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LOMA LINDA UNIVERSITY
School of Behavioral Health
in conjunction with the
Faculty of Graduate Studies

Effects of Proton Radiation on Behavior
in a Mouse Model of Alzheimer's Disease

by

John A. Bellone

A Thesis submitted in partial satisfaction of
the requirements for the degree
Doctor of Philosophy in Clinical Psychology

September 2014

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Each person whose signature appears below certifies that this thesis in his opinion is adequate, in scope and quality, as a thesis for the degree Doctor of Philosophy.

_____, Chairperson
Richard E. Hartman, Associate Professor of Psychology

Paul Haerich, Professor of Psychology

Roman Vlkolinský, Assistant Research Professor, Basic Sciences and Radiation
Medicine, School of Medicine

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Note: For correspondence or questions please contact John A. Bellone at jbellone@llu.edu or johnabellone@gmail.com.

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ABBREVIATIONS

AD	Alzheimer's Disease
APP	Amyloid Precursor Protein
BM	Barnes Maze
CI	Confidence Interval
cm	Centimeter(s)
EB	Escape Box
g	Gram(s)
GCR	Galactic Cosmic Rays
Gy	Gray
HZE	High Charge (Z), High-energy (E) Particle Radiation
mo	Month(s)
LET	Linear Energy Transfer
PSEN1	Presenilin 1
ROS	Reactive Oxygen Species
s	Second(s)
tg	Transgenic
WM	Water Maze
wt	Wild-type
yr	Year
ZM	Zero Maze

ABSTRACT OF THE THESIS

Effects of Proton Radiation on Behavior
in a Mouse Model of Alzheimer's Disease

by

John A. Bellone

Doctor of Philosophy, Graduate Program in Clinical Psychology
Loma Linda University, September 2014
Dr. Richard E. Hartman, Chairperson

Astronauts venturing outside Earth's magnetosphere risk exposure to charged particle radiation that has been shown to cause neurological deficits in rodents via oxidative stress, neuroinflammation, altered neurogenesis, and synaptic changes. Since these responses are similar to those observed in age-related neurodegenerative diseases, we hypothesized that individuals with a propensity toward developing Alzheimer's disease (AD) would be more adversely affected by such exposure. To test this hypothesis, we exposed young double transgenic APP/PSEN1 mice (a commercially available strain engineered to develop AD-like neuropathology) and their wild-type (non-transgenic) counterparts to low doses of 150 MeV proton particle radiation and assessed the effects on hippocampus-dependent behaviors. Spatial learning ability, a sensitive behavioral marker of hippocampal damage, was assessed using the water maze and Barnes maze 3 and 6 months after irradiation. Transgenic mice performed worse than wild-type mice on both behavioral measures, and wild-type mice exposed to 0.5 Gy performed worse than the 0 Gy wild-type mice at 6 months post-irradiation. However, radiation doses up to 1 Gy had no effect on transgenic spatial learning performance. These findings suggest that low doses of proton radiation cause deficits in normal

individuals, but may not exacerbate or accelerate learning and memory deficits in individuals predisposed toward age-related neurological disease.

CHAPTER ONE

INTRODUCTION

NASA has recently been investing in programs and research to assess “the risk of acute or late central nervous system effects from radiation exposure” (from the Human Research Program’s Integrated Research Plan, 2011, p. 26; see also Cucinotta, Wang, & Huff, 2009). Much of the funding is being allocated to animal research focusing on the behavioral effects of prolonged exposure to low doses of radiation. These types of studies attempt to mimic the environment astronauts will be travelling in on extended missions outside the magnetosphere.

The White House’s 2010 national space policy release called for NASA to prepare for a manned mission to an asteroid and Mars within the next two decades (June 28, available through: <http://www.whitehouse.gov/the-press-office/fact-sheet-national-space-policy>). Since the roundtrip journey to Mars will take approximately 2.5 years (Simonsen, Wilson, Kim, & Cucinotta, 2000), astronauts will be exposed to different types and doses of radiation for an extended period. It is estimated that a large number of brain cells (as much as 91% of hippocampal cells and 25% of cell nuclei per year) would be impacted by the particles that make up this radiation environment (Yasuda, Komiyama, & Fujitaka, 2001). These particles collide with other nuclei in the shielding material of the ship or in biological systems and generate secondary particles such as neutrons, α - particles, and electrons. Within the central nervous system, such impacts on neurons and other brain cells can lead to structural and functional changes that may ultimately manifest in cognitive and behavioral dysfunction.

The vast majority of space radiation consists of high-energy protons that originate

from solar activity and galactic cosmic rays (NCRPM, 2006; Zeitlin et al., 2013). These particles are known to cause damage to the central nervous system (CNS) at high doses despite their relatively low mass and low linear energy transfer (LET). Though a large portion of the space radiation environment is represented by protons, little has been investigated as to their effects on cellular mechanisms of learning and memory. Most of the published research has focused on the effects of high charge (Z), high-energy (E) particle radiation (HZE) characterized by high-LET particles, and the research that has been conducted using protons has focused on higher doses than astronauts are likely to receive (Cucinotta, Kim, & Chappell, 2012; Cucinotta, Kim, Chappell, & Huff, 2013).

The present study seeks to determine the effects of low, space-like doses of proton particles on behavior in mice. Additionally, it seeks to determine whether proton radiation affects mice with a predisposition toward developing Alzheimer's disease-like pathology differently than mice without this predisposition. Since radiation exposure and Alzheimer's disease (AD) share many neuropathological similarities, the interaction of radiation dose and genotype may be especially interesting.

Relatively few studies have assessed the effects of low doses of proton radiation on learning and memory function in normal mice, and no known study has measured behavior past 3.5 months post-irradiation or assessed the effects on mice with AD-like pathology. Gaining such an understanding is imperative, being that protons are the dominant ions in the space environment and potential radiation-induced cognitive impairments could jeopardize the astronauts' ability to perform mission objectives and compromise their quality of life.

Specific Aims and Hypotheses

In the present study, we sought to discover the behavioral effects of a low dose of proton radiation on normal mice, as well as the effects of varying doses on transgenic (tg) mice engineered to develop AD-like neuropathology.

Aim 1: To Determine whether a Low Dose of Proton Radiation

Affects Behavior in Normal, Wild-type Mice

Though the results of previous investigation into the behavioral effects of proton radiation has been mixed, data suggest that proton radiation can increase oxidative stress, inhibit neurogenesis, prompt neuroinflammation, cause DNA damage, and activate apoptosis-related genes. Since these can ultimately result in cognitive deficits, it was hypothesized that mice exposed to proton radiation would demonstrate spatial learning and memory deficits in comparison to controls.

Specific Hypothesis

Mice receiving a low dose (0.5 Gy) of proton radiation would perform worse on tests of spatial learning and memory relative to non-irradiated controls (see Figure 1).

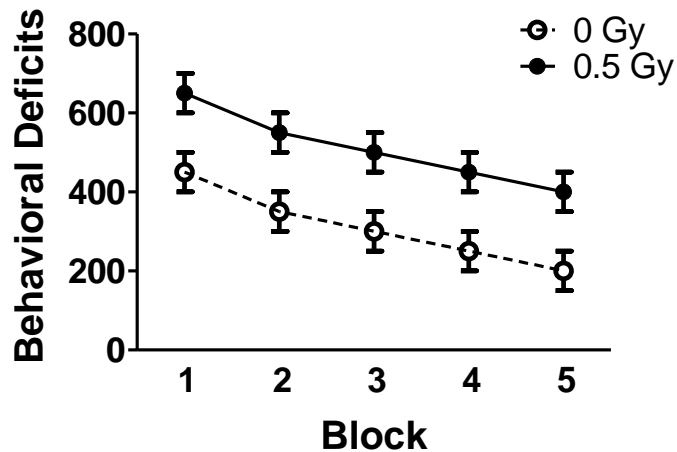


Figure 1. Hypothesized effects of proton radiation on normal mice.

Aim 2: To Determine whether Radiation Affects Behavior in Mice

Predisposed to Developing AD-like Pathology Differently than Wild-type

Mice, and whether this Effect is Dose-dependent

Due to the similarities between the neurological effects of radiation exposure and AD-like processes, it was hypothesized that there would be an additive effect that results in greater behavioral deficits for tg mice. Transgenic mice were hypothesized to have an increased sensitivity to radiation injury compared to wild-type (wt) mice, meaning they would experience greater learning and memory deficits than their irradiated, wt counterparts. Mice receiving higher doses (up to 1 Gy) of proton radiation were hypothesized to exhibit an earlier onset of AD symptomatology and to demonstrate more spatial learning and memory deficits than controls or mice receiving lower doses (e.g., 0.1 or 0.5 Gy).

Specific Hypothesis 1

Irradiated tg mice would perform worse on tests of spatial learning and memory than irradiated wt mice (see Figure 2).

Specific Hypothesis 2

Higher doses of radiation would lead to more severe spatial learning and memory deficits in tg mice compared to non-irradiated mice or those exposed to lower doses (see Figure 2).

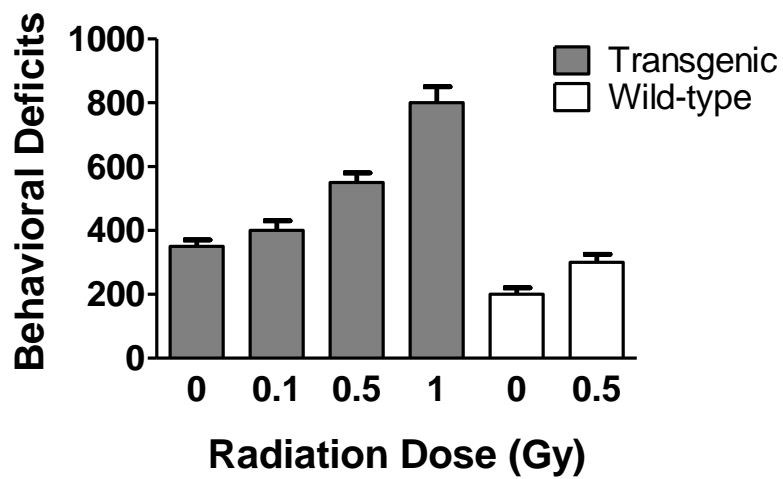


Figure 2. Hypothesized effects of various doses of proton radiation on tg mice compared to wt mice.

