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Early Effects of Whole-Body ^{56}Fe Irradiation on Hippocampal Function in C57BL/6J Mice

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Abstract

Relatively little is known about early irradiation effects on hippocampal function in wild-type mice. In this study, the effects of ^{56}Fe irradiation on hippocampal function were assessed starting 2 weeks after whole-body irradiation. Compared to sham irradiation, radiation impaired novel object recognition in female and male C57BL/6J wild-type mice. There were no effects of irradiation on contextual fear conditioning or spatial memory retention in the water maze. It is possible that oxidative damage might contribute to radiation-induced cognitive changes. Therefore, hippocampal and cortical levels of 3-nitrotyrosine (3NT) and lipid peroxidation, measures of oxidative damage were assessed. There were no effects of irradiation on these measures of oxidative damage. As ^{56}Fe irradiation can increase reactive oxygen species (ROS) levels, which may contribute to the impairments in novel object recognition, the effects of the antioxidant alpha-lipoic acid (ALA) on cognition following sham irradiation and irradiation were also assessed. ALA did not prevent radiation-induced impairments in novel object recognition and impaired spatial memory retention of sham-irradiated and irradiated mice in the probe trial after the first day of hidden platform training in the water maze. Thus, the novel object recognition test is particularly sensitive to detect early cognitive effects of ^{56}Fe irradiation through a mechanism unlikely involving ROS or oxidative damage.

Introduction

High-energy irradiation exposure, including ^{56}Fe , poses a significant risk to the central nervous system. In rodents, ^{56}Fe irradiation affects hippocampus-dependent cognitive function 3 months or longer after irradiation (1–6). In contrast to these studies, few studies have examined potential short-term effects of irradiation on brain function. Manda *et al.* showed that ^{56}Fe (1.5 Gy at 500 MeV/n) increased the time male wild-type mice needed to locate the hidden platform in the water maze 30 days after irradiation, but not at earlier time

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points (7, 8). Recently, we showed early cognitive effects of ^{56}Fe irradiation (0.5 Gy at 500 MeV/n) in human apoE mice (9).

Hippocampus-dependent learning and memory is sex-dependent. Notably, a structural difference is observed in the male and female hippocampus (10). Effects of ^{56}Fe irradiation on hippocampus-dependent cognition in C57Bl6/J wild-type mice 3 months after irradiation (2) and in human apoE mice 13 months after irradiation (11) are sex-dependent, with female mice being more susceptible to radiation-induced cognitive impairments than male mice. Female mice might also be more susceptible to the early effects of ^{56}Fe radiation on hippocampus-dependent cognitive function. As distinct hippocampus-dependent cognitive tests differ dramatically in susceptibility to hippocampal lesions (12), they might also differ in terms of sensitivity to radiation-induced cognitive changes. For example, in rats spatial memory retention in the water maze probe trial 24 h after training was impaired after dorsal hippocampal lesions encompassing 30–50% total volume, yet object recognition memory 3 h after training was only impaired after hippocampal lesions encompassing 75–100% total volume (12). Therefore, other brain regions might be more important for object recognition. Studies involving acute hypoxia in mice following training support an important role for the amygdala in memory recall 4, 5 or 6 h after reoxygenation (13).

Irradiation can increase brain levels of reactive oxygen species (ROS) and oxidative damage, which can be assessed by measuring lipid peroxidation (14) or measuring 3-nitrotyrosine levels (3NT) (14). ROS can affect tissue by damaging DNA and proteins and is associated with behavioral consequences, including hippocampus-dependent cognitive performance (15). Therefore, hippocampus-dependent cognitive changes after ^{56}Fe irradiation might be associated with changes in these markers of oxidative damage.

