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Post-training gamma irradiation-enhanced contextual fear memory associated with reduced neuronal activation of the infralimbic cortex

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HIGHLIGHTS

- Post-training irradiation enhances contextual fear memory.
- This is associated with reduced neuronal activation in the infralimbic cortex.
- This involves reduced GABA-ergic neurotransmission in the infralimbic cortex.

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ABSTRACT

The brain might be exposed to irradiation under a variety of situations, including clinical treatments, nuclear accidents, dirty bomb scenarios, and military and space missions. Correctly recalling tasks learned prior to irradiation is important but little is known about post-learning effects of irradiation. It is not clear whether exposure to X-ray irradiation during memory consolidation, a few hours following training, is associated with altered contextual fear conditioning 24 h after irradiation and which brain region(s) might be involved in these effects. Brain immunoreactivity patterns of the immediately early gene c-Fos, a marker of cellular activity was used to determine which brain areas might be altered in post-training irradiation memory retention tasks. In this study, we show that post-training gamma irradiation exposure (1 Gy) enhanced contextual fear memory 24 h later and is associated with reduced cellular activation in the infralimbic cortex. Reduced GABA-ergic neurotransmission in parvalbumin-positive cells in the infralimbic cortex might play a role in this post-training radiation-enhanced contextual fear memory.

1. Introduction

The brain might be exposed to irradiation under a variety of situations, including clinical treatments, nuclear accidents, dirty bomb scenarios, and military and space missions. Previous studies have investigated short- and long-term effects of pre-training/learning irradiation on learning and memory involving the hippocampus and cortex [1–11] and behaviors mediated by the dopamine reward system [12–15]. Compared to studies assessing cognitive function three months or longer following irradiation [12,14,16–18], fewer studies have examined earlier 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 following irradiation but not at earlier time points [19,20]. Recently, we showed early cognitive effects two weeks following ^{56}Fe irradiation in wild-type (0.1 Gy at 500 MeV/n) [21] and human apoE mice (0.5 Gy at 500 MeV/n) [22].

In contrast to the studies described above, little is known about post-learning effects of irradiation. Correctly recalling tasks learned prior to irradiation is important and pertinent to assess. For example it is not clear whether exposure to X-ray irradiation a few hours following training, during memory consolidation, is associated with altered contextual fear conditioning 24 h after irradiation.

In most studies, there is a bias to study a particular brain region that is pertinent to a specific behavioral or cognitive change seen following radiation exposure. Brain immunoreactivity patterns of the immediately early gene c-Fos, a marker of cellular activity [23–28], can be used to determine which brain areas might be altered in post-training irradiation memory retention tasks. In addition to neurons, glia also expresses c-Fos in brain [24,29–34].

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Cellular activation of c-Fos might be especially important following glutamate activation. C-Fos activation was reported in fibroblasts transfected with the glutamate receptor subunit GluR1 [35] and in glia cells involving activation of the metabotropic glutamate receptor 5 (mGlu5) [36].

GABA-ergic neurons are critical modulators of excitatory neurons in brain and reduced GABA-ergic function can have profound detrimental effects. Dis-inhibition involving reduced GABA-ergic neurotransmission and reduced levels of parvalbumin, a calcium binding albumin protein expressed in fast-spiking GABA-ergic inhibitory inter-neurons [37], is reported following *in utero* irradiation [38–40]. Contextual fear conditioning depends on enhanced synapses of hippocampal mossy fibers onto parvalbumin-positive neurons, resulting in increased feedforward inhibition connectivity and restriction of the number of c-Fos positive post-synaptic neurons at memory retrieval [41] and increases the percentage of neurons with higher parvalbumin reactivity [42]. The increase in feedforward inhibition connectivity involved a majority of the presynaptic terminals, restricted the numbers of c-Fos-expressing postsynaptic neurons at memory retrieval, and correlated temporally with the quality of the memory [42]. We hypothesized that alterations in the number of c-Fos positive cells and parvalbumin-c-Fos positive cells might be involved in the post-training effects of irradiation on contextual fear memory.

In this study, we assessed whether post-training gamma irradiation exposure will affect subsequent contextual fear memory. In addition, immunohistochemistry was used to determine the brain regions involved in the effects of post-training irradiation and whether altered activation of GABA-ergic cells might be involved in these effects.

