Effects of a 33-sequential beam galactic cosmic ray analog on male mouse behavior and evaluation of CDDO-EA as a radiation countermeasure

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ABSTRACT

In long-term spaceflight, astronauts will face unique cognitive loads and social challenges which will be complicated by communication delays with Earth. It is important to understand the central nervous system (CNS) effects of deep spaceflight and the associated unavoidable exposure to galactic cosmic radiation (GCR). Rodent studies show single- or simple-particle combination exposure alters CNS endpoints, including hippocampal-dependent behavior. An even better Earth-based simulation of GCR is now available, including 33-beam GCR (33-GCR) exposure. However, the effect of whole-body 33-GCR exposure on rodent behavior is unknown, and no 33-GCR CNS countermeasures have been tested. Here astronaut-age-equivalent (6mo-old) C57BL/6J male mice were exposed to a 33-GCR (75cGy, a Mars mission dose). Pre-/during/post-Sham or 33-GCR exposure, mice were given a diet containing a 'vehicle' formulation or the antioxidant/anti-inflammatory compound CDDO-EA as a potential countermeasure. Behavioral testing beginning 4mo post-irradiation suggested radiation and diet did not affect measures of exploration/anxiety-like behaviors (open field, elevated plus maze) or recognition of a novel object. However, in 3-Chamber Social Interaction (3-CSI), CDDO-EA/33-GCR mice failed to spend more time exploring a holder containing a stranger mouse vs. nothing, suggesting sociability deficits, and Vehicle/33-GCR and CDDO-EA/Sham mice failed to discriminate between a stranger vs. familiar mouse, suggesting social memory deficits. CDDO-EA given pre-/during/post-irradiation did not attenuate the 33-GCR-induced social memory deficits. Future elucidation of the mechanisms underlying 33-GCR-induced social memory deficits will improve risk analysis for astronauts which may in-turn improve countermeasures.

Keywords: Space Radiation, Charged-Particle, Proton, Countermeasure, 3-Chamber Social Interaction

1. INTRODUCTION

Understanding the biological effects of spaceflight beyond low-Earth orbit remains a pressing priority in order to achieve the safe return of a crewed mission to Mars. A major obstacle to deep spaceflight is the space radiation environment. The interplanetary radiation field consists of energetic charged-particles capable of breaching conventional spacecraft shielding. Charged-particle radiation originates from solar ejecta during periodic yet largely unpredictable solar events, whereas galactic cosmic radiation (GCR) - the remnants of supernovae - circulate through the galaxy and are encountered at a constant fluence (Nelson, 2016). Most space missions to date have occurred within Earth's magnetosphere, and thus astronauts have largely been shielded from these high-energy, potentially-damaging charged particles. As such, there are enormous gaps in knowledge about the human consequences of GCR exposure (Cucinotta, 2007). Filling these knowledge gaps is essential to prepare NASA for successful deep spaceflight missions, but will also help advance science and medicine in other fields that use charged particles, such as in particle cancer therapy (Schaub et al., 2020).

Of NASA's many risk-focused areas, the central nervous system (CNS) is of great concern due to the cognitive demands on astronauts and the social challenges associated with long-term spaceflight. These challenges are exacerbated by the lag in communication associated with increasing distances from Earth leading to lack of immediate ground-based mission support, long-term confinement, and the added psychological stress of no-emergency-return contingencies on a mission to Mars. Thus, learning, memory, and social cognition are among the most important neurocognitive domains to evaluate for their sensitivity to GCR. As modeling GCR on Earth has previously been challenging, our understanding of how CNS function is influenced by space radiation comes primarily from rodents exposed to single charged particles in NASA's Space Radiation Laboratory (NSRL) and similar particle accelerator facilities. These studies show space radiation often (but not always) diminishes aspects of rodent cognitive behavior, including object, fear, and working memory, spatial navigation, attention, anxiety-like behavior, sociability and social memory, with associated changes in cellular and morphological endpoints as well (Kiffer et al., 2019b). A major limitation to these published studies is that most rodents were exposed to monoenergetic, single-ion beams, with only a few recent papers using exposures to simple combinations of varying charged particles (Kiffer et al., 2019b); to date, no studies have examined the behavioral effect of a complex GCR exposure. To address this, recent NSRL upgrades now provide a more Mars-relevant and standardized radiation exposure: 33 beams of charged particles at various energy distributions corresponding more closely to the diverse particle and energy spectra in space (Simonsen et al., 2020). Although this 33-GCR beam is now available, it is currently unknown how exposure to such a complex, mixed radiation field influences the CNS specifically in the context of rodent behavior.

Many single-particle studies of space radiation suggest it damages the CNS (for exceptions, see (Villasana et al., 2010; Kokhan et al., 2019; Liu et al., 2019; Whoolery et al., 2020)) . Therefore, it is reasonable to consider countermeasures to protect the CNS from the charged particle environment of space. Physical countermeasures, such as shielding the spacecraft or spacesuit, currently are cost prohibitive and accompanied by complications. Alternative radiation mitigation strategies, such as pharmacological countermeasures or interventions, are an area of active research (Barthel and Sarigul-Klijn, 2019). Since radiation exposure in space is constant, the best pharmaceutical countermeasures must also be compatible with the quality of life of a space crew. To this end, topical or oral administration of pharmacological countermeasures with long biological half-lives may be preferred. One such compound with a long biological half-life is the triterpenoid 2-cyano-3,

12-dioxooleana-1, 9-dien-28-oic acid (CDDO)-ethylamide (CDDO-EA), which targets the Nrf-2/ARE pathway, upregulates endogenous oxidative stress response elements, and potentially quenches the dense oxidative stress produced by particle radiation (Neymotin et al., 2011). In fact, CDDO-EA and structurally similar variants (such as CDDO-Imidazole or CDDO-Methyl) improve pathological symptoms in mouse models of Huntington's disease, ischemic injury, cerebral malaria, and amyotrophic lateral sclerosis via their anti-inflammatory/antioxidant actions (Stack et al., 2010; Neymotin et al., 2011; Crowley et al., 2017; Xu et al., 2017; Lei et al., 2020). CDDO-Methyl is currently in phase 3 of antioxidant-based clinical trials. Although space radiation has long been known to increase CNS indices of inflammation and reactive oxygen species (Denisova et al., 2002; Suman et al., 2013; Poulose et al., 2014; Cekanaviciute et al., 2018), no work has yet examined the ability of any CDDO variant to act as an effective radiation countermeasure in rodent models of space radiation exposure.

To understand the effects of GCR on the CNS and evaluate the potential behaviorally neuroprotective properties of CDDO-EA, we exposed 6-month(mo)-old male mice to sham radiation or an acute whole-body 75cGy dose of 33-GCR with or without transient co-administration of CDDO-EA. Four months later, we began behaviorally testing the mice in mission-relevant tests spanning the cognitive and social domains. We found that exposure to 33-GCR on average compromised social memory in mice without changing locomotion, anxiety-like behavior, sociability, or hippocampal-dependent memory. We also found CDDO-EA (given at the time of irradiation) did not block the radiation-induced decrease in social memory. While our countermeasure results merit additional study with different CDDO-EA administration parameters, our findings demonstrating radiation-dependent decrease in social memory 5.5mo after exposure show the CNS remains a critical area of concern for charged particle radiation.

2. METHODS

2.1 Animals

6-month-old male C57BL/6J mice (Jackson Laboratory stock #000664, Bar Harbor, Maine) were shipped directly to Brookhaven National Laboratories (BNL). Mice were housed 4 per cage, under a regular 12:12 hour light cycle at 22°C, 30-70% humidity, and given standard rodent chow (LabDiet 5015 #0001328) and water *ad libitum*. After 3 days of acclimation, mice were split into 2 diet groups to receive either a vehicle (Veh: Purina Rodent Diet 5002, 12.5g EtOH, 37.5g Neobee Oil) or a CDDO-EA (Veh + 400mg/kg RTA 405; Reata Pharmaceuticals, Irvine, TX) chow *ad libitum* for 5 days. Both the Veh and CDDO-EA formulations were prepared by Purina Mills, LLC. On day 4 of Veh or CDDO-EA diet, mice in both groups were further subdivided into Sham or 33-GCR groups (n = 22-24 per diet/radiation; **Supp. Fig. 1**), as detailed in Section 2.2. The day after irradiation, mice were shipped to CHOP via ground transport. As part of standard CHOP quarantine procedure, mice were fed 4% fenbendazole chow for 1.5 months and then returned to standard rodent chow (LabDiet 5015 #0001328). For the remainder of the experiment, mice were housed under a 12:12 hour light/dark cycle at 20-23°C and 30-40% humidity. For the duration of the experiment at CHOP, mice were housed in HEPA-filtered, closed airflow vivarium racks (Lab Products Inc., Enviro-Gard™ III, Seaford, DE). Mice were weighed periodically, with one weight bucket per cage to prevent exposure to odors from other cages. After delivery to CHOP, mice that necessitated single housing due to aggression were excluded from behavioral experiments (**Supp. Fig. 1**). All care and procedures were approved by the Institutional Animal Care and Use Committees (IACUC) at BNL and CHOP and were in accordance with the AAALAC and National Institute of Health (NIH) guidelines for the

care and use of laboratory animals. Our scientific reporting adheres to the ARRIVE 2.0 guidelines (du Sert et al., 2020).

2.2 Radiation

All Sham and GCR mice were placed in small well-ventilated holders (10 x 10 x 4.5 cm) paired with cagemates. GCR mice were then given an acute 75cGy whole-body exposure of the NSRL 33-beam GCR simulation over the duration of 2 hours (**Supp. Table 2**). Sham-irradiated mice did not receive charged-particle radiation. Radiation was delivered in an even 60 x 60cm beam distribution in the spring 2019 (19A) campaign. Dosimetry and beam calibration were provided by NSRL staff.

2.3 Overview of Behavioral Testing

Behavioral testing began 4 mo after irradiation and continued for ~6 months to assess mid- to late effects (**Fig. 1A**). Testing was conducted during the light cycle under dim red light conditions (~30-50 Lux) at 72°F and 35-50% humidity. Mice were acclimated to behavior rooms for 1h prior to testing. Recording for most tests was acquired by a ceiling-mounted camera (Ace acA640-90gc, Basler) and tracking was extrapolated with Ethovision XT (Noldus Information Technology). Nose points were used for exploratory measures, and center points for gross locomotor measures. Behavioral tracking for activity chambers was acquired by infrared beam sensors instead of video and processed by Activity Monitoring 5 (Med Associates Inc., #SOF-811). Once mice were placed in testing arenas, the handler left the noise-isolated behavior room and monitored mouse activity from a computer in an adjacent room. All mazes and equipment were disinfected and deodorized with 10% TB-10 (Birex) in between testing trials and allowed time to dry. Mouse handlers were blinded to experimental conditions. At the start of behavioral testing sample sizes per group were N = 13-15 and remained constant for the duration of testing. A sole Veh/33-GCR mouse was lost at ~6mo, and their behavioral data near the time of death was closely analyzed and removed retroactively (**Supp. Table 1**).

2.4 Activity Chambers

Mice were placed in individual, closed (but well-ventilated) sound-isolating activity chambers (Med Associates Inc., #ENV-510, 27 x 27 x 20cm). Gross locomotor activity such as cumulative locomotion, ambulatory time, ambulatory episodes, and mean velocity was measured across a 30 minute (min) trial (Teske et al., 2014).

