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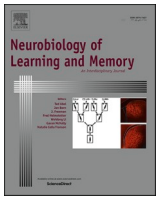

Systemic HDAC3 inhibition ameliorates impairments in synaptic plasticity caused by simulated galactic cosmic radiation exposure in male mice

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ABSTRACT

Deep space travel presents a number of measurable risks including exposure to a spectrum of radiations of varying qualities, termed galactic cosmic radiation (GCR) that are capable of penetrating the spacecraft, traversing through the body and impacting brain function. Using rodents, studies have reported that exposure to simulated GCR leads to cognitive impairments associated with changes in hippocampus function that can persist as long as one-year post exposure with no sign of recovery. Whether memory can be updated to incorporate new information in mice exposed to GCR is unknown. Further, mechanisms underlying long lasting impairments in cognitive function as a result of GCR exposure have yet to be defined. Here, we examined whether whole body exposure to simulated GCR using 6 ions and doses of 5 or 30 cGy interfered with the ability to update an existing memory or impact hippocampal synaptic plasticity, a cellular mechanism believed to underlie memory processes, by examining long term potentiation (LTP) in acute hippocampal slices from middle aged male mice 3.5–5 months after radiation exposure. Using a modified version of the hippocampus-dependent object location memory task developed by our lab termed "Objects in Updated Locations" (OUL) task we find that GCR exposure impaired hippocampus-dependent memory updating and hippocampal LTP 3.5–5 months after exposure. Further, we find that impairments in LTP are reversed through one-time systemic subcutaneous injection of the histone deacetylase 3 inhibitor RGFP 966 (10 mg/kg), suggesting that long lasting impairments in cognitive function may be mediated at least in part, through epigenetic mechanisms.

1. Introduction

Deep space travel presents a number of measurable risks including exposure to cosmic radiation with a spectrum of qualities capable of penetrating the spacecraft, traversing the body and impacting brain function (Cucinotta et al., 2008; Cucinotta, 2014; Nelson, 2016; Norbury et al., 2016). Studies of limited sample sizes of predominantly male astronauts have linked long-duration space flights to changes in brain structure that correlated with altered cognitive performance, although precisely how those changes track with radiation exposure remains uncertain (Roberts et al., 2019). Although available shielding technology is capable of minimizing space radiation health risks for missions in low Earth orbit such as the International Space Station, no existing shielding technology is currently capable of preventing charged-particle radiation exposure that would inevitably occur during a mission to Mars (Nelson et al., 2016). Space radiation includes protons, helium nuclei, and high (H) atomic number (Z) and energy (E), or HZE particles, a mixture of ionized nuclei that defines galactic cosmic radiation (GCR) (Cucinotta, 2014). Direct measurements of the radiation fields in space provide information on the charged particles that make up GCR and contribute to the doses that astronauts would be expected to receive beyond the earth's protective magnetosphere (Nelson, 2016). Such information provides scientists the opportunity to simulate these exposures on earth using animal models (Simonsen et al., 2020) in efforts to

Abbreviations: GCR, galactic cosmic radiation; OUL, objects in updated locations; LTP, long term potentiation; HDAC3, histone deacetylase 3; cGy, centigray; OLM, object location memory; DI, discrimination index.

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extrapolate these findings to human health risks. Based on the duration and specifics of a given round trip mission to Mars, total doses from all space radiation sources (solar and GCR) are not likely to exceed 0.5 Gy (Nelson, 2016; Simonsen et al., 2020).

Estimates on human brain models project that GCR exposures will lead to a direct hit to roughly 25% of the estimated 43 million hippocampus neurons by one or more of these high-energy charged particles (Curtis et al., 1998). Using rodents, studies have reported that exposure to these high-energy charged particle irradiations lead to cognitive impairments that are associated with changes in hippocampus function (Machida et al., 2010; Britten et al., 2012, Britten et al., 2016a, Britten et al., 2016b; Cherry et al., 2012; Haley et al., 2013; Tseng et al., 2014; Acharya et al., 2017; Kiffer et al., 2018; Krukowski et al., 2018b; Parihar et al., 2018), negatively affecting both short (Britten et al., 2012, 2017; Acharya et al., 2017; Kiffer et al., 2018; Krukowski et al., 2018b) and long-term memory (Krukowski et al., 2018a, 2018b; Parihar et al., 2018) in tasks engaging the hippocampus in young to mature adult rodents. Further, these cognitive impairments correspond with a number of physiological changes to the hippocampus as a result of GCR exposure including microglial activation and synaptic loss (Krukowski et al., 2018b; Allen et al., 2020), compromised dendritic morphology (Parihar et al., 2015; Kiffer et al., 2018), reductions in myelination and synaptic density (Dickstein et al., 2019), reduced numbers of NMDA receptor subunits (Machida et al., 2010) and impairments in hippocampal long term potentiation (LTP) (Vlkolinský et al., 2007).

Importantly, impairments in hippocampus function have been shown to persist for at least one-year post single ion GCR irradiation with no evidence of attenuation (Parihar et al., 2018). Although the underlying mechanisms responsible for the long-lasting impact of GCR exposure on hippocampus function are unknown, epigenetic modifications serve as a likely candidate. The ability to form long-term memories is largely reliant on changes in gene expression, which are coordinated, in part, through epigenetic mechanisms that modulate transcriptional processes (Burgess-beusse et al., 2002; Horn and Petersen, 2002; Mozzetta et al., 2014; Korb et al., 2016; Campbell and Wood, 2019). Recent findings suggest a critical role for DNA methylation in the hippocampus of mice exhibiting radiation-induced cognitive impairments where reductions in radiation-induced hypermethylation led to restored short-term object recognition memory (Acharya et al., 2017). Additionally, we have previously reported in non-irradiated young and aging mice, that histone deacetylase 3 (HDAC3) serves as a critical negative regulator of memory formation (McQuown et al., 2011; Malvaez et al., 2013; Rogge et al., 2013; Bieszczad et al., 2015; Alaghband et al., 2017; Kwapis et al., 2019b) as inactivation or deletion of HDAC3 in the hippocampus enables learning in conditions that are normally subthreshold for encoding (Stefanko et al., 2009; McQuown et al., 2011; Malvaez et al., 2013) and allows memory to persist beyond a time when normal memories fail (Stefanko et al., 2009; McQuown et al., 2011). Therefore, manipulation of epigenetic mechanisms that are associated with active transcription faciliate learning and memory in both irradiated and non-irradiated mice.

In this study, we examined whether whole body exposure to a mission-relevant dose of mixed-ion GCR using doses of 5 or 30 cGy interfered with the ability to update an existing memory in middle aged male mice 18–23 weeks after exposure using a modified version of the hippocampus-dependent object location memory task developed by our lab termed “Objects in Updated Locations” (OUL) task (Kwapis et al., 2019a). We find that given sufficient over-training, mice exposed to GCR are able to form a memory for object location but are unable to update this original memory to incorporate new information. We find that exposure to 30 cGy also results in impairments in hippocampal long term potentiation, a proposed cellular mechanism underlying aspects of memory. Given that epigenetic modifications, specifically histone acetylation, are critical in memory formation, we sought to determine whether selective inhibition of the repressive histone deacetylase 3 enzyme is able to improve long term potentiation in male mice exposed to GCR. Disruption of HDAC3 activity via subcutaneous injection of the HDAC3 inhibitor RGFP 966 (10 mg/kg) was found to ameliorate radiation-induced impairments in synaptic plasticity. Together, these data are the first to provide deeper insight into how exposure to GCR impacts the ability to update existing information long after exposure and suggests that impairments in memory updating and synaptic plasticity may be mediated at least in part, through epigenetic mechanisms.

2. Materials and methods

2.1. Subjects

Five-month-old wild-type male mice (C57Bl/6J, Jackson Laboratory, Bar Harbor ME) were ordered to and acclimatized at the NASA Space Radiation Laboratory (NSRL), Brookhaven National Laboratory (BNL, Upton, NY) for ~4 weeks prior to initiation of the study. The mice were group housed under standard conditions (20 °C ± 1 °C; 70% ± 10% humidity; 12 h:12 h light and dark cycle) and provided *ad libitum* access to food and water. Mice were exposed to GCR at 6 months of age and following exposure were transported to University of California, Irvine for behavioral and electrophysiology experiments. Mice were ~9.5 months old at the time of behavioral training in the Objects in Updated Locations (OUL) task. The colony room was adjacent to the behavioral testing room and lights were maintained on a 12 h:12 h light and dark cycle (20 °C ± 1 °C; 70% ± 10% humidity) and provided *ad libitum* access to food and water. All behavioral testing was performed during the light cycle. All experiments were conducted according to US National Institutes of Health guidelines for animal care and use and were approved by the Institutional Animal Care and Use Committee of the Brookhaven National Laboratory and the University of California, Irvine.