Cognitive changes after ^{56}Fe irradiation might involve alterations in the level of reactive oxygen species (ROS), which are a major component of oxidative stress and are increased by irradiation (8). Although ROS are involved in learning and memory, a prolonged increase in ROS can lead to cell damage and cell death through oxidation of cellular components such as lipids, proteins and DNA (16). Due to its high oxygen consumption and relatively low levels of antioxidants, the brain is particularly vulnerable to ROS damage. When the production of ROS exceeds the ability of the cell to repair itself and is greater than that which the antioxidants can scavenge, oxidative stress occurs. Restoration of the balance between ROS and antioxidants may prevent oxidative damage. Therefore, supplementation of antioxidants might also ameliorate the effects of irradiation on cognitive function. The antioxidant, alpha-lipoic acid (ALA) can directly scavenge ROS and appears to restore age-related reductions in the antioxidant glutathione. ALA also participates in the recycling of vitamins C and E, and is able to chelate redox active transition metals, thereby inhibiting production of other ROS such as hydrogen peroxide and the hydroxyl radical. In addition, ALA in its reduced form, dihydrolipoic acid (DHLA), increases acetylcholine production by activation of choline acetyltransferase and appears to increase glucose uptake in insulin-resistant neurons. ALA (200 mg/kg, i.p.) administered to mice prior to whole-body ^{56}Fe irradiation (1.5 Gy), prevented radiation-induced deficits in water maze reference memory and attenuated measures of oxidative stress in the cerebellum (7). In addition, ALA

prevented impairments in memory retention in the water maze of wild-type mice after head-only irradiation (3 Gy, 600 MeV/n) and cognitively tested 3 months after irradiation (1).

In this study, we assessed the short-term effects of ^{56}Fe irradiation on cognitive performance in female and male mice starting 2–4 weeks after irradiation and whether oxidative damage and ROS might be involved in these effects.

Methods

Animals and ^{56}Fe Irradiation

Male and female C57BL/6J wild-type mice ($n = 160$) purchased from the Jackson Laboratory (Bar Harbor, ME) were shipped to Brookhaven National Laboratories (BNL) in Upton, NY. In Experiment 1, after a one-week acclimation period, the mice were either sham irradiated ($n = 8$ mice per group, each sex) or received whole-body irradiation (600 MeV/n; 0.1, 0.2 or 0.5 Gy, $n = 8$ mice per dose and sex). In Experiment 2, the mice were kept on the regular diet or put on a diet containing ALA, as described below, starting 2 weeks before sham irradiation ($n = 8$ mice per diet and sex) or whole-body irradiation (600 MeV/n; 0.1 Gy, $n = 8$ mice per diet and sex). In Experiment 3, after a one-week acclimation period, the mice were either sham irradiated ($n = 8$ mice per group and sex) or received whole-body irradiation (600 MeV/n; 0.5 Gy, $n = 8$ mice per group and sex). One week after irradiation, the mice were shipped to Oregon Health and Science University (OHSU). At BNL and OHSU, the mice were housed under a constant 12 h light:dark cycle. Food (PicoLab Rodent Diet 20, no. 5053; PMI Nutrition International, St. Louis, MO), unless indicated differently and water were provided *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee at OHSU and BNL.

Cognitive Testing

Cognitive testing began 1 week after the mice arrived at OHSU, which was 2 weeks after irradiation. Mice were housed in a single-sex caging situation and singly housed starting 3 days before the first cognitive test. In Experiment 1, mice were tested for novel object recognition (days 1–3), spatial learning and memory in the water maze (days 4–8), and contextual and cued fear conditioning (days 9–10). As sham-irradiated female mice in Experiment 1 did not show spatial memory retention in the first or second probe trial, while they did show spatial memory retention in the first probe trial in Experiment 2, the water maze data of Experiment 1 were excluded from the current study. In Experiment 2, mice were maintained on a regular chow diet (PicoLab Rodent Diet 20, no. 5053; PMI Nutrition International) or were placed on a matched diet to which ALA was added [Rodent Pico Chow 20 + 0.165% ALA, Animal Specialties Inc., Woodburn, OR (a division of PMI)] starting 2 week before irradiation. The concentration of ALA used in the current study was based on previous studies showing neuroprotective effects when this concentration was administered through the diet for up to 3 (1) or 6 months (17). Mice remained on their selected diets throughout behavioral testing. In Experiment 3, mice were tested in the water maze (days 1–5) and for contextual fear conditioning (day 6), but different intervals between training and testing were used compared to those used in Experiment 1. While in Experiment 1 the interval between the last hidden platform training and first probe trial was