2.5 Elevated-Plus Maze (EPM)

Rodent exploratory behavior in the elevated-plus maze (Harvard Apparatus, #760075) revealed another index of anxiety-like behavior. Each mouse was placed on one of the open arms (43 x 33, 6cm [height x arm length x arm width]) pseudorandomly and allowed free exploration for 5 min (Carola et al., 2002). Arena zones were defined as open and closed arm perimeters, a center zone in between the open and closed arms. Measures of total distance moved, entries, and time spent in each zone were taken. The discrimination ratio (DR) between arm exploration was expressed for each mouse as:

$$DR = \frac{(open\ arm\ exploration - closed\ arm\ exploration)}{(open\ arm\ exploration + closed\ arm\ exploration)}$$

2.6 Open Field

Open field exploratory behavior (an index of anxiety-like behavior) was probed by a one-day, 5 min open field paradigm (Seibenhener and Wooten, 2015). Mice were placed in the center of a 42 x 42 x

42cm opaque white polycarbonate arena (Nationwide Plastics) for a five-min trial. General locomotor and exploratory activity was recorded. Behavioral testing was simultaneously conducted on a per cage basis using up to 4 individual adjacent arenas. Mice were placed in the center of the arenas in pseudorandomized orientations. An arena center exploration zone (20 x 20cm) and corner zones (5 x 5cm) were used in analyses to distinguish measures of arena center exploration from time spent in arena corners (thigmotaxis). The discrimination ratio between time spent in arena center and arena corners was calculated for each mouse as:

$$DR = \frac{(center\ exploration - corner\ exploration)}{(center\ exploration + corner\ exploration)}$$

2.7 3-Chamber Social Interaction (3-CSI)

Habituation to a novel area, sociability, and social memory were probed by the Crawley 3-chamber social interaction paradigm (Nadler et al., 2004). Each test mouse was placed in the center chamber of a 3-chamber arena and allowed free exploration for 10 min in each of three consecutive trials (habituation, sociability, and social memory) run on the same day. Inter-trial interval was ~1 min. Stranger mice used for sociability and social memory trials were strain-, age- and sex-matched, were pseudo-randomized for each test subject, had never had previous contact with test mice, and were group-housed in a separate, but identical closed airflow cage system as test mice. Trial 1 (habituation) allowed exploration of the empty three chambers. Trial 2 (sociability) involved placing a novel conspecific stranger (stranger 1) inside one of the two counterbalanced ”holders” in a lateral chamber. Trial 3 (social memory) involved leaving the now-familiar stranger 1 mouse in the same chamber and holder, but another conspecific stranger (stranger 2) is added to the holder in the opposite lateral chamber. Chamber zones were established by the maze walls, and interaction zones were defined as roughly a 3cm area surrounding the holders. Holders were not in direct view from the opposite lateral chamber. Due to the potential confound of remaining olfactory cues in this test, handler gloves were changed in between each test subject and cage, and the mouse holders and arena were thoroughly cleaned in between subjects and cages. Measures of chamber exploration were taken for Trials 1 and 2, and measures of mouse holder perimeter exploration were taken for Trial 3. Data were expressed as chamber or holder perimeter exploration time.

2.8 Novel Object Recognition

Mice were reintroduced to the open field arena for habituation trials across 2 days (Antunes and Biala, 2012) for 10 min free exploration of the empty arena. On day three, mice were introduced to two identical objects (50mL plastic centrifuge tube filled with blue nitrile gloves and water) and allowed free exploration for 10 min. On day four, one object was swapped for a novel object: a 200mL polycarbonate cell culture flask filled with blue aquarium pebbles. For all days, a given mouse was placed in the center of the same arena yet facing a random direction, and on the object recognition test day (day 4) the novel object location was counterbalanced across subjects. Object exploration zones were defined as a ~3 cm perimeter around the external edge of each object. The time each mouse spent in object perimeters was considered as a measure of object exploration time. Object discrimination ratios were calculated as:

$$DR = \frac{(novel\ exploration - familiar\ exploration)}{(novel\ exploration + familiar\ exploration)}$$

2.9 Statistical Anlalyses

Complete details of all analyses excluding PCA are provided in **Supp. Table 1**. Normality of data distribution was first assessed by the Shapiro-Wilk test. Normally-distributed measures were next tested via 2-way ANOVA (main effects: radiation, diet) or 3-way ANOVA (main effects: radiation, diet, [object, or time]), when applicable. Post-hoc multiple pairwise comparisons were performed with

Tukey or Dunn's corrections, when relevant (**Supp. Table 1**). Group differences in non-normally distributed measures were assessed by Kruskal-Wallis tests. For the 3-CSI test, habituation was assessed by within-group 1-way Repeated Measures ANOVA, with chamber exploration (left, center, right) being a repeated measure. Within-group Sociability and Social Memory was assessed using paired t-tests by comparing the exploration time spent between the two lateral chambers. To determine an effect of diet or radiation, and possible interactions in each of the three trials of the assay, we first prepared data by log-transforming the ratio of exploration time of either lateral chamber or stranger for each respective testing phase:

Habituation: $log(\frac{left\ chamber}{right\ chamber})$

Sociability: $log(\frac{stranger\ 1\ chamber}{empty\ chamber})$

Social Memory: $log(\frac{stranger\ 1}{stranger\ 2})$

and of the arena center for all trials:

$$1 - log(\frac{center\ chamber\ exploration}{total\ exploration}).$$

Log exploration ratios of lateral chamber, stranger, and arena center were next used as dependent variables in linear regression models that were unadjusted and adjusted for arena center exploration. Mouse survival was assessed by the Log-rank test. Principal Component Analysis (PCA) and Pearson's correlation matrices were used for multivariable behavioral analyses. Principal components were based on parallel analyses, with loadings as behavioral measures for each sample, and with attention to percent variability per leading principal component. Statistical analyses were performed using Prism 9 (Graphpad). Effect sizes were calculated as percent variance in 2- and 3-way ANOVA analyses, where applicable, R^2 values for repeated measures 1-way ANOVA and multiple linear regressions, and Cohen's d for paired t-tests. All data are expressed as mean +/- SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ for main effects and interactions, and *a* $P < 0.05$, *b* $P < 0.01$, *c* $P < 0.001$, *d* $P < 0.0001$ in post-hoc analyses, where appropriate ($\alpha = 0.05$). Significant *P* values are italicized in the main text.

3. RESULTS

3.1 Study attrition and weights of all mice in study

Male rodent studies that begin at NSRL and end at a home institution can be marked by rates of attrition of 5-15% (Krukowski et al., 2018b, 2018c). Much of the subject number loss can be attributed to the rise of aggression and intra-cage fighting (perhaps due to stress of transport) which requires single-housing and precludes behavioral testing; however, radiation did not affect the weight of mice. In the present study, mice were primarily "removed" from the study when they were singly-housed due to veterinarian recommendations, with **Supp. Fig. 1** showing this attrition over time. Although survival of Veh/33-GCR mice over the entire study visually appears lower than all other groups, analysis shows there are no differences among mouse survival curves ($\chi^2 = 2.03$; $P = 0.57$).

3.2 Weights of behaviorally-tested mice

Mouse weights presented in **Fig. 1B** and data in all subsequent figures are from behaviorally-tested mice who, when examined alone, had a flat survival curve (**Supp. Fig. 1**). Since mice typically gain weight throughout adulthood, a predictable main effect is seen in these behaviorally-tested mice due to time ($F(16, 448) = 213.5$; $P < 0.0001$). However, there is no main effect of diet ($F(1, 406) = 2.208$;

P = 0.14) or radiation (F(1, 28) = 0.9108; *P* = 0.35; **Fig. 1B**), and no interactions among time, diet, or radiation (**Supp. Table 1**).

3.3 Activity Chambers

Four months post-irradiation, mice were tested for gross locomotor activity in fully-enclosed activity chambers (**Fig. 1**). For cumulative locomotion (**Fig. 2A**), there is no main effect of diet (F(1, 54) = 0.2471; *P* = 0.62) or radiation (F(1, 54) = 0.07420; *P* = 0.79), but there is a diet x radiation interaction (F(1, 54) = 4.160; *P = 0.0463*). Post-hoc multiple comparisons reveal no differences beyond chance in cumulative locomotion among the four groups (**Supp. Table 1**). For ambulatory episodes (**Fig. 2B**), there are no main effects of diet (F(1, 54) = 0.6358; P = 0.43) or radiation (F(1, 54) = 0.2526; *P* = 0.62), but there is a diet x radiation interaction (F(1, 54) = 4.268; *P = 0.044)* with no multiple comparisons-based differences evident (**Supp. Table 1**). The significant interaction between diet and radiation suggests that, although the effect of diet is not significant overall, it differs between the radiation groups. In regard to cumulative ambulation (**Fig. 2C**), there is no main effect of diet (F(1, 54) = 0.2024; *P* = 0.66) or radiation (F(1, 54) = 0.03055; *P* = 0.86), but there is a diet x radiation interaction (F(1, 54) = 6.410; *P = 0.01)* yet no post-hoc differences in mean ambulatory time among the four cohorts (**Supp. Table 1**). To further understand potential gross locomotor effects due to treatment, we assessed the mean velocity of mice across the testing period (**Fig. 2D**). No differences are observed among groups (H = 1.381; *P* = 0.71; **Supp. Table 1**).

3.4 Elevated-Plus Maze

Mice are naturally reluctant to leave an enclosed area for an open area, and thus spend more time in the closed vs. open arms in the EPM. However, due to endogenous exploratory drive they will occasionally overcome their putative exposure anxiety and venture into the open arms, providing a quantifiable measure of anxiety-like behavior. In open arm exploration time, there are no main effects of diet (F(1, 54) = 1.398; *P* = 0.24) or radiation (F(1, 54) = 0.01066; *P* = 0.92; **Fig. 3A, Supp. Table 1**). Similarly, in open arm entries (H = 2.675; *P* = 0.45; **Fig. 3B, Supp. Table 1**) and exploration ratios (H = 0.0412; *P* = 0.94; **Fig. 3C, Supp. Table 1**) group medians are not different beyond chance. Since anxiety-like activity often manifests in freezing behavior, cumulative locomotion of mice was also recorded throughout the entire EPM test period (**Fig. 3D**). In cumulative locomotion, there is a main effect of diet (F(1, 54) = 5.134; *P = 0.028*) but not of radiation (F(1, 54) = 0.03087; *P* = 0.86) with no interaction (F(1, 54) = 0.7894; P = 0.37). Mice given CDDO-EA at the time of either 33-GCR or Sham irradiation therefore move more in the EPM vs. mice given Vehicle, though the size of this effect is relatively small. Multiple comparisons reveal no differences beyond chance in mean total distance moved among the four cohorts (**Supp. Table 1**).

3.5 Open Field

Open field behavior was tested next (**Fig. 1**). The open field arenas are distinct from the activity chambers (Section 3.3) as they have no “roof”, and thus promote anxiety-like behavior in a prey species such as mice. Similar to the EPM, mice are reluctant to explore the open portion of the open field and will spend a larger proportion of time in the corners, once more providing a basis for quantifying anxiety-like behavior. There is no evident variation among arena center exploration medians (H = 1.961; *P* = 0.5805; **Fig. 4A, Supp. Table 1**) or of time spent in the corner of arenas (thigmotaxis; H = 2.122; *P* = 0.5475; **Fig. 4B, Supp. Table 1**). Discrimination ratios between time spent in the center vs. corners show no main effect of treatment (H =1.823; *P* = 0.6100; **Fig. 4C, Supp. Table 1**). In line with measures of exploratory or anxiety-like behavior, open field locomotion analysis shows no main effect of diet (F(1, 54) = 0.1548; *P* = 0.6955), radiation (F(1, 54) = 0.5643; *P* = 0.4558), or diet x radiation interaction (F(1, 54) = 1.818; *P* = 0.1832; **Fig. 4D, Supp. Table 1**).

