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Investigation of the Effects of Head Irradiation with Gamma Rays and Protons on Startle and Pre-Pulse Inhibition Behavior in Mice

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With the increased international emphasis on manned space exploration, there is a growing need to understand the impact of the spaceflight environment on health and behavior. One particularly important aspect of this environment is low-dose radiation. In the present studies, we first characterized the γ- and proton-irradiation dose effect on acoustic startle and pre-pulse inhibition behaviors in mice exposed to 0–5 Gy brain-localized irradiation, and assessed these effects 2 days later. Subsequently, we used 2 Gy to assess the time course of γ- and proton-radiation effects on startle reactivity 0–8 days after exposure. Exposures targeted the brain to minimize the impact of peripheral inflammation-induced sickness behavior. The effects of radiation on startle were subtle and acute. Radiation reduced the startle response at 2 and 5 Gy. Following a 2-Gy exposure, the response reached a minimum at the 2-day point. Proton and γ-ray exposures did not differ in their impact on startle. We found there were no effects of radiation on pre-pulse inhibition of the startle response.

INTRODUCTION

In the fall of 2003, a series of Solar Particle Events (SPEs) reached earth in rapid succession (*1, 2*), highlighting the potential radiation danger inherent to the spaceflight environment. Indeed, four of the 10 largest SPEs observed in the space era occurred in the last solar cycle (*2*). In September of 2006, the European Space Agency completed its first dedicated lunar mission, SMART-1 (*3*). The following year, China launched its first lunar orbiter (*4*). Russian, European, Canadian, and Japanese astronauts are also integral to the operation of the International Space Station. NASA has reprioritized the continued support for long-duration low-earth orbit research, and the development of strategies and techniques to send humans to asteroids and Mars within the next two decades. As more and more nations are dedicating significant resources to extraterrestrial colonization, the need for understanding the impact of the spaceflight environment on astronaut health is increasingly obvious (*5, 6*).

Current designs for lunar missions include 6 days in transit over a 90-day total duration. A mission to Mars would likely include 400–500 days in transit over ~1,000-day duration (*6–9*). While current shielding technology does provide some degree of protection, astronauts will inevitably be exposed to significantly increased doses of radiation during long-term missions due to Galactic Cosmic Rays (GCRs) and SPEs (*6, 7*). Total-exposure doses to blood-forming organs over the duration of a mission could be $\geq$2 or $\geq$3 Sv to the skin, depending on shielding conditions and solar activity (*6–8*). Given that access to health care will be limited to what is aboard the spacecraft, particularly on the longer Mars missions, understanding and countering potential threats to astronaut safety prior to liftoff is critical.

Although recent reports indicate that astronauts and cosmonauts report long-term positive psychological response after spaceflight missions, perceiving them as having been meaningful, worthwhile, and beneficial experiences (*10, 11*), there is still concern for behavioral issues in flight (e.g., reaction to stress, cross-cultural differences, personal interactions, group dynamics) (*12–16*). Furthermore, previous work, in this laboratory and others, has shown that even low doses of radiation can impact multiple behavioral end points, albeit with dependencies on both animal model and linear energy transfer (LET) (*17–22*). Understanding the nature of radiation effects on behavior, as well as its potential interactions with other psychological factors, is of critical importance for risk management in spaceflight conditions.

The acoustic startle response is a behavioral reflex evoked by abrupt sounds of high intensity, and is a critical element of a defensive fight-or-flight response. It is a relatively simple behavior that can be elicited in all mammals, making

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it an effective assay for developmental and comparative studies (*23*). The short latency between presentation of an auditory stimulus and the appearance of the startle response indicates that the behavior occurs within a very simple circuit that is mediated by only a few central synapses (*24, 25*).

Inherent in the model are several types of plasticity including habituation, sensitization, pre-pulse inhibition (PPI), and modification by prior associative learning. Sensorimotor gating appears to be responsible for screening out distracting stimuli, preventing sensory overload, and maintaining attentional focus. In humans, sensorimotor gating deficits are integral to schizophrenia and are believed to contribute to a sense of sensory flooding, increased distractibility, and disorganized and fragmented thinking (*26*). Pre-pulse inhibition is one of the most common measures of sensorimotor gating, and involves the inhibition of the startle response by a weaker, preceding stimulus (*26*). Modulation of the startle response is likely due to an enhancement or inhibition of the transfer of information between the sensory receptors and the motor effector systems (*27*).

