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The biobehavioral and neuroimmune impact of low-dose ionizing radiation ☆

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ABSTRACT

In the clinical setting, repeated exposures (10–30) to low-doses of ionizing radiation (⩽200 cGy), as seen in radiotherapy for cancer, causes fatigue. Almost nothing is known, however, about the fatigue inducing effects of a single exposure to environmental low-dose ionizing radiation that might occur during high-altitude commercial air flight, a nuclear reactor accident or a solar particle event (SPE). To investigate the short-term impact of low-dose ionizing radiation on mouse biobehaviors and neuroimmunity, male CD-1 mice were whole body irradiated with 50 cGy or 200 cGy of gamma or proton radiation. Gamma radiation was found to reduce spontaneous locomotor activity by 35% and 36%, respectively, 6 h post irradiation. In contrast, the motivated behavior of social exploration was un-impacted by gamma radiation. Examination of pro-inflammatory cytokine gene transcripts in the brain demonstrated that gamma radiation increased hippocampal TNF-α expression as early as 4 h post-irradiation. This was coupled to subsequent increases in IL-1RA (8 and 12 h post irradiation) in the cortex and hippocampus and reductions in activity-regulated cytoskeleton-associated protein (Arc) (24 h post irradiation) in the cortex. Finally, restraint stress was a significant modulator of the neuroimmune response to radiation blocking the ability of 200 cGy gamma radiation from impairing locomotor activity and altering the brain-based inflammatory response to irradiation. Taken together, these findings indicate that low-dose ionizing radiation rapidly activates the neuroimmune system potentially causing early onset fatigue-like symptoms in mice.

1. Introduction

The impact of ionizing radiation on behavior and neuroimmunity is an emerging field. Currently, the primary focus is on clinically delivered radiation therapy to oncology patients and the consequent adverse biobehavioral impact these critical treatments engender (Bower et al., 2009; Hofman et al., 2007; James, 2006). Therapeutic radiation involves delivery of relatively high doses (30–80 Gy/14–60 days) administered focally and strategically to limit treatment of uninvolved normal tissues (Lawrence et al., 2008). The majority of patients receiving radiotherapy over the past century have been treated with electrons, X-rays (high energy photons) or gamma-rays (high energy photons). The distinction between X- and gamma-rays from a radiotherapy perspective relates to the source of the photons: X-rays originate from outer electrons and gamma rays originate from atomic nuclei. In radiotherapy applications, however, both X- and gamma-rays are photons in the 1–20 MeV energy range. The physical properties of these forms of radiation cause maximal energy deposition (dose) to occur early in the tissue particle track at depths of 0.5–4 cm. Recently, there has been renewed interest in treating patients with heavier charged particles such as protons, which deposit the majority of their dose toward the end of the tissue particle track at depths up to 20–30 cm. This affords more focused delivery of dose to deeply seated neoplasms with less radiation being administered to tissues more distal to the target (Evaluation Subcommittee of ASTRO's Emerging Technologies Committee, 2009). In addition to the differences in macroscopic dose distribution, photons and protons create disparate microscopic dose distributions due to dissimilar linear energy transfer (LET) coefficients. Importantly, differences in microdosimetric track structure may cause photons and protons to have qualitatively and quantitatively unequal dose-toxicity profiles (Cengel et al., 2010).

In contrast, environmental radiation exposure is ubiquitous, of very low-dose (approximately 6.2 mGy/year) (Schauer and Linton, 2009), whole body and comprised of a mix of particle types that in-

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clude photons, electrons, protons and heavy ions. This environmental dosage, however, can jump significantly at altitudes that commercial aircraft fly (approximately 6.30–6.79 μGy/h (Mohler, 2003)), in manned space exploration (approximately 50–100 μGy/day during interplanetary travel and 25–50 μGy/day on planetary surfaces (Cucinotta and Durante, 2006)) or during a severe nuclear reactor accident such as occurred at Fukishima Daiichi Nuclear Power Plant in Japan 2011 (ground air as high as 1 Gy) (Makhijani, 2011; Weissmann, 2011). Environmental radiation can be markedly compounded during a solar particle event (SPE) with doses reaching 1.4 Gy/h for skin, 0.8 Gy/h for eyes and 8 cGy for bone marrow in data modeling studies from the August 1972 SPE (Parsons and Townsend, 2000). In addition, SPE irradiation is primarily comprised of relatively superficially penetrating protons with energies less than 50 MeV. The energy spectra of a specific SPE, however, is highly variable and some SPES have had a greater proportion of deeply penetrating, higher energy protons. With the anticipation of expanded near space/space tourism/travel, nuclear power plant construction and threat of nuclear terrorism, the population at risk for total body radiation exposures in the range of 0.5–2 Gy are likely to increase appreciably.

Total body exposure to ionizing radiation can lead to acute radiation syndrome (ARS) that includes the initial prodromal stage defined by nausea, vomiting and diarrhea (N–V–D stage) (Donnelly et al., 2010). An underappreciated component of the prodromal stage is neuroimmune system-mediated sickness symptoms often described as feelings of unease and weakness with an associated lack of motivation and energy (Hofman et al., 2007; Marquette et al., 2003, Young, 1987). Like other maladies associated with weariness and malaise, radiation-induced fatigue is a complex interplay of mental, emotional and physical biobehaviors that are often ignored due to concerns over the manifest illness stage and, ultimately, survival. The first radiation-induced behavioral effects involving the dose and type(s) of radiation present in SPEs were delineated in animals (predominantly primates) during the 1970s and 80s. Memory and cognition testing in monkeys irradiated at dose rates of 0.3, 0.8 and 1.8 Gy/min, (total dose of 10.0 Gy) showed that hampered performance occurred in 81% of animals at 1.8 Gy/min but only in 7% of animals at 0.3 Gy/min. Thus, the effective dose for radiation-induced performance deficits was estimated to occur at doses of 3 Gy or less (Bogo, 1988). In addition, behavioral test complexity appeared impacted by ionizing radiation with tasks requiring greater physical exertion being affected more (Bogo, 1988). As for rodents, conditioned taste aversion could be induced at doses as low as 0.25 Gy (Bogo, 1988). Interestingly, 3 Gy of proton radiation caused a decrease in latency to fall in rotarod testing and loss of acoustic startle habituation (Pecaut et al., 2002). In sum, almost all studies reporting on the behavioral impact of low-dose radiation ($\leqslant$10 Gy) examined endpoints of days to weeks post radiation. Therefore, almost nothing is known about the immuno behavioral impact of low-dose ionizing radiation within hours after exposure.

2. Methods

2.1. Materials

All reagents and chemicals were purchased from Sigma–Aldrich (St. Louis, MO) except as noted. RNAlater (AM7021) and RiboPure Blood Kits (AM1928) were purchased from Ambion (Austin, TX). QIAGEN RNeasy Lipid Tissue Mini Kits (Cat No. 74804) were purchased from QIAGEN (Valencia, CA). Reverse transcription kits and primers for qPCR were purchased from Applied Biosystems (Foster City, CA). Plastic containment cubes (AMAC530C) were purchased from AMAC Plastic Products (Petaluma, CA).