3.6 Three-Chamber Social Interaction

Three-CSI consists of three sequential trials - habituation, sociability, and social memory - tested on a single day, with slight differences in chamber set-up in each trial (**Fig. 5**). In the habituation trial, exploration (time spent in) each of the three empty chambers was measured to assess possible chamber or testing environment bias (**Fig. 5A**). During habituation, mice may spend more time in the more enclosed lateral (left and right) chambers vs the center chamber, but a more important metric for later stages of the 3-CSI test is that during habituation the mice do not spend unequal time in the left vs right chambers. Within-group ANOVA on the 3-CSI habituation data suggests no main effect of chamber exploration time for Veh/Sham mice ($P = 0.08$) or CDDO-EA/Sham mice ($P = 0.17$; **Fig. 5A**). However, a main effect of chamber exploration time is detected in Veh/33-GCR mice ($P < 0.05$, **Fig. 5A**; medium effect size, **Supp. Table 1**) and CDDO-EA/33-GCR mice ($P < 0.01$, **Fig. 5A**; medium effect size, **Supp. Table. 1**). Post-hoc multiple-comparisons suggest that Veh/33-GCR mice spend more time exploring the left vs center chamber ($P < 0.05$) and more time exploring the right vs center chamber ($P < 0.01$), but do not spend more time exploring the left vs right chambers ($P = 0.93$; **Fig. 5A**). CDDO-EA/33-GCR mice also spend more time exploring the left vs center chamber ($P < 0.001$) and more time exploring the right vs center chamber ($P < 0.05$), but do not spend more time exploring the left vs. right chambers ($P > 0.99$; **Fig. 5A**). Given that the within-group analyses show a main effect (medium effect size) of chamber exploration time in the Veh/33-GCR and CDDO-EA/33-GCR groups, we next analyzed if during habituation, diet or radiation influenced the ratio of time exploring the left:right chamber independent of (unadjusted for) time spent in the center chamber; this analysis was not performed after adjusting for time in center since the 1-way ANOVA RM accounts for time spent in the center. Multiple linear regressions suggest during habituation there are no effects of and no interactions between diet and radiation on left:right chamber exploration time ratio ($P = 0.67$; **Supp. Table. 1**).

In the sociability 3-CSI trial, mice are tested for their willingness to approach an unfamiliar or “stranger” conspecific mouse enclosed in a holder in one of the lateral chambers. As a measure of sociability, the time a mouse spent exploring the chamber containing the holder with stranger 1 versus the opposite chamber with an empty holder was quantified (**Fig. 5B**). Within-group analysis of 3-CSI sociability data suggests that Veh/Sham, Veh/33-GCR, and CDDO-EA/Veh mice spent more time exploring the holder containing stranger 1 vs the empty holder ($P < 0.001$, $P < 0.0001$, $P < 0.01$, respectively) with large effect sizes (**Supp. Table 1**). However, CDDO-EA/33-GCR mice show no difference in time spent exploring the holder containing stranger 1 vs the empty holder ($P = 0.1252$), with a medium effect size (**Supp. Table 1**). Because time in the lateral chambers is quantified without considering time spent in the center, and to probe the role of diet or radiation on sociability we ran regressions that were unadjusted as well as adjusted for proportion of time spent in the center chamber, with lateral chamber exploration as the outcome variable (**Supp. Table 1**). Unadjusted analyses suggest an effect of lateral chamber exploration ($P < 0.01$), but not of diet, radiation, or a diet x radiation interaction. When adjusted for center chamber exploration, there are no main effects of chamber exploration ratio, diet, radiation, center exploration ratio and no interactions between chamber exploration ratio, diet, or radiation. These results suggest exploration of the center chamber is an influencing factor in lateral chamber exploration among groups.

Finally, in the 3-CSI social memory trial, measurement of the time the test mouse spent exploring the chamber containing the holder with now-familiar stranger 1 vs. a holder containing a novel stranger 2 gives us an index of social novelty and thus social memory. Mice have an endogenous drive to

explore novel mouse odors, and unless adversely affected, mice will spend more time around a novel vs. familiar mouse (**Fig. 5C**). Within-group analyses of 3-CSI social memory data show Veh/Sham mice spent more time exploring the holder containing stranger 2 vs. stranger 1 beyond chance ($P = 0.041$) with a moderate effect size ($d = 0.5361$; **Supp. Table 1**), suggesting normal social memory. In contrast, there is a trend toward Veh/33-GCR mice spending more time exploring the holder containing stranger 2 vs. stranger 1 and this difference approximated statistical significance ($P = 0.053$), but the effect size was extremely small ($d = 0.0936$). These data suggest stranger discrimination was impaired in Veh/33-GCR mice. CDDO-EA/Sham and CDDO-EA/33-GCR mice do not spend more time exploring the holder containing stranger 2 vs. stranger 1 ($P = 0.10$, $P = 0.75$, respectively; both small effect sizes [$d = 0.0007$ and $d = 0.1702$, respectively]). These data suggest CDDO-EA itself has a small negative effect on social memory and CDDO-EA does not prevent the 33-GCR-induced deficits in social memory. When assessing the relationship in 3-CSI social memory data between diet and radiation via multiple linear regression, there is a main effect of stranger exploration ratio ($P < 0.01$), the outcome variable, but not of diet, radiation, and there are no interactions among chamber exploration, diet, and radiation (**Supp Fig. 1**). However, when adjusting for center chamber exploration, there are main effects of stranger exploration ratio ($P < 0.01$), diet ($P < 0.05$), but no main effects of radiation, center chamber exploration ratio, and no interactions among main variables. These results suggest no influence of center chamber exploration on stranger exploration across the four groups (**Supp Fig. 1**).

3.7 Novel Object Recognition

Novel Object Recognition is another task that takes advantage of a mouse's exploratory drive to investigate unfamiliar over familiar objects. When analyzing the time mice spent exploring a 3cm margin around each object during the novel object testing trial (**Fig. 6A**), a main effect of object exploration ($F(1, 53) = 78.75$; $P < 0.0001$), but not of diet ($F(1, 53) = 0.0013$; $P = 0.97$) or radiation ($F(1, 53) = 1.149$; $P = 0.23$) is observed, with no interactions between or among the three main variables (**Supp. Table 1**). Multiple comparisons suggest all four groups explore the novel object more than the familiar object (Veh/Sham: $P < 0.0001$; Veh/33-GCR: $P = 0.023$; CDDO-EA/Sham: $P = 0.0005$; CDDO-EA/33-GCR: $P = 0.002$). To further assess the influence of treatment on object recognition, discrimination ratios were plotted (**Fig. 6B**). All mean object discrimination ratios are positive, there are no main effects of diet ($F(1, 53) = 0.4505$; $P = 0.51$) or radiation $F(1, 53) = 0.0754$; $P = 0.71$), and no diet x radiation interaction, suggesting intact and similar object discrimination across cohorts (**Fig. 6B**).

3.8 Multivariable Behavioral Analyses

PCA determines possible linear combinations of behavioral variables that account for the most variance by reducing the dimensionality of a multivariable dataset. This method allows for the visualization of related behaviors in a treatment-based manner. Individual samples are also displayed by reduced dimensionality of all behavioral values, allowing visualization of relating individuals by diet and radiation such that clusters emerge in similarly-performing individuals in a manner that does not factor in treatment in the calculation of plot coordinates. The total number of behaviorally-tested mice included in the analyses after outliers exclusions is 47, and the number of behavioral variables is 19. PC1 accounts for a relatively low 25.9% of the total variance, followed by 15.6% in PC2. A loadings plot reveals several relationships between behavioral measures (**Fig. 7A**). Anxiety-like and activity measures generally align in a cluster along the PC1 axis (PC1 = -0.45 to -0.85) and appear to be somewhat separate from object and stranger exploratory measures (PC1 = -0.2 to 1.0), with open field thigmotaxis and stranger 2 exploration isolated. Interestingly, however, PC2 clearly separates anxiety-like measures from activity measures (PC2 = 0 to -0.35, and 0.5 to

0.65, respectively). PC Loadings reveal several close variance relationships. For example, EPM exploration ratio is highly predictive of Open Field exploration ratio, suggesting the variances in height-dependent and open field exposure anxiety-like behavior are highly similar. An unexpected pattern that emerges is that the variance in Open Field exploration is tightly related to that of the time spent with a familiar stranger in the Social Memory trial of the 3-CSI test, though the two measures do not appear to correlate (**Fig. 7A, 7B**). Indeed, anxiety-like measures broadly show a moderate correlation, whereas no such observation is noted for exploratory behaviors (**Fig. 7B**). PC scores for individual mice reveal no treatment-based clustering, suggesting low general behavioral predictability due to treatment (**Fig. 8**).

4. DISCUSSION

To investigate the potential effects of a ground-based 33-beam GCR analog exposure, we compared the behavioral performance of mature male mice on a variety of tasks starting 4mo after exposure to an acute, 75cGy, Mars mission-relevant dose of whole-body 33-GCR or Sham irradiation. We additionally assessed whether a transient, five-day diet of CDDO-EA (400mg/Kg) or Vehicle given before, during, and immediately after irradiation prevented or attenuated behavioral changes associated with radiation. Behavioral testing beginning 4mo post-irradiation revealed no effects of radiation or diet on exploration or anxiety-like behaviors (assessed in open field, EPM, and 3-CSI habituation trial) or recognition of a novel object (NOR). However, in 3-CSI, sociability was compromised in CDDO-EA/33-GCR mice and social memory was blunted in Vehicle/33-GCR and CDDO-EA/Sham mice. CDDO-EA given before, during, and immediately after irradiation did not attenuate the 33-GCR-induced social memory deficits.

A consistent pattern in the literature across variations in particle, energy, and dose and multiple behavioral domains is that radiation often (but not always) leads to deficits in rodent behavior. The most commonly researched and reported effect is that high-energy charged-particle radiation decreases object recognition in rodents. This may reflect the abundance of literature on object recognition testing, the higher radiosensitivity of the hippocampus (the brain region often linked to object recognition ability), or a combination of these (Kwak et al., 2016; Kiffer et al., 2019b). Prior work assessing hippocampus-dependent behavior following irradiation paradigms most similar to the 33-GCR we used here (50cGy of protons, 1hr break, 10cGy of ^{16}O) found radiation decreased object-dependent short-term spatial memory in male mice 3 and 9 months later, and decreased object memory 9 months later (Kiffer et al., 2018a, 2020). It is therefore notable that here, we report no 33-GCR-induced changes in object recognition.

To assess why we observed relatively few GCR-dependent behavioral differences relative to what has been outlined in the literature, it's instructive to compare our behavioral findings to that of similar work. Whereas worse object recognition is relatively widely reported after exposure to a range of particle types, changes in open field anxiety-like behavior is not as commonly reported. Whole-body exposure to 50cGy of monoenergetic protons decreased open field activity when assessed in males 9 months later (Kiffer et al., 2018b). However, a sequential 50cGy exposure to protons and 10cGy ^{16}O did not change open field behavior in male mice 9 months later (Kiffer et al., 2020). A 50cGy proton exposure has also been reported to decrease spatial memory and disrupt hippocampal signaling in male mice (Bellone et al., 2015; Lee et al., 2017; Rudobeck et al., 2017). The 33-GCR paradigm in the current study consists of approximately 55cGy of protons at multiple energies (Supp. Table 2), and did not change anxiety-like behavior in open field or elevated plus maze behavior nor object exploration. However, a recent study using a 30cGy 5-GCR paradigm where the proton

contribution was 18cGy reported radiation-dependent reductions in object and spatial memory, and increased elevation-based anxiety 6 weeks after exposure in male mice (Klein et al., 2021). The inconsistencies in behavioral outcomes among studies using similar dose contributions by particle, and time from irradiation may be indicative of laboratory variations in housing conditions and testing, but could also suggest a complex mechanism between radiation complexity (number and energy of particles) and behavior.