2.2. Irradiation

During whole body irradiation mice at 6 months of age were loosely restrained in Lucite containers with breathing holes (3 in. × 1.5 in. × 1.5 in.) and oriented with their long axis parallel to the beam for exposure to 5 cGy or 30 cGy using the simplified 6-beam GCR simulation (experimental cycle NSRL18B). The 6 charged particle species were delivered in rapid succession to simulate the spectrum of radiations experienced during a deep space mission while inside of a vehicle (Nelson, 2016; Norbury et al., 2016). The 6 exposures were delivered in the order, energy, linear energy transfer (LET) and dose fractions as shown.

Ion (energy)	LET	Dose Fraction	5 cGy (Approx Total Dose)	30 cGy (Approx Total Dose)
H (1 GeV)	0.2	0.35	1.75	10.50
Si (600 MeV/n)	50.4	0.01	0.05	0.30
He (250 MeV/n)	1.6	0.18	0.90	5.40
O (350 MeV/n)	20.9	0.06	0.30	1.80
Fe (600 MeV/n)	173.8	0.01	0.05	0.30
H (250 MeV)	0.4	0.39	1.95	11.70

The NSRL physics staff performed all radiation dosimetry and confirmed spatial beam uniformity. Concurrent control mice were placed in the Lucite boxes at the NSRL for the same length of restraint time as required for the radiation exposure, approximately 20 min. Following irradiation or sham (non) irradiation mice were returned to their respective home cages and 5 days later shipped to the University of California, Irvine.

2.3. Memory updating OUL task

2.3.1. Apparatus

The Objects in Updated Locations (OUL) task took place in an apparatus consisting of four identical chambers (61 × 46 × 27 cm) constructed of opaque white Plexiglas. Each box included an identically placed strip of black duct tape to serve as the orienting mark. The boxes were open in the room, allowing for extra-maze cues to be used for spatial orientation (shelf, walls, lighting placement, etc.) and these cues remained stable and un-moved throughout the experiment. Boxes were cleaned with 10% ethanol in between animals and were cleaned with 70% ethanol following behavioral experiments each day. House lights remained off with lamps positioned to allow each box to be equally illuminated at ~50 lx. Identical 200-mL tall-form glass beakers filled with cement were used in the OUL task. All sessions were recorded for offline analysis of object exploration using anymaze tracking software.

2.3.2. Experimental design

Prior to the beginning of behavioral experiments mice were handled for 2 min each day for four consecutive days. This handling protocol has been successfully implemented for both young and aging mice prior to the start of behavior (Vogel-Ciernia and Wood, 2015; Kwapis et al., 2018, 2019a, 2019b; Butler et al., 2019). Mice were then habituated to the context (in the absence of objects) for 6 consecutive days and were allowed to explore the context for 5 min each day (Fig. S1). Distance

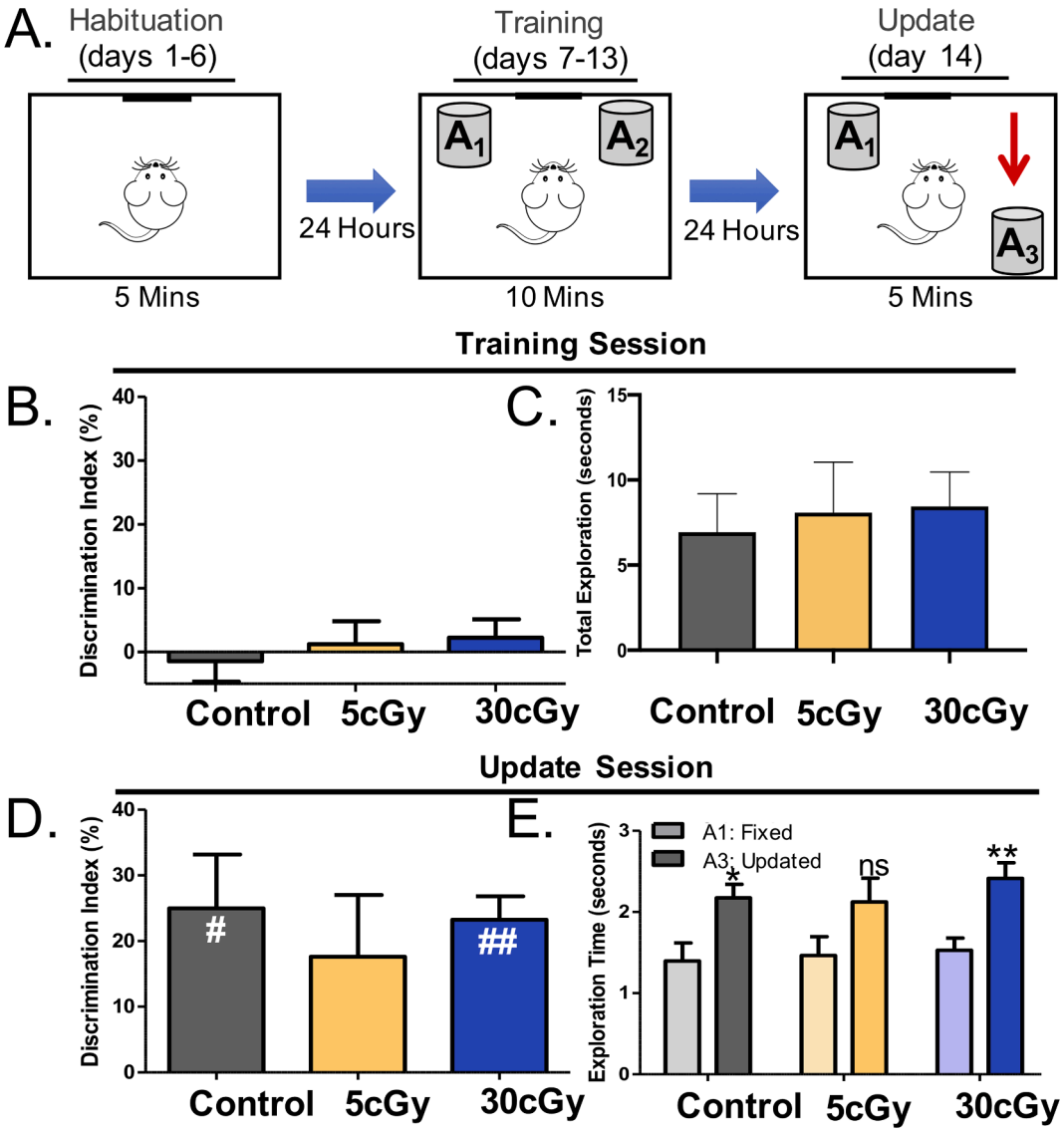

Fig. 1. Extensive over-training enables long term memory formation in mice exposed to galactic cosmic radiation (GCR). A. Experimental design. Black tape serves as the spatial navigation cue and denotes orientation in the box. Red arrow denotes that an object has been moved. B. Discrimination index (DI) scores during training day 1. C. Total amount of time in seconds exploring objects during training. Mice from all 3 groups display a low DI, indicating no preference for object A1 or A2 and have similar levels of total object exploration (C). D. DI scores during update session. With 7 days of training, GCR exposed mice perform equally well in the object location memory task compared with control. E. Amount of time in seconds exploring the original object position (A1) or the updated position (A3). Control and the 30 cGy GCR dose group spent more time exploring the updated object location (A3) compared to the original object location (A1). Data are presented as mean ± SEM; (control: n = 10, 5 cGy: n = 9, 30 cGy n = 11), # $p < 0.05$, ## $p < 0.01$ compared with training day 1 (within group). * $p < 0.05$, ** $p < 0.01$ compared to original object location (A1).

traveled and speed was examined during habituation sessions using ANY-maze behavioral analysis software (Stoelting Co). Following handling and habituation mice underwent object location memory training for 10 min per day for 7 consecutive days in which 2 identical objects (200-mL tall-form glass beakers filled with cement) were positioned in distinct locations (location A1 and location A2). Following the last training day, mice were given a 5-minute update session in which one object was moved to a new location in a counterbalanced fashion (location A3). The update session used in OUL is identical to a test session of a traditional object location memory experiment (Vogel-Ciernia and Wood, 2015; Lopez et al., 2016; Alaghband et al., 2017; Kwapis et al., 2018) where time spent exploring the updated (location A3) vs. familiar/ fixed location (A1) is examined (Fig. 1) to assess memory for the original training session. To keep measurements as objective as possible, we employed a fairly strict definition of object exploration; although mice may have spent more time around a particular object, exploration was scored only when the mouse directly interacted with the object with the head oriented toward the object and came within 1 cm or when the nose touched the object (Vogel-Ciernia and Wood, 2015); time spent standing on the object or rearing on the object was not counted. Strong memory for the training session is evidenced by greater exploration of the object in a new location given a preference for novelty in mice. Therefore, the update session allowed us to examine whether mice learned the original training information in addition to updating the original memory. Preference for the novel location was expressed as a discrimination index (DI): (tA3 − tA1)/(tA3 + tA1) × 100%, where t indicates the time spent exploring the designated object.