CHAPTER TWO

REVIEW OF THE LITERATURE

Various types of radiation have been shown to cause deleterious neurological effects in rodents. Much of the literature on low-dose effects has focused on high-LET iron (^{56}Fe) particles, since they make up a relatively large portion of galactic cosmic rays. Doses of ^{56}Fe radiation at 1 Gy and higher can produce hippocampal changes that result in behavioral deficits (Shukitt-Hale, Szprengiel, Pluhar, Rabin, & Joseph, 2004; Haerich, Nelson, & Pecaut, 2005), and one study even showed impairment at doses as low as 0.1 Gy (Shukitt-Hale, Casadesus, McEwen, Rabin, & Joseph, 2000). Other studies have used X-rays (Rola et al., 2004) and gamma radiation (Pellmar & Lepinski, 1993) to show similar results, though typically at higher doses.

Though the symptoms of radiation exposure may partially remit following the acute period, long-lasting changes have been noted. For example, mutations can occur for several generations in DNA-damaged cells (a process termed “genomic instability”), leading to an accumulation of genetic abnormalities (Bassing et al., 2002). Additionally, reduced immune functioning has been identified several months following radiation exposure in mice (Gridley, Pecaut, & Nelson, 2002).

Interestingly, some research has focused on the effects of low doses of radiation on humans. Though the doses of radiation that adult cancer patients often receive are too high and localized to pertain to the space environment (Nelson, 2011), cognitive deficits have been found in children exposed to radiation. For example, one cohort of children around 1 year of age received X-ray treatment to diminish the appearance of facial birthmarks. Doses above 0.1 Gy contributed to cognitive impairment and decreased

school attendance later in life (Hall et al., 2004). Another cohort of children in Israel received an average of 1.3 Gy of X-radiation to treat fungal infections. These children later had lower IQs and lower high school aptitude scores than peers who did not receive such treatment (Ron, Modan, Floro, Harkedar, & Gurewitz, 1982). Findings from these studies suggest that humans can be cognitively and behaviorally affected by low doses of radiation.

There are relatively few studies that have investigated the effects of proton radiation on behavior, and the results of those studies have been mixed. Shukitt-Hale and colleagues (2004) showed that rats given proton radiation at doses of 1.5, 3, and 4 Gy did not differ from the control group at 1.5 months post-irradiation. As a result, the authors suggested that proton radiation may not be as deleterious as high-LET particles. Dulcich and Hartman (2013) also found no spatial learning and memory deficits by 2 months after exposure to 2 Gy. Another study observed no differences between control rats and those given 4 Gy of proton radiation in a bar press task that assesses changes in reinforcement contingencies (Rabin et al., 2002). These findings indicate that proton radiation may not affect a rat's ability to respond appropriately in response to increased work demands.

In contrast to these studies, Pecaut and colleagues (2002) found that mice irradiated with 3 and 4 Gy of protons showed reduced habituation to an acoustic startle stimulus and acute learning and memory deficits in an object recognition test. Findings from another study showed that protons at doses of 2 and 5 Gy acutely attenuated startle reactivity (Haerich et al., 2012). While most of these studies have primarily focused on relatively high doses (up to 5 Gy) to maximize potential effects, doses at or below 1 Gy are more applicable to space travel under normal circumstances (Cucinotta et al., 2012; Cucinotta et al., 2013), and their effects need to be examined in detail.

Though the behavioral effects of proton radiation are still largely unknown, evidence suggests that molecular changes can occur. For example, Hada and Sutherland (2006) showed that proton radiation induced complex DNA damage (e.g., oxidized base and abasic clusters) *in-vitro*. A similar study found that the number of double-strand breaks increased with increasing doses of radiation (Friedland, Jacob, Bernhardt, Paretzke, & Dingfelder, 2003).

Discovering the neuropathological and behavioral effects of proton radiation is vital, since astronauts may not be safe even in their spacecraft. For example, reports of astronauts being impacted by particles while in their ship are well documented (Pinsky, Osborne, Bailey, Benson, & Thompson, 1974). The stimulation of retinal cells by radiation has been described as “flashes of light,” and is termed “anomalous phosphene perception.” Some studies have shown that shielding may even exacerbate the damage since the particles collide with the atoms of the shielding material to scatter other ions, a phenomenon referred to as “secondary radiation.” For example, one study assessed physiological changes following proton radiation exposure and found that aluminum shielding enhanced the detrimental effects in shielded animals (Pecaut et al., 2003). Another study found that shielding increased the average LET of the particles, and showed that shielding did not offer any protection when mice were measured behaviorally by the open-field and acoustic startle habituation tests (Pecaut et al., 2002).

Radiation produces effects similar to those seen in neurodegenerative disorders and aging (Casadesus et al., 2005), such as AD. Several studies have used the APP/PSEN1 tg mouse to demonstrate behavioral deficits related to spatial learning and memory. For example, 7-month-old APP/PSEN1 mice show impairments in the Barnes maze (BM; Reiserer, Harrison, Syverud, & McDonald, 2007), a task that involves

learning spatial association rules. Spatial learning and memory deficits are also observed in the water maze (WM). Though one study did not find such impairments in 6-month-old mice, deficits were apparent when the animals were re-tested at 18 months of age (Savonenko et al., 2005). In that study, deficits found in APP/PSEN1 mice were worse than those found in just APP or PSEN1 strains.

Neurological Mechanisms

Alzheimer's Disease Model

The APP/PSEN1 tg mice have neurological characteristics that lead to behavioral deficits similar to those seen in human AD. One of the hallmarks of AD is the presence of extracellular senile (neuritic) plaques (Zubenko, 1997) containing amyloid-beta ($A\beta$) that aggregate (into oligomers) and impair synaptic transmission (Walsh et al., 2002). The increased accumulation of these $A\beta$ plaques is largely a consequence of the altered metabolism of the amyloid precursor protein (APP; a string of amino acids embedded in the cellular membrane) being clipped at a certain point in its amino acid chain.

APP, a protein that is prevalent in nearly all mammalian tissue, is metabolized by gamma-secretase and either alpha- or beta-secretase. It is typically processed by one of two pathways: the non-amyloidogenic pathway (cleaved by gamma- and alpha-secretase) that has neuroprotective properties, and the amyloidogenic pathway (cleaved by gamma- and beta-secretase) that often results in the production of $A\beta$ peptides. In the amyloidogenic processing of APP, beta-secretase cleaves APP further along its amino acid chain than alpha-secretase typically cleaves it. The resulting fragment, $A\beta$, is longer and more prone to clinging together to form the plaques pathognomonic of AD. An abnormality in the functioning of a sub-component of the gamma-secretase enzyme

(presenilin) can also result in this undesired fragmentation of APP (Zubenko, 1997). Refer to Hartman (2008) and Hartman (2009) for a more in-depth explanation of APP processing.

Three identified genetic mutations can lead to altered APP metabolism, ultimately resulting in increased A β (specifically, the “long form” typically consisting of a 42 amino acid chain) and early-onset AD pathology (Hardy, 1997). The APP/PSEN1 tg mice contain two of these predispositions: the over-expression of both the APP gene (located on chromosome 21) and the presenilin-1 gene (located on chromosome 14). Together, these genes act as a “double hit,” increasing the likelihood of developing relatively large amounts of A β plaque and subsequent behavioral deficits (Jankowsky et al., 2004).

Though the mechanisms involved in A β 's toxicity remain uncertain, there are several well-supported processes by which it occurs. For example, free radicals are often produced when intracellular A β enters mitochondria (Reddy, 2006), resulting in oxidative stress. Oxidative damage is also triggered by extracellular complexes forming within plaques and can damage muscarinic acetylcholine receptors (Fawcett et al., 2002). Inflammation generally occurs as a result of the plaque deposition and degeneration of tissue (Akiyama et al., 2000). The role of oxidative stress and inflammation in AD pathology is further substantiated by studies demonstrating that antioxidant intake can decrease plaque deposition and ameliorate behavioral deficits (Hartman et al., 2006; Fawcett et al., 2002). Synaptic loss (Hamos, DeGennaro, & Drachman, 1989), altered neurogenesis (Ziabreva et al., 2006), and necrosis (Smale, Nichols, Brady, Finch, & Horton, 1995) also frequently occur.

As will be further discussed (see Chapter 3), the APP/PSEN1 tg mice do not develop the neurofibrillary tangles or neuronal loss also characteristic of AD, but do develop the amyloid plaques, synaptic loss, and cognitive deficits.

Proton Particle Radiation

The traversal of high-energy protons through biological matter has been hypothesized to accelerate the onset of neurological dysfunction via multiple cellular and molecular processes. This triggers the formation of reactive oxygen species (ROS) that may lead to acute and/or chronic oxidative stress. While the production of ROS by irradiated cells is adaptive at low levels (e.g., triggers DNA repair and secretion of growth factors; Spitz, Dornfeld, Krishnan, & Gius, 2012), its persistence can lead to impairments in hippocampal synaptic transmission (Pellmar, 1995). Giedzinski and colleagues (2005) showed that low doses of proton radiation (1 and 2 Gy) increase ROS levels in neuronal precursor cells in the acute post-irradiation phase (6-12 hours). Another study (Baluchamy et al., 2012) demonstrated that doses as low as 0.1 Gy can increase ROS levels.