Due to increasing interest in the social challenges associated with spaceflight, several groups have recently demonstrated charged-particle radiation-induced deficits in social memory in male rats and male and female mice from 1 to 9 months after exposures to doses of 10-50 cGy of either ^{16}O (0.1, 0.25 cGy) or ^{1}H+^{16}O+^{28}Si, a pattern that is emerging with the increased use of the 3-chamber social interaction test (Krukowski et al., 2018b; Mange et al., 2018; Kiffer et al., 2019a). Here we observed that radiation exposure blunted social memory. Non-irradiated mice given CDDO-EA similarly spent equal time exploring both stranger mice. Finally, mice given CDDO-EA and exposed to 33-GCR also spent similar time exploring stranger mice, suggesting the transient prior administration of CDDO-EA impairmed social memory and did not prevent 33-CGR-induced social memory deficits. To our surprise, unlike the three other cohorts, which demonstrated a significantly higher proportion of exploratory time with stranger 1 vs the empty chamber, CDDO-EA/33-GCR mice displayed similar stranger 1 and empty chamber exploration. Neither diet nor radiation changed gross locomotor activity, anxiety-like behavior, or object recognition. Furthermore, PCA indicated no relationship between treatment and overall behavioral measures across the tested domains. These results indicate that 1) 33-GCR radiation is detrimental to social memory when tested months after exposure, 2) transient prior administration of CDDO-EA did not block the radiation-induced social memory deficit, and 3) short-term CDDO-EA given months prior can itself decrease social memory.

Why might the 75cGy dose of 33-GCR paradigm used here not cause changes in anxiety, object recognition memory, or sociability relative to paradigms using similar doses of single- or simple particle combinations at similar time points following exposures (Kiffer et al., 2019b)? One reason could be that social memory is one of the first behavioral phenotypes to decline with age in mice (Boyer et al., 2019), and as previously suggested, charged-particle radiation has been argued to accelerate aging (Joseph et al., 1993; Shukitt-Hale et al., 2003; Casadesus et al., 2004; Vlkolinsky et al., 2010). Another possible reason is that a complex mixed radiation field such as the one we used for the present study has distinct radiation properties relative to single- or simple mixed-particle exposures. In this regard, it is useful to consider a peculiar aspect of radiation interaction with tissues: the dependence of Linear Energy Transfer (LET) of specific particles on type of DNA damage and damage response. Low-LET ^{4}He radiation induces a far lower proportion of clustered lesions when compared to high-LET ^{4}He, the damage of which consists almost entirely of clustered lesions as shown by exposure modelling (Nikjoo et al., 2001; Watanabe et al., 2015). In addition, proton-induced DNA double-strand breaks are also LET-dependent, which correlates with the relative biological effectiveness of the cell (Chaudhary et al., 2016). DNA damage response is likewise LET-dependent. When comparing low- to high-LET proton and ^{4}He exposures on varying non-neuronal cancerous cells, there are distinct LET-dependent mechanisms of recognition and response to DNA damage (Carter et al., 2018; Roobol et al., 2020). With respect to particles of similar LET and energies, double-stranded DNA breaks increase as particle track radius increases, which is dependent on the particle's atomic number, Z (Jezkova et al., 2018). Based on these observations, a complex particle field such as that of 33-GCR - which involves a combination of low- and high-LET radiation and particles of varying Z - is expected to recruit repair machinery associated with the high-LET components of the radiation field. This may help explain why mice exposed to

75cGy of 33-GCR have fewer changes in behavior relative to mice exposed to similar doses of high- or low-LET radiation (Cekanaviciute et al., 2018). Furthermore, the cell-based studies showing LET-dependent differences in DNA repair used very high doses. It is also possible that there are "upside-down-U" dose-dependent effects of radiation repair where lower doses are not as effective at DNA repair response, as previously suggested (Carr et al., 2018). This may explain why a 30cGy dose of 5-GCR decreased performance of mice in several behavioral domains (Klein et al., 2021) whereas the 75cGy dose of 33-GCR used here only decreased social memory. Future work using 33-GCR at different doses should elucidate the potential dose- and LET-properties of a complex radiation field on DNA damage and response, central nervous system response due to LET-dependence being characterized in non-nervous tissues and cognitive behavior.

While mechanistic underpinnings of the 33-GCR-induced deficits in social memory were outside the scope of this study, it is reasonable to consider that these social memory deficits were due to hippocampal radiosensitivity following a rich literature on charged-particle radiation-dependent changes to the hippocampus and hippocampus-relevant behavior. Even though we did not observe treatment-based changes to NOR, which is at least in part hippocampus-dependent, emerging work points to the hippocampal CA2 as being critical for the social memory of mice under the 3-chamber social recognition test (Hitti and Siegelbaum, 2014; Meira et al., 2018). Importantly, the CA2 is uninvolved in the classical hippocampal pathways associated with acquisition and recall of episodic memory including object-dependent memory, and has been recently implicated in its own hippocampal and non-hippocampal pathways (Kohara et al., 2014). This may help explain why stranger 2 exploration in the social memory phase of the 3-chamber social recognition test was the most variance-isolated principal component loading. Given this distinction, future work should interrogate the potential effects of 33-GCR on the hippocampal CA2 and its associated regions, due to previous work showing that 25cGy of ^{16}O impaired social memory, profoundly reduced the length of CA2 pyramidal neuron dendrites, while reducing dendritic complexity and lowered CA2 mushroom spine density of mature female mice (Kiffer et al., 2019a). Furthermore, slightly higher doses (100cGy) of ^{12}C or ^{28}Si have been demonstrated to reduce hippocampal neurogenesis in the dentate gyrus of male and female mice, a region that has also been recently shown to project directly to the CA2 (Rola et al., 2005; Kohara et al., 2014; Whoolery et al., 2017; Zanni et al., 2018). The hippocampal CA2 may therefore be a more radiosensitive subregion than other hippocampal areas.

Due to the complexities and costs associated with radiation shielding, pharmacological countermeasures are being considered to mitigate radiation exposures. One promising family of antioxidant compounds is the triterpenoid oleanolic acid derivative CDDO, which can be orally administered. Several CDDO variations exist with differing moieties on carbon 28. One such compound, CDDO-Me, has gone through a number of clinical trials and at the time of preparation of this manuscript in anti-inflammatory therapy phase-3 trials (NCT03749447) for chronic kidney disease. CDDO is of particular CNS interest due to proven therapeutic intervention for mouse models of Huntington's disease, malaria, and ischemic injury (Stack et al., 2010; Crowley et al., 2017; Xu et al., 2017; Lei et al., 2020). In addition, dietary CDDO-EA (400mg/Kg) has proven as an effective countermeasure against GCR-induced lung tumors in tumorigenic mice exposed to 30cGy of a 3-beam mixed field (Luitel et al., 2020). The exact mechanism of charged-particle radiation toxicity on neurons remains unclear, though oxidative stress, being the primary source of radiation interaction with tissues remains a primary therapeutic target. CDDO-EA was therefore a promising intervention as it offers a multifaceted protective approach by targeting the Nrf-2 pathways, which have protective downstream effects on oxidative stress management, microglial activation, and blood-brain barrier integrity, all of which have been demonstrated to be adversely affected in the

central nervous system of mice following radiation (Mao et al., 2016; Krukowski et al., 2018a). Contrary to our hypothesis, CDDO-EA did not exert a protective effect in male mice exposed to 33-GCR. Whereas CDDO-EA may be effective at quenching membrane and cytosolic oxidative stress, we speculate that it might be incapable of preventing the sudden DNA damage that high-energy radiation produces. However, previous studies including dietary antioxidants have demonstrated protective behavioral outcomes in otherwise impaired male rats and mice but only at the relatively high doses of 150cGy or greater (Rabin et al., 2005; Shukitt-Hale et al., 2013; Villasana et al., 2013; Poulose et al., 2014). Interestingly, in our study neither diet nor radiation affected sociability individually in mice, but CDDO-EA in combination with 33-GCR exposure did. Future work is warranted to understand the intricacies of the interactions between 33-GCR exposure, CDDO-EA and sociability behavior.

While some of the group differences in behavior approached critical alpha significance, particularly within Sham/33-GCR social memory, effect sizes suggest no meaningful differences in stranger exploration; therefore, replication of this experiment at different time points from irradiation is warranted. In addition, it's important to point out that CDDO-EA was only provided transiently, while in ongoing human clinical trials with CDDO, volunteers are provided daily oral CDDO-Methyl (Bardoxylone) without any reported behavioral changes. It's possible that CDDO-EA may exert a radioprotective effect if given for a longer duration following radiation exposures. Similarly, the GCR fluence in space is on the order of 0.01 cGy/day, and the dose-rate effects of 33-GCR should similarly be elucidated. Future studies examining the effects of space radiation on the brain should pursue mechanisms of action related to DNA damage recognition and repair promotion in an LET-basis. Whereas much attention has been placed on cognitive behaviors (Kiffer et al., 2019b), more attention to social behaviors is warranted due to the increasing body of literature outlining radiation-dependent changes in sociability and social memory following exposure to charged-particle radiation (Krukowski et al., 2018b; Mange et al., 2018; Kiffer et al., 2019a), as well as the relevance of social behavior to prolonged spaceflight (Landon, L. B., Vessey, W. B., Barrett, J. D., 2016). This study, which to our knowledge is the first to assess the effects of 33-GCR on the central nervous system, suggests the brain remains a relevant area of concern for Mars-relevant spaceflight.

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Declaration of Interest

JWS serves on the scientific advisory board of Reata Pharmaceuticals (Irvine, TX). Neither the funding bodies nor Reata Pharmaceuticals were involved in the study design, manuscript preparation, or decision to publish.

Data Availability Statement

Experiment data will be made available upon reasonable request.