The next day, mice were tested for 5 min with all 4 identical objects in distinct locations (A1, A2, A3 and a fourth object in a novel location, called A4) to determine whether the original memory was updated to incorporate the new location information as described previously in our studies (Kwapis et al., 2019a). Time spent exploring the novel object location (A4) compared with the updated (A3), fixed (A1) and moved (A2) object location was examined (Fig. 2). Memory for the original training information was assessed by calculating a DI comparing exploration of the novel location (A4) to objects in the original training locations (A1): DI = (tA4 − tA1)/(tA4 + tA1) × 100% and (A2): DI = (tA4 − tA2)/(tA4 + tA2) × 100%. Memory for updated information was assessed by calculating a DI comparing exploration of the novel location (A4) to the updated location (A3): DI = (tA4 − tA3)/(tA4 + tA3) × 100%. All habituation, training, testing, and scoring were performed by

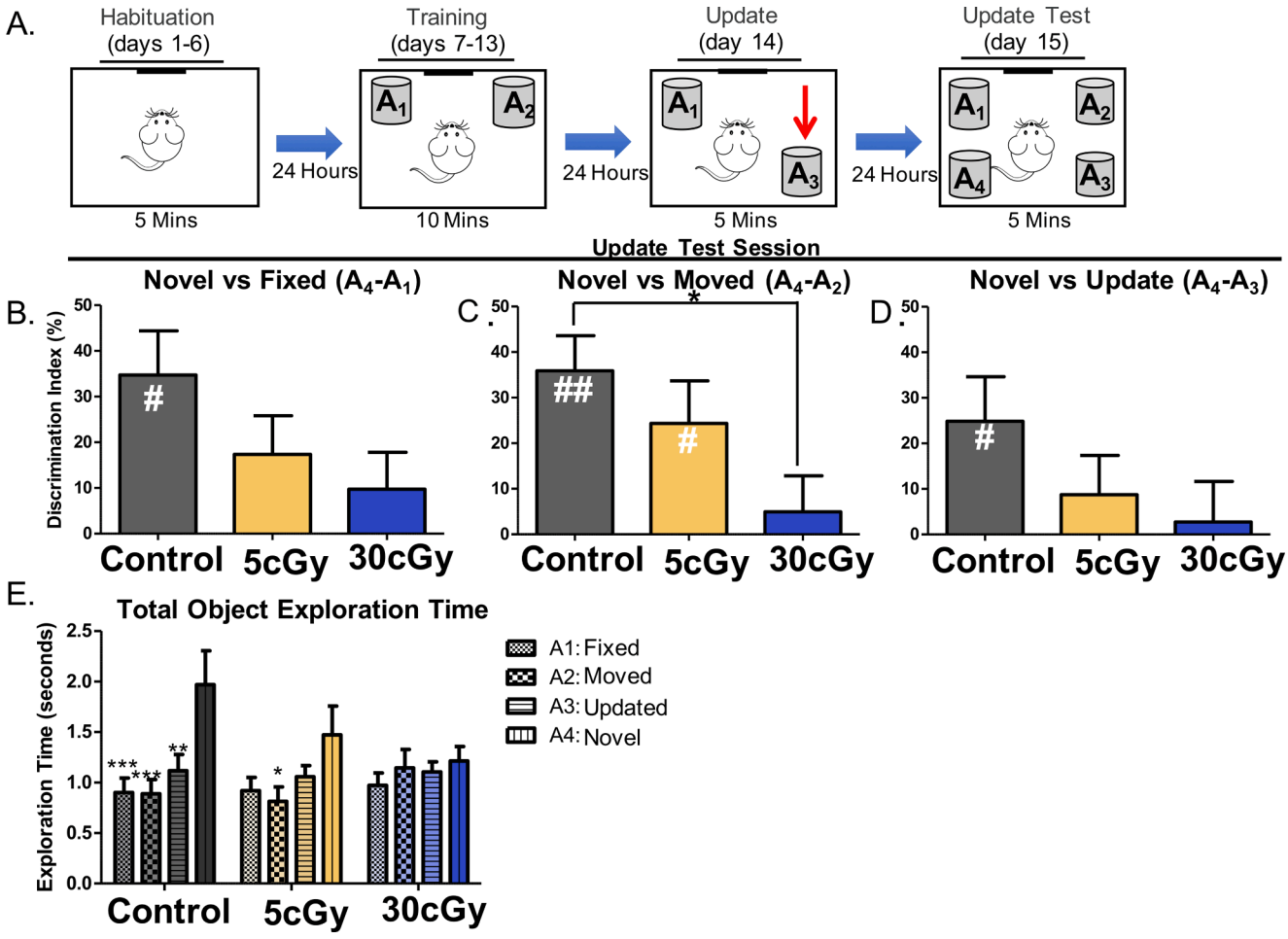

Fig. 2. Memory updating is impaired in mice exposed to galactic cosmic radiation (GCR). A. Experimental design. Black tape serves as the spatial navigation cue and denotes orientation in the box. Red arrow denotes that an object has been moved. B-D. Discrimination index (DI) scores during update test day. Mice from the 30 cGy GCR dose group had a significantly lower DI for the moved novel object (A4) vs the moved object (A2) location (C) compared with control mice. E. Amount of time in seconds exploring the original object position (A1), moved object position (A2), updated object position (A3) or novel object position (A4). Group differences were not observed in overall exploration time. However, the control group spent significantly more time exploring the novel object location compared with the original (A1), moved (A2) and updated (A3) object locations. The 5 cGy group spent significantly more time exploring the novel object location compared with the moved (A2), but not the updated object locations (A3). The 30 cGy group did not differ in the amount of time spent exploring the novel object location compared with original, moved or updated object locations. Data are presented as mean ± SEM; (control: n = 10, 5 cGy: n = 11, 30 cGy n = 12), # $p < 0.05$, ## $p < 0.01$ compared with training day 1 (within group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to novel object location (A4).

experimenters blinded to the experimental conditions using a scoring app to allow for precise exploration time measurements. 3 mice (2 control, 1 5 cGy) that didn't explore all objects for a combined period of at least 2 s were excluded from the study, criteria which is consistent with our previous OUL study (Kwapis et al., 2020). Following completion of behavior, mice were transferred back to their home cage and were used in electrophysiology studies five weeks later.

2.4. Drug administration

RGFP 966 (Abcam), a selective HDAC3 inhibitor was dissolved in DMSO and diluted in a vehicle of 30% hydroxypropyl-β-cyclodextrin and 100 mM sodium acetate, pH 5.4. The final DMSO concentration was 5% for drug and vehicle. Mice were injected with RGFP 966 (10 mg/kg, s.c.) or vehicle and brains were extracted 30 min following injection and hippocampal slices were prepared for electrophysiology experiments as described below. We have previously observed maximum drug concentration in the mouse brain 30 min following subcutaneous injection of RGFP 966 with this effective 10 mg/kg dose (Malvaez et al., 2013), therefore we have adhered to this dosing regimen.

2.5. In vitro hippocampal slice preparation

Five weeks after cessation of behavior, hippocampal slices were prepared as previously described (Vogel-Ciernia et al., 2013; Acharya et al., 2017; Kwapis et al., 2018) from mice exposed to 5 or 30 cGy of GCR for comparison to concurrent controls, some of which received subcutaneous injections of RGFP 966 (Abcam), a selective HDAC3 inhibitor 30 min prior to brain dissection as described above. Following isoflurane anesthesia, mice were decapitated, and the brain was quickly removed and submerged in ice-cold, oxygenated dissection medium containing (in mM): 124 NaCl, 3 KCl, 1.25 KH_2PO_4, 5 $MgSO_4$, 0 $CaCl_2$, 26 $NaHCO_3$, and 10 glucose. Coronal hippocampal slices (320 μm) were prepared using a Leica vibrating tissue slicer (Model:VT1000S) before being transferred to an interface recording containing preheated artificial cerebrospinal fluid (aCSF) of the following composition (in mM): 124 NaCl, 3 KCl, 1.25 KH_2PO_4, 1.5 $MgSO_4$, 2.5 $CaCl_2$, 26 $NaHCO_3$, and 10 glucose and maintained at 31 ± 1 °C. Slices were continuously perfused with this solution at a rate of 1.75–2 ml/min while the surface of the slices were exposed to warm, humidified 95% O_2 / 5% CO_2. Recordings began following at least 2 h of incubation.