2.2. Animals

Animal use was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council). Male 6-wk old CD-1 mice (n = 632) were obtained from Taconic (Hudson, NY). Mice were group housed (4× cage) in standard shoebox cages (length 28 cm; width 17 cm; height 12.5 cm) and allowed water and standard rodent chow *ad libitum*. Mice where maintained in an environmentally controlled room on a 12 h dark/light cycle (1900–0700 h) at a temperature of 72 F and a humidity of 45–55%. Mice tested were 7–8 wks of age. Video recording of animal behavior was performed under red light using a Sony HDR-XR500 V Night Shot capable video camera (San Diego CA, USA).

2.3. Radiation exposure

Mice were singly housed for 12 h prior to irradiation or sham irradiation. ^{137}Cs irradiated mice were exposed to gamma radiation for no more than 10 min at a dose rate of 44.50 ± 0.1 cGy/min (high dose rate) to doses of 50 cGy or 200 cGy. ^{137}Cs radiation was delivered using a Nordion GammaCell 40-Series 1 Irradiator (Ottawa, Canada). ^{60}Co irradiated mice were exposed to gamma radiation at a dose rate of 0.5 ± 0.01 cGy/min (low-dose rate). ^{60}Co radiation was delivered using an Atomic Energy of Canada Eldorado Model 'G' irradiator (Ottawa, Canada). Proton irradiated mice were exposed to proton radiation at a dose rate of either 50.0 ± 0.1 cGy/min (high-dose rate) or 0.5 ± 0.01 cGy/min (low dose rate). Proton radiation was delivered using the horizontal clinical beam line at the Loma Linda University Medical Center (LLUMC, Loma Linda CA). Protons were tailored to have a similar macroscopic dose distribution as ^{60}Co produced photons, which are considered to be the standard by which other forms of therapeutic radiation are compared (Cengel et al., 2010; Maks et al., 2011). Sham irradiated mice were treated identically to irradiated mice except the radiation source remained shielded. Irradiation occurred 3 h prior to the start of the dark cycle (1600 h).

2.4. Restraint

Immediately before radiation exposure or sham irradiation exposure, all subject mice were individually placed inside 7.25 × 4.10 × 4.10 cm well-ventilated containment cubes. These cubes did not provide absolute restraint and mice were able to move minimally. In minimally restrained mice, time of confinement did not exceed 10 min (restraint-10 mice). For mice subjected to more sustained restraint, confinement in the containment cube was 240 min (restraint-240 mice). Therefore, restraint-10 mice were irradiated while simultaneously confined (total confinement/irradiation duration $\leqslant$10 min). Restraint-240 mice were either: (1) irradiated while simultaneously confined (confined/irradiation duration, 10 min) then allowed to remain in the containment cube sans irradiation for an additional 230 min (total confinement, 240 min) or (2) irradiated while simultaneously confined (total confinement/irradiation duration, 240 min). Following any form of restraint, mice were returned to their home cage.

2.5. Social exploration

Social exploration was performed as we have previously described (Johnson et al., 2007). Social exploration testing was initiated 10 min after irradiation/restraint exposure. At the time points indicated a 3–4 wk-old novel conspecific juvenile mouse of the same sex (challenge mouse) was introduced into the home cage of the subject mouse. The challenge mouse was confined in a 7.62 × 7.62 × 7.62 cm square metal mesh enclosure. Testing

duration was 5 min and a new challenge mouse was used to test each subject mouse at every time point examined. Investigation/exploration was evaluated from the video record and was considered as nose-to-enclosure contact.

2.6. Locomotion

Spontaneous locomotor activity was measured as we have previously described (Lavin et al., 2011). Immediately after irradiation/restraint exposure and at the time points indicated mice were video recorded in their home cage for 5 min. Movement was quantified using EthoVision XT 7 (Noldus Information Technology, Leesburg VA). Parameters examined included distance moved (cm) and velocity of movement (cm/s).

2.7. qPCR

Following behavioral testing, mice were sacrificed via CO_2 asphyxiation. For blood collection, cardiac puncture was executed using a Becton Dickinsin (BD) 26G × 3/8 inch needle (Franklin Lakes, NJ). Drawn blood was anti-coagulated in EDTA containing Microtainers (BD, Cat No. 365974). After anticoagulation, 0.4 mL of blood was mixed in 1.3 mL of RNAlater. Total RNA was extracted using RiboPure Blood Kits per manufacturer's instructions. For brain collection, brains were harvested and either perfused or not perfused (un-perfused) (as indicated) with ice cold PBS to remove blood as we have done previously (Lavin et al., 2011). Perfusion studies were performed to determine the contribution, if any, of blood based gene transcripts to brain biomarker detection. Where indicated cortex, hippocampus, hypothalamus and cerebellum were separately dissected from perfused brains as we have done previously (Lavin et al., 2011). In all brain isolations, olfactory bulb was not included for study. Total RNA was extracted using a QIAGEN RNeasy Lipid Tissue Mini Kit per the manufacturer's instructions. After all RNA extractions reverse transcription was performed with an Applied Biosystem high-capacity cDNA reverse transcription kit (Cat No. 4368813). As indicated, qPCR utilized the following TaqMan (Applied Biosystems) gene expression primers: IL-1β (Mm99999061_mH), TNF-α (Mm00443258_m1), IL-1RA (Mm01337566_m1), activity-regulated cytoskeleton-associated protein (Arc) (Mm00479619_m1), IL-6 (Mm01210733_m1), IL-1α (Mm_99999060_m1) and IFN-γ (Mm99999071_m1). qPCR was performed on a 7900 HT Fast real-time PCR system (Applied Biosystems) using TaqMan universal PCR master mix (Cat No. 4318157). To normalize gene expression, a parallel amplification of endogenous glyceraldehydes-3-phosphate dehydrogenase (Mm03302249_g1) was performed. Relative quantitative evaluation of target gene levels was performed by comparing ΔC_t's, where C_t was the threshold concentration.

2.8. Statistics

All data are presented as mean ± SEM. Data were analyzed using SAS 9.2 (SAS Institute, Inc., Cary NC). To test for statistical differences, a one-way or two-way ANOVA was used with or without repeated measurements where needed. Tukey's test was used for post hoc pair-wise multiple comparison procedures. Where needed and indicated, raw data was transformed to attain normal distribution. Also, where indicated, a Freidman's two-way ANOVA for non-parametric analysis was used when nonparametric data was unable to be transformed to a normal distribution. All statistical analysis included testing for time point × dose, restraint × dose or perfusion × dose interactions. Statistical significance was denoted at $P < 0.05$.

3. Results

3.1. Gamma radiation but not proton radiation reduces mouse locomotor activity

Fig. 1A shows that restraint-10 mice exposed to 50 or 200 cGy of gamma radiation (44.5 ± 0.1 cGy/min) had, respectively, a 33.8% and 35.1% reduction in spontaneous distance moved (locomotion) 6 h post irradiation compared to sham irradiated mice. Fig. 1B shows that mean velocity of movement (velocity) was reduced 6 h post irradiation at both 50 and 200 cGy of gamma radiation by 34.7% and 35.7%, respectively. When 50 or 200 cGy of gamma radiation was delivered at approximately 1/100 the dose rate (0.5 ± 0.01 cGy/min), mouse locomotion/velocity was not impacted at 0, 2, 4, 6, 8 or 24 h after irradiation (data not shown). Similarly, when 50 or 200 cGy of proton radiation was used (dose rate of either 0.5 ± 0.01 cGy/min or 50.0 ± 0.1 cGy/min), mouse locomotion/velocity was not impacted at 0, 2, 4, 6, 8 or 24 h after irradiation (data not shown). Social exploration was not affected by either 50 or 200 cGy of gamma or proton radiation (regardless of dose rate) at 0, 2, 4, 6, 8 and 24 post irradiation (Table S1).