1 h, it was 24 h in Experiment 3. Conversely, while the interval between training and contextual fear conditioning was 24 h in Experiment 1, it was 1 h in Experiment 3. As we can test up to 25 mice in a single water maze experiment, the 64 mice of Experiment 1 were tested in three cohorts, with the sexes and the four treatments equally represented in each cohort. Subsequently, the 32 mice of Experiment 3 were tested in 2 cohorts with the sexes and two treatments were equally represented in each cohort.

Novel Object Recognition

In Experiments 1 and 2, the novel object recognition test was performed as described (16). On day 1, the mice were habituated to an open field (16" × 16" Kinder Scientific, Poway, CA) for 3 times for 10 min each. On day 2, the mice were placed in the open field containing two identical objects and were allowed to freely explore for 15 min. On day 3, the mice were again placed in the open field, but one familiar object was replaced with a novel object. The mice were allowed to explore for 15 min. Movement and time spent exploring each object was recorded and analyzed using EthoVision XT video tracking system (Noldus Information Technology, Sterling, VA). The percentage time exploring the novel object on day 3 was used to assess novel object recognition. For each group, the preference for the novel object versus the familiar object was assessed. The open field arena and objects were cleaned with 5% acetic acid between mice groups and trials.

Water Maze

Hippocampus-dependent spatial learning and memory was assessed in the water maze. A circular pool (diameter: 140 cm) was filled with water made opaque with nontoxic chalk (24°C) and mice were trained to locate a submerged platform. To determine whether irradiation affected the ability to swim or to learn the water maze task, mice were first trained to locate a clearly marked platform (visible platform, days 1 and 2). In Experiments 1 and 2, the mice were subsequently trained to locate the platform when it was hidden beneath the surface of opaque water (days 3–5). Training during the hidden platform sessions (acquisition) required the mice to learn the location of the hidden platform based on extra-maze cues. For both visible and hidden sessions, there were two daily sessions, morning and afternoon, which were 2 h apart. Each session consisted of three trials (with 5 min intertrial intervals). A trial ended when the mice located the platform. Mice that failed to locate the platform within 60 s were led to the platform by placing a finger in front of their swim path. The mice were taken out of the pool after they were physically on the platform for a minimum of 3 s. During visible platform training, the platform was moved to a different quadrant of the pool for each session. For the hidden platform training, the platform location was kept constant. The mice were placed into the water facing the edge of the pool in one of nine randomized locations, and the start location was changed for each trial. The swimming patterns of the mice were recorded with Noldus EthoVision XT video tracking software (Noldus Information Technology) set at six samples/s. The time to locate the platform (latency) was used as a measure of performance for the visible and hidden sessions. As swim speeds can influence the time it takes to reach the platform, they were also analyzed to assess whether there were genotype or treatment differences in this measure.

To measure spatial memory retention in Experiments 1 and 2, probe trials (platform removed) were conducted 1 h after the last hidden trial of each mouse on each day of hidden platform training (i.e., a total of three probe trials). In Experiment 3, the mice were trained to locate a hidden platform, as described above, followed by a probe trial 24 h later. The time spent in the target quadrant, the quadrant where the platform was previously located during hidden platform training, was compared to the time spent in the three nontarget quadrants. In addition, the time spent in the target quadrant only was compared across the three probe trials as well as with all groups of mice. For the probe trials, mice were placed into the water in the quadrant opposite the target quadrant.