The goal of these studies was to characterize the dose response and timing of radiation effects on the startle response including startle reactivity, habituation, and pre-pulse inhibition. From 1 to 8 days following exposure to ionizing radiation, all mice were exposed to a series of startle trials. A white-noise startle probe was delivered on each trial and the amplitude of the whole-body startle response was measured. One quarter of the trials included only this startle probe. The remainder of the trials included a non-startle-eliciting noise pulse presented just prior to the startle probe. This ''pre-pulse'' was expected to produce a substantial inhibition of the startle response elicited by the startle probe. We measured startle reactivity on the startle probe-alone trials, and the amount of (pre-pulse) inhibition produced on the pre-pulse + probe trials, as well as habituation occurring from the early trials to the later ones. To minimize the impact of peripheral tissue damage and any subsequent ''sickness behavior'' due to peripheral inflammatory events, all irradiations were localized to the head. We utilized both γ and proton radiation for these studies.

METHODS

All methods and procedures were approved by the Loma Linda University Animal Care and Use Committee.

Animals

Male C57BL6 mice were purchased from Charles River Breeding Laboratories (Hollister, CA) at eight-weeks-old, and were allowed to acclimate and recuperate from shipping stresses for at least one week before testing. Mice were irradiated at age 9–10 weeks. The mice were housed in large shoebox cages in groups of eight or less and were maintained on a 12-h light/dark cycle (lights on at 0700 h). Humidity and temperature were maintained at 30–40% and 65–70°F. Food and water were available *ad libitum*. All mice were tested during the light phase between 0900 and 1400 hours.

Sedation

Isoflurane sedation was used during all animal irradiations because isoflurane is the recommended anesthesia for C57BL6 mice (*28*). (This noninvasive technique is a very fast-acting anesthetic for light sedation.) The sedation was used to minimize the movements of mice during irradiation, thus allowing for accurate placement of the beam and ensuring minimal exposure to noncerebral tissues. Each mouse was sedated with isoflurane (3–4% in air) and placed in positional cradles to stabilize their position during irradiation. The cradles were then placed in a box designed for the beam line, and 1–2% isoflurane gas was administered until irradiation was complete. Four animals were irradiated simultaneously.

Head-Only Irradiation

For proton irradiations, the whole head of each mouse was irradiated with a 250 MeV proton beam aimed horizontally using the synchrotron and a rotating gantry at the Loma Linda University Medical Center. The beam was spread laterally using a double-scattering system, giving a virtual source distance of 285 cm. A cast Cerrobend collimator 3-inches thick with four 1.2-cm holes was placed between the source and the mice. A 1.2-cm diameter aperture collimated the beam 19.4-cm upstream of isocenter, giving a 1.5-cm diameter field at the mouse. At this depth, the average energy and stopping power in water of the primary protons was approximately ~237 MeV and ~0.4 keV/mm (*29*). The lineal-energy spectrum at this depth was described by Robertson *et al.* (*30*). Calibration was performed using a Scantronik's small diode detector s/n DEB 050-1038 and Kodak X-Omat V film in conjunction with the Vidar VXR-16 film scanner and RIT 113 dosimetry software, these were cross-calibrated with an NIST traceable thimble ionization chamber and the ICRU Report no. 59, dosimetry protocol. Reported doses were delivered to the center of the mouse brain 1.58 cm below the surface and at a water-equivalent depth of 1.44 cm. Radiation was delivered in a single fraction at a dose rate of approximately 0.53 Gy/min (average across all proton runs) to total doses of 1, 2, 3 and 5 Gy. Dose due to backscatter from the table was negligible and the penumbra of the beam was ~0.45 cm (data not shown).

For γ irradiations, exposures were performed using a horizontal beam from an Eldorado therapy unit (Atomic Energy of Canada, Ltd., Commercial Products Division, Ottawa, Canada) containing a ^{60}Co source. The collimator used in this experiment was identical to that used for proton irradiation. A plastic plate 0.8-cm thick was placed immediately upstream of the boxes so that the front surface of each box was at the depth of maximum dose. Dose was calibrated using film. Kodak X-Omat V was placed at 80 cm from the source with a PTW Markus parallel plate ion chamber (on center axis). The dose rate was calculated to be 18.3 cGy/min.

Procedures and Design

Isoflurane and restraint. The irradiation protocol included at least three components that might affect startle responding: (1) handling and transportation to and from the radiation room; (2) placement in the radiation box cradles that included bite-bar restraint to maintain accurate targeting; and (3) the isoflurane sedation. To test the effect of our sedation protocol on startle behavior, mice ($n = 20$/group) were randomly assigned to one of three groups: isoflurane-only, isoflurane + restraint, or transportation-only. Isoflurane-only mice were sedated for approximately 5 min but not placed on cradles in the radiation box. Mice in the isoflurane + restraint group were sedated and placed on cradles in the radiation box for 5 min. Transportation-only mice were transported to and from the radiation

room, but did not receive isoflurane sedation and were not restrained or placed in the radiation box. Startle and pre-pulse inhibition were tested approximately 24 h after transport and/or sedation.