3.2. Gamma radiation up-regulates gene transcripts for TNF-α and Arc in whole brains 6 h post irradiation

Fig. 2A shows that unperfused and perfused brains from restraint-10 mice exposed to 200 cGy of gamma radiation (44.5 ± 0.1 cGy/min) had a 3.2-fold and 2.1-fold increase in TNF-α gene transcripts, respectively, 6 h post irradiation. Whole brain gene transcript expressions for IL-1α, IL-1β, IL-1RA, IL-6 and IFN-γ were not impacted by gamma radiation at this time point. Fig. 2B demonstrates that in perfused brains restraint-10 mice exposed to 200 cGy of gamma radiation (44.5 ± 0.1 cGy/min) had a 0.37-fold decrease in Arc 6 h post irradiation.

3.3. Gamma radiation up-regulates gene transcripts for IL-1β and IL-1RA in blood 8 h post irradiation

Fig. 3A and B show that blood from restraint-10 mice exposed to 200 cGy of gamma radiation (44.5 ± 0.1 cGy/min) had a 3.3-fold and 3.3-fold increase in IL-1β and IL-1RA gene transcripts, respectively, 8 h post irradiation. Blood gene transcript expressions for IL-1α, TNF-α, IL-6, and IFN-γ were not impacted by gamma radiation at this time point or at 4 h post gamma irradiation. IL-1α was increased 2.1-fold at 6 h by 200 cGy of gamma radiation (data not shown), however, IL-1β, IL-1RA, TNF-α, IL-6, and IFN-γ were not. Fig. 3C and D demonstrate that up-regulation of IL-1β and IL-1RA gene transcripts in whole brain at 8 h post gamma irradiation are due to blood in the brain.

3.4. Restraint inhibits the impact of 200 cGy gamma radiation on locomotor activity

Fig. 4A demonstrates that in restraint-240 mice 200 cGy of radiation (44.5 ± 0.1 cGy/min) increased locomotion 4 h post irradiation by 20.3% and 25.4%, respectively, compared to sham irradiated restraint-240 mice and restraint-240 mice exposed to 50 cGy of gamma radiation. Likewise, 4 h post irradiation velocity was increased in restraint-240 mice exposed to 200 cGy of gamma radiation by 25.7% and 45.7%, respectively, compared to sham irradiated restraint-240 mice and restraint-240 mice exposed to 50 cGy of gamma radiation. At 6 h post irradiation mice exposed to 50 cGy had a 29.2% and 35.3% decrease in locomotion compared to sham and 200 cGy gamma irradiated mice and a 29.6%

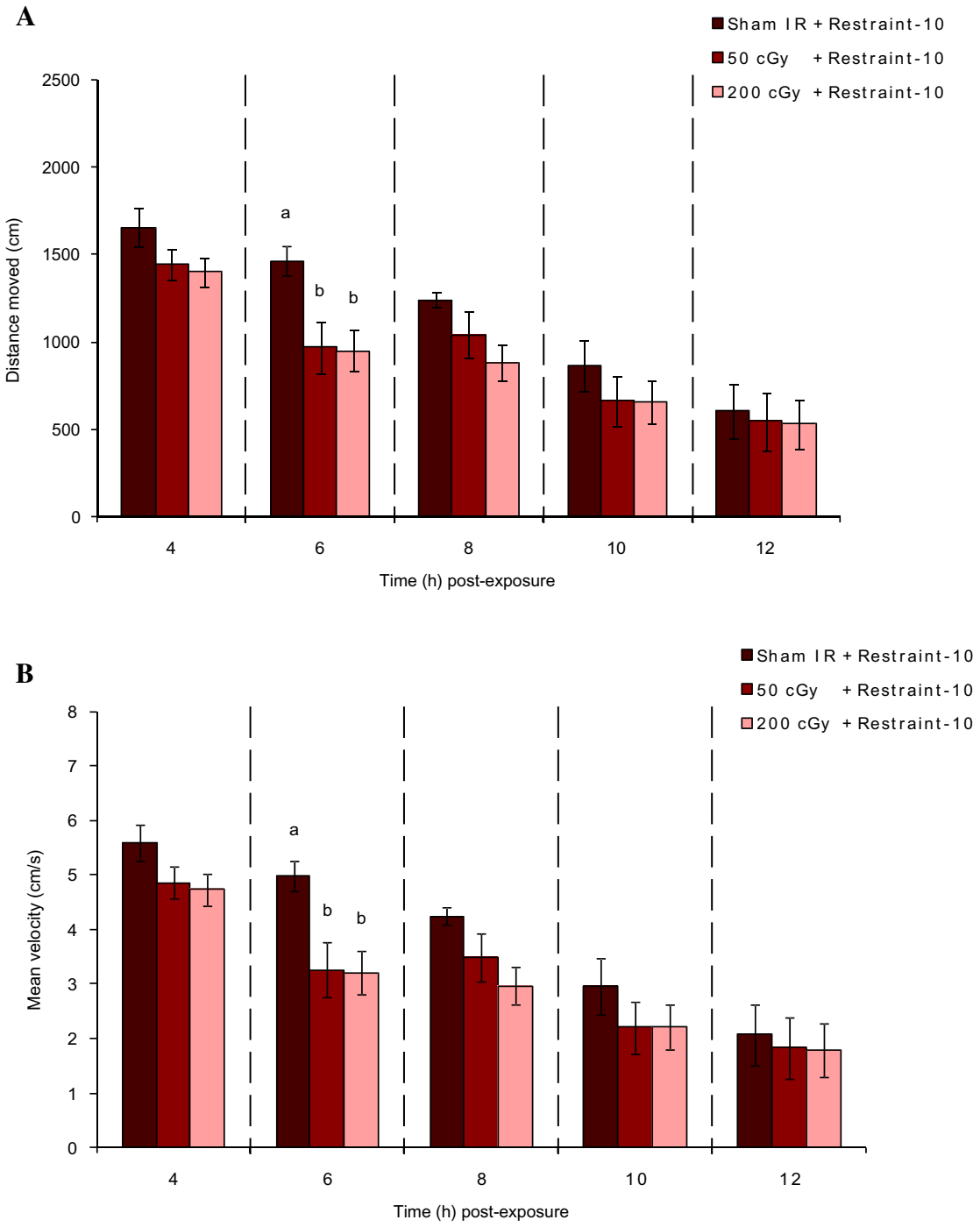

Fig. 1. Gamma radiation but not proton radiation reduces mouse locomotor activity. Restraint-10 mice were exposed to 50 or 200 cGy of gamma radiation (44.5 ± 0.1 cGy/min) as indicated. Spontaneous locomotor activity and velocity of movement were measured at the time points indicated post irradiation. Results are expressed as means ± s.e.m.; $n = 8$. (A) Distance moved (cm): main effects of dose ($P < 0.001$) and time point ($P < 0.001$); 6 h time point: $P < 0.05$, sham IR *v* 50 cGy (1463.8 ± 83.7 *v* 968.4 ± 147.6) and sham IR *v* 200 cGy (1463.8 ± 83.7 *v* 950.5 ± 117.2). (B) Velocity of movement (cm/s): main effects of dose ($P < 0.001$) and time point ($P < 0.001$); 6 h time point: $P < 0.05$, sham IR *v* 50 cGy (5.0 ± 0.3 *v* 3.3 ± 0.5) and sham IR *v* 200 cGy (5.0 ± 0.3 *v* 3.2 ± 0.4). Bars without a common superscript are different ($P < 0.05$).

and 35.6% reduction in velocity, respectively. When restraint-240 mice were compared to restraint-10 mice, restraint-240 mice exposed to 200 cGy of gamma radiation (44.5 ± 0.1 cGy/min) moved 39.5%, 83.5% and 59.1% farther at 4, 6, and 8 h post irradiation, respectively (Table S2). Non-irradiated restraint-240 mice and restraint-10 mice moved similarly except at the 10 h time point (Table S2).