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Fig. 1

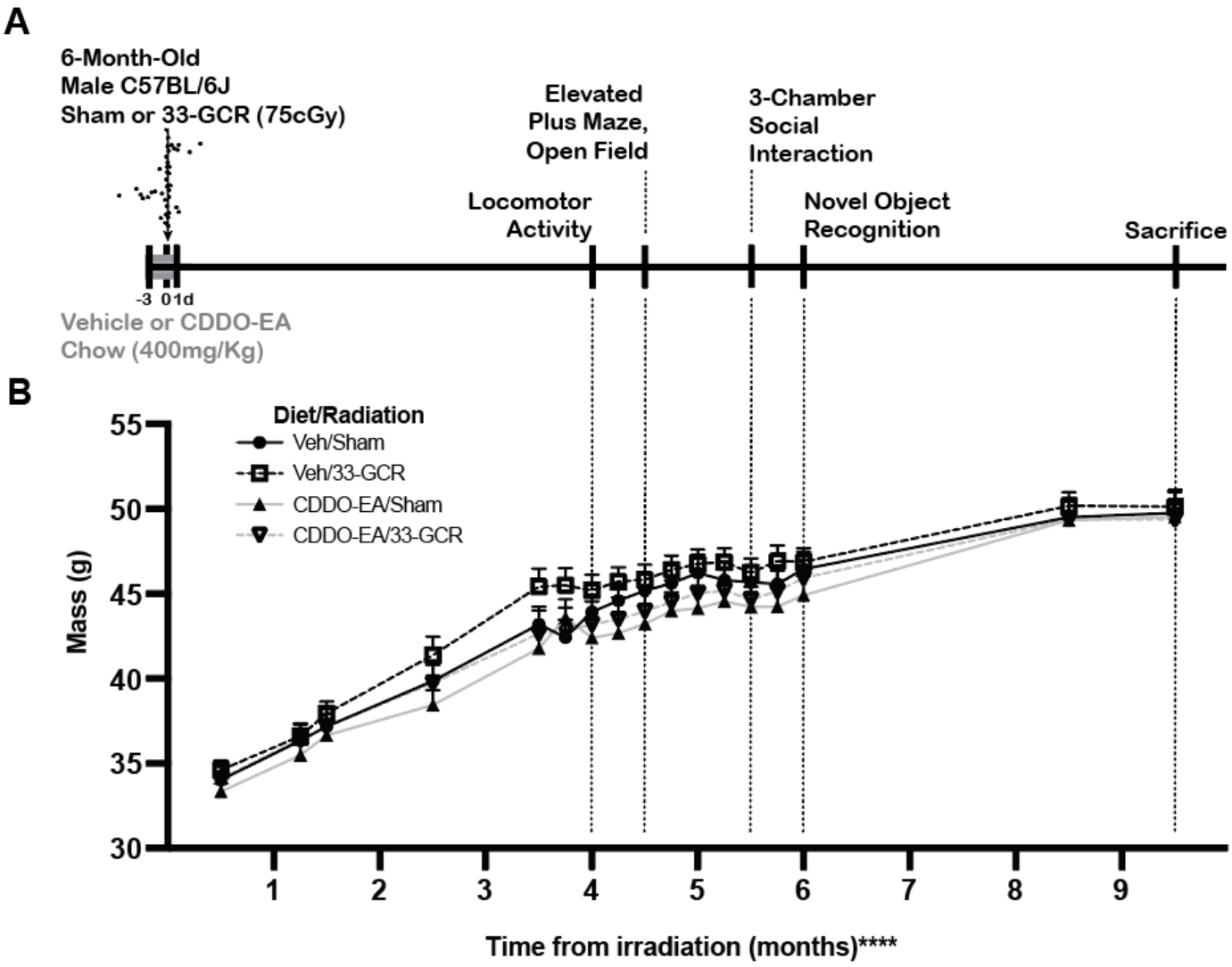


Figure 1. Experimental timeline and weights of behaviorally tested mice. A) Six-month-old male C57BL/6J mice received either a Mars-mission-relevant 75cGy whole-body exposure to ground-based galactic cosmic radiation consisting of 33 unique particle types of varying "Z" (atomic weight) and energies (33-GCR, represented by the arrow with a particle track) or sham irradiation (Sham; **Supp Table 2**). A subset of each group was given the naturally-derived, candidate dietary countermeasure 2-cyano-3, 12-dioxooleana-1, 9-dien-28-oic acid-ethylamide (CDDO-EA) or a vehicle (Veh) diet for 5 consecutive days before, during, and immediately after irradiation such that Day 4 of CDDO-EA or vehicle coincided with 33-GCR or Sham. Beginning 4 months following irradiation, mice were tested for gross locomotor and exploratory activity (Locomotor Activity, Open Field, *habituation trial* of the 3-Chamber Social Interaction [3-CSI] task), anxiety-like behavior (Elevated Plus Maze, Open Field), sociability (3-CSI *sociability* trial), social memory (3-CSI *social memory* trial), and exploratory cognitive behavior (Novel Object Recognition). Sacrifice occurred 39 weeks post-irradiation. **B**) Mouse mass in all cohorts increased over time with no difference among groups. Details on statistics provided in **Supp. Table 1**.

Fig. 2

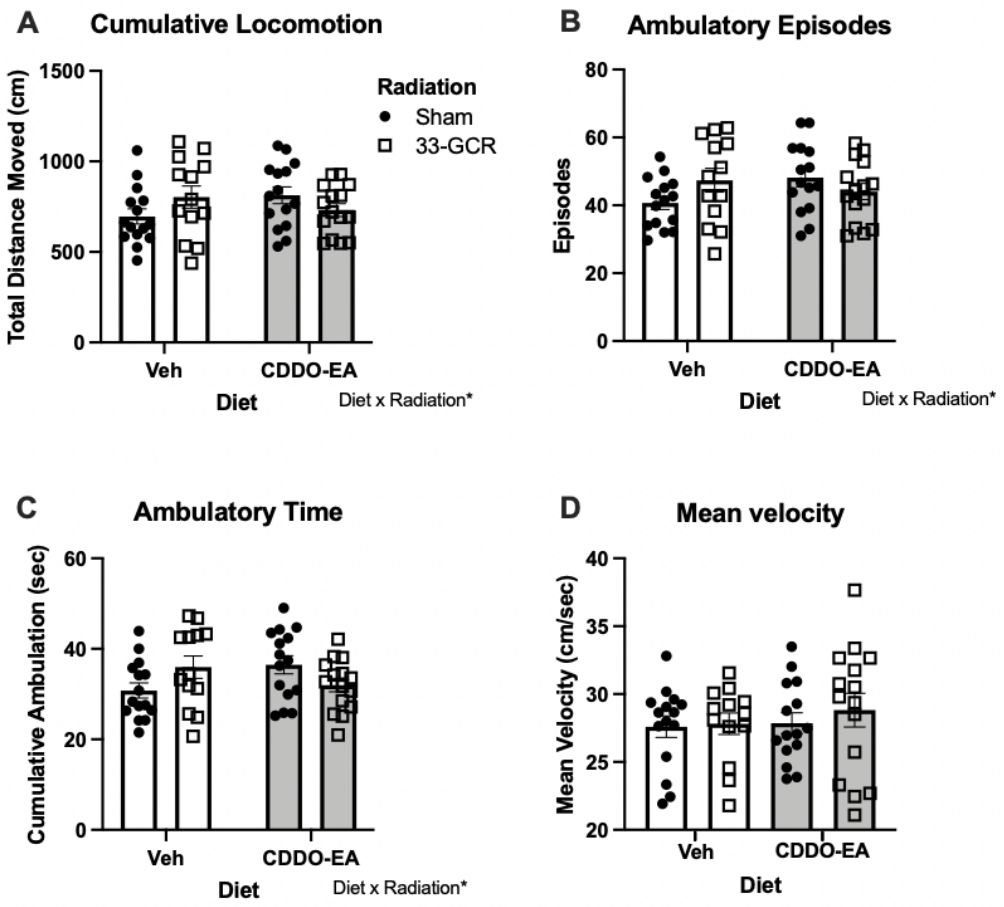


Figure 2. Exposure to 33-GCR and CDDO-EA did not change gross locomotor activity measures 4 months post-irradiation. Despite a significant interaction between diet and radiation (**A-C**), mice in all groups had similar **A**) Cumulative Locomotion, **B**) Ambulatory Episodes, and **C**) Ambulatory Time. **D**) Mean Velocity was also unchanged. Details on statistics provided in **Supp. Table 1**.

Fig. 3

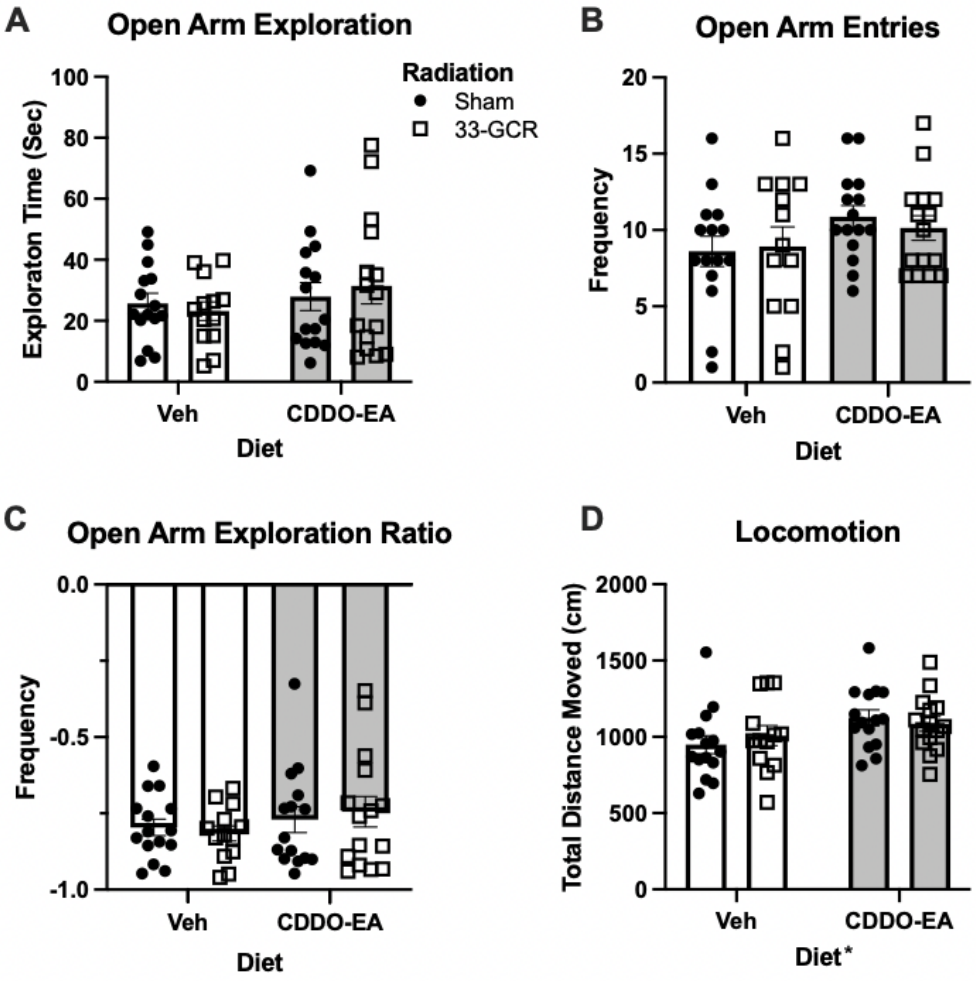


Figure 3. Exposure to 33-GCR and CDDO-EA did not change measures in the Elevated Plus Maze 4.5 months post-irradiation. Open Arm **A**) Exploration and **B)** Frequency of entries were similar among experimental groups. **C**) No difference in exploration ratios between Open and Closed arms were noted among treatment groups. **D**) There was a main effect of diet; mice that received CDDO-EA 4.5 months prior (and either 33-GCR or Sham) moved more in the EPM vs. mice that received Sham. Post-hoc analyses revealed no differences in Total Distance Moved among the four groups. Details on statistics provided in **Supp. Table 1**.