Fear Conditioning

Hippocampal function was also assessed using the contextual fear-conditioning task (days 9–10). In this task, mice learned to associate the environmental context (fear-conditioning chamber) with a mild foot shock (unconditioned stimulus, US). Contextual fear conditioning is hippocampus- and amygdala-dependent. The mild foot shock was also paired with a tone (conditioned stimulus, CS) to allow assessment of cued fear conditioning, which is amygdala-dependent but not hippocampus-dependent. When the mice were re-exposed to the context or the tone, conditioned fear resulted in freezing behavior. Mice displayed this conditioned fear by ceasing all movement except for respiration (i.e., freezing). On day 1 of fear conditioning (day 9 of cognitive testing), each mouse was placed in a fear-conditioning chamber (Med Associates, St. Albans, VT) and allowed to explore for 2 min before delivery of a 30 s tone (80 dB), which was immediately followed by a 2 s foot shock (0.76 mA). The 28 s tone and 2 s foot shock were repeated 2 min later for a total of 2 tones and 2 foot shocks. On day 1 of fear conditioning, motion index was measured prior to the first tone to assess potential baseline differences and during the foot shock to assess potential differences in perception of it. In Experiment 1, on day 2 of fear conditioning (day 10 of behavioral testing), each mouse was first placed in the fear-conditioning chamber containing the exact same context, but there was no administration of a tone or foot shock. Freezing was analyzed for 3 min. One hour later, the mice were placed in a new context (containing a different odor, cleaning solution, floor texture, walls and shape) where they were allowed to explore for 3 min before being re-exposed to the fear-conditioning tone and freezing was assessed for an additional 3 min. Cued fear conditioning was assessed by measuring the amount of freezing prior to and following the tone that was originally paired with the foot shock. In Experiment 3, mice were placed in the fear-conditioning chamber that contained the same context as during training 1 h after the initial training. The fear conditioning chambers were cleaned with 5% acetic acid between mice.

Western Blot Analysis

Following cognitive testing in Experiment 1, the mice were euthanized by cervical dislocation and their brains were removed. The hippocampus and cortex were dissected and immediately frozen in liquid nitrogen for lipid peroxidation (see below) or Western blot analysis. For Western blot analysis, the cortex and hippocampus were homogenized separately in 1 ml or 300 μ l of RIPA buffer (Pierce Pharmaceuticals, Rockford, IL) containing 10% Halt Protease Inhibitor Cocktail (Pierce Pharmaceuticals). Homogenized tissue was spun at 12,000g for 15 min, and protein concentrations were determined in the

supernatant using a Nanodrop Spectrometer (NanoDrop ND-1000). The samples were stored at -80°C until use.

Proteins were denatured by boiling for 5 min at 99°C in a solution of Laemmli buffer containing 5% 2-mercaptoethanol (Bio-Rad, Hercules, CA). For each sample, 40 μg of protein was loaded in a lane of pre-prepared gels (Criterion Bio-Rad Ready Gels, 4–15% Tris-HCl, 18-well). For each gel, one lane was loaded with Precision Plus Protein WesternC Standard (Bio-Rad). The gels were placed in an electrophoresis apparatus and were run with a Bio-Rad Power Pac for 60 min at 175 V. Proteins were transferred to PVDF membranes for 30 min at 100 V.