3.5. Restraint of gamma irradiated mice impacts TNF-α, IL-1RA and Arc gene expression differentially in cerebral hippocampus, hypothalamus, cortex, and cerebellum at 4, 8, 12 and 24 h post irradiation

Table 1 shows that when restraint-10 mice and restraint-240 mice were exposed to either 50 or 200 cGy of gamma radiation

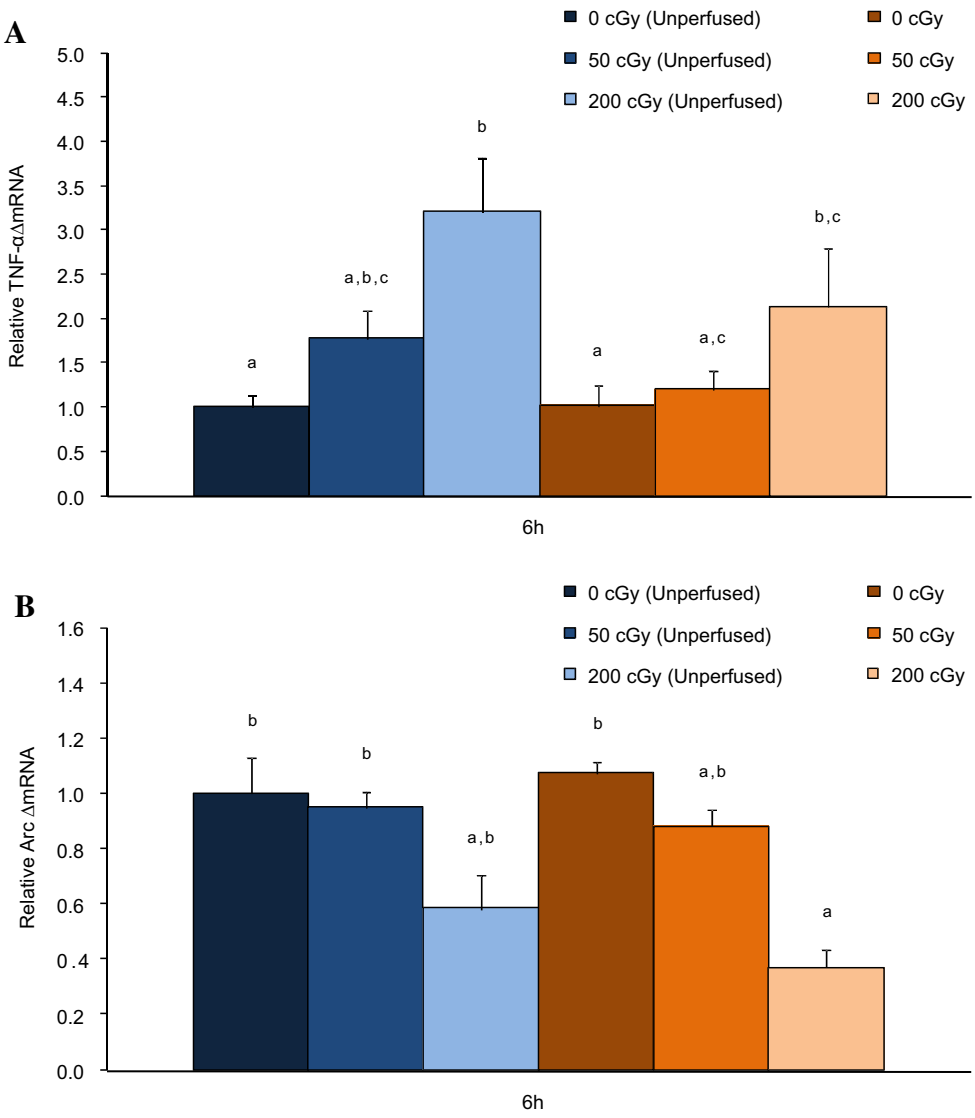

Fig. 2. Gamma radiation up-regulates gene transcripts for TNF-α and Arc in whole brain 6 h post irradiation. Restraint-10 mice were exposed to 50 or 200 cGy of gamma radiation (44.5 ± 0.1 cGy/min) as indicated. qPCR was used to quantify mRNAs from un-perfused and perfused whole brains as indicated 6 h post irradiation. Results are expressed as relative fold change in mRNA expression (ΔmRNA), means ± s.e.m.; $n = 4$–6. (A) TNF-α: main effect of dose ($P < 0.001$) and perfusion ($P = 0.042$); $P < 0.05$, 200 cGy (unperfused) *v* sham IR (unperfused), sham IR, 50 cGy (3.205 ± 0.252 *v* 1.000 ± 0.166, 1.017 ± 0.286, 1.201 ± 0.241, respectively); $P < 0.05$, 200 cGy *v* sham IR (unperfused), sham IR (2.139 ± 0.387 *v* 1.000 ± 0.166, 1.017 ± 0.286, respectively). (B) Arc: main effect of dose ($P = 0.003$); $P < 0.05$, 200 cGy *v* sham IR (unperfused), 50 cGy (unperfused), sham IR (0.370 ± 0.076 *v* 1.000 ± 0.239, 0.951 ± 0.211, 1.074 ± 0.107, respectively).

(44.5 ± 0.1 cGy/min) differential gene transcript expression of TNF-α, IL-1RA and Arc occurred that was dependent on brain region, restraint duration and dose of gamma radiation.

4. Discussion

Near continuous exposure to environmental ionizing radiation of a very low-dose rate is omnipresent. With certain occupations and in certain circumstances, dose rate can increase such that a modest dose of radiation (200 cGy) is received in a relatively short period of time (>8 h). In humans, these exposures are increasing in frequency (nuclear accidents) and becoming better recognized (SPEs). When ionizing radiation doses are significant in duration or energy to cause ARS, prodromal stage symptoms of nausea, vomiting and diarrhea (NVD) (Donnelly et al., 2010; James, 2006) occur manifesting within hours (Donnelly et al., 2010). Fatigue is either underappreciated or unreported because prodromal stage research has focused on NVD as this symptom triad is perceived as predicative of severe organ damage and demise (Donnelly et al., 2010). In humans, radiation-induced NVD typically requires a minimum dose of 70 cGy, although mild symptoms may be observed at doses of 30 cGy (Centers for Disease Control, 2010). Radiation-induced fatigue has been best studied in relationship to radiation therapy where loss of energy and malaise is a common side effect (Bower, 2007). Serious cancer treatment-associated fatigue, however, usually manifests gradually (Portenoy and Itri, 1999) compounding with delivery of repeated fractionated doses of 200 cGy that over the course of therapy (up to 8 wks), can deliver up to 80 Gy. In general, CNS function is not felt to be impacted at single radiation doses of ⩽200 cGy (Porter and Kaplan, 2006) and if fatigue is documented in a single 100–200 cGy exposure it is usually tallied during the illness phase which for a dose ⩽200 cGy would occur nearly a month post exposure (Porter and Kaplan, 2006). Overall, a single isolated exposure of less than ⩽200 cGy is deemed recoverable without supportive care (Porter and Kaplan,

Table 1
Impact of high-dose rate gamma irradiation on TNF-α, IL-1RA and Arc gene transcripts in cerebral hippocampus, hypothalamus, cortex, and cerebellum at 4, 8, 12 and 24 h post irradiation.