The increase in ROS can inhibit neurogenesis (Limoli et al., 2004) and result in impaired long-term potentiation (LTP; Serrano & Klann, 2004), which has been associated with cognitive impairment (Raber et al., 2004; Snyder, Hong, McDonald, & Wojtowicz, 2005). Other potential mechanisms of neurological dysfunction include neuroinflammation (Monje, Toda, & Palmer, 2003; Rola et al., 2005), DNA damage (Hada & Sutherland, 2006; Baluchamy et al., 2010a), and the activation of apoptosis-related genes (Baluchamy et al., 2010b).

Even cells that are not directly impacted can be affected by the release of bioactive molecules from damaged cells (called the “bystander effect”; Kobayashi et al., 2004), furthering the neurological impairment by way of inflammation (Hein & O'Banion, 2009) and possibly by the mechanism involved in glutamate-mediated excitotoxicity (Rothman & Olney, 1987; Waxman & Lynch, 2005). Increased cytokine and prostaglandin expression (from the inflammation) can also promote neurodegeneration by preventing the integration of new neurons (Jakubs et al., 2008).

CHAPTER THREE

METHODS

Subjects

A total of 80 male mice (64 APP/PSEN1 tg and 16 wt) were purchased from Jackson Laboratories at 2.5 months of age and housed individually. Seven batches of approximately 12 mice each were delivered at one-month intervals over the course of the year. Half of the wt mice were randomly assigned to receive 0.5 Gy of proton radiation and the remaining half comprised the control group. Similarly, tg animals were randomly assigned to one of 4 radiation groups (0-1 Gy). Table 1 shows the breakdown of sample size by radiation dose and genotype. The mice were kept on a 12:12 hour light-dark cycle, given clean bedding once per week, provided water and chow *ad libitum*, and kept in a temperature and humidity-controlled room. Using only males avoided any potential differences brought about by females' estrous cycles. We chose a smaller sample size for wt mice as compared to tg mice, as well as only using 2 radiation groups, because funding was limited and NASA's interest mainly lay in the tg mice for aforementioned reasons (see Chapters 1 and 2).

Table 1

Total sample size of group by radiation dose and genotype.

		Genotype	
		tg	wt
Radiation Dose (Gy)	0	16	8
	0.1	16	--
	0.5	16	8
	1	16	--

Genetic Background

Double tg mice that express a mutant mouse/human amyloid precursor protein (Mo/HuAPP695swe) and a mutant human presenilin-1 (PSEN1- Δ E9) were used in the present study (Jackson Laboratories stock # 004462). Such tg animal models are often used for studying AD, since they closely mimic the pathological processes involved, including the early onset of amyloidosis in the cortex and the hippocampus. As aforementioned, this APP/PSEN1 tg model acquires hippocampal plaque deposition, synaptic loss, and behavioral deficits characteristic of AD. This pathology typically begins developing at 6 months of age, with an abundance of deposits found at 9 months of age (Jankowsky et al., 2004). Double tg mice were preferred to other single tg strains (e.g., APP or PSEN1 alone) since the pathology typically develops earlier and is more pronounced (Savonenko et al., 2005).

While the APP/PSEN1 strain of mice is considered to be an appropriate model of AD-like pathology, mice of this strain do not develop the neurofibrillary tangles or

neuronal loss associated with AD. While there is currently no available murine AD model that develops all of the pathognomonic signs of AD, the APP/PSEN1 tg mice were chosen as a relatively good animal model (e.g., presence of plaque, synaptic loss, neuroinflammatory changes, and electrophysiological and cognitive deficits). Additionally, the strain is commercially available and is widely used by other laboratories (Cherry et al., 2012; Harrison, Hosseini, & McDonald, 2009; Jankowsky et al., 2004).

The mice were a strain of B6C3F1/J (Jackson, Stock# 100010), where female C57BL/6J (Stock#000664) were crossed with male C3H/HeJ (Stock#000659) mice. This strain has typically been used as the wt strain to generate APP/PSEN1 double tg mice provided by Jackson Laboratories. We used these wt mice to determine baseline behavioral performance and electrophysiological data for the APP/PSEN1 tg mice.

Relevance of Using an Animal Model

In vivo rodent models are used very frequently in scientific experiments to study various disease processes and conditions. Though they do not translate perfectly, rodent brains are structurally and functionally similar to humans, with comparable neurological correlates of memory impairment. Also, they are relatively easy to manipulate genetically (e.g., double transgenic APP/PSEN1), and researchers have the ability to control extraneous variables (e.g., gender, strain, experience, etc.) that would not be realistically attainable in clinical trials. Additionally, many behavioral measures have been developed for rodents.

Procedures

Mice arrived at Loma Linda University's Behavioral Neuroscience Laboratory (BNL). On the day following arrival, the experimenter held each mouse for two minutes to decrease anxiety brought about by the transfer process and facilitate acclimation of the mice to the experimenter. Over the course of the following 2 weeks, the experimenter assessed baseline spatial learning and memory ability with the water maze. Upon completion of these tests, animals were transferred to a different site on campus for irradiation and then re-transferred to the BNL for testing at the 3 and 6 month post irradiation time-points. Anxiety levels were assessed using the zero maze following water maze and Barnes maze testing for these last 2 time-points (see Figure 3). The experimenter was blind to the group breakdown.

All procedures were conducted in accordance with the Loma Linda University Institutional Animal Care and Use Committee's (IACUC) guidelines and approval, and animals were handled by individuals who have received certification from the university's Animal Care Facility.

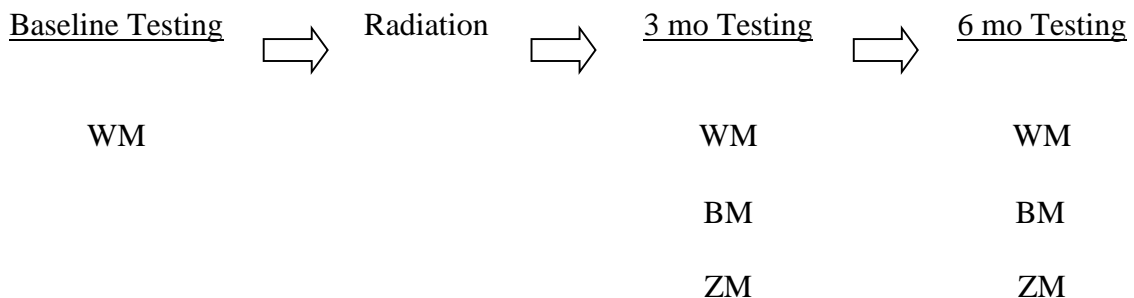


Figure 3. Timeline and sequence of behavioral testing. WM: water maze; BM: Barnes maze; ZM: zero maze.

Irradiation

Mice were irradiated at Loma Linda University Medical Center's Proton Treatment and Research Center (Coutrakon et al., 1997; see Figure 4 for a picture of the irradiation room). They were placed in ventilated acrylic boxes (3 x 3 x 8cm) to minimize movement (see Figure 5). They were aligned with the beam line with their heads located in the center of the trajectory. Those mice set to receive 0.1, 0.5, and 1 Gy of whole-body proton radiation were then given the appropriate dose at a rate (amount of joules deposited) of 1.5-2.5 Gy/min. The energy of the beam was 150 MeV. The entire procedure lasted approximately 10 min. Control mice were also placed in the clear acrylic boxes for approximately the same amount of time as the irradiated mice to control for any potential effects of constraint-induced stress.

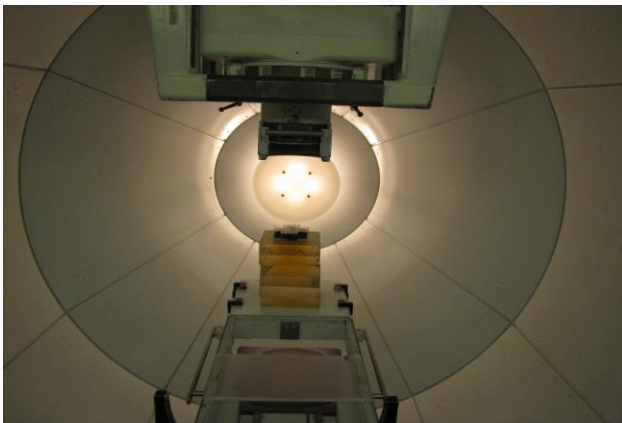


Figure 4. LLUMC's Proton Treatment and Research Center.

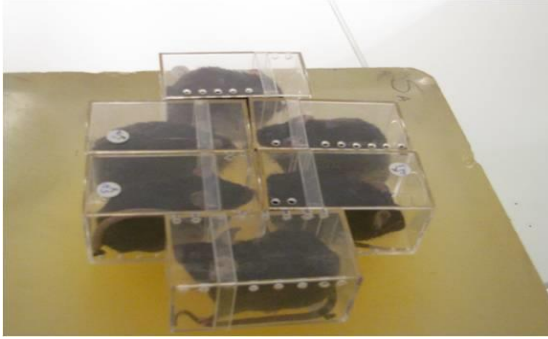


Figure 5. Mice placed in ventilated acrylic boxes to limit movement.

Dose and Energy Justification

NASA has recently been hesitant to fund anything above 1 Gy, since higher doses do not realistically model the radiation levels astronauts will be exposed to (Cucinotta et al., 2012; Cucinotta et al., 2013). Therefore, doses between 0.1 and 1 Gy were chosen. A single, as opposed to fractionated, dose was used because of the limited access to the Proton Treatment Center and recent findings demonstrating that acute and fractionated exposures cause similar decrements (Rivera et al., 2013). An energy level of 150 MeV/n has been chosen since it is one of the most common energy levels in the space environment and particles travelling at this speed will homogeneously traverse the brain.

