain injury. Prog. Neurobiol. 157, 188–211.

Galea, L.A.M., Frick, K.M., Hampson, E., Soihrabji, F., 2017. Why estrogens matter for behavior and brain health. Neurosci. Biobehav. Rev. 76, 363–379.

Gervais, N.J., Hamel, L.M., Brake, W.G., Mumby, D.G., 2016. Intra-perirhinal cortex administration of estradiol, but not an ERβ agonist, modulates object-recognition memory in ovariectomized rats. Neurobiol. Learn. Mem. 133, 89–99.

Gilles, G.E., McArthur, S., 2010. Independent influences of sex steroids of systemic and central origin in a rat model of Parkinson's disease: a contribution to sex-specific neuroprotection by estrogens. Horm. Behav. 57, 22–34.

Inagaki, T., Gautreaux, C., Luine, V., 2010. Acute estrogen facilitates recognition memory consolidation and alters monoamine levels in memory-related brain areas. Horm. Behav. 58, 415–426.

Jenrow, K.A., Brown, S.L., Lapanowski, K., Naei, H., Kolozvary, A., Kim, J.H., 2013. Selective inhibition of microglia-mediated neuroinflammation mitigates radiation-induced cognitive impairment. Radiat. Res. 179, 549–556.

Joseph, J.A., Hunt, W.A., Rabin, B.M., Dalton, T.K., 1992. Possible "accelerated aging" induced by 56Fe heavy particle irradiation: implications for manned space flights. Radiat. Res. 130, 88–93.

Krukowski, K., Grue, K., Frias, E.S., Pietrykowski, K., Jones, T., Nelson, G., Rosi, S., 2018a. Female mice are protected from space radiation-induced maladaptive responses. Brain Behav. Immun. 74, 106–120.

Krukowski, K., Feng, X., Paladini, M.S., Chou, A., Sacramento, K., Grue, K., Riparip, L.K., Jones, T., Campbell-Beachler, M., Nelson, G., Rosi, S., 2018b. Temporary microglia-depletion after cosmic radiation modifies phagocytic activity and prevents cognitive deficits. Sci Rep. https://doi.org/10.1038/s41598-018-26039-7.

La Tessa, C., Sivertz, M., Chiang, I-H., Lowenstein, D., Rusek, A., 2016. Overview of the NASA space radiation laboratory. Life Sci. Space Res. 11, 18–23.

Li, M., Gonon, G., Buonanno, M., Autsavapromporn, N., de Toledo, S.M., Pain, D., Azzam, E.I., 2014. Health risks of space exploration: targeted and nontargeted oxidative injury by high-charge and high-energy particles. Antioxid. Redox Signal. 20, 1501–1523.

Lindner, M.D., Plone, M.A., Francis, J.M., Blane, T.J., Salmone, J.D., Emerich, D.F., 1997. Rats with partial striatal dopamine depletions exhibit robust and long-lasting behavioral deficits in a simple fixed-ratio bar pressing task. Behav. Brain Res. 86, 25–40.

Luine, V., 2015. Recognition memory tasks in neuroendocrine research. Behav. Brain Res. 285, 158–164.

McEwen, B.S., Milner, T.A., 2017. Understanding the broad influence of sex hormones and sex differences in the brain. J. Neurosci. Res. 95 (, 1–2), 24–39.

Nilson, J., 2008. Estradiol and neurodegenerative oxidative stress. Front. Neuroendocrinol. 29, 463–475.

Norbury, J.W., Slaba, T.C., 2014. Space radiation experiments - the role of neutrons and light ions. Life Sci. Space Res. 3, 90–94.

Pal, R., Tiwari, P.C., Nath, R., Pant, K.K., 2016. Role of neuroinflammation and latent transcription factors in pathogenesis of Parkinson's disease. Neurol. Res. 38, 1111–1122.

Parihar, V.K., Allen, B.D., Caressi, C., Kwok, S., Chu, E., Tran, K.K., et al., 2016. Cosmic radiation exposure and persistent cognitive dysfunction. Sci. Rep. 6. https://doi.org/10.1038/srep34774.

Pratap, U.P., Patil, A., Sharma, H.R., Hima, L., Chockalingam, R., et al., 2016. Estrogen-induced neuroprotective and anti-inflammatory effects are dependent on the brain areas of middle-aged female rats. Brain Res. Bull. 124, 238–253.

Poulose, S.M., Bielinski, D.F., Carrihill-Knoll, K., Rabin, B.M., Shukitt-Hale, B., 2011. Exposure to oxygen (^{16}O) particle irradiation causes age-like decrements in rats through increased oxidative stress, inflammation and loss of autophagy. Radiat. Res. 176, 761–769.

Raber, J., Allen, A.R., Rosi, S., Sharma, S., Dayger, C., Davis, M.J., Fike, J.T., 2013. Effects of whole body ^{56}Fe radiation on contextual freezing and arc-positive cells in the dentate gyrus. Behav. Brain Res. 246, 162–167.

Raber, J., Weber, S.J., Kronenberg, A., Turker, M.S., 2016. Sex- and dose-dependent effects of calcium ion irradiation on behavioral performance of B6D2F1 mice during contextual fear conditioning training. Life Sci. Space Res. 9, 59–61.

Rabin, B.M., Carrihill-Knoll, K., Hinchman, M., Shukitt-Hale, B., Joseph, J.A., Foster, B.C., 2009. Effects of heavy particle irradiation and diet on object recognition memory in rats. Adv. Space Res. 43, 1193–1199.

