Behavioural Brain Research 319 (2017) 181–187

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Enhanced cued fear memory following post-training whole body irradiation of 3-month-old mice

Reid H.J. Olsen[a], Sydney J. Weber[a], Tunde Akinyeke[a], Jacob Raber[a,b,*]

[a] Department of Behavioral Neuroscience, Oregon Health & Science University, Portland, OR 97239, USA
[b] Departments of Neurology and Radiation Medicine, Division of Neuroscience, ONPRC, Oregon Health & Science University, Portland, OR 97239, USA

HIGHLIGHTS

- Post-training irradiation increases freezing levels.
- Post-training irradiation reduces motion in extinction trials.
- Post-training irradiation reduces activity levels in the zero maze.
- Post-training irradiation transiently reduces body weights.

ARTICLE INFO

Article history:
Received 18 May 2016
Received in revised form
12 November 2016
Accepted 15 November 2016
Available online 16 November 2016

Keywords:
Cued fear
Body weight
Zero maze
Irradiation
Post-training

ABSTRACT

Typically, in studies designed to assess effects of irradiation on cognitive performance the animals are trained and tested for cognitive function following irradiation. Little is known about post-training effects of irradiation on cognitive performance. In the current study, 3-month-old male mice were irradiated with X-rays 24 h following training in a fear conditioning paradigm and cognitively tested starting two weeks later. Average motion during the extinction trials, measures of anxiety in the elevated zero maze, and body weight changes over the course of the study were assessed as well. Exposure to whole body irradiation 24 h following training in a fear conditioning resulted in greater freezing levels 2 weeks after training. In addition, motion during both contextual and cued extinction trials was lower in irradiated than sham-irradiated mice. In mice trained for cued fear conditioning, activity levels in the elevated zero maze 12 days after sham-irradiation or irradiation were also lower in irradiated than sham-irradiated mice. Finally, the trajectory of body weight changes was affected by irradiation, with lower body weights in irradiated than sham-irradiated mice, with the most profound effect 7 days after training. These effects were associated with reduced c-Myc protein levels in the amygdala of the irradiated mice. These data indicate that whole body X ray irradiation of mice at 3 months of age causes persistent alterations in the fear response and activity levels in a novel environment, while the effects on body weight seem more transient.

1. Introduction

Consequences of radiation exposure are perhaps most typically thought of in terms of cancer risk [1]. However, a growing body of evidence supports a pernicious influence of irradiation on neurobiological systems with profound behavioral and cognitive consequences [2]. As environmental whole body irradiation exposure might occur as part of a natural disaster, an accident at a nuclear facility, a military mission, or radiological terrorism, it is important to establish a body of knowledge from which to predict and anticipate important consequences and outcomes [3]. This is also important absent catastrophe when considering the incidence of radiological exposure of cancer patients. Radiological effects on behavioral and cognitive performance appear to be long-lasting. For example, following total body irradiation (8 Gy) of 8-week-old mice, activity in the open field was reduced at 3 h following irradiation, partially recovered within 24 h, followed by a later decrease 4 days after irradiation with a slow recovery starting at 17 days

* Corresponding author at: Departments of Behavioral Neuroscience and Neurology, L470, Oregon Health and Science University, 3181 S.W. Sam Jackson Park Road, Portland, OR 97201, USA.
E-mail address: raberj@ohsu.edu (J. Raber).

http://dx.doi.org/10.1016/j.bbr.2016.11.031
0166-4328/

[4]. Locomotion and body temperature, as assessed by telemetry, decreased rapidly following irradiation reaching a minimum between 13 and 17 days following irradiation and full recovery starting at 27 days [4]. Radiation effects on learning and memory have been reported 30 days or more after whole body irradiation of 3- or 8-week-old male mice (4 Gy) [5,6]. Diffusion tension imaging (DTI) and *in vivo* proton nuclear magnetic resonance spectroscopy (MRS) performed 2 or more days following irradiation showed that the hippocampus and frontal cortex are especially sensitive to these early radiation effects [7,8] thus implicating potentially retrograde mnemonic processes as being particularly sensitive. These effects might be age-dependent, as activity in the open field was not altered in 6-month-old mice 24 h following receiving whole body irradiation at 2, 5, or 8 Gy [9].