Dose response. To characterize the dose response to proton or γ radiation, mice were randomly assigned to one of 4 dose groups: 1, 2, 3 and 5 Gy ($n = 20$/group). The control groups were transported to the radiation facilities and sedated, but were not exposed to radiation. Mice were tested approximately 36 h after irradiation.

Time course. To characterize the time course of the radiation response after exposure to protons or γ rays, mice were randomly assigned to 4 time-point groups: 18 h, 2, 4 and 8 days ($n = 20$/group). The control group ($n = 20$/group) was transported to the radiation facilities and sedated, but was not exposed to radiation; five animals from each sham-exposure control group were tested at each of the 4 time points to control any effects of latency between sedation and testing. All controls were pooled together in the final analysis. The dose for the time course experiments was set at 2 Gy.

Acoustic startle and pre-pulse inhibition. The acoustic startle response was quantified using the SR-LAB system (San Diego Instruments, Inc., San Diego, CA). Briefly, mice were placed into one of 4 identical Plexiglas chambers. Any movement of test mice resulted in the displacement of a piezoelectric cartridge secured to the Plexiglas base, generating voltage proportional to the amount of displacement. The mean increase in the rectified signal during a 100 ms epoch beginning 20 ms following the onset of the startle probe was recorded as the startle response.

One-to-two days before each experiment, each mouse was weighed and placed in the startle chamber for a 5-min acclimation period without any system-generated noise (*31*). Exposure to the apparatus prior to the testing session results in less anxiety regarding a new environment because anxiety and fear can potentiate startle responding (*32*).

At the beginning of each test session, animals were placed in the testing apparatus for a 5-min acclimation period. During this period, there was a constant background white noise of 78 ± 4 dB and this background noise remained throughout the test session. The test included 64 trials, which were semi-randomized within 8 blocks of 8 trials. Each block included two (startle) probe-alone trials, as well as two trials at each pre-pulse intensity, 80, 85 and 90 dB. The startle probe was a 25 ms, 120 dB white noise; the pre-pulse was white noise presented for 100 ms with an onset 125 ms prior to the onset of the probe. Prior to each experiment, the piezoelectric sensors and speakers were calibrated using a Quest Model 2900 Integrating/Logging Sound Level Meter (Quest Technologies, Onconomowoc, WI). The startle chambers were cleaned between mice to minimize the effects of olfactory cues on behavior.

Analysis. Three outcome measures were of interest in this study: startle reactivity, pre-pulse inhibition, and startle habituation.

Startle reactivity was defined as the amplitude of the startle response on probe-alone trials. Startle reactivity was assessed using a trials 2 (trials 1–32, trials 33–64) × radiation type 2 (protons, γ radiation) × group analysis of variance (ANOVA) with trials as a repeated factor and between group factors of radiation type and group. The trials factor was not included in the analysis focusing on the first 32 trials. The groups factor varied across experiments. In the first experiment it included three groups: isoflurane only, isoflurane + restraint, and transport only. The dose experiment included five groups: nonirradiated controls, and animals receiving 1, 2, 3 and 5 Gy exposures. There were also five groups in the time course experiment: nonirradiated controls, and animals tested 18 h, 2, 4 and 8 days after irradiation. Tukey HSD pairwise comparisons were employed to examine follow-up group differences.

Pre-pulse inhibition was defined as the percentage reduction in startle amplitude on pre-pulse + probe trials compared with probe-alone trials. We used the following equation to calculate %PPI:

$$\%\mathrm{PPI} = \frac{(\mathrm{NoPP} - \mathrm{PP})}{(\mathrm{NoPP})} \times 100.$$

Because a relatively intense background noise was required to mask ambient sounds arising outside the lab, there were no significant increases in PPI associated with increases in pre-pulse intensity. Therefore, the three pre-pulse conditions were combined and are reported as a single pre-pulse + probe condition. Analysis of PPI began with a trials × PPI (probe alone, pre-pulse + probe) × radiation type × group ANOVA on mean startle amplitudes (in a–d units) to examine the main effect of the pre-pulse. This was followed with a trials × radiation type × group ANOVA on the PPI data to assess the effect of radiation and the group manipulation.