Fig. 4

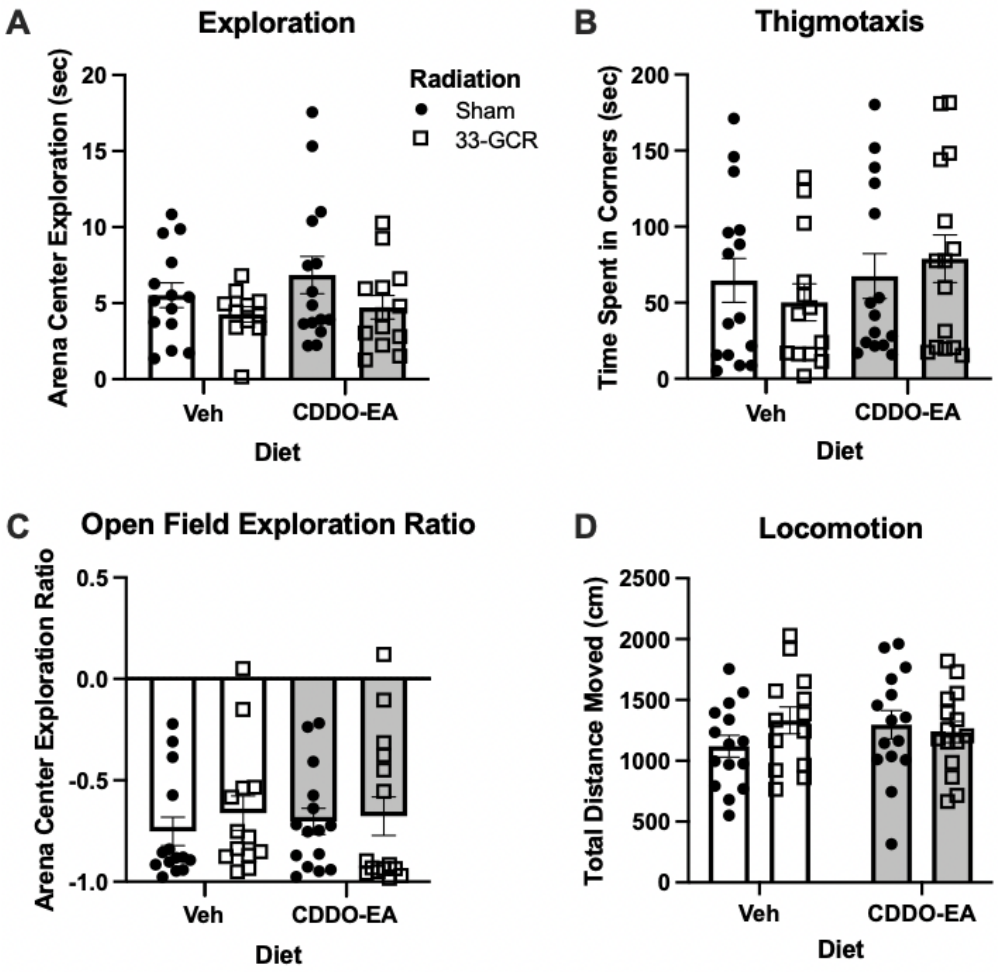


Figure 4. Open field exploration is unaffected by 33-GCR or CDDO-EA when tested 4.5months post-IRR. Measures of **A**) Arena Center Exploration, **B**) Arena Corner Exploration, **C**) Exploration Ratio between Arena Center and Corner were statistically similar across experimental groups. **D**) No difference in Total Distance Moved was observed during open field testing. Details on statistics provided in **Supp. Table 1**.

Fig. 5

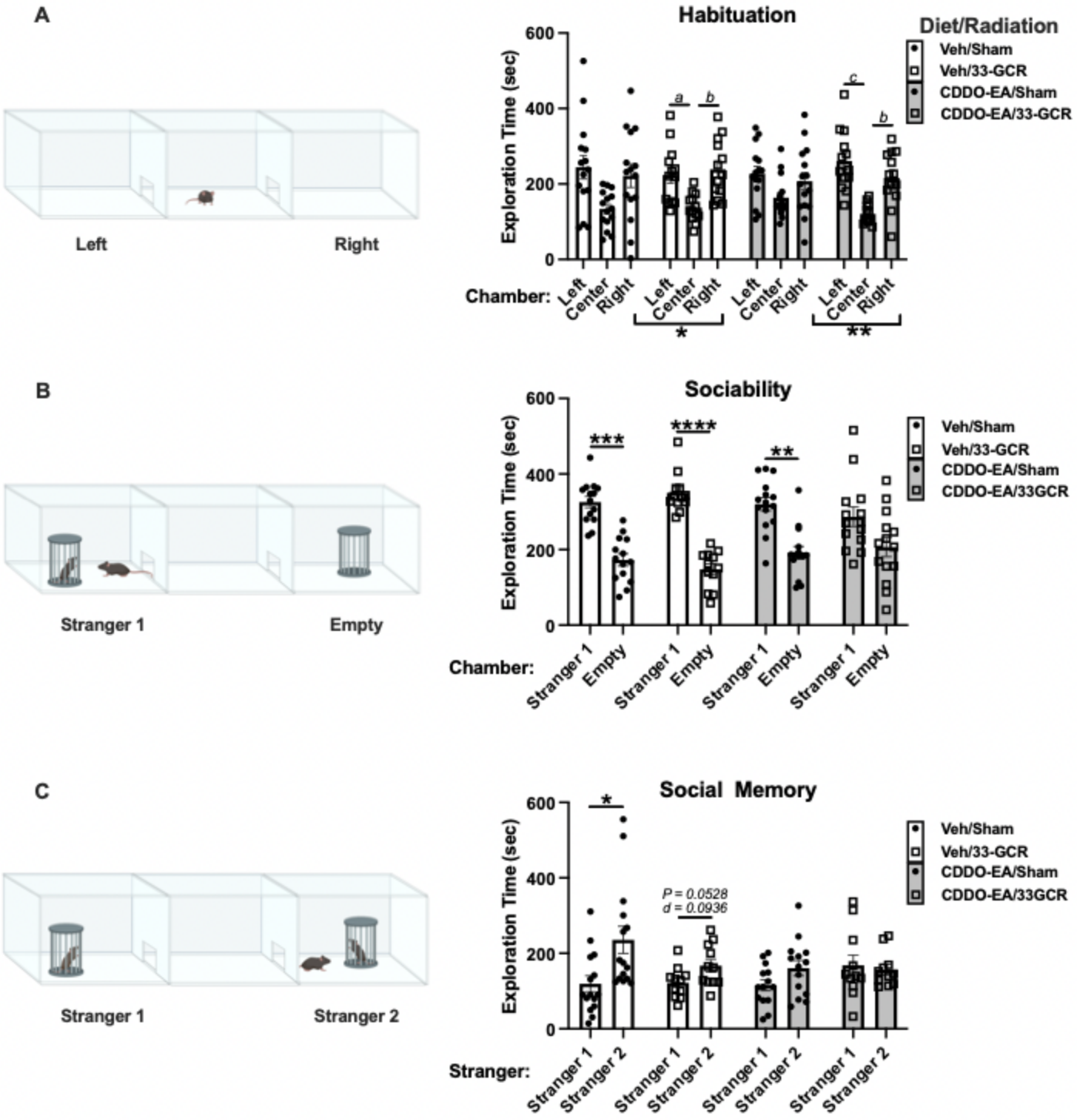


Figure 5. Social behavior was altered by radiation and CDDO-EA in mice when tested by the 3-chamber social interaction (3-CSI) paradigm. Schematics depicting experimental conditions in each of the three trials of 3-CSI: **A**) Habituation**, B**) Sociability and **C**) Social Memory. **A**) Habituation to the arena (left, center, right chambers) was uniform in each treatment group. **B**) When tested for their propensity to explore a stranger conspecific, mice of three treatment groups (Veh/Sham, Veh/33-GCR, CDDO-EA/Sham) spent a significantly larger proportion of exploration time in the chamber containing the stranger vs. the empty chamber. CDDO-EA/33-GCR mice did not. **C**) When adding yet another conspecific stranger to the arena,

only Veh/Sham mice explored the novel stranger (stranger 2) for a significantly longer proportion of the exploration duration than the previous stranger (stranger 1), with Veh/33-GCR mice approaching significance. A CDDO-EA diet appears to have compromised social memory in both the Sham and 33-GCR groups. Schematics generated with biorender. Details on statistics provided in **Supp. Table 1**. Main effects: *P = < 0.05, **P = < 0.01; Multiple Comparisons: *a* P = < 0.05, *b* P = < 0.01, *c* P = < 0.001; *d* = effect size (Cohen's d).

Fig. 6

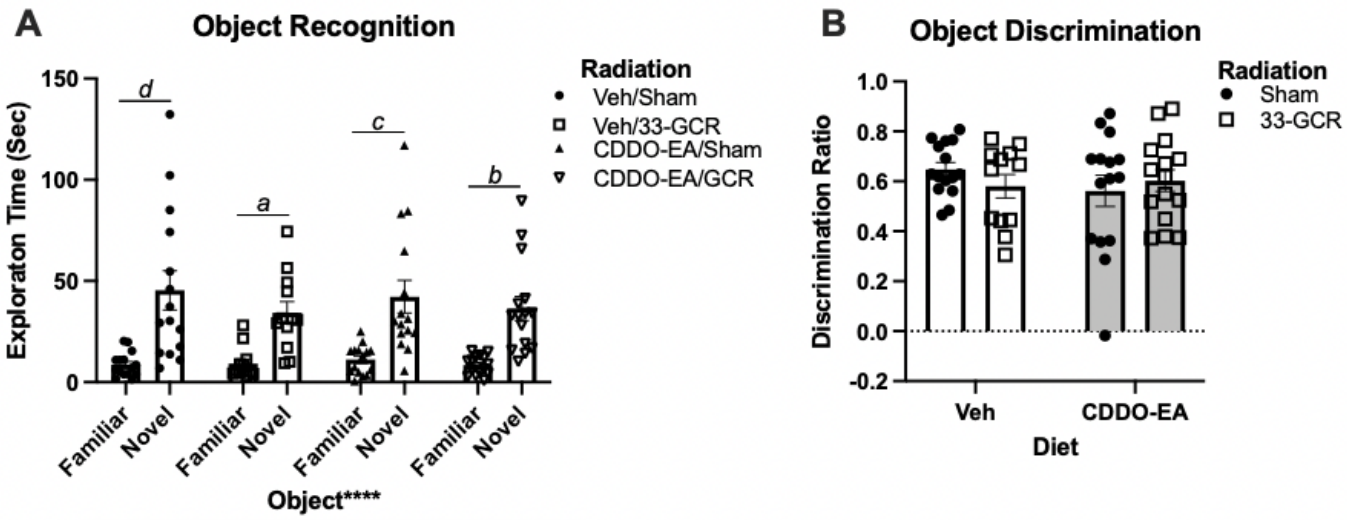


Figure 6. No effect of 33-GCR or CDDO-EA on Novel Object Recognition was observed during testing. **A**) Mice across treatment groups spent significantly more time exploring the Novel than the Familiar Object during the testing session. **B**) A similar mean positive Discrimination Ratio was observed across mice in all groups, indicating intact object memory. Details on statistics provided in **Supp. Table 1**.