Head vs. Whole Body Radiation

In addition to the practical limitations of only irradiating the head of each mouse to isolate radiation-induced effects, exposing the mouse's entire body relates more closely to the type of exposure astronauts will experience. The effects of peripheral tissue response (e.g., carcinogenesis, loss of bone and muscle density, etc.) and radiation sickness were not expected at the doses being administered.

Behavioral Tests

Water Maze

The WM is one of the most widely used tests of spatial learning and memory ability in rodents (Morris, 1981; Klapdor & Van Der Staay, 1997). The WM was comprised of three tests: cued (day 1), spatial (days 2 and 3), and probe (days 2 and 3). The maze consisted of a metal tub (110 cm diameter) filled with water made opaque using white, non-toxic paint (see Figure 6 for a depiction). A circular platform (11 cm diameter) onto which mice could step to escape the water was located in one of four quadrants. Mice were released into the water with their noses facing the wall at one of four release points. Each mouse was given 60 s to find the platform. The experimenter manually guided mice to the correct location if the time elapsed and they had not found the platform. Once on the platform, mice were allowed to remain there for 5 s. Swim path was recorded by a computerized tracking system (Noldus Ethovision, Wageningen, The Netherlands) that uses an overhead camera to quantify distance moved and other parameters. Ten trials were given in blocks of 5 each day (2 trials per block), with an interval of approximately 20 minutes between blocks. Less distance moved generally indicates better performance.



Figure 6. Picture of the water maze used to assess spatial learning and memory.

The water maze consisted of three tests:

Cued (Day 1)

The cued test is a control task that assesses sensorimotor (e.g., locomotion and vision) and motivational deficits that could alter performance on the spatial and probe tests (see below). For this task, the escape platform was visible just above the surface of the water and a pole sticking off the top made its location even more salient. Mice were released into the pool opposite the location of the platform, which was moved to a different quadrant after each block.

Spatial (Days 2-3)

The spatial test is a measure of spatial learning ability. During this test the escape platform was submerged just below the surface of the opaque water and mice relied on spatial cues to find the platform, since they could not directly see it. The platform location changed to a different quadrant each day. The second spatial day is considered

the “reversal learning phase” since it requires that the mice disregard previously learned information (i.e., that the platform is in one location) and re-learn a new platform location.

Probe (Days 2-3)

The probe test is a measure of memory consolidation. We conducted the probe trial one hour after the completion of block 5 of each spatial test. For these trials, the platform was removed from the tub and mice were allowed to search the pool for 60 s. We measured the amount of time mice spent searching the quadrant where the platform was previously located, as well as the total number of times they crossed over the former location of the platform.

Our lab has successfully used the WM in many studies over the past several years to assess functional/behavioral differences and impaired hippocampal functions using a variety of brain injury models: Ashwal et al., 2014; Kamper et al., 2013; Fukuda et al., 2013; Pop et al., 2013; Ajao, Pop, Kamper, Hartman, & Badaut, 2012; Hartman, Kamper, Goyal, Stewart, & Longo, 2012; Lekic et al., 2011; Chen et al., 2009.

Barnes Maze

The BM also assesses spatial learning and memory ability (Barnes, 1979), and is used as the land version of the WM. The test consists of a dry, circular table with 20 holes along the outer surface and a hidden box located under one of the holes (see Figure 7 for a depiction). Mice were placed in the center of the table and were motivated to find the hole with the box to escape exposure. The entire table is wiped with a 70% alcohol solution after each trial to remove any remnant olfactory cues. We conducted the test

over 3 days, with each mouse receiving 5 trials per day. A trial was completed when a mouse either found the escape box or 5 minutes elapsed, after which the experimenter manually guided the mouse to the hole with the escape box. Like the WM, an overhead camera recorded the mouse's movements, allowing for quantification of distance moved and other parameters. Cued, spatial, and probe tests were conducted in a manner very similar to the WM.

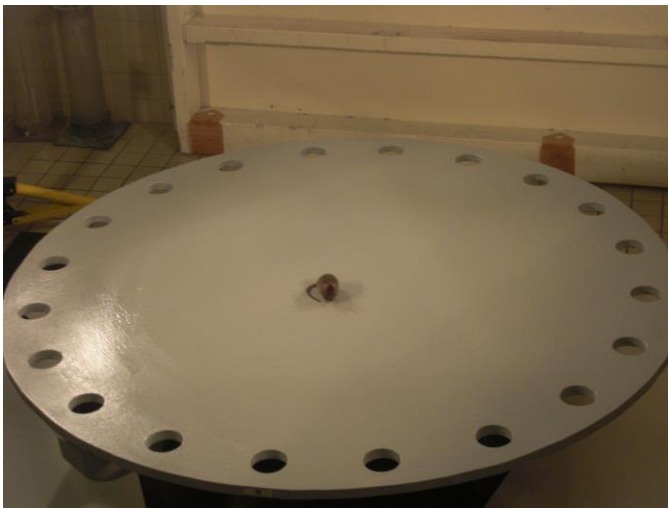


Figure 7. Picture of the Barnes maze used to assess spatial learning and memory.

Though this test measures the same construct as the WM, it had been added to the battery because it is another widely used measure of spatial learning and memory ability in mice. Some authors have indicated that neither test is superior to the other, but that each uses a different motivational technique and may produce varying results (Gerlai & Clayton, 1999; Patil, Sunyer, Höger, & Lubec, 2009). For this reason, they recommend that the tests be used as parallel measures of spatial learning and memory.

Elevated Zero Maze

The ZM consisted of a thin, horizontal ring (100 cm outer diameter, 10 cm wide) half exposed and half partially enclosed by walls (35 cm high). Halogen lights directly illuminated the exposed areas of the maze. Mice were placed in the center of one of the exposed areas at the start of the test, and were given five minutes to explore the maze. The amount of time a mouse spends in the dark, enclosed space was measured. Since mice typically explore novel environments, spending relatively more time in the dark part of the test is associated with greater levels of anxiety (Shepherd, Grewal, Fletcher, Bill, & Dourish, 1994; refer to Figure 8 for a depiction of the test).

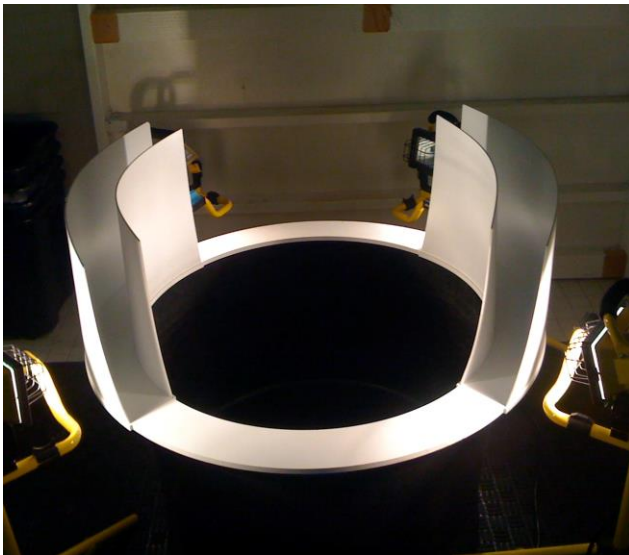


Figure 8. Picture of the zero maze used to assess anxiety-like behavior.

Statistical Analysis

We used a number of statistical techniques to discover significant differences or trends in the data. For Aim 1, we used a mixed design ANOVA with 1 between-subjects

factor (radiation group) and 1 repeated measure (block) to assess main effects in the WM and BM. For Aim 2, we used a mixed design ANOVA with 2 between-subjects factors (radiation and genotype group) and 1 repeated measure (block) to assess main effects in the WM and BM. The Greenhouse-Geiser correction to the degrees of freedom was used for both Aim 1 and 2 analyses to control for sphericity and compound symmetry since there were repeated measures (5 blocks). We used a one-way ANOVA to assess main effects in the ZM. An ANCOVA technique was used to control for confounding variables where appropriate. Radiation groups were pooled when assessing genotype effects. Alpha level was set at 0.05 for all tests of statistical significance. Error bars represent \pm standard error of the mean, unless otherwise stated.

Only the comparisons of interest were analyzed to limit the experiment-wise error probability (alpha). Data were screened for potential outliers by generating scatterplots and observing whether any subject's data consistently and significantly (± 2 standard deviations) deviated from the group's data. No data met these criteria and thus no subjects were excluded from any analysis. Only the last 30 s of the 60 s probe trials were used for the wt mice, since floating behavior was observed during the first half of the trials. No other alterations were made. The statistical program *Statistica* was used for all analyses, and *Prism* was used to create the figures.

CHAPTER FOUR

RESULTS

Mortality

Eight mice died over the course of the experiment. All 8 mice were tg and were from batches 1-4 of 7. Table 2 shows the breakdown of mortality per radiation dose.

Table 2

Breakdown of mortality by radiation dose.

Radiation Dose (Gy)	Number Deceased
0	1
0.1	1
0.5	3
1	3

Aim 1

Aim 1 sought to determine whether a low dose of proton radiation affects behavior in normal, wt mice. The specific hypothesis held that mice receiving a low dose (0.5 Gy) of proton radiation would perform worse on tests of spatial learning and memory relative to non-irradiated controls. Wild-type mice were tested in the WM prior to being irradiated. They were then tested in the WM, BM, and ZM 3 and 6 months post-irradiation. Figures 9-12 depict WM data, Figures 13-14 depict the BM data, and Figure 15 depicts the ZM data.

No significant differences were observed at the pre-irradiation or 3-month time-point on any spatial test. However, at 6 months post-irradiation, the group that received 0.5 Gy performed significantly worse than the control group (swim distance, Mean [cm] \pm SEM: 294.44 ± 35.75 vs. 188.08 ± 13.40) in the WM during the second spatial test ($F_{1,14} = 7.76, p < .02$; see Figure 11). Figure 12 shows a representative swim path from an irradiated mouse that perseverated on the previous platform location as compared to the path of a control mouse during the first trial of the reversal learning phase. Further analysis showed that irradiated mice spent a larger percentage of their time in the previous platform quadrant (93.89% in the 1st 15 s of the 1st trial vs. 69.73% for controls) and crossed the previous platform location more frequently (1.5 vs. 0.38 crosses) during the first trial of this phase. However, these differences were not significant.