Finally, habituation was examined by comparing startle reactivity and PPI data from trials 1–32 with trials 33–64. Trial effects in the analyses of startle reactivity and PPI were used to assess the effect of radiation and the group manipulation on habituation. Because 64 startle probes were presented over the course of the experiment, we suspected that the largest effects of startle reactivity and pre-pulse inhibition would be observed in the earlier trials. Therefore, trials 1–32 were analyzed separately in each experiment.

If a mouse was moving immediately prior to the onset of the startle probe, this produced a nonzero output by the piezoelectric sensor. Data were excluded for individual trials in which the pre-stimulus baseline activity was nonzero because this precluded the measurement of an accurate baseline and calculation of startle magnitude. Trials in which no measurable startle response was detected, defined as an average activity following the startle probe of less than 4 a–d units, were assigned a value of 0 and thereby excluded from calculations of startle amplitude. To ensure accurate estimates of the startle response, a minimum of 6 valid trials was required in each condition. Mice not meeting this criterion were excluded from the analyses for lack of data. Finally, mice notably injured due to infighting were excluded because further testing would be neither reliable nor ethical. Analyses were conducted using SYSTAT (v.10, SPSS Inc., Chicago, IL). The criterion for significance was set at $P = 0.05$.

There was a large fraction of mice that did not react to the startle probe after exposure to the pre-pulse stimulus. Because this resulted in the lack of a measurable signal in the PPI trials, many of these mice ended up being excluded from the final statistical analysis. Although radiotherapy has been shown to lead to hearing loss (*33–34*), it is unlikely that this explained the lack of a response in our mice because the drop-out rate appeared to be unrelated to radiation dose. Another possible explanation involved age-dependent hearing loss. Several investigators have reported progressive hearing loss with age in C57BL6 mice (*35, 36*). This too is unlikely to explain the lack of response because mice were all less than 4 months old at testing and should have experienced minimal hearing deficits at that time. While we have no definitive indication, we suggest the most likely cause is that early onset of C57-strain in some mice, the relatively intense pre-pulses and background noise required to mask extra-laboratory acoustic intrusions, along with the comparative low sensitivity of our startle chamber, combined to produce the lack of a measurable response. Nevertheless, there remained a sufficient number of mice contributing data in sufficient volume to produce reliable results.

RESULTS

Isoflurane and restraint. No mice were excluded from analyses for infighting or as outliers; 10 mice from the transport-only controls and eight from each of the 2 isoflurane groups were excluded from the omnibus analyses because of insufficient data from PPI trials.

The primary goal of this experiment was to examine the effect of the immobilization procedures associated with

brain-localized irradiation on the startle response measures. In particular, we focused on isoflurane sedation and restraint. Sedation appeared to have a negligible effect on its own; however, sedation combined with the restraint necessary for radiation exposure potentiated startle reactivity during the first 32 trials. Analyses of these probe-alone trials revealed a treatment group main effect, $F(2, 52) = 3.89$, $P = 0.02$ (Fig. 1A). Pairwise comparisons indicated that the amplitude of the startle response was significantly greater for mice receiving the isoflurane + restraint than for either the isoflurane-only or the transportation-only groups ($P = 0.03$ and 0.01, respectively). The latter two groups did not differ from each other, $P = 0.80$.

PPI was observed for all treatment groups with an overall mean of 66.6%, $F(1, 31) = 53.66$, $P < 0.001$, with no evidence of an interaction with sedation or restraint procedures, $F < 1$ (Fig. 1B).