Fig. 7

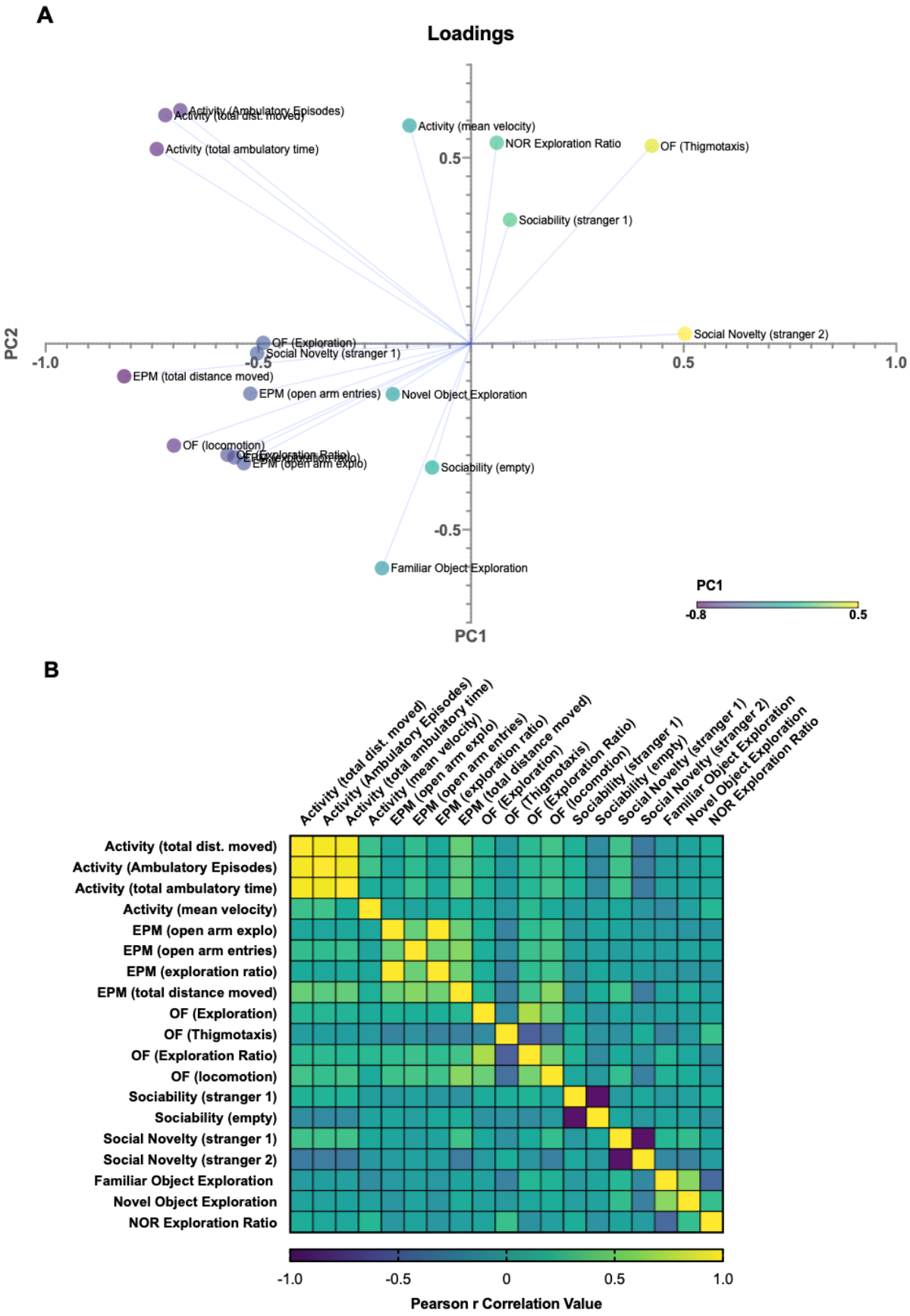


Figure 7. Relationship between behavioral performances between behavioral tests. A) Principal components of loadings reveal several clusters relating to Anxiety-like, Gross Locomotor Activity, and Exploration behaviors. **B**) A correlation matrix suggests strong correlations between Activity measures, and moderate correlations between Anxiety measures, and Exploratory measures.

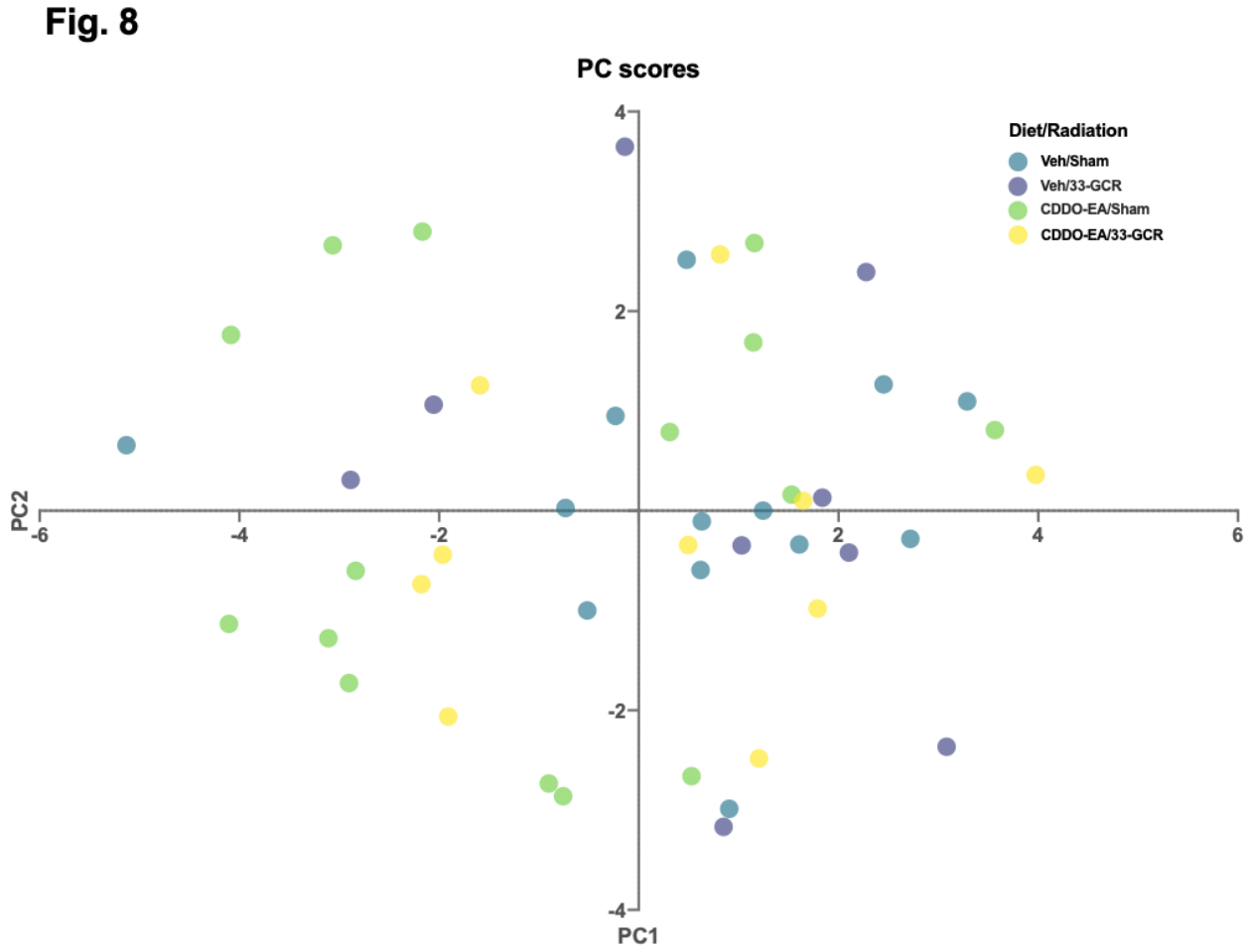


Figure 8. Principal Component (PC) Analysis of behavioral measures between treatments. Individual behavioral measures were used as loadings. A lack of treatment-dependent clusters suggests no gross variance-based behavioral relationships due to specific CDDO-EA or 33-GCR treatments. *N* = 12-16; PC1 = 25.9%, PC2 = 15.56%.

SUPPLEMENTARY DATA

Supplementary Table 1. (Statistical Analyses)