Rabin, B.M., Carrihill-Knoll, K.L., Long, L.V., Pitts, S.C., Shukitt-Hale, B., 2013. Effects of 17β-estradiol on cognitive performance of ovariectomized female rats exposed to space radiation. J. Behav. Brain Sci. 3, 67–73.

Rabin, B.M., Carrihill-Knoll, K.L., Miller, M.G., Shukitt-Hale, B., 2018. Age as a factor in the responsiveness of the organism to the disruption of cognitive performance by exposure to HZE particles differing in linear energy transfer. Life Sci. Space Res. 16, 84–92.

Rabin, B.M., Carrihill-Knoll, K.L., Shukitt-Hale, B., 2011. Operant responding following exposure to HZE particles and its relationship to particle energy and linear energy transfer. Adv. Space Res. 48, 370–377.

Rabin, B.M., Carrihill-Knoll, K.L, Shukitt-Hale, B., 2015. Comparison of the effectiveness of exposure to low LET helium particles (^{4}He) and gamma rays (^{137}Cs) on the disruption of cognitive performance. Radiat. Res. 184, 266–272.

Rabin, B.M., Joseph, J.A., Shukitt-Hale, B., 2005a. Effects of age and diet on the heavy particle-induced disruption of operant responding produced by a ground-based model for exposure to cosmic rats. Brain Res. 1036, 122–129.

Rabin, B.M., Joseph, J.A., Shukitt-Hale, B., 2005b. A longitudinal study of operant responding in rats irradiated when 2 months old. Radiat. Res. 164, 552–555.

Rabin, B.M., Polouse, S., Bielinski, D.F., Shukitt-Hale, B., 2019. Effects of head-only or whole-body exposure to very low doses of 4he (1000 mev/n) particles on neuronal function and cognitive performance. Life Sci. Space Res. 20, 85–92.

Rabin, B.M., Shukitt-Hale, B., Gomes, S., Carrihill-Knoll, K.L., 2014. Comparison of the effects of partial and whole body exposures to ^{16}O particles on cognitive performance in rats. Radiat. Res. 181, 251–257.

Rola, R., Sarkissian, V., Obenhaus, A., Nelson, G.A., Otsuka, S., Limoli, C.L., Fike, J.R, 2005. High-LET radiation induces inflammation and persistent changes in markers of hippocampal neurogenesis. Radiat. Res. 164, 556–560.

Salamone, J.D., 1994. The involvement of nucleus accumbens dopamine in appetitive and aversive motivation. Behav. Brain Res. 61, 117–133.

Salamone, J.D., Correa, M., 2002. Motivational reviews of reinforcement: implications for understanding the behavioral functions of nucleus accumbens dopamine. Behav. Brain Res. 137, 3–25.

Salamone, J.D., Correa, M., Yang, J.H., Rotolo, R., Presby, R., 2018. Dopamine, effort-based choice, and behavioral economics: basic and translational research. Front. Behav. Neurosci. 12, 52. https://doi.org/10.3389/inbeh.2018.00052.

Schimmerling, W., Cucinotta, F.A., Wilson, J.W., 2003. Radiation risk and human space exploration. Adv. Space Res. 31, 27–34.

Shukitt-Hale, B., Casadesus, G., Cantuti-Castelvetri, I., Rabin, B.M., Joseph, J.A., 2003. Cognitive deficits induced by ^{56}Fe radiation exposure. Adv. Space Res. 31, 119–126.

Shukitt-Hale, B., Casadesus, G., McEwen, J.J., Rabin, B.M., Joseph, J.A., 2000. Spatial learning and memory deficits induced by ^{56}Fe radiation exposure. Radiat. Res. 154, 28–33.

Simpkins, J.W., Dykens, J.A., 2008. Mitochondrial mechanisms of estrogen neuroprotection. Brain Res. Rev. 57, 421–430.

Stephenson, J., Nutma, E., van der Valk, P., Amor, S., 2018. Inflammation in CNS neurodegenerative diseases. Immunology 154, 204–219.

Strom, J.O., Theodorsson, E., Theodorsson, A., 2008. Order of magnitude differences between methods for maintaining physiological 17B-oestradiol concentration in ovariectomized rats. Scand. J. Clinical Lab. Invest. 68, 814–822.

Tenkorang, M.A., Snyder, B., Cunningham, R.L., 2018. Steroids 133, 21–27.

Tuscher, J.J., Fortress, A.M., Kim, J., Frick, K.M., 2015. Regulation of object recognition and object placement by ovarian sex steroid hormones. Behav. Brain Res. 285, 140–157.

Vegeto, E., Benedusi, V., Maggi, A., 2008. Estrogen anti-inflammatory activity in brain: a therapeutic opportunity for menopause and neurodegenerative diseases. Front. Neuroendocrinol. 29, 507–519.

Villasana, L., Dayger, C., Raber, J., 2013. Dose- and ApoE isoform-dependent cognitive injury after cranial 56Fe irradiation in female mice. Radiat. Res. 179, 493–500.

Villasana, L., Rosenberg, J., Raber, J., 2010. Sex-dependent effects of ^{56}Fe irradiation on contextual fear conditioning in C57BL/6J mice. Hippocampus 20, 19–23.

Walf, A.A., Rhodes, M.E., Frye, C.A., 2006. Ovarian steroids enhance object recognition in naturally cycling and ovariectomized, hormone-primed rats. Neurobiol. Learn. Mem. 86, 35–46.

Zeitlin, C., Hassler, D.M., Cucinotta, F.A., Ehresmann, B., Wimmer-Schweingruber, R.F., Brinza, D.E., et al., 2013. Measurements of energetic particle radiation in transit to Mars on the Mars science laboratory. Science 340, 1080–1084.