Irradiated mice also traveled a longer distance to find the escape box in the BM than controls (468.91 ± 50.31 vs. 382.93 ± 40.72 cm) during the second day of spatial testing (reversal learning) 6 months after irradiation. However, the differences in BM performance were not significant. Probe data for both the WM and BM also trended in the same direction (worse spatial memory performance for irradiated mice), but they were not significantly different. No differences were observed during the cued test in either the WM or BM, suggesting that there were no differences in vision, locomotion, or motivation between the control and irradiated mice.

There were no differences among radiation groups in the amount of time spent in the dark on the ZM. However, there was a significant change across time, where both groups spent more time in the dark area the second time they were tested. This finding was expected, since the mice have already explored the open section of the maze,

detracting from its novelty. Additionally, while a greater degree of change over time was observed in the wt mice relative to the tg mice, the effect was not significant ($p = .059$).

Water Maze Figures

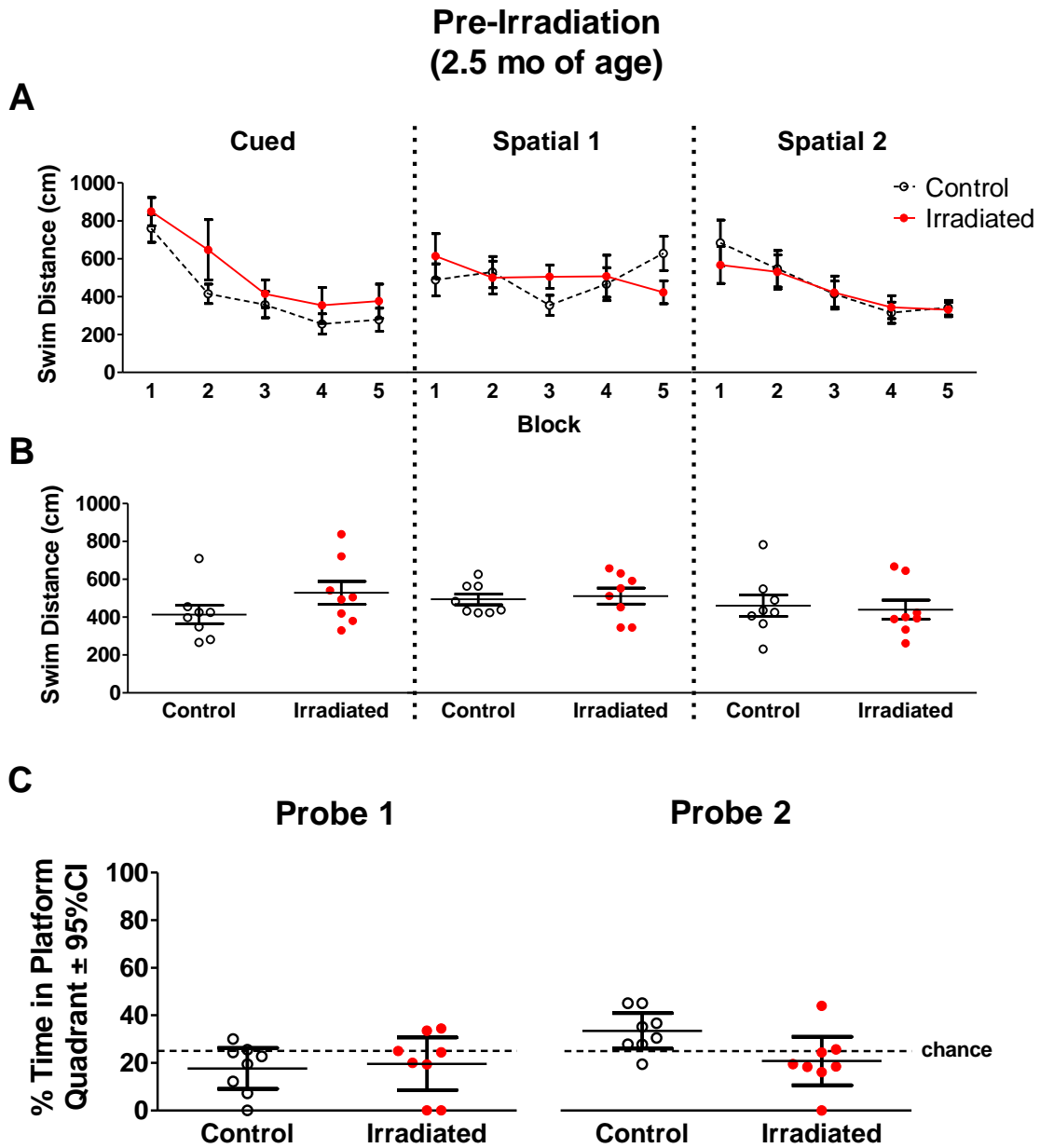


Figure 9. WM wt pre-irradiation behavior. Panel A shows performance on each of the 5 blocks, while panel B shows performance averaged over the blocks. Panel C shows percentage of time spent in the platform quadrant for probe trials. The probe data are for the last 30 s of the 60 s trial (to account for initial floating behavior). No significant differences were observed on any test during the pre-irradiation time period. Error bars represent \pm SEM for A and B, 95% confidence interval for C.

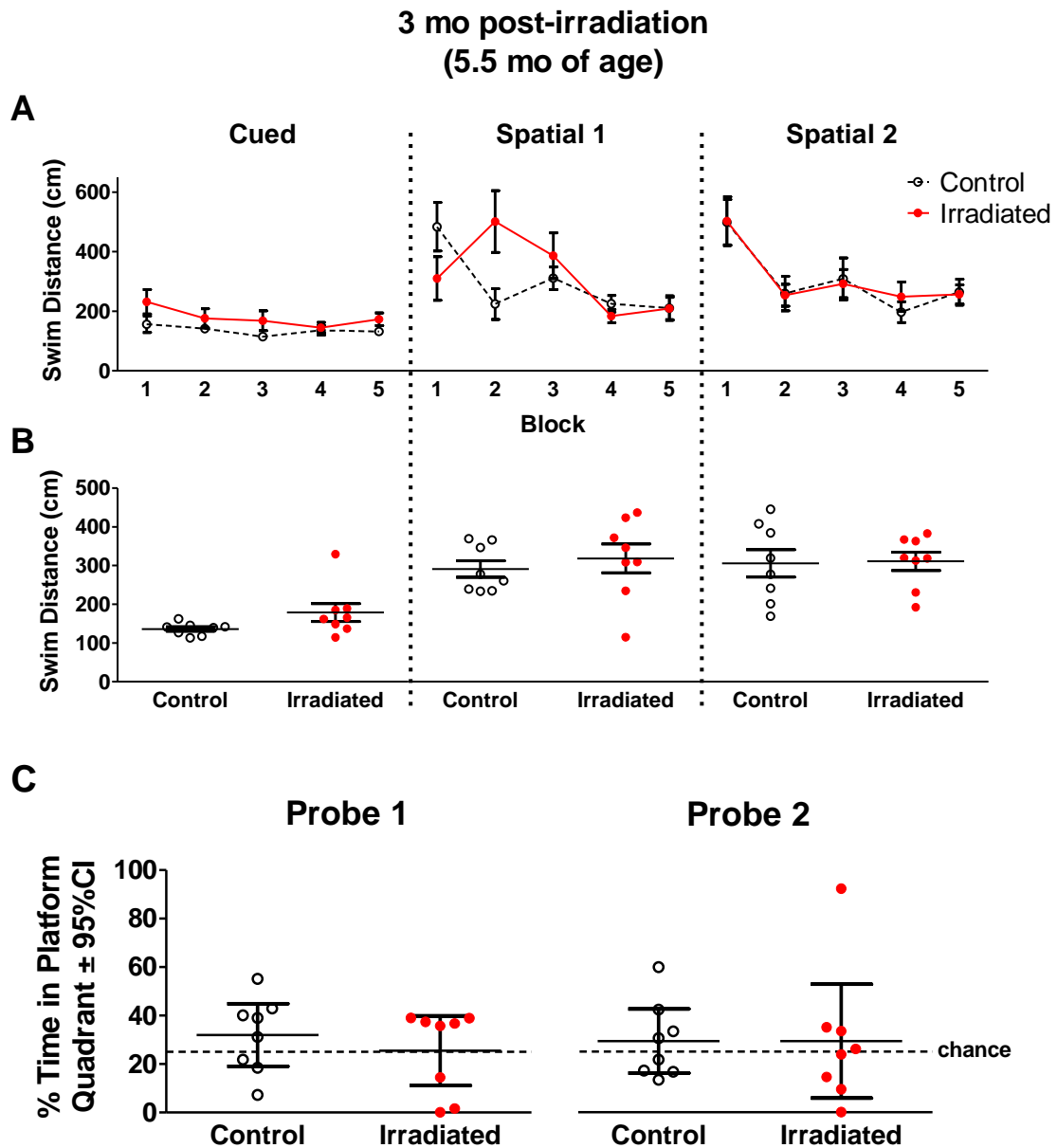


Figure 10. WM wt 3-month post-irradiation behavior. Panel A shows performance on each of the 5 blocks, while panel B shows performance averaged over the blocks. Panel C shows percentage of time spent in the platform quadrant for probe trials. The probe data are for the last 30 s of the 60 s trial (to account for initial floating behavior). No significant differences were observed on any test during the 3-month post-irradiation time period. Error bars represent \pm SEM for A and B, 95% confidence interval for C.