Field excitatory postsynaptic potentials (fEPSPs) were recorded from CA1b stratum radiatum apical dendrites using a single glass pipette filled with 2 M NaCl (2–3 MΩ) in response to orthodromic stimulation (twisted nichrome wire, 65 μm diameter) of Schaffer collateral-commissural projections in CA1c stratum radiatum. Pulses were administered 0.033 Hz using a current that elicited a 50% maximal spike-free response. After establishing a 10–20-minute stable baseline, long-term potentiation (LTP) was induced by delivering a single episode of 5 'theta' bursts, each burst consisting of four pulses at 100 Hz and the bursts themselves separated by 200 ms (*i.e.*, theta burst stimulation or TBS). The stimulation intensity was not increased during TBS.

Data in the text are presented as means ± SD, while in the figures as mean ± SEM. The fEPSP slope was measured at 10–90% fall of the slope and data in figures on LTP were normalized to the last 20 min of baseline. Electrophysiological measures were analyzed using a 2-way ANOVA unless otherwise specified in the text and the level of significance was set at $p \leq 0.05$.

2.6. Western blot

Tissue from whole hippocampus was homogenized in RIPA buffer (sc-24948, Santa Cruz Biotech). Protein concentrations were measured using a Bradford protein assay (BioRad) and 30–60 μg total protein lysates were loaded into each lane of a 10% acrylamide gel (BP1408-1, Fisher Sci). Gels were run in Tris/Glycine/SDS running buffer for 60 min at 120 V and blots and transferred in Tris/Glycine/Methanol transfer buffer at 220 mA for 90 min at RT with an ice pack onto PVDF membranes (IPFL 10,100 Millipore). After transferring, membranes were incubated in blocking buffer (5% dry milk/washing buffer) for 1 h, washed in dry milk/Tris/NaCl washing buffer and were then incubated in primary antibodies (1:1000, rabbit anti-cofilin (phospho S3), Abcam, ab12866); (1:5000, mouse anti- ß-Actin (AC-15), Abcam, ab6276) in 1.5% BSA/washing buffer overnight at 4 °C on shaker. The membranes were then washed and incubated in their respective secondary antibodies (1:5,000) from LiCOR: rabbit IRDye 680RD and mouse IRDye 800CW. Proteins were detected with LiCor Image System (scanner) and quantification of protein expression was performed using LiCor Image Studio Light software.

2.7. Statistical analysis

Sample sizes in this study were similar to those generally used in the field, including those reported in previous publications (Vogel-Ciernia et al., 2013; Kwapis et al., 2018, 2019a, 2019b; López et al., 2018; Butler et al., 2019) although no statistical methods were used to predetermine sample sizes. Statistical analyses were performed using either one-way ANOVA (Figs. 1B-D, 2B-D, 4) or two-way ANOVA (Figs. 1E, 2E) followed by Sidak- corrected t tests to compare individual groups or a two-tailed Student's *t* test (Fig. 3B). Simple planned comparisons to assess discrimination index (DI) scores were conducted within group to compare training and test DI using Student's *t* test (Figs. 1, 2). Two-way ANOVA had factors of Object and Group (Figs. 1E, 2E). All statistics were performed with GraphPad Prism 7 software. Main effects and interactions for all ANOVA are described in the text. All analyses were two-tailed and required an α value of 0.05 for significance. Error bars in all figures represent SEM.

3. Results

3.1. Mice exposed to GCR form a memory for object location with extensive over-training

To examine whether exposure to 5 cGy or 30 cGy doses of mixed-ion GCR impacts hippocampus-dependent long-term memory with extensive training, mice received 7 consecutive days of 10-minute training in the OUL task where mice were exposed, to 2 identical objects in distinct locations (A1 and A2) of a familiar context (Fig. 1A-C). Seven days is considered extensive training given our previous studies indicating long-term memory in young adult male mice after only 1 training session (Stefanko et al., 2009; Roozendaal et al., 2010; Barrett et al., 2011; Reolon et al., 2011; Vogel-Ciernia et al., 2013) and aging male mice after 3 training sessions (Kwapis et al., 2018, 2019a). Following the last training day, mice were given a 5-minute update session that is identical to a test session of a traditional object location memory experiment, where one object was moved to a new location (A3) and time spent with each object location was examined (Fig. 1). Given that mice exhibit an innate preference for novelty, formation of the original memory for the object locations from training is evidenced by greater exploration of the object placed in the new, updated location (A3) compared with the familiar, fixed location (A1).

Mice exposed to low and high dose mixed-ion GCR perform equal to non-irradiated controls with no measurable differences in DI on the update session (Fig. 1D-E; one-way ANOVA, DI: Group $F(2, 29) = 0.272$, $p = 0.763$). Overall object exploration during the update session was also similar between groups (Fig. 1E; two-way ANOVA, significant main effect of Object Location $F(1, 54) = 20.44$, $p < 0.0001$, but not Group $F(2, 54) = 0.5307$, $p = 0.591$). Control and 30 cGy mice spent significantly more time exploring the updated location (A3) compared with the fixed location (A1) (Fig. 1E; Sidak's post hoc test, control: $p = 0.032$, 5 cGy: $p = 0.112$, 30 cGy: $p = 0.008$), indicating robust memory for training. Within group comparisons (Fig. 1D, significance denoted with

symbol within bars) of the DI scores from training day 1 compared with the update session reveal greater DI scores during the update session compared with training day 1 for the control group (t (9) = 2.866, p = 0.018) and 30 cGy group (t (10) = 4.312, p = 0.001), but not the 5 cGy group (t (8) = 1.534, p = 0.163), further indicating that the original object location memory was successfully acquired. Therefore, while differences in DI or overall exploration time are not observed between groups (Fig. 1D-E), post-hoc tests reveal greater time exploring the updated (A3) vs fixed (A1) object location in only the control and 30 cGy group (Fig. 1E).

3.2. Memory updating is impaired in mice exposed to GCR

To assess whether mice exposed to 5 cGy or 30 cGy doses of mixed-ion GCR are able to update an existing memory, mice were tested the day following the update session by placing 3 identical objects in distinct locations (A1, A2, A3) and a fourth object in a novel location (A4) and measuring time spent with the new object location (A4) compared with the fixed (A1), moved (A2) and updated (A3) locations as indicated by a higher score on the DI (Fig. 2A). Preferential exploration of novel object location A4 compared with original object locations A1 or A2 indicate strong memory for the original information presented, whereas greater exploration of novel object location A4 compared with the updated object location A3 is indicative of successful updating of the original object location memory. As in Fig. 1, DI scores were calculated to compare exploration of novel object location A4 with locations A1, A2 and A3 (Fig. 2B-D) and within group comparisons were made to examine DI scores between training day 1 vs update test (significance denoted with # symbol within bars). Total time exploring each object location was also examined (Fig. 2E).

All groups showed intact memory for the original information, with no group differences in DI scores when assessing exploration of the fixed location A1 compared with the novel object location A4 (Fig. 2B; one-way ANOVA, Group F (2, 30) = 2.114, p = 0.138). However, within group comparisons of the DI scores from training day 1 compared with A4 vs A1 update test reveal greater DI scores during the update test compared with training day 1 for only the control group (t (9) = 3.309, p = 0.009), but not the 5 cGy group (t (10) = 1.834, p = 0.096) or the 30 cGy group (t (11) = 0.709, p = 0.492). Memory for the moved object location (A2) differed between the groups (Fig. 2C; one-way ANOVA, Group F (2, 30) = 3.504, p = 0.042) where mice exposed to 30 cGy of GCR displayed similar amounts of time exploring both the moved object location A2 and novel object location A4, resulting in a significantly lower DI compared to the control group (Fig. 2C; Sidak's post hoc test, p = 0.042), but not the 5 cGy GCR group (Fig. 2C; Sidak's post hoc test, p = 0.726). Within group comparisons of the DI scores from training day 1 compared with A4 vs A2 update test revealed greater DI scores during the update test compared with training day 1 for only the control group (t (9) = 4.503, p = 0.001) and the 5 cGy group (t (10) = 2.403, p = 0.037) but not the 30 cGy group (t (11) = 0.179, p = 0.860). Overall, these data suggest that, although all aspects of the original memory were retained in the control and the 5 cGy mixed-ion GCR group, only partial aspects of the original memory are retained in mice exposed to 30 cGy.