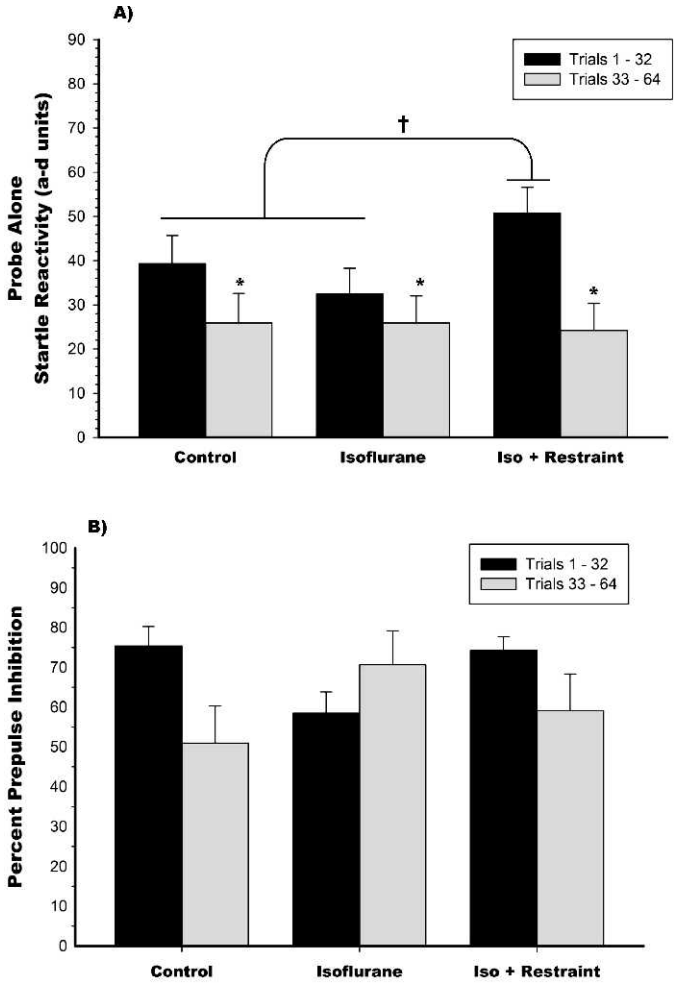

FIG. 1. Impact of isoflurane and restraint on startle and PPI responses. Panel A: Startle reactivity on probe-alone trials. For the first 32 trials, there was a main effect of group (†$P < 0.02$); Reactivity for the Iso + restraint group was greater than the Isoflurane only and control groups (post hoc Tukey $P < 0.05$); there was a main effect of habituation (*) across all 64 trials, which was similar across the three groups ($P < 0.001$). Panel B: Percent PPI. Neither the effect of habituation or group was significant. All values represent means; error bars are SEM. $n = 9$ to 12/group. Iso = Isolfurane.

Habituation was observed in startle reactivity from trials 1–32 to trials 33–64, $F(1, 52) = 21.09$, $P < 0.001$, but not in PPI, $F(1, 31) = 1.5$, $P = 0.23$. The pattern of habituation in startle reactivity, but not the PPI trials, also appeared as a trials × PPI interaction in the mean startle amplitude analysis, $F(1, 31) = 9.7$, $P = 0.004$, that did not interact with the sedation or restraint procedures, $F(1, 31) = 2.54$, $P = 0.09$. This interaction appears to result from a decline in probe-alone response amplitudes across trials, whereas the amplitude on pre-pulse + probe trials remained relatively constant. For the transportation-only and isoflurane + restraint groups, this resulted in a decrease in the percentage of PPI; however, the relatively small decrease in isoflurane group probe-alone responding resulted in an increase in the percentage of PPI.

The impact of the combination of isoflurane and restraint on startle reactivity was unexpected and will be considered in the discussion. However, it is important to note here that the potentiation of startle reactivity observed in the sedation + restraint group did not appear to affect PPI and habituation.

Dose response. Ten mice were excluded from analyses for infighting, and an additional 37 animals were excluded across analyses for insufficient data. At least 75 γ-irradiated animals (16, 17, 15, 17, and 10 in the 0, 1, 2, 3, and 5 Gy groups, respectively) contributed data to the analyses. At least 78 proton-irradiated animals contributed data (18, 16, 17, 13, and 14 in the 0, 1, 2, 3, and 5 Gy groups, respectively).

Startle reactivity appeared to decline with increasing dose across the first 32 trials (see Fig. 2A). Analysis of only those trials confirmed a significant effect of dose $F(4, 177) = 2.44$, $P = 0.05$. Proton and γ radiation produced similar effects because there was neither a main effect of radiation nor a radiation and dose interaction, F's < 1. Pairwise comparisons indicated that startle reactivity was reduced at 2 and 5 Gy compared with the sham-irradiated control group, $P < 0.05$; however, this is a weak effect, $\eta^2 = 0.05$, observable only in the early trials When all 64 trials were included in the analysis, the dose effect was no longer reliable, $F(4, 166) = 1.68$, $P = 0.16$. Although the main effect of trial remained significant, $F(4, 166) = 88.2$, $P < 0.001$, the trial × dose interaction was not, $F(4, 166) = 1.66$, $P = 0.16$.

PPI was robust at an overall mean of 67%, $F(1, 138) = 251.9$, $P < 0.001$ (see Fig. 2B). Importantly, $F(1,143) = 8.75$, $P = 0.004$, there was no evidence that PPI was affected either by the type of radiation or dose $F < 1.2$, $P > 0.36$, although habituation was observed.