Brain region	Time post-irradiation (h)	Restraint-10				Restraint-240		
		Gene	Sham IR	50 cGy	200 cGy	Sham IR	50 cGy	200 cGy
Hippocampus	4	TNF-α[*,#]	1.000 (0.253)[a,c]	1.144 (0.133)[a,c]	1.535 (0.211)[a]	0.394 (0.261)[b,c]	0.599 (0.075)[c]	1.326 (0.450)[a]
Hippocampus	4	Arc[#]	1.000 (0.132)[a]	0.886 (0.098)[a]	0.974 (0.147)[a]	1.730 (0.172)[b]	1.303 (0.184)[a,b]	1.326 (0.177)[a,b]
Hypothalamus	4	TNF-α[*,#]	1.000 (0.320)[a]	0.985 (0.260)[a,b]	1.019 (0.180)[a]	1.235 (0.355)[b,c]	1.248 (0.127)[c]	1.260 (0.339)[a]
Hypothalamus	4	IL-1RA[#]	1.000 (0.437)[a,b]	0.668 (0.199)[a,b]	1.313 (0.137)[a]	0.630 (0.381)[a,b]	0.560 (0.194)[b]	0.683 (0.275)[a,b]
Cortex	4	TNF-α[*,#,†]	1.000 (0.237)[a]	1.076 (0.137)[a]	1.226 (0.184)[a]	0.420 (0.220)[b]	0.516 (0.110)[b]	1.096 (0.356)[a]
Cortex	4	Arc[*,#]	1.000 (0.154)[a,b,c]	0.573 (0.157)[a]	0.667 (0.127)[a,c]	1.732 (0.375)[b]	1.426 (0.227)[b]	1.199 (0.272)[b,c]
Cerebellum	4	TNF-α[*,#]	1.000 (0.121)[a]	0.963 (0.131)[a]	1.164 (0.198)[a]	0.522 (0.146)[b]	0.465 (0.233)[b]	0.822 (0.352)[a,b]
Cerebellum	4	Arc[*,§]	1.000 (0.062)[a]	0.923 (0.042)[a]	0.895 (0.092)[a]	0.532 (0.083)[c]	0.573 (0.130)[b,c]	0.786 (0.147)[a,b]
Hippocampus	8	TNF-α[*]	1.000 (0.127)[a]	1.338 (0.094)[a]	2.093 (0.136)[b]	1.002 (0.146)[a]	1.200 (0.131)[a]	1.965 (0.138)[b]
Hippocampus	8	IL-1RA[*]	1.000 (0.491)[a,c]	0.909 (0.204)[a]	1.880 (0.203)[b,c]	1.537 (0.249)[a,b,c]	1.008 (0.203)[a,c]	2.280 (0.103)[b]
Hippocampus	8	Arc[#]	1.000 (0.127)[a,b]	0.972 (0.108)[a,b]	1.124 (0.159)[a]	0.904 (0.141)[a,b]	0.782 (0.094)[b]	0.869 (0.106)[a,b]
Hypothalamus	8	Arc[#,†]	1.000 (0.088)[a]	1.066 (0.087)[a]	0.770 (0.194)[a,b]	0.610 (0.111)[b,c]	0.473 (0.205)[c]	0.674 (0.118)[b,c]
Cortex	8	TNF-α[*]	1.000 (0.224)[a,b]	1.092 (0.094)[a,b]	1.645 (0.264)[a]	0.732 (0.140)[b]	1.395 (0.336)[a]	1.454 (0.212)[a]
Cortex	8	IL-1RA[*,§]	1.000 (0.159)[a]	1.109 (0.067)[a]	3.043 (0.196)[b]	1.199 (0.183)[a]	1.188 (0.157)[a,b]	2.630 (0.085)[b]
Hippocampus	12	TNF-α[*,†]	1.000 (0.176)[a]	1.497 (0.146)[b,c]	1.890 (0.136)[c]	1.123 (0.123)[a,b]	1.173 (0.111)[a,b]	2.409 (0.152)[c,d]
Hippocampus	12	IL-1RA[*]	1.000 (0.261)[a]	1.873 (0.241)[b]	2.391 (0.210)[b]	1.731 (0.167)[a,b]	1.692 (0.275)[a,b]	2.991 (0.123)[b]
Cortex	12	TNF-α[*,#]	1.000 (0.191)[a]	1.131 (0.172)[a,b]	1.732 (0.149)[b]	1.131 (0.202)[a,b]	1.154 (0.165)[a,b]	2.899 (0.205)[c]
Cortex	12	IL-1RA[*,†,§]	1.000 (0.076)[a]	1.157 (0.055)[a]	2.454 (0.067)[b]	1.374 (0.113)[a]	1.228 (0.117)[a]	3.499 (0.113)[b]
Hippocampus	24	TNF-α[*]	1.000 (0.110)[a]	1.109 (0.071)[a,b]	1.529 (0.158)[b,c]	1.050 (0.070)[a,b]	1.166 (0.263)[a,b]	1.807 (0.147)[c]
Hippocampus	24	IL-1RA[*]	1.000 (0.218)[a,b]	0.804 (0.306)[a]	1.133 (0.159)[a,b]	0.894 (0.175)[a,b]	1.049 (0.337)[a,b]	1.524 (0.097)[b]
Cortex	24	TNF-α[*]	1.000 (0.227)[a]	1.108 (0.100)[a,c]	1.803 (0.154)[b,c]	1.073 (0.146)[a]	1.139 (0.254)[a,c]	2.332 (0.184)[b]
Cortex	24	IL-1RA[*,†,]	1.000 (0.215)[a,b]	0.662 (0.169)[a]	1.054 (0.141)[a,b]	0.697 (0.164)[a]	0.855 (0.304)[a]	1.580 (0.123)[b]
Cortex	24	Arc[*,†]	1.000 (0.191)[a]	0.648 (0.123)[a,b,c]	0.431 (0.060)[c]	0.564 (0.126)[b,c]	0.678 (0.170)[a,b]	0.747 (0.258)[a,b]

Results are expressed as relative fold change in mRNA expression (ΔmRNA), means (s.e.m.); $n = 6$. Results within individual rows without a common superscript letter are different ($P < 0.05$).
[*] $P < 0.05$, significant main effect of dose.
[#] $P < 0.05$, significant main effect of restraint-240.
[†] $P < 0.05$, significant dose-restraint-240 interaction.).
[§] Indicates data was transformed using $((\text{Original value})^2)^{1/4}$.