To test whether the original memory was updated, exploration of the novel location A4 was compared to the updated location A3 during the test session. Assessment of DI scores revealed no group differences (Fig. 2D; one-way ANOVA, Group F (2, 29) = 1.771, p = 0.188). However, within group comparisons of the DI scores from training day 1

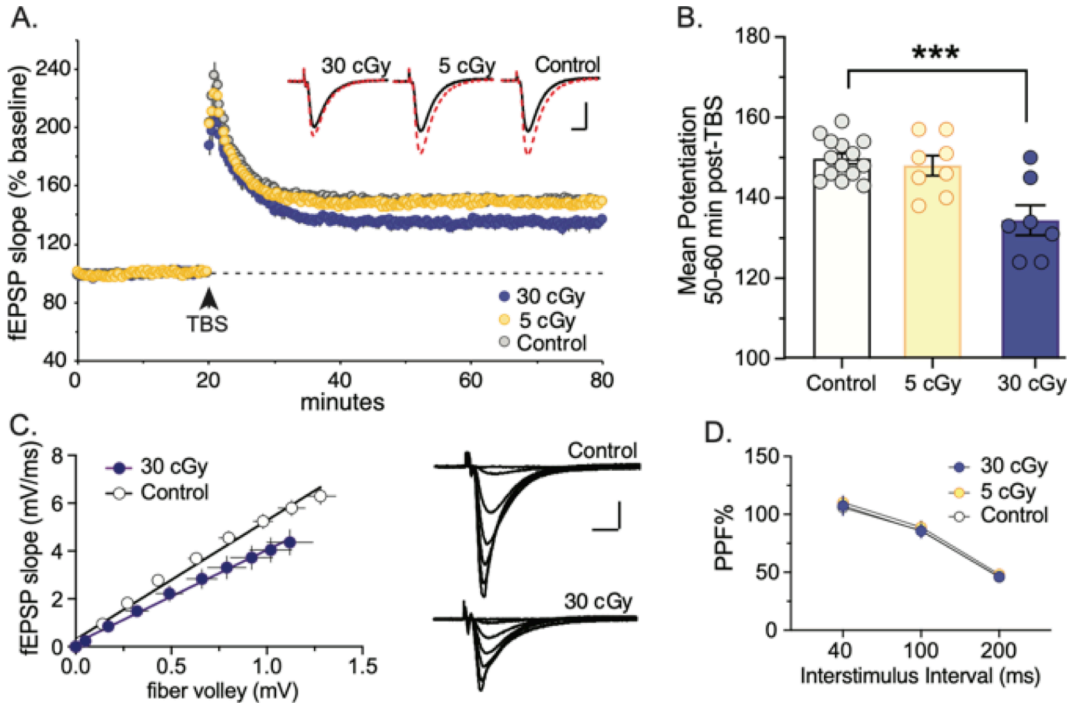

Fig. 3. Long term potentiation (LTP) is impaired in hippocampal slices from mice irradiated using 30 cGy of mixed-ion galactic cosmic radiation (GCR). A. Extracellular field recordings following stimulation of the Schaffer-commissural projections to the proximal apical dendrites of the CA1b field of the dorsal hippocampus, ~4.5 months following irradiation using 5 cGy or 30 cGy of GCR. Following a stable 20-minute baseline recording, a single train of TBS (black arrow) was applied, and baseline recordings were resumed for an additional 60 min. The time course shows that theta burst stimulation (TBS) -induced LTP was markedly reduced in slices from 30 cGy treated mice compared with slices from unirradiated control mice. Inset, Representative traces collected during baseline (black line) and 60-minute post-TBS (red line). B. Summary graph showing mean fEPSP slope 50–60 min after stimulation. Potentiation was significantly lower in slices from 30 cGy mice compared with non-irradiated control mice. C. Plot of fEPSP slope against corresponding fiber volley amplitude reveals that the slopes of the individual linear regression lines were significantly different. Inset; Representative traces collected during generation of input/output curve in slices from 30 cGy and unirradiated control mice. D. Paired-pulse facilitation (PPF) was comparable between all three groups. Data are presented as mean ± SEM; (n = 14 (control), 8 (5 cGy), 7 (30 cGy) slices from 8, 4, and 4 mice). *** $p < 0.001$, compared to control.

compared with A4 vs A3 update test reveal greater DI scores during the update test compared with training day 1 only in the control group ($t(9) = 2.657$, $p = 0.026$), but not the 5 cGy ($t(10) = 0.737$, $p = 0.477$) or 30 cGy group ($t(11) = 0.059$, $p = 0.953$). Further analyses examined total object exploration time for object locations A1, A2, A3 and A4. Group differences were not observed in overall object exploration time (Fig. 2E; two-way ANOVA, Group $F(2, 120) = 0.756$, $p = 0.471$), indicating that differences in DI scores between groups were not due to differences in overall exploration time but rather in time spent with each object. Indeed, a difference in the amount of time spent exploring each object during the update test was observed (Fig. 2E; two-way ANOVA, Object $F(3, 120) = 8.003$, $p < 0.0001$, Group × Object interaction $F(6, 120) = 1.648$, $p = 0.139$). Sidak's post hoc test revealed that during the update test session mice from the control group spent significantly more time exploring the novel object location A4 compared with objects A1 ($p = 0.0002$), A2 ($p = 0.0002$), or A3 ($p = 0.0046$), indicating successful recall of both the original information and the updated information at test (Fig. 2E), consistent with previous reports (Kwapis et al., 2019a). Mice exposed to a 5 cGy dose of GCR only displayed greater exploration for the novel object location A4 compared with object locations indicative of the original memory: A1 ($p = 0.0838$) and A2 ($p = 0.0282$), but not for the updated information A3 ($p = 0.2705$, Fig. 2E). Mice exposed to the higher 30 cGy dose of GCR showed equal time exploring the novel object location compared with objects A1 ($p = 0.6832$), A2 ($p = 0.9885$), or A3 ($p = 0.9589$, Fig. 2E). Together, these findings indicate impaired memory updating in male mice 18 weeks after exposure to mixed-ion GCR.

3.3. LTP in hippocampal field CA1 is impaired in male mice exposed to 30 cGy of 6-beam GCR

The observed differences in memory updating between mice exposed to GCR compared with controls led us to ask whether male mice exposed to 5 or 30 cGy doses of GCR are also impaired in hippocampal synaptic plasticity. Acute hippocampal slices were used to measure field excitatory postsynaptic potentials (fEPSP) recordings from stratum radiatum of the CA1b in response to stimulation of the Schaffer collateral-commissural projections in the CA1c. To examine changes in long-term synaptic plasticity, we applied theta burst stimulation (TBS) to induce long-term potentiation (LTP) in slices 5 weeks following cessation of behavior. A single train of five theta bursts to Schaffer collateral inputs has been previously reported as the threshold for inducing stable potentiation in mice (Vogel-Ciernia et al., 2013; White et al., 2016; Acharya et al., 2017; Kwapis et al., 2018). Field EPSP slopes begin to stabilize 30 min after the delivery of TBS, when the consolidation of LTP has been shown to occur (Lynch, 1998; Kramár and Lynch, 2003). During this time period synaptic events are engaged that induce long-term synaptic strength, a critical cellular mechanism of learning and memory (Silva, 2003). Therefore, as in our previous studies (Vogel-Ciernia et al., 2013; White et al., 2016; Acharya et al., 2017; Kwapis et al., 2018), the mean fEPSP slope was analyzed using a one-way ANOVA during this plateau phase (50–60 min after induction) to assess consolidation (Fig. 3B). TBS produced robust potentiation in non-irradiated control and 5 cGy GCR slices, which briefly decayed over a period of 10 min and stabilized at approximately 50% above the pre-TBS baseline (Fig. 3A-B). No significant differences in potentiation were observed between control and 5 cGy slices 50–60 min post-TBS (panel B; one-way ANOVA, Group $F(2, 26) = 11.97$, $p = 0.0002$; post hoc test 5 cGy vs control: $p = 0.844$). In contrast, TBS delivered to slices from mice exposed to the 30 cGy dose of GCR produced a notable decrease in LTP. Short-term potentiation was comparable to non-irradiated controls, but the level of potentiation 50–60 min post TBS was significantly reduced compared with controls (Fig. 3B, one-way ANOVA, Group $F(2, 26) = 11.97$, $p = 0.0002$; post hoc test 30 cGy vs control: $p = 0.0002$). Collectively, these findings suggest that exposure to a 30 cGy dose of mixed-ion GCR leads to impairments in long term synaptic plasticity in the CA1 region of the hippocampus 4.5 months after radiation exposure.