Once proteins were transferred to the membranes, the membranes were placed in 3% bovine serum albumin (BSA) Tris-buffered saline containing 5% Tween (TBST) blocking buffer for 1 h. Membranes were washed in TBST buffer (4×5 min) and were incubated in 3% BSA TBST with one of the following primary antibodies for 12 h at 4°C : antibodies against 3-nitrotyrosine (raised in mouse, 1 $\mu\text{g}/\text{ml}$; Millipore, Billerica, MA) or β -actin antibody (raised in mouse, 0.5 $\mu\text{g}/\text{ml}$; Santa Cruz Biotechnology, Santa Cruz, CA). There were no effects of irradiation on β -actin levels. Membranes were washed in the TBST buffer (4×5 min) and were incubated in secondary antibody (donkey anti-mouse-HRP, 1 $\mu\text{g}/\text{ml}$; Santa Cruz) in addition to a secondary antibody for the protein standards (1:10000; Precision Protein StrepTactin-HRP, Bio-Rad) in the 3% BSA TBST buffer for 1 h. Membranes were incubated in SuperSignal West Pico Solution (Bio-Rad) for 5 min. Pixel densities of specific bands of 3NT and of β -actin for each sample were imaged and quantified using densitometry with FluorChem Q Software (FluorChem Q, Alpha Innotech, San Leandro, CA). Background levels were automatically determined by the software using upper- and lower-edge interpolation. The 3NT and β -actin bands were measured for each sample. Antibodies were stripped from the membranes using Restore Plus Stripping Buffer (Pierce Protein Biology Products) for 5 min at room temperature and re-blocked in 3% BSA TBST blocking buffer for 1 h. β -actin was used as a loading control for each membrane. Data were analyzed as a ratio between the 3NT and β -actin bands compared to sham-irradiated levels for a specific blot. As indicated above, the mice were tested in three different cohorts. As there was a significant effect of cohort on protein levels, but not of radiation or sex, the data were analyzed as a comparison to the sham-treated animals in the same cohort.

Lipid Peroxidation

The extent of lipid peroxidation was estimated as the concentration of two common polyunsaturated fatty acid peroxides, malondialdehyde (MDA) and 4-hydroxyalkenals (HAE), using a microplate assay (Oxford Biochem). Aliquots of hippocampal and cortical homogenates (25 μl) were incubated with N-methyl-2-phenylindole (80 μl) and methanesulfonic acid (20 μl) for 1 h at 45°C , and absorbance of the samples was read at 586 nm using a spectrophotometer.

Statistical Analysis

In the object recognition test, a two-way ANOVA was used to assess group differences in exploratory times, with irradiation and sex as between subject factors. Student's *t* test was

used to analyze preference for the novel object. For the water maze, learning curves were analyzed using two-way repeated measures ANOVA. For the analysis of spatial memory retention in the probe trials, one-way ANOVA was used to assess the effect of quadrant in each group, followed-up by post hoc tests when appropriate. To assess contextual and cued fear responses in the conditioned fear tasks, two-way ANOVA was used with irradiation and sex as between subject factors. Repeated measures were used to assess pre- and post-tone cued freezing. In addition, for sham-irradiated mice and mice irradiated at a dose of 0.5 Gy, contextual freezing 1 and 24 h after training were compared. For the Western blot analysis, pixel intensities for each marker and brain area were analyzed using two-way ANOVA and Bonferroni's post hoc tests. Cognitive tests and protein levels were also assessed with an ANCOVA, using the cohort as a covariate. All statistical tests were conducted using SPSS (SPSS Inc., Chicago, IL) and were considered significant at $P < 0.05$.

Results

Overall Health

Irradiated mice did not show any signs of distress or physical impairment throughout testing. Furthermore, no motor deficits were observed in the irradiated mice during any of the cognitive tests.

Novel Object Recognition

All groups spent a comparable amount of time exploring the two identical objects (day 2, not shown). On day 3, sham-irradiated male and female mice spent significantly more time exploring the novel object compared to the familiar object (Fig. 1A and B). In contrast, no group of irradiated male or female mice showed a preference for the novel object (Fig. 1A and B). This radiation effect was not due to potential group differences in the overall time spent exploring the objects (Fig. 1A). As there were no sex differences, we also analyzed the data collapsed by sex (Fig. 1C and D). These results show that the effects of ^{56}Fe irradiation on novel object recognition were independent of dose or sex. A diet containing ALA (Experiment 2) did not prevent this radiation-induced cognitive impairment (Fig. 2). In addition, the ALA-containing diet impaired novel object recognition in sham-irradiated mice (Fig. 2).