Experiment	Behavior	Figure Panel	Test Statistic	Main Effect	Dist.-Value (Dfn, Dfd)	Main Effect P-Value (bold text: $P<0.05$)	Effect Size (% variance, R^2, or Cohen's d)	Post-hoc Test	Group	Sample Size (n)	Gaussian?	Mean (predicted or rank)	Group Difference P-Value
Animal Weight		1B	Mixed Effects 3-way ANOVA	Time from Radiation (months)	F (16, 448) = 213.5	***P<0.0001***			Veh/Sham	15			
				Diet	F (1, 406) = 2.208	*P=0.1381*			Veh/33-GCR	13			
				Radiation	F (1, 28) = 0.9108	*P=0.3481*			CDDO-EA/Sham	15			
				Time from Radiation (months) x Diet	F (16, 406) = 0.9906	*P=0.466*			CDDO-EA/33-GCR	15			
				Time from Radiation (months) x Radiation	F (16, 406) = 0.3613	*P=0.9898*							
				Diet x Radiation	F (1, 406) = 0.05553	*P=0.8138*							
				Time from Radiation (months) x Diet x Radiation	F (16, 406) = 0.9120	*P=0.5555*							
Locomotor Activity Chambers	Cumulative Locomotion	2A	2-way ANOVA	Radiation x Diet	F (1, 54) = 4.160	***P=0.0463***	% Var = 7.107		Veh/Sham	15	Yes	696.2	
				Diet	F (1, 54) = 0.2471	*P=0.6212*	% Var = 0.4221	Mult. Comp/ w/ Tukey Corr.	Veh/33-GCR	13	Yes	802.8	All Comparisons *P > 0.05*
				Radiation	F (1, 54) = 0.07420	*P=0.7864*	% Var = 0.1268		CDDO-EA/Sham	15	Yes	813.1	
									CDDO-EA/33-GCR	15	Yes	731.7	
	Ambulatory Episodes	2B	2-way ANOVA	Radiation x Diet	F (1, 54) = 4.268	***P=0.0436***	% Var = 7.204		Veh/Sham	15	Yes	40.67	
				Diet	F (1, 54) = 0.6358	*P=0.4287*	% Var = 1.073	Mult. Comp/ w/ Tukey Corr.	Veh/33-GCR	13	Yes	47.38	All Comparisons *P > 0.05*
				Radiation	F (1, 54) = 0.2526	*P=0.6173*	% Var = 0.4264		CDDO-EA/Sham	15	Yes	48.16	
									CDDO-EA/33-GCR	15	Yes	44.07	
	Ambulatory Time	2C	2-way ANOVA	Radiation x Diet	F (1, 54) = 6.410	***P=0.0143***	% Var = 10.56		Veh/Sham	15	Yes	30.79	
				Diet	F (1, 54) = 0.2024	*P=0.6546*	% Var = 0.3333	Mult. Comp/ w/ Tukey Corr.	Veh/33-GCR	13	Yes	35.95	All Comparisons *P > 0.05*
				Radiation	F (1, 54) = 0.03055	*P=0.8619*	% Var = 0.05031		CDDO-EA/Sham	15	Yes	46.48	
									CDDO-EA/33-GCR	15	Yes	31.98	
	Mean Velocity	2D	Kruskal-Wallis		H = 1.381	*P=0.7101*			Veh/Sham	15	No	27.47	
									Veh/33-GCR	13	No	28.54	
									CDDO-EA/Sham	15	Yes	28	
									CDDO-EA/33-GCR	15	Yes	33.87	
Elevated Plus Maze	Open Arm Exploration	3A	2-way ANOVA	Radiation x Diet	F (1, 54) = 0.4639	*P=0.4987*	% Var = 0.8309		Veh/Sham	15	Yes	25.74	
				Diet	F (1, 54) = 1.398	*P=0.2422*	% Var = 2.504		Veh/33-GCR	13	Yes	23.17	
				Radiation	F (1, 54) = 0.01066	*P=0.9181*	% Var = 0.0191		CDDO-EA/Sham	15	Yes	27.96	
									CDDO-EA/33-GCR	15	Yes	31.44	
	Open Arm Entries	3B	Kruskal-Wallis		H = 2.675	*P=0.4445*			Veh/Sham	15	Yes	24.9	
									Veh/33-GCR	13	Yes	28.12	
									CDDO-EA/Sham	15	Yes	34.7	
									CDDO-EA/33-GCR	15	No	30.1	
	Open Arm Exploration Ratio	3C	Kruskal-Wallis		H = 0.0412	*P=0.9400*			Veh/Sham	15	Yes	29.47	
									Veh/33-GCR	13	Yes	27.15	
									CDDO-EA/Sham	15	No	29.93	
									CDDO-EA/33-GCR	15	No	31.13	
	Locomotion	3D	2-way ANOVA	Radiation x Diet	F (1, 54) = 0.7894	*P=0.3782*	% Var = 1.313		Veh/Sham	15	Yes	948.5	
				Diet	F (1, 54) = 5.134	***P=0.0275***	% Var = 8.538	Mult. Comp/ w/ Tukey Corr.	Veh/33-GCR	13	Yes	1008	All Comparisons *P > 0.05*
				Radiation	F (1, 54) = 0.03087	*P=0.8612*	% Var = 0.0513		CDDO-EA/Sham	15	Yes	1125	
									CDDO-EA/33-GCR	15	Yes	1085	
Open Field	Arena Center Exploration	4A	Kruskal-Wallis		H = 1.961	*P=0.5805*			Veh/Sham	15	Yes	28.61	
									Veh/33-GCR	13	Yes	23.86	
									CDDO-EA/Sham	15	No	30.57	
									CDDO-EA/33-GCR	15	Yes	23.81	
	Thigmotaxis	4B	Kruskal-Wallis		H = 2.122	*P=0.5475*			Veh/Sham	15	Yes	27.53	
									Veh/33-GCR	13	No	25.04	
									CDDO-EA/Sham	15	No	31.37	
									CDDO-EA/33-GCR	15	No	33.47	
	Exploration Ratio	4C	Kruskal-Wallis		H =1.823	*P=0.6100*			Veh/Sham	15	No	26.5	
									Veh/33-GCR	13	No	33.31	
									CDDO-EA/Sham	15	No	30.6	
									CDDO-EA/33-GCR	15	No	26	
	Locomotion	4D	2-way ANOVA	Radiation x Diet	F (1, 54) = 1.818	*P=0.1832*	% Var = 3.215		Veh/Sham	15	Yes	1120	
				Diet	F (1, 54) = 0.1548	*P=0.6955*	% Var = 0.2738		Veh/33-GCR	13	Yes	1333	
				Radiation	F (1, 54) = 0.5643	*P=0.4558*	% Var = 0.9983		CDDO-EA/Sham	15	Yes	1296	
									CDDO-EA/33-GCR	15	Yes	1236	
3-Chamber Social Interaction	Habituation	5A	1-way Repeated ANOVA	Time Spent in Chamber	F (1.256, 17.59) = 3.249	*P=0.0811*	R^2 = 0.0000		Veh/Sham	15	Yes		
			1-way Repeated ANOVA		F (1.346, 16.15) = 5.887	***P=0.0200***	R^2 = 0.3291 (medium)	Mult. Comp/ w/ Tukey Corr.	Veh/33-GCR	13	Yes	Left (223.6) vs. Center (137.4)	***P=0.0131***
												Left (223.6) vs. Right (238.7)	*P=0.9303*
												Center (137.4) vs. Right (238.7)	***P=0.0059***
			1-way Repeated ANOVA		F (1.531, 21.44) = 1.955	*P=0.1725*	R^2 = 0.1225		CDDO-EA/Sham	15	Yes		
			1-way Repeated ANOVA		F (1.182, 14.18) = 10.96	***P=0.0038***	R^2 = 0.4774 (medium)	Mult. Comp/ w/ Tukey Corr.	CDDO-EA/33-GCR	15	Yes	Left (260.8) vs. Center (120.5)	***P=0.0040***
												Left (260.8) vs. Right (216.5)	*P=0.5476*
												Center (120.5) vs. Right (216.5)	***P=0.0018***
			Mult. Linear Regression	Chamber Exploration Ratio	F (3, 49) = 0.3827	*P=0.7109*	Adj. R^2 = -0.0369				Yes		
				Diet		*P=0.8391*							
				Radiation		*P=0.6324*							
				Diet x Radiation		*P=0.5384*							
	Sociability	5B	2-Tailed Paired T-Test			***P=0.0002***	*d* =1.4028 (large)		Veh/Sham	15	Yes	Empty (173) vs Stranger 1 (325.9)	
						P<0.0001	*d* =2.0616 (medium)		Veh/33-GCR	12	Yes	Empty (147.8) vs Stranger 1 (350.1)	
						P=0.0020	*d* =0.9757 (large)		CDDO-EA/Sham	15	Yes	Empty (191.6) vs Stranger 1 (320.3)	
						P=0.1252	*d* =0.4379		CDDO-EA/33-GCR	15	Yes	Empty (206.7) vs Stranger 1 (287)	
			Mult. Linear Regression (unadjusted)	Chamber Exploration Ratio	F (3, 50) = 1.252	***P=0.0010***	Adj. R^2 = 0.0141				Yes		
				Diet		*P=0.7629*							
				Radiation		*P=0.2381*							
				Diet x Radiation		*P=0.2833*							
			Mult. Linear Regression (adjusted for center exploration)	Chamber Exploration Ratio	F (3, 49) = 0.7074	*P=0.2822*	Adj. R^2 = -0.0343				Yes		
				Diet		*P=0.9853*							
				Radiation		*P=0.5786*							
				Center Exploration Ratio		*P=0.4365*							
				Diet x Radiation		*P=0.2571*							
				Diet x Center Exploration Ratio		*P=0.9951*							
				Radiation x Center Exploration Ratio		*P=0.4894*							
	Social Memory	5C	2-Tailed Paired T-Test			***P=0.0406***	*d* =0.5361		Veh/Sham	15	Yes	Stranger 1 (119.4) vs Stranger 2 (235.6)	
						P=0.0528	*d* =0.0936		Veh/33-GCR	11	Yes	Stranger 1 (121.9) vs Stranger 2 (167.1)	
						P=0.1005	*d* =0.0007		CDDO-EA/Sham	14	Yes	Stranger 1 (115.2) vs Stranger 2 (160.8)	
						P=0.7523	*d* =0.1702		CDDO-EA/33-GCR	11	Yes	Stranger 1 (168) vs Stranger 2 (156.9)	
			Mult. Linear Regression (unadjusted)	Stranger Exploration Ratio	F (3, 47) = 1.336	***P=0.0014***	Adj. R^2 = 0.0198				Yes		
				Diet		*P=0.2066*							
				Radiation		*P=0.1893*							
				Diet x Radiation		*P=0.6547*							
			Mult. Linear Regression (adjusted for center exploration)	Stranger Exploration Ratio	F (6, 44) = 1.434	***P=0.0040***	Adj. R^2 = 0.0495				Yes		
				Diet		***P=0.0267***							
				Radiation		*P=0.2625*							
				Center Exploration Ratio		*P=0.1442*							
				Diet x Radiation		*P=0.6147*							
				Diet x Center Exploration Ratio		*P=0.0520*							
				Radiation x Center Exploration Ratio		*P=0.2111*							

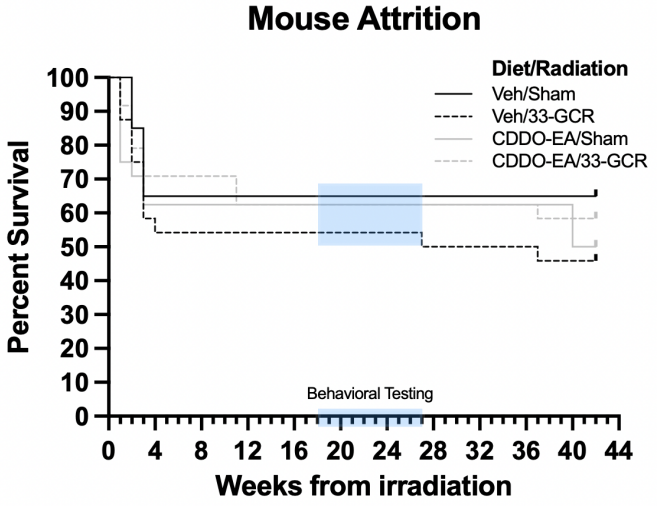
Novel Object Recognition	Object Exploration	6A	3-way ANOVA	Object	F (1, 53) = 78.75	***P=0.0001***	% Var = 34.60	Mult. Comp/ w/ Tukey's Test, Within Group	Veh/Sham (Familiar)	15	Yes	8.67	***P=0.0001***
				Diet	F (1, 53) = 0.001295	*P=0.9714*	% Var = 0.0009681		Veh/Sham (Novel)	15	No	45.41	
				Radiation	F (1, 53) = 1.149	*P=0.2887*	% Var = 0.8585		Veh/33-GCR (Familiar)	12	No	9.227	***P=0.0266***
				Object x Diet	F (1, 53) = 0.04101	*P=0.8403*	% Var = 0.01801		Veh/33-GCR (Novel)	12	Yes	34.3	
				Object x Radiation	F (1, 53) = 1.176	*P=0.2832*	% Var = 0.5165		CDDO-EA/Sham (Familiar)	15	Yes	11.14	***P=0.0005***
				Diet x Radiation	F (1, 53) = 0.01377	*P=0.9070*	% Var = 0.01029		CDDO-EA/Sham (Novel)	15	Yes	42.22	
				Object x Diet x Radiation	F (1, 53) = 0.3964	*P=0.5316*	% Var = 0.1742		CDDO-EA/33-GCR (Familiar)	15	No	8.453	***P=0.0022***
									CDDO-EA/33-GCR (Novel)	15	Yes	36.43	
	Object Discrimination	6B	2-way ANOVA	Radiation x Diet	F (1, 53) = 1.317	*P=0.2563*	% Var = 2.397		Veh/Sham	15	Yes	40.67	
				Diet	F (1, 53) = 0.4505	*P=0.5050*	% Var = 0.8203		Veh/33-GCR	12	Yes	47.38	
				Radiation	F (1, 53) = 0.07541	*P=0.7847*	% Var = 0.1373		CDDO-EA/Sham	15	Yes	48.16	
									CDDO-EA/33-GCR	15	Yes	44.07	
Survival		Supp. 1	Log-rank (Mantel-Cox)		χ^2 = 2.03	*P=0.565*			Veh/Sham	22			
									Veh/33-GCR	24			
									CDDO-EA/Sham	24			
									CDDO-EA/33-GCR	24			

Supplementary Table 2. 33-GCR paradigm in order of sequential delivery

Particle	Energy (MeV/n)	Dose Fraction	Dose (cGy)	Range in H2O (Cm)
^{1}H	1000	24.71%	18.5325	322
^{4}He	1000	4.98%	3.735	37.8
^{28}Si	600	1.62%	1.215	0.08
^{1}H	20	6.08%	4.56	0.42
^{1}H	23	1.34%	1.005	0.54
^{4}He	20	2.20%	1.65	0.04
^{4}He	23	0.42%	0.315	0.05
^{48}Ti	1000	0.90%	0.675	0.06
^{4}He	27	0.44%	0.33	0.06
^{4}He	32	0.46%	0.345	0.08
^{1}H	27	1.48%	1.11	0.72
^{1}H	32	1.60%	1.2	0.98
^{1}H	37	1.74%	1.305	1.28
^{1}H	43	1.86%	1.395	1.68
^{4}He	37	0.50%	0.375	0.12
^{4}He	43	0.52%	0.39	0.14
^{16}O	350	3.08%	2.31	0.13
^{4}He	50	0.54%	0.405	0.18
^{4}He	59	0.54%	0.405	0.24
^{1}H	50	2.00%	1.5	2.2
^{1}H	59	2.12%	1.59	2.97
^{1}H	69	2.22%	1.665	3.93
^{1}H	80	2.24%	1.68	5.13
^{4}He	69	0.54%	0.405	0.32
^{4}He	80	0.54%	0.405	0.42
^{12}C	1000	2.34%	1.755	1.84
^{4}He	100	1.22%	0.915	0.63
^{1}H	100	5.44%	4.08	7.64
^{1}H	150	7.00%	5.25	15.63
^{4}He	150	1.50%	1.125	1.32
^{56}Fe	600	0.82%	0.615	0.02
^{4}He	250	3.28%	2.46	3.3
^{1}H	250	13.77%	10.3275	37.6
		Total	**75**	

Supplemental Fig. 1

Fig. 1



Supplemental Figure 1. Mouse attrition among groups was not significantly different throughout the study. A Kaplan-Meier survival curve of mice throughout the duration of the study with the behavioral testing period shaded light blue. Mice that were singly-housed at the recommendation of veterinarians were counted as losses. The remaining mice were sacrificed on week 42 of the study. Despite a visually lower percent survival in Veh/33-GCR mice, survival curves are not significantly different. Details on statistics provided in **Supp. Table 1**.