Next, we generated input/output curves and measured changes in paired-pulse facilitation to determine whether radiation exposure would alter baseline neuronal function within the hippocampus that could contribute to the deficit in LTP. The fEPSP slope and amplitude of the nonsynaptic fiber volley responses across a range of stimulation currents were collected and plotted (Fig. S2A). Relative to control, we found that as stimulation intensity increased, fEPSP slope decreased in slices from mice exposed to 30 cGy of mixed-ion GCR, while no significant change was detected in mice exposed to 5 cGy (Fig. S2A top panel, 2-way ANOVA, Group $F(2, 26) = 3.233$, $p = 0.0557$; Current $F(1.187, 30.84) = 345$, $p < 0.0001$; Current × Group $F(18, 234) = 4.289$, $p < 0.0001$), indicating that the 30 cGy radiation exposure decreased synaptic transmission. We also measured fiber volley amplitude among the 3 groups to determine whether a decrease in afferent activation (presynaptic fiber input) contributed to the decrease in transmission in 30 cGy irradiated mice (Fig. S2A, bottom panel). We found no significant difference between groups (2-way ANOVA, Group $F(2, 26) = 0.3426$, $p = 0.7131$).

Since exposure with 5 cGy did not affect baseline transmission or LTP, we decided to plot the fEPSP slope against corresponding fiber volley amplitude to normalize values across slices from control and 30 cGy mice. Fig. 3C confirms that the difference between the slopes of the linear regression line for 30 cGy and control were significantly different ($p = 0.0008$), suggesting that the decrease in synaptic transmission in mice exposed to 30 cGy of GCR may have contributed to the observed LTP deficit. Next, we tested for differences in transmitter release kinetics using paired-pulse facilitation. The slices from mice exposed to either GCR dose did not differ from control slices (Fig. 3D). Taken together, these results indicate that only the higher, 30 cGy dose of mixed-ion GCR elicits changes in select measures of baseline synaptic transmission and LTP in the hippocampus.

3.4. Disruption of HDAC3 activity ameliorates irradiation-related impairments in synaptic plasticity

Given previous reports from our lab using unirradiated mice indicating that HDAC3 serves as a critical negative regulator of memory formation (McQuown et al., 2011; Malvaez et al., 2013; Rogge et al., 2013; Bieszczad et al., 2015; Alaghband et al., 2017; Kwapis et al., 2019b), we next tested whether HDAC3 inhibition can mitigate radiation-induced deficits in synaptic plasticity long after exposure. We subcutaneously injected mice with a selective HDAC3 inhibitor RGFP 966 (10 mg/kg) or vehicle (30% hydroxypropyl-β-cyclodextrin, 5% DMSO and 100 mM sodium acetate, pH 5.4) 30 min prior to brain extraction, a time point where maximum drug concentration is observed in the mouse brain (Malvaez et al., 2013). We then evaluated LTP in acute hippocampal slices for long-term synaptic changes. Fig. 4A-B summarizes the effects of RGFP 966 on theta burst-LTP in slices from non-irradiated mice. The mean fEPSP slope as a percentage from baseline measured 50–60 min after induction was + 83 ± 11% for the slices from mice injected with RGFP 966 and + 54 ± 14% for controls (Fig. 4B; $p < 0.001$; *t*-test; two-tailed), strongly suggesting that HDAC3 inhibition dramatically enhances theta burst-induced LTP. We then tested whether HDAC3 inhibition can overcome LTP deficits in in mice exposed to 30 cGy of mixed-ion GCR. Fig. 4C-D summarizes these results. Surprisingly, slices from mice injected with RGFP 966 produced a profound increase in the level of potentiation 50–60 min after induction (mean fEPSP slope = 81, ± 12%) relative to controls (mean fEPSP slope = 39 ± 2%, $p < 0.0001$; *t*-test; two-tailed). Taken together, these results suggest that systemic inhibition of HDAC3 significantly enhances theta burst-induced LTP in the hippocampus relative to controls, and most notably, can overcome the deficit in LTP in slices from mice exposed to 30 cGy of GCR.

Next, we sought to determine whether RGFP 966 alters neuronal function by generating input/output curves and measuring paired-pulse

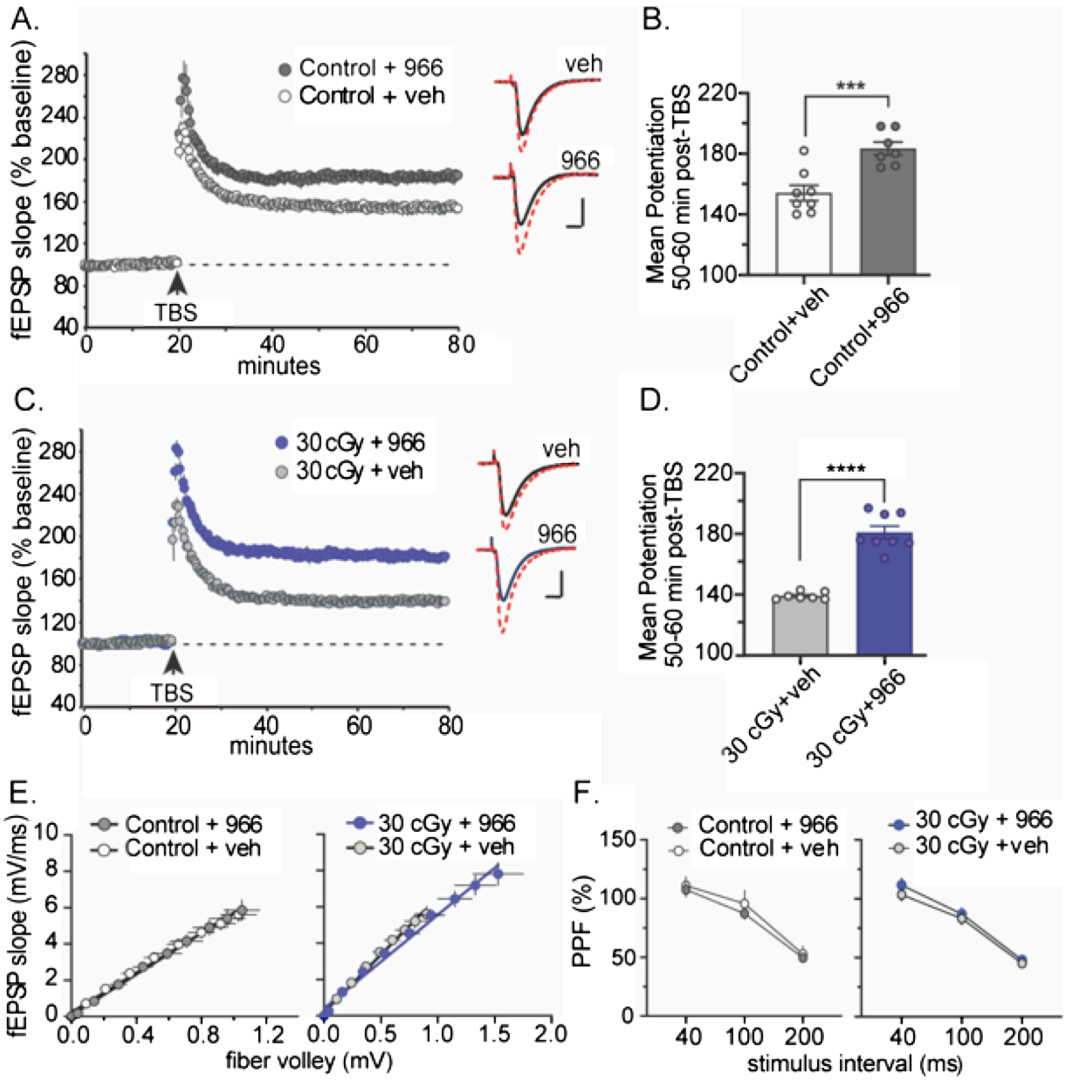

Fig. 4. Disruption of HDAC3 activity via a single subcutaneous injection ameliorates irradiation-related impairments in synaptic plasticity. A-B. Disruption of HDAC3 activity (966) significantly enhanced theta burst-induced LTP (TBS) 50–60 min post induction in the hippocampus of unirradiated control mice. Inset, representative traces collected during baseline (black line) and 60 min post-TBS (red line) C-D. HDAC3 inhibitor 966 rescued the deficit in LTP in slices from 30 cGy GCR irradiated mice, ameliorating irradiation-related impairments in synaptic plasticity 50–60 min post-TBS. Inset, representative traces collected during baseline (black line) and 60 min post-TBS (red line). E. The difference between the slopes of the linear regression line in the input/output curve in slices from unirradiated mice treated with 966 and vehicle were not significantly different (left panel). In contrast, a significant difference between slopes were found in slices from 30 cGy irradiated mice treated with 966 relative to vehicle controls (right panel). F. Paired-pulse facilitation (PPF) were comparable between groups at each interval test (nonirradiated control vs nonirradiated 966 (left panel) and 30 cGy slices from mice injected with RGFP 966 vs 30 cGy veh controls (right panel)). Data are presented as mean ± SEM; (control + veh: n = 8, control + 966 n = 7, 30 cGy + veh n = 7, 30 cGy + 966 n = 8 slices from 4, 4, 3, 4 mice). *** $p < 0.001$, **** $p < 0.0001$, compared to control.