Habituation occurred in both startle reactivity and PPI. The main effect of habituation across all trials was significant, $F(1, 138) = 84.0$, $P < 0.001$. Moreover, a significant trials × PPI interaction, $F(1, 138) = 69.7$, $P < 0.001$, reflected the greater habituation effect for probe-

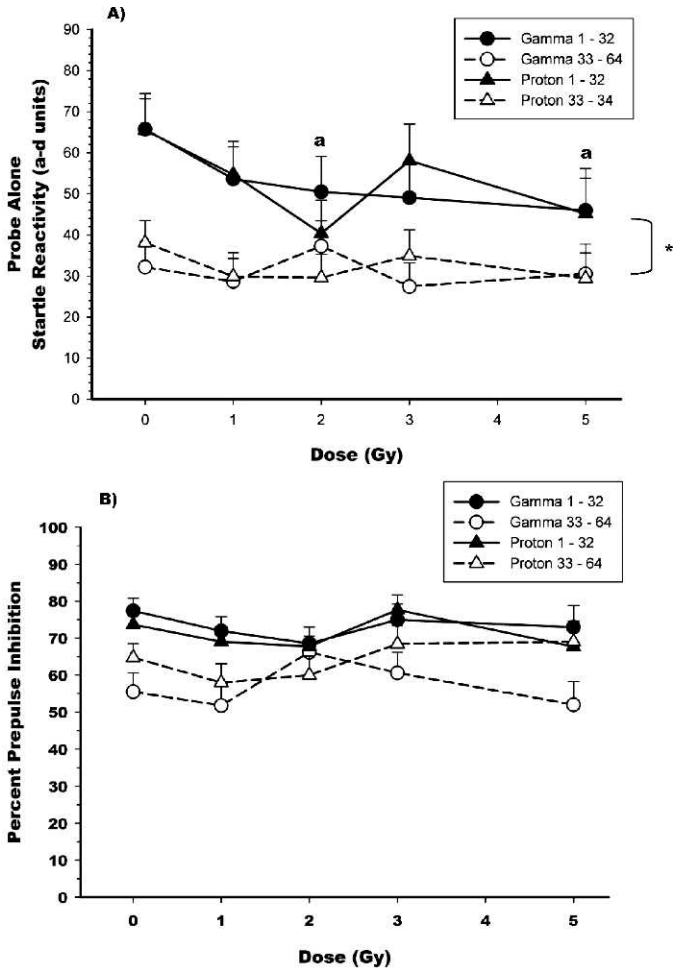

FIG. 2. Impact of radiation dose on startle and PPI responses. Panel A: Startle reactivity on probe-alone trials. For the first 32 trials, there was a main effect of dose ($P = 0.05$); reactivity at 2 and 5 Gy was less than the 0 Gy controls (**a**, post hoc Tukey $P < 0.05$); across all 64 trials there was a main effect of Habituation (*$P < 0.001$). Panel B: Percent PPI. Neither the effect of trials or dose was significant. All values represent means; error bars are SEM. $n = 10$ to 18/group.