2006) which is underscored by the establishment of 5 cSv (equivalent to 5 cGy of gamma radiation) as the annual occupational radiation exposure limit by the Nuclear Regulatory Commission (NRC) (Code of Federal Regulations, 1998).

As delineated above, little work has been performed examining the early impact (<24 h) of low-dose (⩽2 Gy) ionizing radiation on the neuroimmune system. Fig. 1 demonstrates that a single dose of gamma radiation as low as 50 cGy reduces mouse locomotor activity 6 h after exposure. When social exploration was examined at radiation doses of 50 cGy and 200 cGy neither dose impaired mouse exploratory behavior (Table S1). These findings indicate that low-dose ionizing gamma radiation appears to perturb unmotivated behaviors to a greater extent than motivated behaviors. Such results are similar to findings we have observed in high-fat diet (HFD) fed mice where the HFD state causes a decrease in spontaneous locomotion (Lavin et al., 2011) that is not reflected by a loss of social exploration (Sherry et al., 2009). Customarily, strong activators of the neuroimmune system like lipopolysaccharide (LPS) induce both locomotor retardation and social withdrawal (Dantzer, 2004). As we have shown, HFD-feeding appears to be a very weak stimulator of CNS inflammation (Lavin et al., 2011). Consequently, from a biobehavioral standpoint, low-dose radiation is at best a very weak immediate activator of neuroimmunity.

To demonstrate that the irradiation given could activate the neuroimmune system, pro-inflammatory cytokine gene transcripts were measured in whole brains from mice administered both 50 cGy and 200 cGy of gamma radiation. Fig. 2 shows that TNF-α is increased 6 h post irradiation in mice receiving 200 cGy. When un-perfused brains were compared to perfused brains, 50 cGy increased TNF-α transcripts in un-perfused brains. To determine if this boost in transcripts was due to an increase in blood TNF-α transcripts, whole blood TNF-α transcripts were examined and found to be unaffected by gamma radiation. Blood, however, did show significant transcript up-regulation of the inflammatory bioactives IL-1β and IL-1RA 8 h post 200 cGy gamma irradiation (Fig. 3) and these blood transcripts were present in the brain (Fig. 3). Taken together these findings indicate that low-dose gamma radiation activates the neuroimmune system relatively rapidly. How radiation triggers this response is not clear.

Previous work with high-dose radiation in mice (15 Gy) has demonstrated that irradiation of the body without irradiating the head induces up-regulation of proinflammatory cytokine transcripts in the brain (Marquette et al., 2003). This work was designed to support the concept that a stimulated peripheral innate immune system could communicate with the brain as is seen with peripheral LPS administration (Dantzer, 2004). Although not designed to test this question, our work is supportive in that blood present in the brain contains cells with increased IL-1β gene transcripts. Further support for this concept was seen in the proton radiation experiments because proton irradiation at both 50 cGy and 200 cGy did not disturb either locomotion or social exploration (data not shown, Table S1, respectively). Proton radiation is of higher energy than gamma radiation (Ni et al., 2011) and contributes more significantly to SPEs (Cengel et al., 2010; Ni et al., 2011). Given this higher energy, we expected proton radiation to impact behavior more than gamma radiation. Proton radiation, however, has a different linear energy transfer profile than gamma radiation. The Bragg peak for gamma radiation is likely more toward the skin surface as opposed to proton radiation where the Bragg peak is likely skewed to the interior of the animal. This difference in ionization location further supports the potential importance of peripheral immune activation to radiation-induced neuroimmune activation. Interestingly, blood also showed an up-regulation of IL-1RA transcripts indicating (in conjunction with the IL-1β transcript data noted above) that the IL-1 arm of the innate immune system is an early pathway activated by radiation. Organ systems with high radiation sensitivity include the hematopoietic system where radiation increases mitochondrial-dependent reactive