6 mo post-irradiation
(8.5 mo of age)

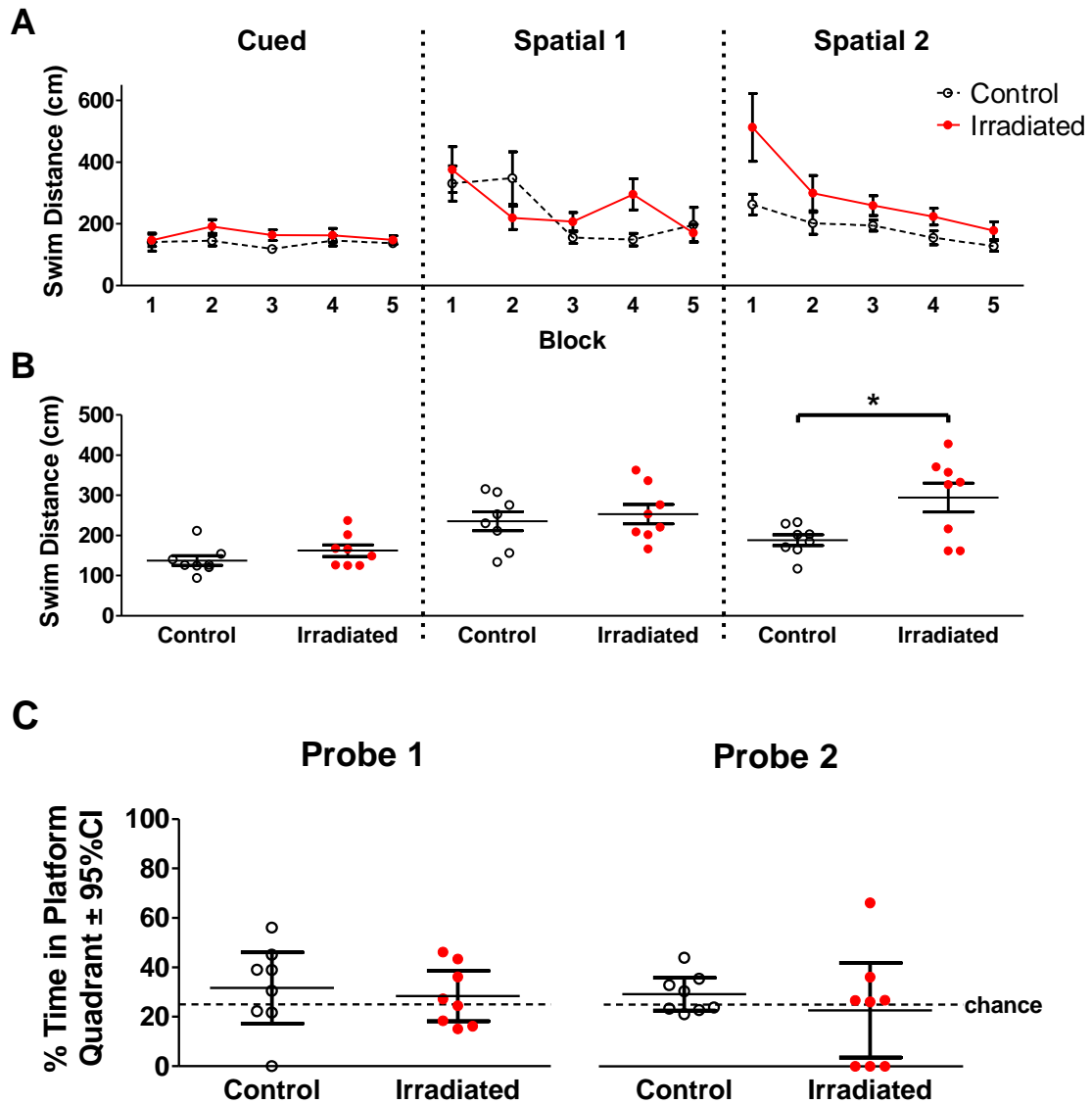


Figure 11. WM wt 6-month post-irradiation behavior. Panel A shows performance on each of the 5 blocks, while panel B shows performance averaged over the blocks. Panel C shows percentage of time spent in the platform quadrant for probe trials. The probe data are for the last 30 s of the 60 s trial (to account for initial floating behavior). No differences were observed during the cued or spatial 1 tests. However, non-irradiated controls swam significantly less distance to the hidden platform than mice exposed to 0.5 Gy during the spatial 2 (reversal learning) test. Error bars represent \pm SEM for A and B, 95% confidence interval for C. * represents $p < .02$.

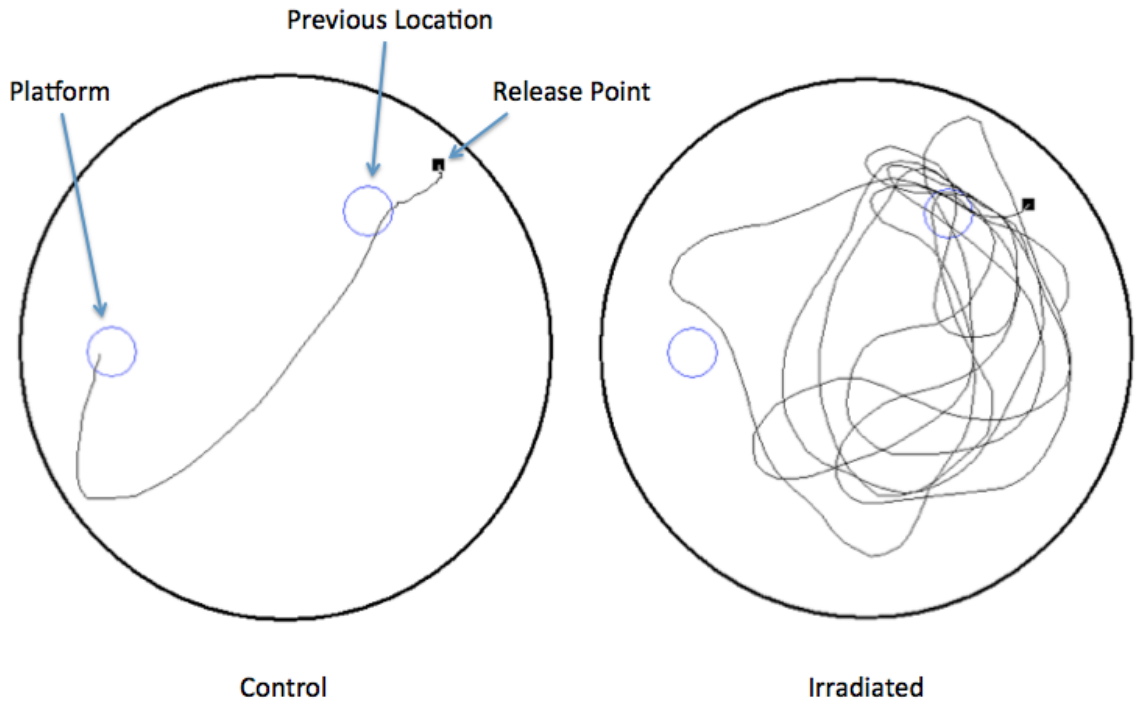


Figure 12. Representative trials of reversal learning at 6 months in the WM. The control mouse first swam to where the platform was located on the previous day, and then searched for the new location upon discovering that the platform was no longer where it previously had been. The irradiated mouse perseverated on searching for the platform at its previous location, indicating an impaired ability to find the new location.