2. Material and methods

2.1. Animals

Five-week-old male C57Bl6/J wild-type mice ($n = 52$) purchased from the Jackson Laboratory (Bar Harbor, ME) were used for this study 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 Institutional Animal Care and Use Committee at the Oregon Health & Science University (OHSU, Portland, Oregon).

2.2. Contextual fear conditioning

Sixteen mice were cognitively trained in a contextual fear conditioning paradigm, involving a five-shock paradigm, consisting of 2-s 0.35 mA shocks, separated by 2-min inter-shock-intervals (ISI), with the first shock at 2 min from the beginning of the trial. Sixteen mice were cognitively trained in an object recognition test (see below). The total length of the training session was 10 min. Two 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 ($n = 8$ mice) were placed in a new mouse cage fitting in the irradiator and received whole body irradiation at a dose of 1 Gy (dose rate: 1.25 Gy/min). This dose and exposure time could be relevant to nuclear accidents, dirty bomb scenarios, and military missions. This is a relative low dose and not expected to cause significant cell death but has been shown to induce DNA damage that is repaired within 24 h [43]. The other half of the mice ($n = 8$ mice) were placed in a new mouse cage and received a sham-irradiation procedure by being placed into the new cage for the same duration of time. Mice were randomly assigned to experimental group (irradiated or sham-irradiated). After fear-conditioning training, and prior to irradiation, mice were randomly sorted until all initial values (body-weight, baseline-freezing, freezing levels after acquisition, etc.,) were not significantly different between groups. The next day, or 24 h after training, the mice were tested for recall of conditioned fear during a 6 min trial. All freezing data were analyzed using Med Associates software (Georgia and St. Albans, Vermont), as previously described [44]. The software analyzes freezing based on a proprietary algorithm scoring with freezing defined as no movement except respiration.

2.3. Object exploration test

To compare to the training and testing received for contextual fear conditioning, sixteen mice were cognitively trained and tested for novel object recognition test performed as described [45] but without habituation of the mice three days prior to the training day. In an independent experiment, twenty mice were cognitively trained and tested for novel object recognition following habituation of the mice three days prior to the training day. Mice were placed in an open field (16 × 16 in., Kinder Scientific, Poway, CA) containing two identical objects and they were allowed to freely explore for 15 min. The next day, mice were placed again in the open field, but one familiar object was replaced with a novel object. 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 open field arena and objects were cleaned with 5% acetic acid between mice and trials.

2.4. C-Fos immunohistochemistry

Two hours following testing for contextual fear conditioning or novel object recognition, thirty-two mice (sixteen mice per cognitive test) were intracardially perfused with 20 ml phosphate-buffered saline (PBS) followed by 40 ml 4% paraformaldehyde. Brains were removed, stored overnight in 4% paraformaldehyde, and then transferred to 30% sucrose. The 2-h time point was chosen based on previous studies demonstrating extensive induction of c-Fos in the mouse brain following an environmental exposure at that time point [46,47]. Fixed brains were sectioned coronally into three-series of free-floating sections at 40 μm using a cryostat (Microm HM505E, MICROM international GmbH, Walldorf, Germany). One series of sections, containing a 1/3 representation of the brain with sections 120 μm apart was processed for immunohistochemical detection of c-Fos. A second series of sections was processed for c-Fos and parvalbumin double labeling (see below). For c-Fos alone immunohistochemistry, sections were rinsed in phosphate buffered saline (PBS), incubated in 1% hydrogen peroxide and 0.3% Triton-X (TX) in PBS (PBS-TX, Sigma T-9284) for 10 min, again rinsed in PBS, then incubated in 10% normal goat serum (NGS) in PBS-TX for 1 h. After rinsing in PBS, sections were incubated in primary antisera (c-Fos rabbit polyclonal: 1:5000, Santa Cruz Biotechnology, sc52, Billerica, MA, USA) in 4% Normal Goat Serum (NGS) and PBS-TX overnight at room temperature. Sections were rinsed in PBS and incubated for 1 h in biotinylated goat-anti rabbit antibody in PBS-TX (1:500, Vector Laboratories, Burlingame, CA) followed by rinses in PBS and a 1 h incubation in avidin–biotin peroxidase complex (ABC Elite kit PK-6100 standard, Vector Laboratories). Following rinses in Tris buffered saline (TBS), sections were developed for visualization of c-Fos positive cells in a hydrogen peroxide/diaminobenzidine/TBS solution for 10 min, after which sections were rinsed in PBS and immediately mounted on slides. The following day, sections were dehydrated in ethanol,