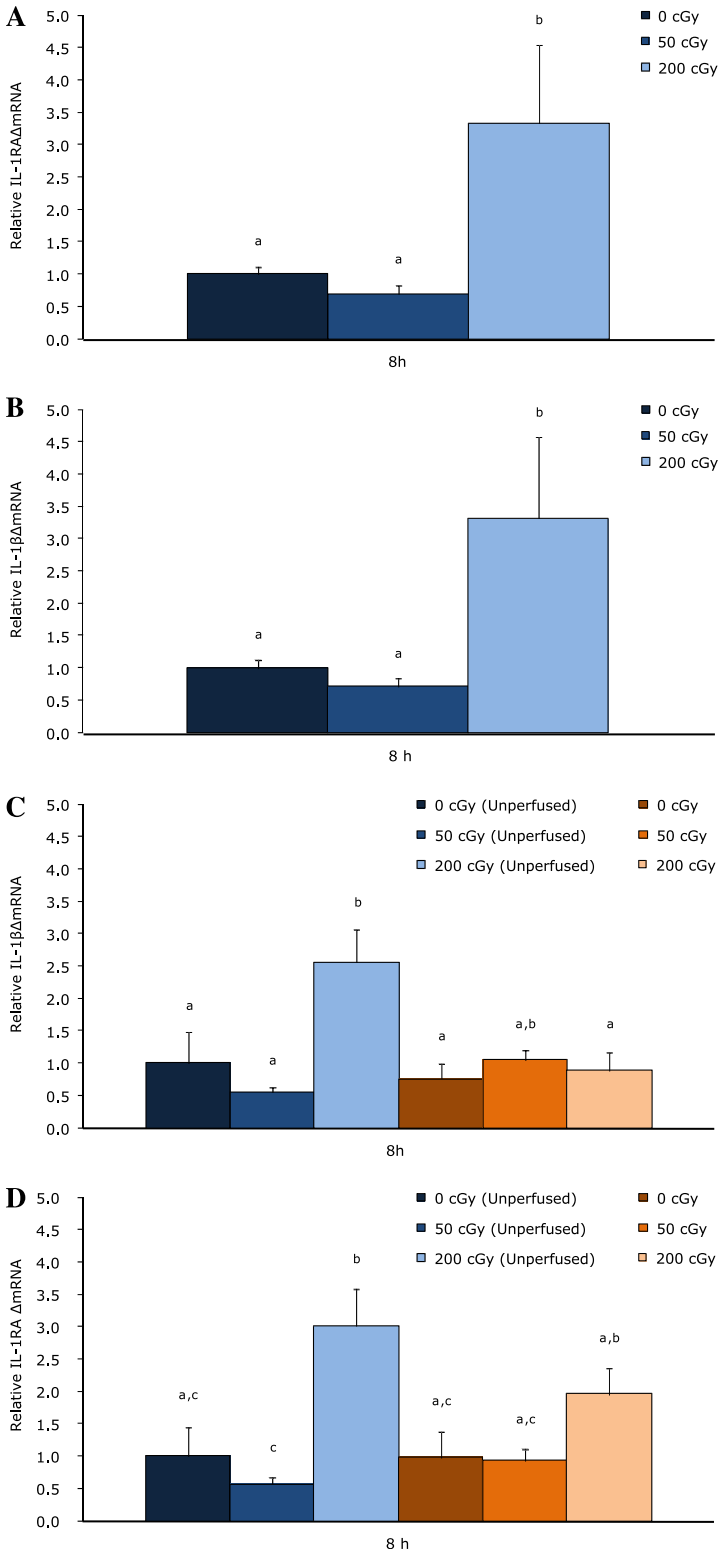

Fig. 3. Gamma radiation up-regulates gene transcripts for IL-1β and IL-1RA in blood 8 h post irradiation. Restraint-10 mice were exposed to 50 or 200 cGy of gamma radiation (44.5 ± 0.1 cGy/min) as indicated. qPCR was used to quantify mRNAs from blood and un-perfused and perfused whole brains as indicated 8 h post irradiation. Results are expressed as relative fold change in mRNA expression (ΔmRNA), means ± s.e.m.; $n = 4–6$. (A) IL-1β: main effect of dose ($P < 0.001$); $P < 0.05$, 200 cGy *v* sham IR, 50 cGy (3.312 ± 0.468 *v* 1.000 ± 0.182, 0.711 ± 0.254, respectively). (B) 8 h IL-1RA: main effect of dose ($P < 0.001$); $P < 0.05$, 200 cGy *v* sham IR, 50 cGy (3.335 ± 0.446 *v* 1.000 ± 0.146, 0.690 ± 0.273, respectively). (C) IL-1β: main effect of dose ($P = 0.006$), dose-perfusion interaction ($P = 0.002$); $P < 0.05$, 200 cGy (unperfused) *v* sham IR (unperfused), 50 cGy (unperfused), sham IR, 50 cGy (2.555 ± 0.267 *v* 1.000 ± 0.570, 0.557 ± 0.182, 0.755 ± 0.407, 1.052 ± 0.189, respectively). (D) IL-1RA: main effect of dose ($P < 0.001$); $P < 0.05$, 200 cGy (unperfused) *v* sham IR (unperfused), 50 cGy (unperfused), sham IR, 50 cGy (3.013 ± 0.255 *v* 1.000 ± 0.531, 0.570 ± 0.244, 0.982 ± 0.488, 0.935 ± 0.258, respectively); $P = 0.009$, 200 cGy *v* 50 cGy (unperfused) (1.961 ± 0.267 *v* 0.570 ± 0.244). Bars without a common superscript are different ($P < 0.05$).

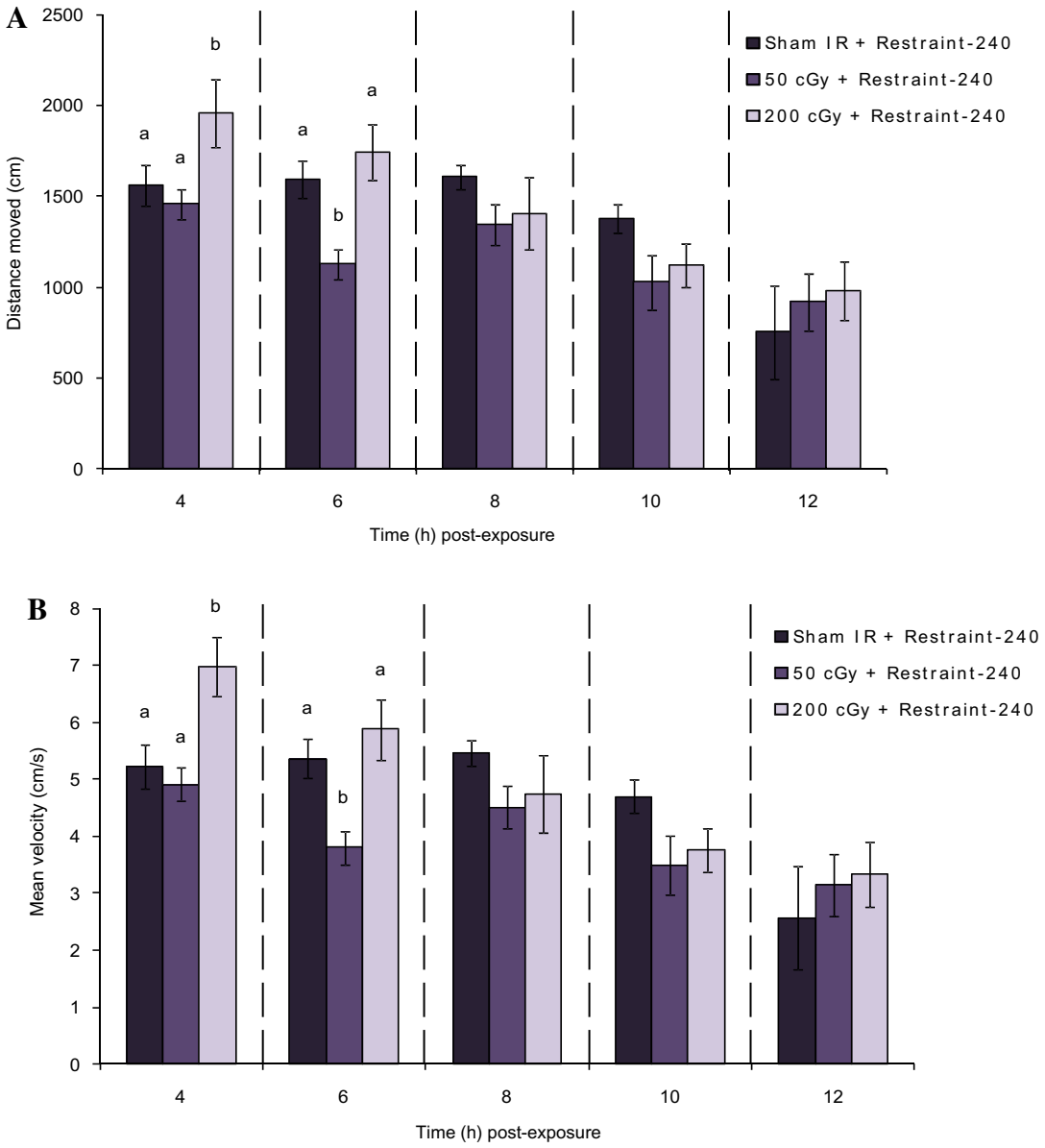

Fig. 4. Restraint-240 inhibits the impact of 200 cGy gamma radiation on locomotor activity. Restraint-240 mice were exposed to 50 or 200 cGy of gamma radiation (44.5 ± 0.1 cGy/min) as indicated. Spontaneous locomotor activity and velocity of movement were measured at the time points indicated post irradiation. Results are expressed as means ± s.e.m.; n = 8–16. (A) Distance moved (cm): main effects of dose (P = 0.004) and time point (P < 0.001), dose-time point interaction (P = 0.036); 4 h time point: $P \leqslant 0.05$, sham IR + restraint-240 *v* 200 cGy + restraint-240 (1559.1 ± 111.5 *v* 1955.8 ± 188.1), 50 cGy + restraint-240 *v* 200 cGy + restraint-240 (1457.3 ± 85.6 *v* 1955.8 ± 188.1); 6 h time point: P < 0.05, sham IR + restraint-240 *v* 50 cGy + restraint-240 (1592.9 ± 101.8 *v* 1127.7 ± 82.0), 50 cGy + restraint-240 *v* 200 cGy + restraint-240 (1127.7 ± 82.0 *v* 1743.9 ± 151.2). (B) Velocity of movement (cm/s): main effects of dose (P = 0.003) and time point (P < 0.001), dose-time point interaction (P = 0.013); 4 h time point: P < 0.05, sham IR + restraint-240 *v* 200 cGy + restraint-240 (5.2 ± 0.4 *v* 7.0 ± 0.5), 50 cGy + restraint-240 *v* 200 cGy + restraint-240 (4.9 ± 0.3 *v* 7.0 ± 0.5); 6 h time point: P < 0.05, sham IR + restraint-240 *v* 50 cGy + restraint-240 (5.4 ± 0.4 *v* 3.8 ± 0.3), 50 cGy + restraint-240 *v* 200 cGy + restraint-240 (3.8 ± 0.3 *v* 5.9 ± 0.5). Bars without a common superscript are different (P < 0.05).