In all these studies, the animals were trained and tested for cognitive function following irradiation. Thus, neurobehavioral processes reliant on previous experiences, conditioning, and memory were not assessed in isolation. Post-training irradiation is translational relevant. For example, soldiers, physicians and nurses, astronauts, emergency responders, and cancer patients can be exposed to irradiation following learning experiences and formation of memories. Therefore, it would be important to assess whether memory alterations due to radiation exposure could be retrograde. Previously, we showed enhanced contextual and cued fear memory prior to and during extinction following post-training irradiation of one-month-old male mice [10]. Due to the established age-sensitivity of radiation effects in mice with increased sensitivity in younger mice, the prior observed enhanced contextual and cued fear memory prior to and during extinction following post-training irradiation following post-training irradiation of one-month-old male mice could be age-dependent. Thus, more adult mice might be conferred relative protection against effects of irradiation on contextual and cued fear memories. This could have significant consequences when anticipating potential human corrolaries, with possible focus on specific sequelae and subsequent intervention in younger or older populations. Therefore, in the current study 3-month-old male mice were irradiated with X-rays 24 h following training in a fear conditioning paradigm and tested two weeks later for retention and extinction of hippocampus-dependent contextual fear memory or hippocampus-independent cued fear memory. Average motion during the extinction trials was analyzed as well, to determine whether these measures of the fear (escape) response were affected by post-training irradiation. As measures of anxiety might affect retention and extinction of fear, performance in the elevated zero maze was assessed as well. Finally, effects of irradiation on body weight changes over the course of the study were assessed.

Irradiation affects the levels of the v-myc avian myelocytomatotsis viral oncogene homolog (c-Myc) protein [11]. Recently, we reported that c-Myc plays an important role in the response of the brain in substance abuse [12]. Therefore, in this study we also assessed whether the behavioral changes were associated with alterations of c-Myc protein levels in pertinent brain regions.

2. Material and methods

2.1. Animals

Three-month-old male C57Bl6/J wild-type mice ($n = 70$) purchased from the Jackson Laboratory (Bar Harbor, ME) were used for the experiments of this study, as described below in detail. The mice were housed under a constant 12 h light: 12 h dark cycle. Food (PicoLab Rodent Diet 20, no. 5053; PMI Nutrition International, St. Louis, MO) and water were provided *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee of Oregon Health & Science University (OHSU), Portland, Oregon.

2.2. Experiment 1: contextual fear conditioning

Forty mice were cognitively trained in a contextual fear conditioning paradigm, involving a ten-shock paradigm, consisting of 2-s 0.35 mA shocks, separated by 60-s inter-shock-intervals (ISI), with the first shock at 60 s from the beginning of the trial. The total length of the training session was 10 min. Twenty-four hours after training, all mice were brought to a room within the animal facility containing an X-ray irradiator (Rad Source RS2000 Biological Research Irradiator, Suwanee, GA). Half of the mice (randomly sorted into two groups until any differences in baseline measurements were non-significant) were placed in a new mouse cage fitting in the irradiator and received whole body irradiation at a dose of 4 Gy (dose rate: 1.25 Gy/min). This dose was selected as that was the dose we used previously to assess post-training irradiation effects on cognitive performance in one-month-old mice [10]. This dose could be relevant in the context of radiation therapy in cancer patients, nuclear contamination from power plants, military conflicts, and bioterrorism. The other half of the mice was placed in a new cage and received a sham-irradiation procedure by being placed into a new cage for the same duration of time. Fourteen days after training (or thirteen days after irradiation or sham-irradiation), the mice were tested for recall and extinction of conditioned fear, over a period of six days. The mice remained in the testing environment for an additional eight minutes to maintain the same 10-min trial length in all trials. For assessment of contextual fear memory, the same environment was used as during training. For assessment of cued fear memory, a different environment was used compared to the one used during training. On day ten, recall of post-reinstatement hippocampus-dependent contextual fear memory was assessed by exposure to the training context. Mice were weighed the day after training (before irradiation), and every three days thereafter.

2.3. Experiment 2: cued fear conditioning

In a separate experiment, to evaluate the contribution of non-hippocampus-dependent memory processes, thirty male mice were cognitively trained using a cued fear conditioning paradigm consisting of ten shocks. A 60-s habituation period was followed by 30-s tones (2800 Hz, 80 dB), co-terminating with 2-s 0.35 mA shocks, separated by 60-s inter-shock-intervals (ISI), and with a final 2-min post-shock acquisition period. Twenty-four hours after training, the mice were irradiated with 4 Gy or sham-irradiated as described in Experiment 1. Two weeks (14 days) after training (13 days after irradiation), the mice were tested for recall and extinction of cued fear over eight days. Cued extinction trials consisted of the mouse being placed into an environment distinct from the one used during training (rounded walls, novel floor texture, cleaning with a 10% isopropanol solution). A 60-s baseline/habituation period was followed by five 60-s tone-presentations separated by 60-s inter-stimulus-intervals. Mice were weighed the day after training, and every three days thereafter.

2.4. Elevated zero maze

To determine whether potential differences in measures of anxiety might contribute to altered performance in fear conditioning tests, a subgroup of 20 mice from the cued fear experiment was tested for anxiety-like phenotype in the elevated zero maze. Because the potential anxiety phenotype in question required a temporal proximity to the fear conditioning extinction testing, mice