Water Maze

In all experiments, there were no effects of sex or irradiation on swim speeds during the visible platform sessions. Therefore, time to locate the platform (latency) was used as a performance measure. There was no effect of sex or irradiation on the ability to locate the visible or hidden platform sites (data not shown). When spatial memory retention was assessed in Experiment 1, sham-irradiated male mice showed spatial memory retention and spent more time in the target quadrant where previously the platform was hidden than in any other quadrant in the first probe trial. However, it took sham-irradiated female mice until the third probe trial to show spatial memory retention (data not shown). As that is not typically seen, we repeated the water maze test in the presence or absence of the ALA-containing diet (Experiment 2). Sham-irradiated female and male mice showed spatial memory retention in the probe trial following the first day of hidden platform training (Fig. 3A). In contrast,

sham-irradiated and irradiated male and female mice on an ALA-containing diet did not show spatial memory retention in the first probe trial (Fig. 3A). As there were no sex differences, we also analyzed the data collapsed by sex (Fig. 3B). After an additional day of training, in the second probe trial all groups showed spatial memory retention and spent more time searching in the target quadrant than in any other quadrant.

Fear Conditioning

There was no effect of irradiation on baseline motion index, motion index during foot shock, or on contextual fear conditioning (Table 1). In cued fear conditioning, post-tone freezing was significantly greater than pre-tone freezing ($P < 0.0001$), but there was no effect of irradiation on pre- or post-tone freezing (data not shown).

Experiment 3: Variable Memory Retention

Radiation effects were detected in novel object recognition 24 h after training, but not in water maze performance 1 h after training or in contextual fear conditioning 24 h after training. In these distinct hippocampus-dependent tests, different time intervals between training and testing modulate test difficulty. Therefore, in Experiment 3 we assessed effects of irradiation in sham-irradiated and irradiated mice (600 MeV, 0.5 Gy) in a probe trial 24 h after the first day of hidden platform water maze training and in contextual fear conditioning 1 h after training. In the water maze, none of the groups showed spatial memory retention 24 h after hidden platform training (not shown). In contextual fear conditioning, the male mice showed higher levels of freezing at 1 h than at 24 h after training ($P < 0.05$) (Fig. 4). This was not seen in female mice. In neither female nor male mice was there an effect of irradiation on contextual fear conditioning 1 h after training. These data indicate that the inability of contextual fear conditioning to detect short-term effects of ^{56}Fe irradiation is unlikely due to the time interval between training and testing.

Oxidative Damage

There was no effect of irradiation on lipid peroxidation (Table 2) or 3NT levels in the hippocampus or cortex (not shown).

Discussion

In this study, we show the short-term effects of ^{56}Fe irradiation on only novel object recognition, but not on spatial memory retention in the water maze or in contextual fear conditioning. The early effects of relatively low doses of whole-body ^{56}Fe irradiation on novel object recognition were not dose- or sex-dependent. In contrast to novel object recognition, there were no significant short-term effects of ^{56}Fe irradiation on contextual fear conditioning 1 or 24 h after training, indicating that interval differences between training and testing in this test unlikely contributed to the lack of sensitivity to detect the effects of ^{56}Fe irradiation.