oxygen species (ROS) generation (Xiao and Whitnall, 2009). Importantly, ROSs have been shown recently to stimulate the NALP3 inflammasome (one of three inflammasomes responsible for activation of caspase-1) which is required for the conversion of pro-IL-1β into mature secretable IL-1β (Schroder et al., 2010). Curiously, little work has been performed examining radiation and inflammasome activation. Given the reduction of locomotion observed after 50 cGy of radiation and the lack of pro-inflammatory transcript changes, radiation-dependent triggering of the inflammasome with production of mature IL-1β, as seen with the NALP3 inflammasome and uric acid (Martinon et al., 2006), seems likely. TNF-α gene transcript up-regulation may be secondary to the IL-1β signal if that signal is significantly robust.

How pro-inflammatory cytokines induce fatigue is still imprecise. Although CNS IL-1 (Patarca, 2001) and TNF-α are implicated (Cavadini et al., 2007; Foley, 2007), the mechanistic connection remains elusive. Some believe the indoleamine-2,3-dioxygenase (IDO) pathway may be important (Capuron et al., 2011) because altered serotonergic (5-hydroxytryptamine, (5-HT)) neurotransmission is identified in patients with chronic fatigue syndrome (Smith et al., 2008). In addition, pro-inflammatory cytokines, especially TNF-α, provoke the brain-based IDO pathway to convert the serotonin precursor tryptophan to kynurenine which affords production of potential neurotoxic kynurenine metabolites (Dantzer et al., 2008, 2011). With radiation-induced fatigue, however, the IDO pathway seems an unlikely player because blockade of 5-HT

is a key first line defense for the inhibition of radiation-induced nausea and vomiting in radiotherapy patients (Monroe et al., 2008) and there is no clear evidence that inhibition of these prodromal ARS symptoms with 5-HT antagonists ameliorates subsequent radiation-induced fatigue.

Low dose-rate gamma radiation was also examined and found not to perturb locomotor or social exploratory behavior (data not shown, Table S1, respectively). The most likely explanations for these observations were that low-dose rate radiation (50 cGy and 200 cGy of radiation delivered over 240 min as opposed to 10 min as in Fig. 1 (high-dose rate)) did not impart significant energy to activate the neuroimmune system or that the mice in the low-dose rate experiments were restrained for 240 min as opposed to 10 min. To investigate these possibilities, more prolonged restraint experiments were performed in which mice were delivered 50 cGy and 200 cGy (both at high-dose rate) during the first 10 min of restraint then left restrained for an additional 230 min. Fig. 4 reveals that restraint-240 ameliorated the effect of 200 cGy on locomotion but not that of 50 cGy. Unexpectedly, restraint-240 produced hyper-mobility in mice irradiated with 200 cGy suggesting that restraint-240 may sensitize mice to biobehavioral stimuli like it does for cutaneous hypersensitivity (Flint and Tinkle, 2001). Importantly, restraint-240 had no impact on locomotor activity of sham irradiated mice (data not shown) which is consistent with the majority of work examining restraint (Buynitsky and Mostofsky, 2009). Taken together these findings indicate that early radiation-induced alterations in biobehaviors requires a threshold dose rate that can be potentially modulated by the stress response. Furthermore, since the bulk of the time restrained was spent after irradiation these results point to a radiation priming-like interaction which occurs at 200 cGy but not at 50 cGy because the 50 cGy irradiated restraint-240 mice behaved like restraint-10 50 cGy irradiated mice (Fig. 1).

In general, the overall impact of restraint tends towards immune suppression (Buynitsky and Mostofsky, 2009). Table 1 shows that immediately after restraint-240 (4 h post-irradiation) sham irradiated restraint-240 mice had a decrease in hippocampal, hypothalamic, cortical and cerebellar TNF-α transcripts compared to sham irradiated restraint-10 mice. 200 cGy irradiation prevented this down-regulation while 50 cGy had little effect. As time post-irradiation progressed, TNF-α transcripts increased showing a movement toward resolution by 24 h post irradiation. Unlike whole brain at 8 h post irradiation (Fig. 3D), brain regions showed region-specific increases in IL-1RA gene transcripts. At 8 h post-irradiation, hippocampal IL-1RA gene expression was increased especially in the cortex (Table 1). Peak IL-1RA expression occurred near 12 h post-irradiation with resolution before 24 h. IL-1RA expression patterns somewhat mimicked that of TNF-α but were expressed in the brain later and resolved quicker. This pattern fits with a NALP3 inflammasome driven biobehavioral process (Johnson et al., 2007).

Finally, activity-regulated cytoskeletal-associated protein (Arc) was up-regulated by restraint-240 in the hippocampus as previously reported (Mikkelsen and Larsen, 2006). Arc is an immediate early gene induced in hippocampal and parietal neurons following behavioral experiences best tied to maintenance of long-term potentiation and spatial memory consolidation (Rosi et al., 2008). Inflammation and proinflammatory cytokines, including TNF-α, are shown to reduce Arc gene transcript expression (Centonze et al., 2010). Unexpectedly, restraint-240 led to decreased Arc in the cerebellum. What function Arc has in the cerebellum is not clear but it may play a role in cerebellar associative learning as evoked in such responses as eyeblink conditioning (Kim and Thompson, 2011). 200 cGy gamma irradiation led to a decrease in whole brain Arc gene transcripts at 6 h. Brain region analysis (Table 1) showed that this effect was transient in that it was not evident at 4 or 8 h post irradiation. At 24 h post irradiation (restraint-10 200 cGy), Arc transcripts were reduced in the cortex which may fit with post-radiation cognitive deficits that can manifest after whole brain irradiation, although acute radiation encephalopathy is rare at doses under 300 cGy (Soussain et al., 2009). Taken together these findings indicate that low-dose gamma radiation affects the cortex and hippocampus of mice with changes that can last at least 24 h post-irradiation. Up-regulated TNF-α appears linked to down-regulated Arc. Since manned space and commercial air travel carry a significant risk of SPE exposure in conjunction with physical and psychological stress (extensively studied in astronauts (Morphew, 2001) and more recently recognized in airline passengers (economy class syndrome (Dalen, 2003; Grajewski et al., 2011))), restraint stress may modulate early radiation-induced fatigue due to brain-based immunosuppression (DeLano and Mallery, 1998). However, this restraint-240-dependent reduction in TNF-α gene expression is short-lived and cortical TNF-α is higher in irradiated restraint-240 animals at 12 and 24 h post irradiation when compared to restraint-10 animals. More work is required to understand if and/or how early neuroimmune activation contributes to radiation-induced fatigue-like responses, especially when conjoined to the stress response.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbi.2011.09.006.

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