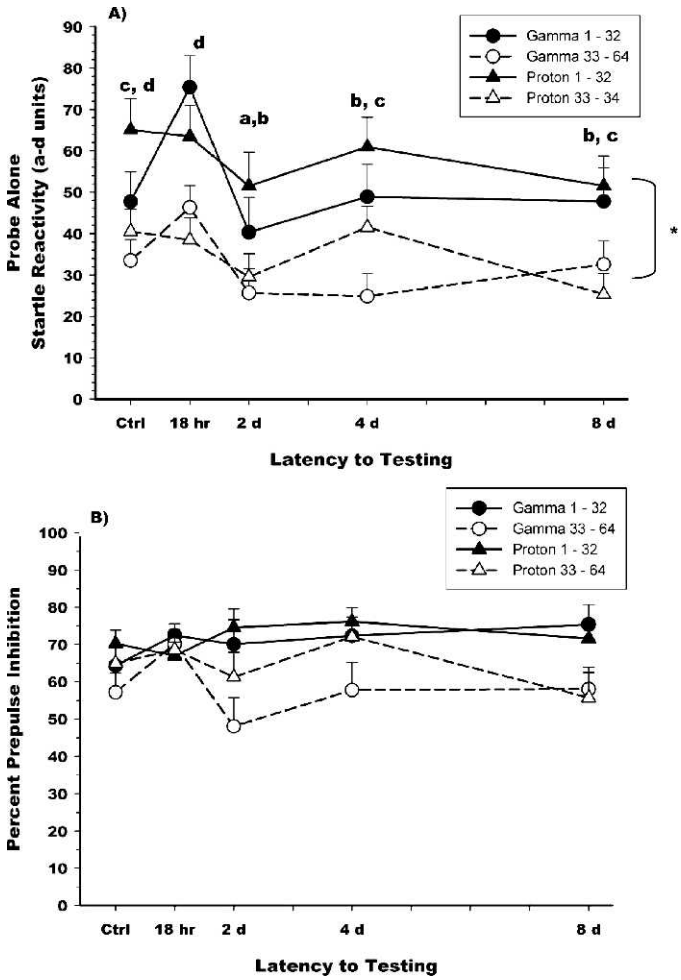

FIG. 3. Time course of radiation effects on startle and PPI responses. Panel A: Startle reactivity on probe-alone trials. For all 64 trials, there was a main effect of Day ($P = 0.007$); reactivity on day 2 was least, followed by days 4 and 8, the control group and the 18 h group. Significant differences (post hoc Tukey, $P < 0.05$) are indicated by lower-case letters (**a**, **b**, **c**, **d**). Across all 64 trials, there was also a main effect of habituation (*$P < 0.001$. Panel B: Percent PPI. Neither the effect of trials or day was significant. All values represent means; error bars are SEM. $n = 15$ to 19/group.

alone, as compared with pre-pulse + probe trials, $\eta^2 = 0.35$ and 0.06, respectively.

Time course. One mouse was excluded from analyses for infighting and 28 mice were excluded for insufficient data. At least 83 γ-irradiated mice (19, 17, 14, 17, and 16 in the control, 18 h, 2, 4 and 8 days groups, respectively) contributed data to the analyses. At least 88 proton-irradiated animals contributed data (18, 17, 15, 19, and 19 in the control, 18 h, 2, 4 and 8 day groups, respectively).

Examination of Fig. 3A suggests that startle reactivity was at a minimum at day 2, increased nonsignificantly at days 4 and 8, and was greatest at 18 h; this is supported by a main effect of the group variable, time, in both the first 32 trials, $F(4, 189) = 3.37$, $P = 0.01$ and across all 64 trials, $F(4, 184) = 3.66$, $P = 0.007$. Follow-up comparisons also support this interpretation revealing that startle reactivity at day 2 was significantly less than the control group, $P <$ 0.05. Mean startle reactivity on days 4 and 8 was not significantly different from day 2, the control group, or each other. Finally, startle reactivity at 18 h was greater than days 2, 4, and 8, but not significantly greater than the control group. This pattern of pairwise results was the same for both the first 32 trials and across all 64. Neither the main effect of Radiation nor its interaction with Time reached significance, $P < 0.14$, indicating that both proton- and γ-radiation were associated with similar effects across time.

As may be seen in Fig. 3B, PPI was robust at an overall mean of 68% and varied across time points, appearing as a main effect of PPI, $F(1, 158) = 361.1$, $P < 0.001$, and a PPI × time interaction, $F(4, 158) = 2.44$, $P = 0.05$; however, in the PPI analysis (using %PPI as the dependent variable), the effect of time was not significant ($F < 1$). Follow-up comparisons suggested that the greatest PPI occurred at 18 h

and on day 4. However, although mean amplitude PPI at both of these time points was greater than that displayed by the control group, $P < 0.05$, the change in percentage of PPI was not significant.

Habituation was apparent across all conditions, but was greater for probe-alone trials than for pre-pulse + probe trials, appearing as a main effect of Trials, $F(1, 158) = 93.6$, $P < 0.001$ and a trials × PPI interaction, $F(1, 158) = 68.3$, $P < 0.001$. There was no evidence of a radiation effect on habituation because no test day differed from the control group, and none of the interactions involving trials and time reached significance. Moreover neither the main effect nor any interaction of Radiation was significant.

DISCUSSION

The results of these studies may be considered as four primary observations. First, the expected effects of habituation and pre-pulse inhibition were successfully produced, validating the experimental paradigm. Second, exposure to ionizing radiation produced both dose- and time-course effects. These included a decrease in startle reactivity appearing within the first 48 h. Third, there were no differences between proton and γ-ray exposures. Finally, although startle reactivity was affected, irradiation produced no observable effect on pre-pulse inhibition.

We can be confident in the basic experimental manipulations used in this study. The startle probe produced reliable startle responses, which habituated across trials. The addition of a pre-pulse 125 ms prior to the startle probe produced a robust inhibition of the response, ranging from 50%–70%. Although we were unable to reproduce the intensity-associated increments in PPI noted by other investigators (*37, 38*), we did observe habituation of the PPI response. As has been observed in previous studies, including studies with humans (*39*), this habituation was the result of decreases (habituation) in the Probe Alone response amplitude. Without a corresponding decrease in the response on pre-pulse + probe trials, this produced the reduction in percent pre-pulse inhibition.