facilitation in slices collected from RGFP 966 and vehicle injected mice. For non-irradiated mice, there were no significant differences between groups in fEPSP slope (2-way ANOVA, Group F (1, 13) = 0.3567, p = 0.5606), or fiber volley amplitude (2-way ANOVA, Group F (1, 13) = 0.3528, p = 0.5627) across stimulation intensities (Figs. 3-1B, top and bottom panels, respectively). Accordingly, as shown in Fig. 4E (left panel), plotting the fEPSP slope against fiber volley amplitude revealed there was no significant difference between the slopes of the linear regression lines (p = 0.61). In contrast, 30 cGy irradiated mice that had been injected with RGFP 966 showed a marked increase in fEPSP slope (2-way ANOVA, Group F (3, 26) = 6.437, p = 0.0021), and also fiber volley amplitude (2-way ANOVA, Group F (1, 13) = 6.174, p = 0.0274) across stimulation intensities (Figs. 3-1C, top and bottom panels, respectively). Plotting the fEPSP slope against corresponding fiber volley amplitude revealed that the difference between the slopes of the linear regression lines were significantly different (Fig. 4E, right panel; p = 0.001). These data suggest the possibility that RGFP 966 alters, not only baseline synaptic transmission, but also the excitability of afferent inputs to the CA1b stratum radiatum. Finally, we evaluated whether RGFP 966 alters presynaptic release properties. Fig. 4F shows that RGFP 966 does not cause any disruptions in transmitter release kinetics at any stimulus interval tested in slices from non-irradiated or irradiated mice, relative to vehicle controls. Taken together, these results indicate that one-time systemic disruption of HDAC3 can improve neuronal function and ameliorate irradiation-related impairments in hippocampal LTP.

3.5. Phosphorylated cofilin is decreased in the dorsal hippocampus of mice exposed to high-dose GCR

The observed impairments in LTP in male mice exposed to 30 cGy of mixed-ion GCR compared with controls led us to test whether exposure to GCR is also associated with reductions of an actin-regulatory protein,

cofilin, in the hippocampus and whether this accompanies irradiation-related impairments in memory updating and synaptic plasticity. We have previously reported punctate labeling of phosphorylated Cofilin (p-cofilin) within the stratum radiatum of the CA1 (Vogel-Ciernia et al., 2013), the area from which fEPSPs were recorded in the present study. LTP is accompanied by phosphorylation of cofilin (Fukazawa et al., 2003; Kramár et al., 2009) and it prompts spine formation (Yuste and Bonhoeffer, 2001, 2004). The stratum radiatum of the CA1 region of the hippocampus is also the area where phosphorylation of cofilin is the final signaling step in the actin remodeling pathway that underlies LTP maintenance (Chen et al., 2007). Therefore, we used Western blot to assess p-cofilin expression in the hippocampus of mice exposed to 5 cGy or 30 cGy doses of GCR as compared to controls. This analysis revealed an effect of GCR exposure (Fig. 5, one-way ANOVA, Group $F(2, 15) = 10.24$, $p = 0.0016$), where a significant decrease in p-cofilin was observed in mice exposed to 5 cGy ($p = 0.0283$) and 30 cGy of mixed-ion GCR ($p = 0.0014$) relative to control. These findings are consistent with observations regarding LTP impairment and provide further mechanistic insight corroborating radiation-induced impairments in memory updating.

4. Discussion

This study reports that whole body exposure to 6-beam GCR interferes with the ability to update an existing memory and leads to impairments in synaptic plasticity in middle aged male mice 4.5 months after GCR exposure. Further, we find that radiation-induced impairments in synaptic plasticity were ameliorated with systemic inhibition of the repressive histone deacetylase 3 (HDAC3) enzyme, RGFP 966. Together, these findings suggest that GCR exposures may impair specific aspects of cognitive function. Epigenetic modifications that promote an open chromatin state through selective HDAC3 inhibition yield promise for preventing cognitive impairments resulting from irradiation, warranting further behavioral investigation.

Despite a number of reports having found exposure to GCR or even single ions to elicit impairments in short term memory (Britten et al., 2012, 2017; Acharya et al., 2017; Kiffer et al., 2018; Krukowski et al., 2018b), few studies have examined long-term hippocampus-dependent memory using mission-relevant doses of GCR (Krukowski et al., 2018b). A novelty in our study is the use of mission-relevant doses incorporating multi-ion GCR exposure to examine memory updating and LTP in male mice. While exposure to single ions from the GCR spectrum have been used to examine spatial memory in middle aged rats on a Barnes Maze task (Wyrobek and Britten, 2010; Britten et al., 2016a) and a number of other tasks assessing short-term memory (Shukitt-Hale et al., 2003; Carr et al., 2018; Howe et al., 2019), the impact of mission-relevant GCR exposure on long term spatial memory updating has not been examined until now. Here, we observed that middle-aged GCR exposed mice were capable of forming long-term hippocampus-dependent memories with sufficient training. To our knowledge, this is the first report indicating that hippocampus-dependent long-term memory formation is still possible with extensive training following mixed-ion GCR exposure; however, as we did not examine memory in mice after only 1 training session in our study, we cannot rule out the possibility that learning would have occurred in a shorter period of time.

In order to probe whether exposure to GCR impairs updating this original memory we utilized our newly established OUL paradigm which serves as a simple task that allows assessment of memory for the original and updated information in a single test session (Kwapis et al., 2019a). Memory updating is important to examine as most adult memories are not new associations but are alterations (updates) to existing memories, making this task relevant to the human condition. The OUL paradigm has previously been validated by our lab to confirm that the update session requires retrieval of the original memory rather than two distinct memories via two complementary methods: intra-hippocampal anisomycin injections and Arc CatFISH. The study by Kwapis et al. (2019a) revealed that intra-hippocampal injection of the protein synthesis inhibitor anisomycin following the update session disrupted the updated information as well as the original memory at test, suggesting that memory updating in OUL engages the original memory. Similarly, Arc CatFISH revealed a largely overlapping set of neurons in the hippocampus as the original memory. Therefore, we are confident that the observed robust performance in non-irradiated controls in the current study is reflective of effective memory updating rather than formation of two distinct associations.

We find that despite robust performance on the update session after 7 days of training, the ability to update this memory to incorporate information of the moved object location is impaired in mice exposed to a 30 cGy dose of GCR. Evidence for this is indicated by similar time exploring original object locations A1 and A2, updated object location A3 and novel object location A4 (Fig. 2E) in addition to low DI scores and non-significant DI scores between training and test session (Fig. 2B-D). Given our findings of similar distance traveled during habituation and similar overall object exploration times during the training, update and test session between groups, it is unlikely that differences in locomotion or object exploration account for differences in performance. Given that extensive overtraining was likely required for GCR-exposed mice to acquire an original memory for object location, it is perhaps not surprising that memory updating was impaired in these mice. The inability to update the original object location memory is likely due to impairments in synaptic plasticity that would prevent incorporation of new information during the update session. Previous reports observe that synaptic reactivation, which takes place when retrieval and novelty detection occur concomitantly (such is the case during the update session), re-sensitizes LTP to protein synthesis inhibition (Fonseca et al., 2006) allowing for restabilization mechanisms through a protein synthesis-dependent reconsolidation process (Gonzalez et al., 2019)). This possibility falls in line with our observed impairments in protein synthesis-dependent hippocampal LTP in male mice irradiated using 30 cGy of mixed-ion GCR (Fig. 3), opening up the possibility that the adverse impact of radiation on synaptic plasticity may be contributory if not causal to impaired memory updating in male mice exposed to

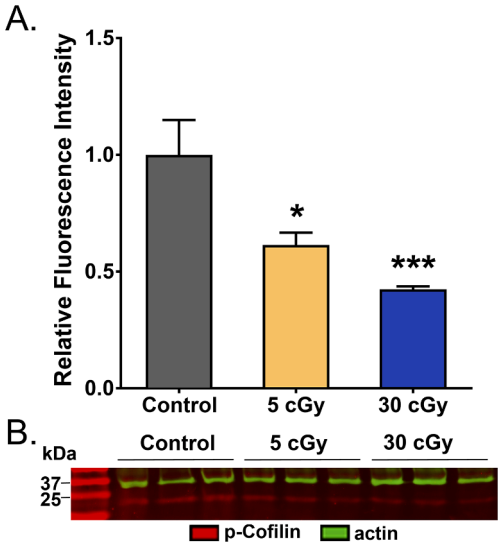

Fig. 5. Exposure to 30 cGy GCR is associated with lower levels of p-cofilin relative to non-irradiated controls in the hippocampus. a. Western blot analysis of p-cofilin and actin levels in the dorsal hippocampus in mice exposed to 5 and 30 cGy doses of mixed-ion GCR compared with unirradiated control mice. b. Representative Western blot of p-cofilin and actin levels in the dorsal hippocampus. Data are presented as mean ± SEM; (n = 6/group), * $p < 0.05$, *** $p < 0.001$ compared to control.