Barnes Maze Figures

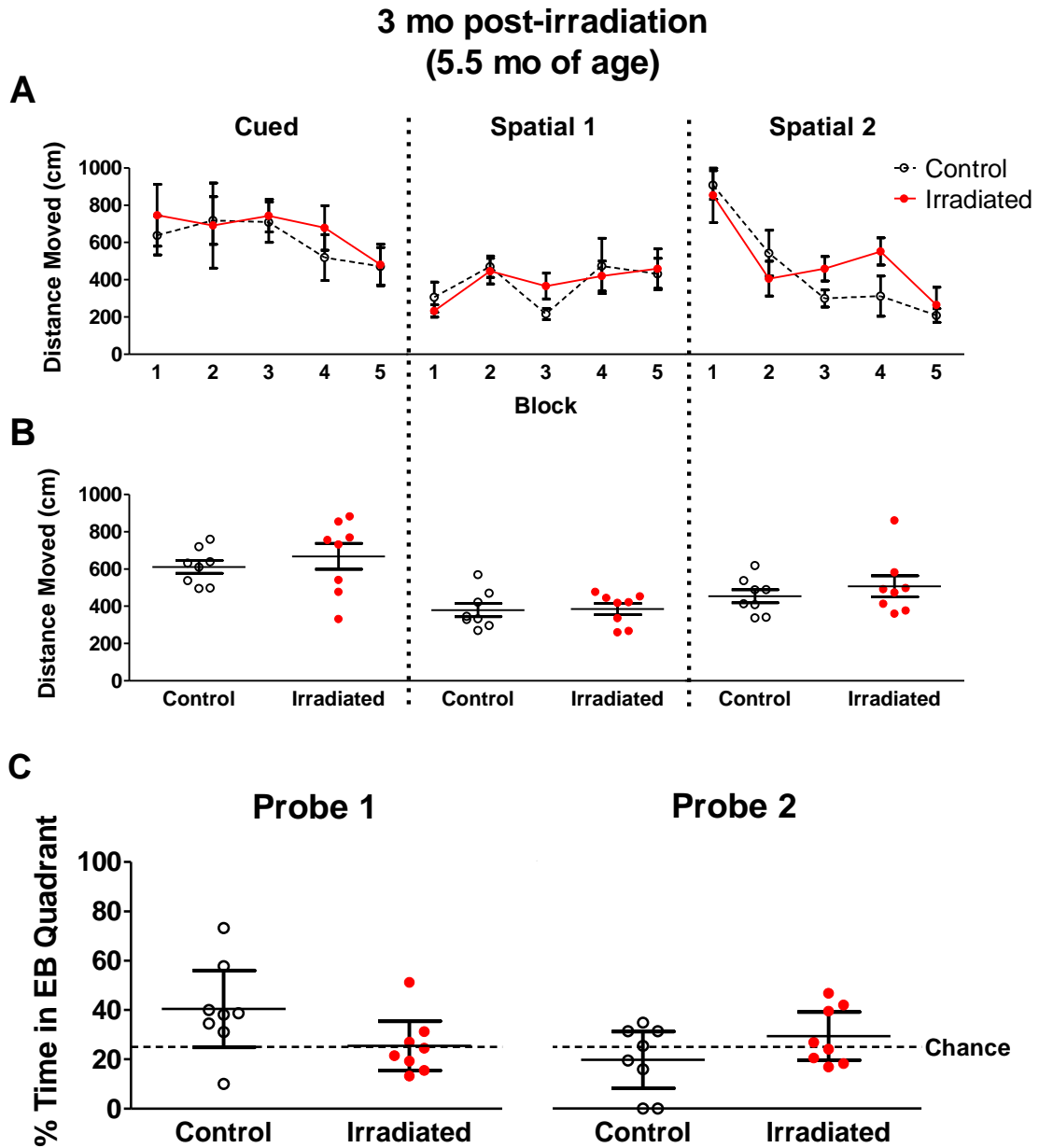


Figure 13. BM wt 3-month post-irradiation behavior. Panel A shows performance on each of the 5 blocks, while panel B shows performance averaged over the blocks. Panel C shows percent time spent in the quadrant where the escape box (EB) was previously located. No significant differences were observed on any test during the 3-month post-irradiation time period. Error bars represent \pm SEM for A and B, 95% confidence interval for C.

Zero Maze Figure

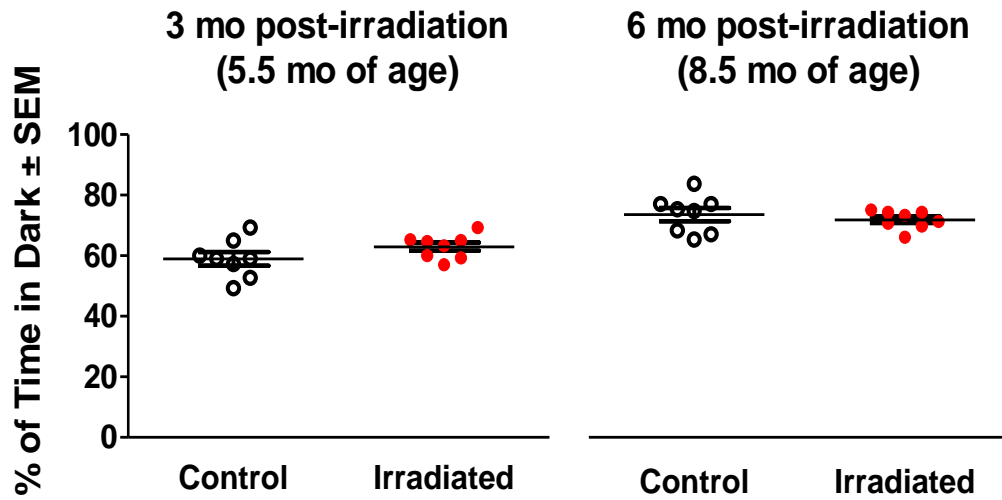


Figure 15. ZM wt behavior. No significant differences were observed at either the 3 or 6-month post-irradiation time-point. However, a significant change over time was observed in both groups. Error bars represent \pm SEM.

Aim 2

Aim 2 sought to determine whether radiation affects behavior in mice predisposed to AD-like pathology differently than wt mice, and whether this effect is dose-dependent. The first specific hypothesis was that irradiated tg mice would perform worse on tests of spatial learning and memory than irradiated wt mice. The second hypothesis held that higher doses of radiation would lead to more severe spatial learning and memory deficits in tg mice compared to non-irradiated tg mice or those exposed to lower doses. Transgenic mice were given the same battery as wt mice, where they were tested in the WM prior to being irradiated and then tested in the WM, BM, and ZM 3 and 6 months post-irradiation. Figures 16-19 depict WM data, Figures 20-22 depict the BM data, and

Figure 23 depicts the ZM data. The wt data that was previously shown (under the “Aim 1” section of this Chapter) was added to most of these figures for comparison.

Genotype effects were the first to be analyzed in order to confirm that the double tg mice elicited the behavioral deficits expected. As hypothesized, the tg mice performed significantly worse than the wt mice at 3 and 6 months post-irradiation on the WM and BM (see Figures 17, 18, 20, and 21, respectively). There were even genotype differences at the pre-irradiation time-point on the more difficult spatial 2 (reversal learning) test, and on the cued WM test at 3 and 6 months post-irradiation. To ensure that the differences observed in the spatial tests were not due to visual, locomotor, or motivational factors, an ANCOVA technique was used to vary out cued differences from the spatial analyses. All but the 6-month spatial 1 genotype effect remained statistically significant after controlling for these cued differences. No genotype differences were observed at the pre-irradiation time-point, most likely because the neuropathological processes in the tg mice had not yet had time to present phenotypically.

Transgenic mice spent significantly less time in the exposed section of the ZM compared to wt mice at both the 3 and 6 month time-points, demonstrating reduced anxiety-like behavior (see Figure 23). Additionally, wt mice were significantly heavier, on average, than tg mice (31.61 grams compared to 28.44 grams, respectively; see Figure 24).

Once deficits in tg mice were confirmed relative to wt mice, attention was shifted to the effects of radiation dose among tg mice. No significant differences were observed at any time-point in the WM among these mice. However, all groups showed a significant learning curve across repeated blocks on most tests. In the BM, the only significant difference was observed during the spatial 1 test of the 6 month time-point,

where the 0 and 0.5 Gy group performed significantly better than the 1 Gy group ($F_{3,52} = 2.85$, $P < .05$; see Figure 21). While no differences were found between groups on any probe test, it was found that the 1 Gy group was the only group that spent a less-than-chance percentage of time in the platform quadrant during probe 1 at 6 months post-irradiation in the WM (Mean = 29.12 ± 6.45 CI). In the BM, it was found that tg controls were the only group that spent a greater-than-chance percentage of time in the platform quadrant during probe 1 at 3 months post-irradiation (Mean = 38.32 ± 12.63 CI). No significant differences were observed during the cued test at any time-point on either the WM or BM, suggesting that there were no differences in vision, locomotion, or motivation between the control and irradiated tg mice.

For tg mice, there were no differences among radiation groups in the amount of time spent in the dark on the ZM. However, there was a significant change across time, where all groups spent more time in the dark area the second time they were tested. This finding was expected, since the mice have already explored the open section of the maze, detracting from its novelty. There were no differences in the degree of change over time by radiation dose.

Water Maze Figures

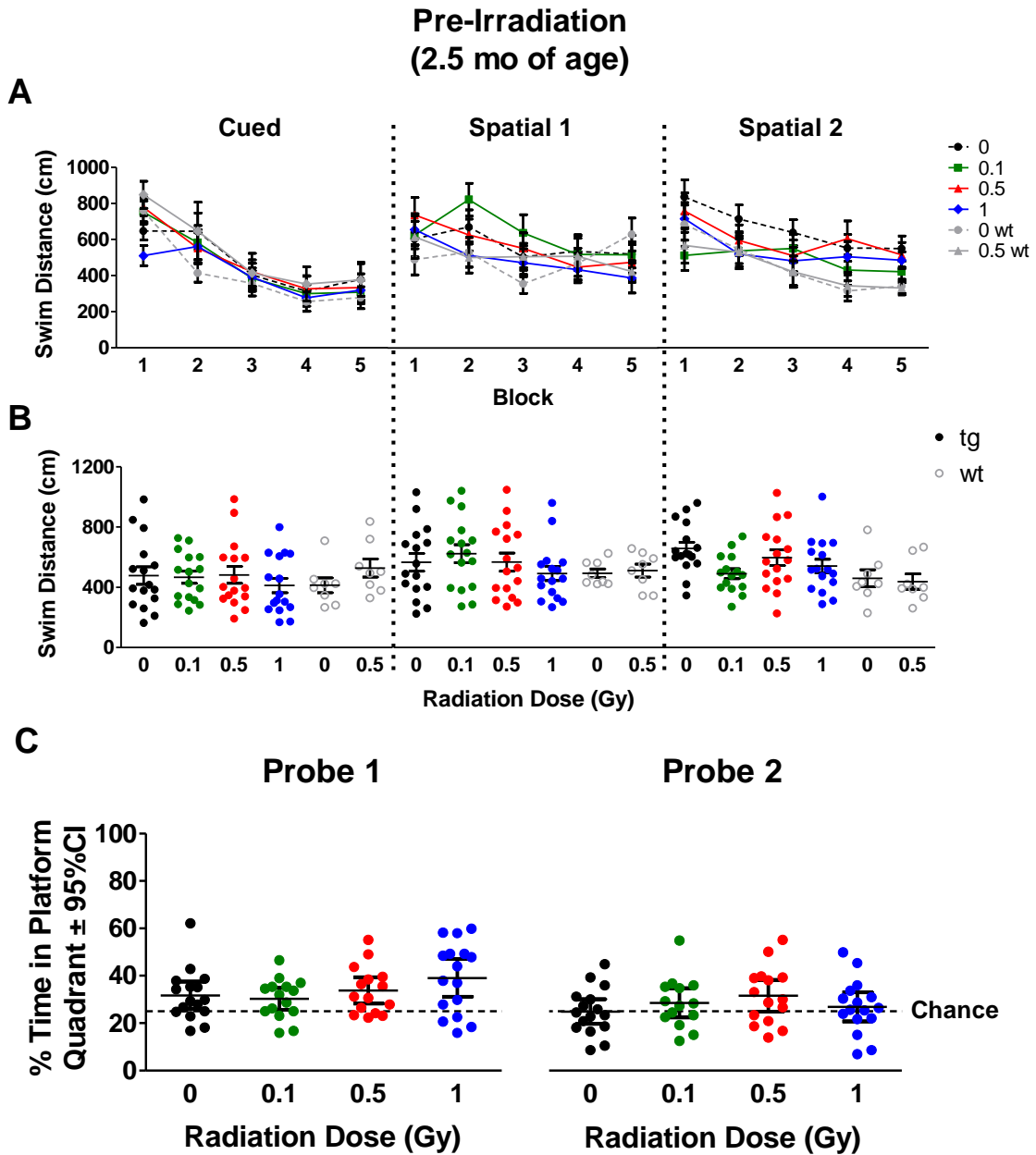


Figure 16. WM tg and wt pre-irradiation behavior. Panel A shows performance on each of the 5 blocks, while panel B shows performance averaged over the blocks. Genotype effects were observed on the spatial 2 test. Panel C shows percentage of time spent in the platform quadrant for probe trials. No significant differences were observed among the tg mice on any test during the pre-irradiation time period. Error bars represent \pm SEM for A and B, 95% confidence interval for C.