Surprisingly, we also found a statistically reliable potentiation of startle in mice that experienced both anesthesia and placement in the bite bar. It is unclear why this effect occurred; however, it is possible that mice partially regained consciousness while being loaded into the bite bar resulting in an activation of aversive response pathways in the amygdala, and thus led to an enhanced startle response (*40, 41*). In at least one study, rats exposed to restraint stress displayed an exaggerated startle response up to 24 h later (*42*). Without further study, this specific mechanism of the potentiated startle effect remains speculative. Nevertheless, it is important to emphasize that the sedation + restraint group did not differ from the others in PPI or habituation. These data support the concept that startle reactivity, startle habituation, and pre-pulse inhibition involve separate but parallel system from the acoustic startle response (*43*).

Having established that the experimental manipulations of the startle response produced the expected results, we turn to the critical variable of radiation exposure. In these experiments, both the radiation dose and the latency-to-testing affected startle reactivity. Dose was inversely related to startle amplitude. The decrease in startle amplitude appeared as radiation exposure increased to 2 Gy, but did not decrease with greater exposure (up to 5 Gy). This result is in line with our previous observation using protons (*17*) in which that mice tested within 24 h of proton irradiation displayed a reduction in startle reactivity at the 3 and 4 Gy doses. Moreover, the time course results suggest that our radiation exposure produced an acute effect. Minimum startle reactivity was measured at testing latency of 48 h and did not significantly change or return to the level of the controls though latencies of 4–8 days. These data, too, are in line with our previous observations in which radiation reduced startle reactivity when tested at 36 h (*17*). Moreover, the reduction appears to be transient as well. When testing was delayed for more than 2 days, startle reactivity increased to a level intermediate between day 2 and the control level. Taken together, the dose and time-course results indicate that low-LET radiation exposure of as little as 2 Gy may alter some neural information processing mechanisms and that the effects may appear within 48 h of exposure. However, these acute effects also appear to be transient, receding within 1–2 weeks (*17*).

At no point in any of our studies did we find a statistically reliable difference between proton and γ-ray exposures for either the startle or PPI groups. By definition, the relative biological effectiveness (RBE) of γ rays is 1. The RBE of protons is generally accepted to be about 1.1 (*44*). This would suggest that the biological consequences of similar doses of each radiation type would be similar. However, others and we have found significant differences between these radiation types in immune-related parameters (*45, 46*) suggesting at least the potential that there may be a differential response in behavior. This difference in effect may be due to differences in their physical properties and how they react at the tissue level. Unlike photons (γ rays), protons can have a finite range in tissue with the LET being strongly energy-dependent and the greatest dose delivered at the peak of the Bragg curve. In contrast, the greatest dose for γ rays is at the surface of the tissue, and decreases with depth (*47*). Because of these differences, and because most of the radiation in space is protons (~80%) (*48*), using γ rays to model spaceflight radiation may not always be adequate. However, as we report here, we found no significant differences between protons and γ rays in any of our end points.

Although robust PPI was observed throughout the study, we failed to observe any effect of irradiation on PPI. This is in line with our previous study in which mice exposed to 5 Gy ^{56}Fe or ^{21}Si particle irradiation displayed no differences

in PPI compared with sham-irradiated controls (*18*). A similar lack of differences has also been reported after exposure to 10 Gy X radiation (*49*) and only following a more substantial, 15 Gy X irradiation has a reduction in PPI been reported (*50*). Taken together, these data suggest that the impact of low-dose, low-LET radiation on the fundamental circuitry serving PPI is, at most, minimal. Nevertheless, we cannot conclude that sensorimotor gating as measured by PPI is completely unaffected by ionizing radiation. For example, the dopamine agonist apomorphine inhibits PPI; and this effect may be blocked following 5 Gy ^{56}Fe or ^{21}Si particle irradiation (*51*). Another study with mice demonstrated that 3 days after whole-body γ irradiation at 1.5 Gy, dopamine levels in the hippocampus were 144% higher than in sham-irradiated controls (*52*). Taken together, since the hippocampus and striatum are a source of significant modulation to the pre-pulse inhibition circuit, and that are possible radiation effects on dopaminergic functions, it will be important for future research to explore the impact of radiation on dopaminergic and other systems that modulate the expression of PPI.

CONCLUSIONS

The effects of radiation were subtle and acute, affecting the startle response within 2 days following irradiation of at least 2 Gy without any apparent statistically identifiable differences in the response to proton compared to γ rays. The current studies complement and extend our work (*17*) and that of others (*22*) on the effect of low-dose proton radiation on the central nervous system function, and indicate similarities and contrasts with the effects of heavy particles such as ^{56}Fe, which have been described as similar to age-related changes (*53*). Both may impact startle responding; however low-LET radiation produces subtle and transient effects that only appear shortly after exposure.

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