Although we did not see effects of radiation on water maze learning and memory in the current study, effects of whole-body ^{56}Fe irradiation (1.5 Gy) on spatial learning in the water maze 30 days after irradiation were observed in male mice (8). Additionally, the

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detrimental effects of higher doses of brain only ^{56}Fe irradiation on hippocampus-dependent contextual fear conditioning 3 months after irradiation were reported (1, 2). Differences in the time intervals between irradiation and cognitive testing, in the type (whole body versus brain only), doses of exposure and degree of hippocampal dependency of the distinct cognitive test designs might have contributed to these divergent findings. Object recognition was less sensitive to hippocampal lesions than spatial memory retention in the water maze (12) and hypoxia affected more object recognition and amygdala-dependent cued fear conditioning than in the spatially cued Y-maze performance (13). In addition, in the latter study hypoxia/reoxygenation more profoundly activated caspase 1 in the amygdala than in the hippocampus (13). These data indicate that, while the cognitive tests used in this study are all hippocampus-dependent to some degree, the fact that the novel object recognition test seems particularly sensitive to detect early cognitive effects of ^{56}Fe irradiation suggests that the amygdala might be an important target for early radiation effects.

The mechanism contributing to these early cognitive effects of irradiation are unknown. One hypothesis is that these effects are at least in part due to an increase in ROS (19) and can be attenuated by pretreatment with α -lipoic acid, which is a potent anti-oxidant (8). Beneficial effects of ALA were also seen in cognitively-impaired 12-month-old SAMP8 mice (20) and other cognitive impairments (21). However, in the current study a diet containing ALA did not prevent the radiation-induced impairments in novel object recognition. In addition, there were no effects of irradiation on hippocampal or cortical levels of 3-nitrotyrosine (3NT) or lipid peroxidation. These data indicate that the early impairments in novel object recognition unlikely involve ROS or oxidative damage. As these measures of oxidative damage were assessed in the whole hippocampus and cortex, we cannot exclude that there might be oxidative damage in distinct subregions of either of these brain region. Alternatively, as indicated earlier, there might be important changes in oxidative damage in the amygdala. The ALA-containing diet impaired spatial memory retention of sham-irradiated and irradiated mice in the first water maze probe trial. This might be related to the dual role of ROS in the brain, having both positive and negative effects on cognition (22).

Another mechanism contributing to the cognitive effects of irradiation might involve the acetylcholine system. The acetylcholine system, particularly choline acetyl transferase (ChAT) and M1 receptors, is implicated in learning and memory (23) and in age-related cognitive decline (24). However, we did not see effects of irradiation on levels of ChAT or M1 receptors (not shown).

In summary, whole-body ^{56}Fe irradiation has short-term effects on novel object recognition, but not on spatial memory retention in the water maze or on contextual fear conditioning. These early CNS effects on novel object recognition might not be limited to ^{56}Fe . Gamma irradiation reduced activity in the home cage in cortical levels of activity-regulated cytoskeleton-associated protein (Arc), and increased hippocampal levels of tumor necrosis factor (TNF)- α and hippocampal and cortical levels of Interleukin 1 Receptor Antagonist (IL1RA) within 24 h after irradiation (25). Future studies are warranted to determine the mechanisms that contribute to these early radiation effects on novel object recognition.

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Figure 1.

Figure 2.

Figure 3.

Figure 4.

Table 1

	Males	Females
Sham irradiation	5.5 ± 1.4	12.3 ± 4.6
0.1 Gy	6.9 ± 2.4	8.3 ± 2.7
0.2 Gy	11.9 ± 3.2	17.5 ± 6.8
0.5 Gy	7.4 ± 3.0	11.7 ± 6.1

¹
n = 8 mice/dose/sex.

Table 2

Hippocampus					Cortex				
Sham (n = 8)	0.1 Gy (n = 9)	0.2 Gy (n = 11)	0.5 Gy (n = 9)	Sham (n = 4)	0.1 Gy (n = 6)	0.2 Gy (n = 7)	0.5 Gy (n = 5)		
16.25 ± 2.17	14.91 ± 2.06	13.40 ± 0.968	14.44 ± 1.92	11.60 ± 1.83	14.82 ± 2.68	14.03 ± 1.90	12.18 ± 0.718		

¹ Lipid peroxidation is expressed as mM/mg protein. Mean ± SEM values are shown.