6-beam GCR, though further investigation is warranted.

Despite memory for the original object location during the update session in mice exposed to 30 cGy GCR after 7 days of training, we did not observe greater exploration of either original object location A1 or A2 when compared with either the updated location A3 or novel location A4 (Fig. 2E). These findings are also consistent with our previous study utilizing the OUL task where aging male mice, impaired in memory updating, also did not show preferential exploration of either original object (Kwapis et al., 2019a). These findings may be explained by either disruption of the original memory by the update session or interference with retrieval of the original memory (retroactive interference) or may instead be explained by an inability of the original memory to persist or be retained beyond a 24-hour testing interval. Although, mice exposed to a 5 cGy dose of GCR perform well in aspects of the update test (Fig. 2C), some features are indicative of subtle impairments in memory updating compared with non-irradiated controls. This was revealed as within-group comparisons examining the DI scores from the training compared with the update test, showing a significantly higher DI in 5 cGy mice only when assessing memory for the novel object location A4 compared with the moved object location A2, suggesting a possible dose-dependent effect of GCR exposure on memory updating. That hippocampus-dependent memory updating is impaired in GCR-exposed male mice is not surprising given the severe impact of mixed-ion GCR exposure on hippocampus function resulting in microglial activation, synaptic loss, reductions in AMPA expressing synaptic terminals (Krukowski et al., 2018b, 2018a), and alternations in glutamatergic transmission (Machida et al., 2010; Britten et al., 2012). Therefore, we also analyzed the impact of GCR exposure on hippocampal long-term potentiation, a form of synaptic plasticity, almost 5 months following exposure.

In this study, we find that exposure to 30 cGy results in impaired LTP compared to robust potentiation observed in control, non-irradiated male mice (Fig. 3A-B). These findings are in line with similar reports showing that whole brain exposure to accelerated iron particles, a component of GCR, was associated with impaired synaptic plasticity in CA1 neurons 1 month following exposure (Vlkolinský et al., 2007). Our study adds to these findings by reporting lasting impairments induced by mixed-ion GCR exposure on hippocampal synaptic plasticity almost 5 months following exposure to 30 cGy of GCR and finds that exposure to the combination of 6 charged particle species that make up GCR results in impaired LTP. Additionally, when plotting the fEPSP slope against corresponding fiber volley amplitude we observed a difference between the slopes of the linear regression line for 30 cGy and control mice (Fig. 3C) which may suggest that impaired LTP may partly be attributed to decreased synaptic transmission. We also observed effects on baseline transmission where mice exposed to 30 cGy of GCR had a severe reduction in the size and slope of the fEPSP, with no changes in paired-pulse facilitation (PPF) (Fig. 3D), suggesting that the higher dose of GCR may have interfered with neuronal excitability including reduction in either synapse number or reduced AMPA receptor function. These possibilities fall in line with previous reports of synaptic loss in hippocampus and reductions in AMPA expressing synaptic terminals in the hippocampus of male mice exposed to 3-ion GCR (Krukowski et al., 2018b). Another possibility for impaired LTP are reductions in NMDA receptors as significant decreases in the levels of the glutamatergic NMDA receptors NR1, NR2A and NR2B were observed following a single dose of 60 cGy HZE-particle radiation in male rats (Machida et al., 2010). Further, microglial activation may also play a role in LTP impairments given reports showing prevention of cosmic-radiation induced cognitive deficits through microglia depletion (Krukowski et al., 2018a). In line with impaired LTP in our study, we also observed lower levels of p-cofilin expression in the hippocampus of the 30 cGy irradiated male mice as compared with non-irradiated controls. Maintenance of LTP requires dynamic actin filaments for stable long-term potentiation in the CA1 region of the hippocampus (Krucker et al., 2000) and in the CA1, phosphorylation of cofilin is the final signaling step in the actin remodeling pathway that underlies LTP maintenance (Chen et al., 2007) and LTP maintenance is accompanied by phosphorylation of cofilin (Fukazawa et al., 2003; Kramár et al., 2009). Phosphorylation of cofilin also prompts dendritic spine formation (Yuste and Bonhoeffer, 2001, 2004) and we have previously reported significant reductions in the number and density of dendritic spines along hippocampal neurons of the dentate gyrus in male mice that underwent whole-body proton irradiation (Parihar et al., 2015). Therefore, low levels of p-cofilin expression in the hippocampus as a result of GCR exposure may contribute to observed long lasting impairments in synaptic plasticity.

A key finding in our study was the ability to reverse impairments in LTP in male mice exposed to a 30 cGy dose of GCR through a single systemic subcutaneous administration of the HDAC3 inhibitor RGFP 966. HDACs act by removing acetyl groups from histones, which facilitates a condensed chromatin structure and represses transcription. Histone acetyltransferases (HATs) serve to add acetyl groups to histones and facilitate a relaxed chromatin structure (for reviews see: Kouzarides, 2007; Barrett and Wood, 2008; Keiser and Wood, 2019) and generally, increasing HAT activity or reducing HDAC activity leads to improved long-term memory formation (for reviews see: Marmorstein and Roth, 2001; Barrett and Wood, 2008; Gräff and Tsai, 2013) by increasing histone acetylation (Levenson et al., 2004; Vecsey et al., 2007; McQuown et al., 2011; Kwapis et al., 2017). The particular HDAC discussed in this study, HDAC3, serves as a negative regulator of memory. Consistent with findings of the present study where HDAC3 inhibition ameliorates impairments in LTP long after exposure to GCR, our lab has previously reported that inactivation or deletion of HDAC3 in the hippocampus of non-irradiated mice reverses age-related impairments in hippocampal LTP (Kwapis et al., 2018), enables learning in conditions that are normally subthreshold for encoding (McQuown et al., 2011; Malvaez et al., 2013; Rogge et al., 2013) and allows memory to persist beyond a time when normal memories fail (Stefanko et al., 2009; McQuown et al., 2011). In addition to ameliorating impairments in LTP, systemic HDAC3 inhibition also reversed potential impairments in synaptic transmission, as a marked increase in fEPSP slope and fiber volley amplitude was observed across stimulation intensities in slices from mice injected with RGFP 966 (Fig. S2C). Further, plotting the fEPSP slope against corresponding fiber volley amplitude revealed that the difference between the slopes of the linear regression lines were significantly different, suggesting that HDAC3 inhibition may not just alter baseline synaptic transmission, but also enhance the excitability of afferent inputs to the CA1b stratum radiatum and enhance the ceiling for producing stable LTP. Although we hypothesize that in addition to reversing impairments in synaptic plasticity, HDAC3 inhibition would facilitate memory updating in mice exposed to GCR, the present study design precluded our ability to obtain additional animals for these investigations.

To our knowledge, this is the first study showing that the administration of a single dose (10 mg/kg, s.c) of an HDAC3 inhibitor completely reverses GCR-linked impairments in LTP almost 5 months after exposure. These data point to the possibility that long-lasting impairments in memory and synaptic plasticity as a result of GCR exposure may be mediated via epigenetic modifications. Specifically, exposure to GCR may facilitate a closed chromatin structure associated with repressing transcription through increased HDAC activity by promoting removal of acetyl groups from histones that generally relax chromatin structure and facilitate gene expression. While additional work in GCR-exposed mice is required to substantiate the potential benefits of HDAC3 inhibition on a range of cognitive tasks as well as any potential sex-specific differences resulting from mixed-ion GCR exposure, future studies implementing such strategies hold promise for exploiting epigenetic mechanisms for mitigating space radiation-induced neurological complications.

CRediT authorship contribution statement

A.A. Keiser: Conceptualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Project administration, Funding acquisition. **E.A. Kramár:** Formal analysis, Investigation, Writing - review & editing. **T. Dong:** Validation, Investigation. **S. Shanur:** Validation, Investigation. **M. Pirodan:** Validation, Investigation. **N. Ru:** Investigation, Formal analysis. **M.M. Acharya:** Conceptualization. **J.E. Baulch:** Conceptualization, Writing - review & editing, Supervision. **C.L. Limoli:** Conceptualization, Resources, Writing - review & editing, Supervision, Funding acquisition. **M.A. Wood:** Conceptualization, Resources, Writing - review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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