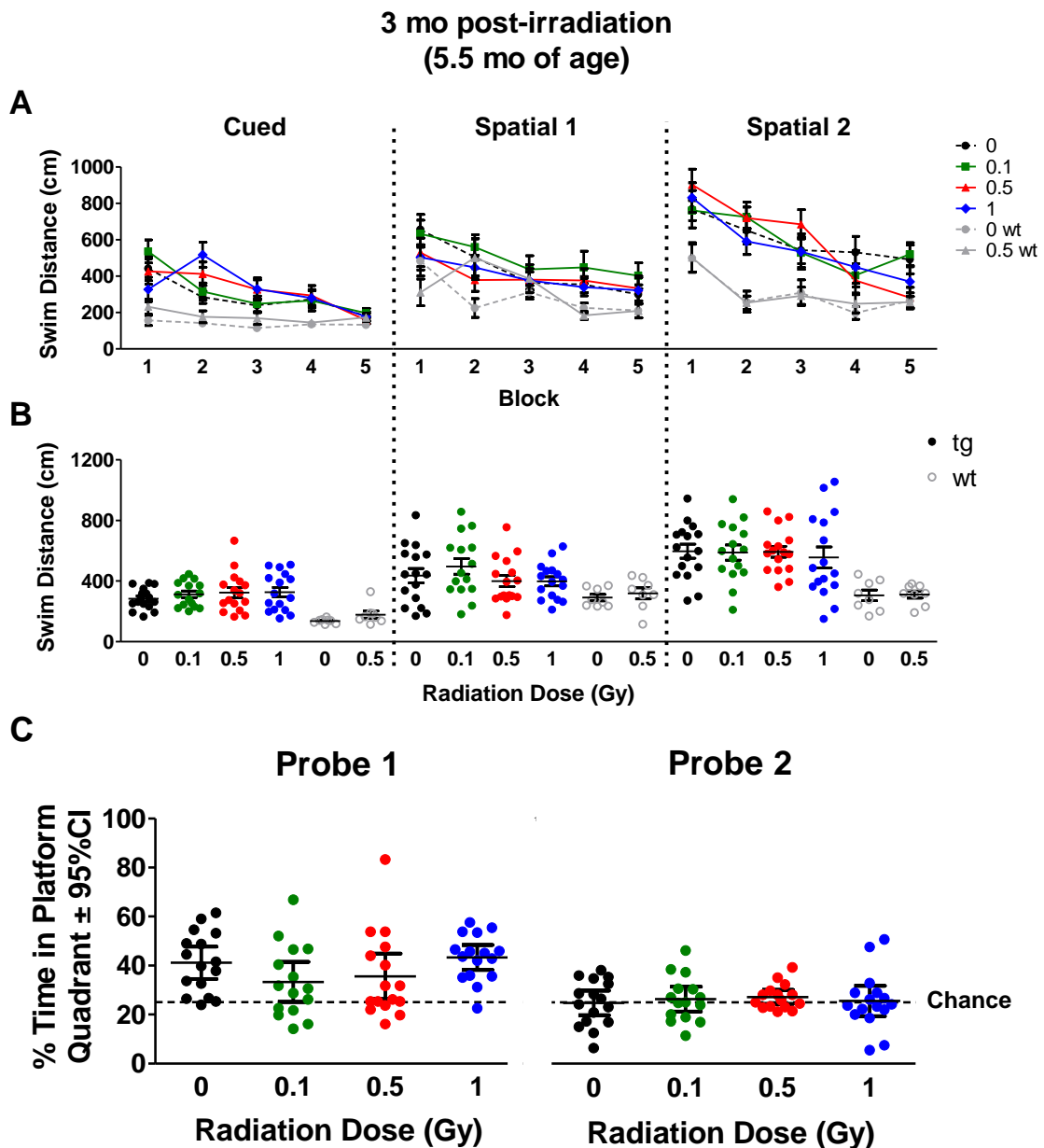


Figure 17. WM tg and wt 3-month post-irradiation behavior. Panel A shows performance on each of the 5 blocks, while panel B shows performance averaged over the blocks. Genotype effects were observed on all three tests. Panel C shows percentage of time spent in the platform quadrant for probe trials. No significant differences were observed among the tg mice on any test during the 3-month post-irradiation time period. Error bars represent \pm SEM for A and B, 95% confidence interval for C.

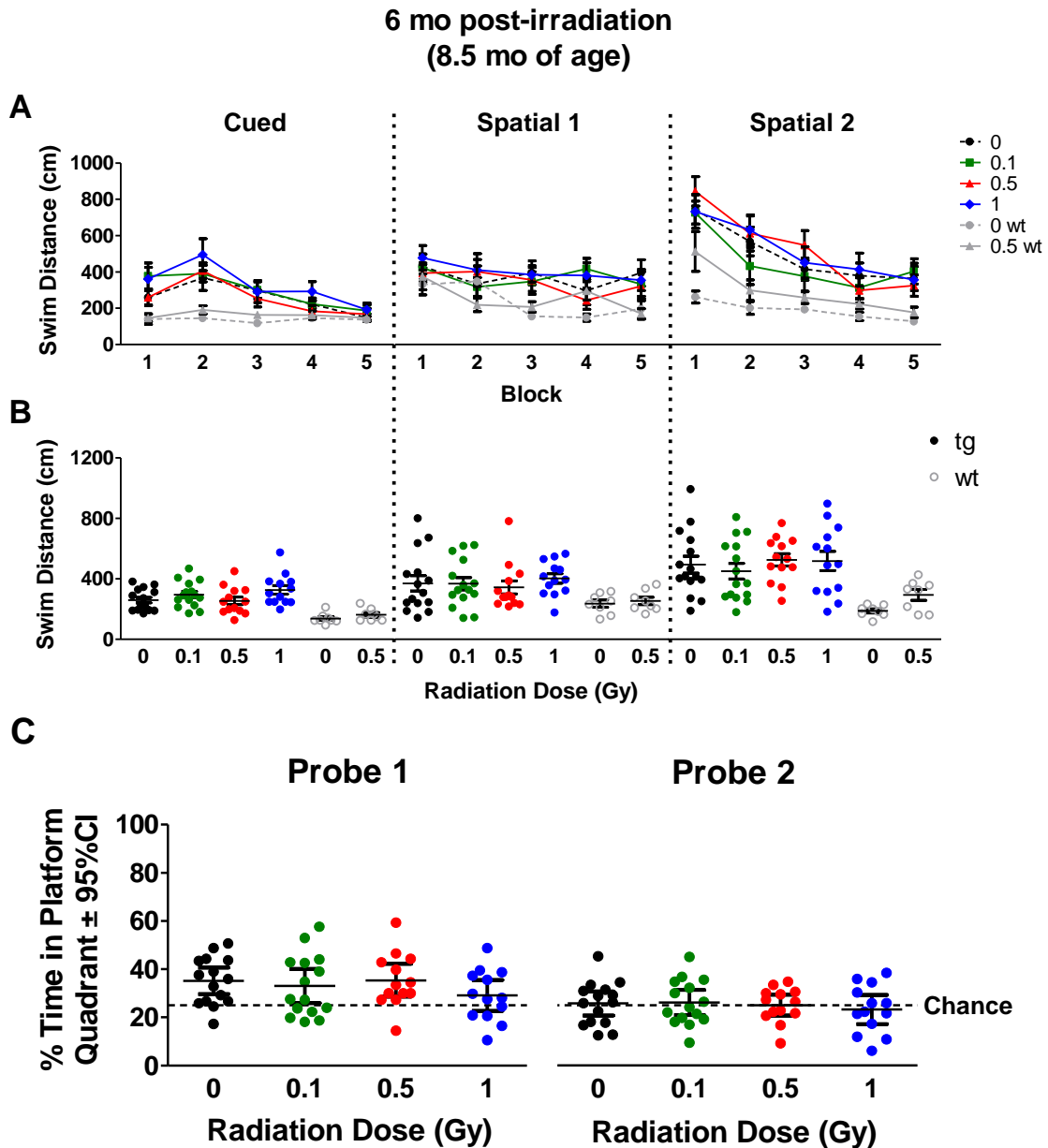


Figure 18. WM tg and wt 6-month post-irradiation behavior. Panel A shows performance on each of the 5 blocks, while panel B shows performance averaged over the blocks. Genotype effects were observed on the cued and spatial 2 tests. Panel C shows percentage of time spent in the platform quadrant for probe trials. No significant differences were observed among the tg mice on any test during the 6-month post-irradiation time period. Error bars represent \pm SEM for A and B, 95% confidence interval for C.

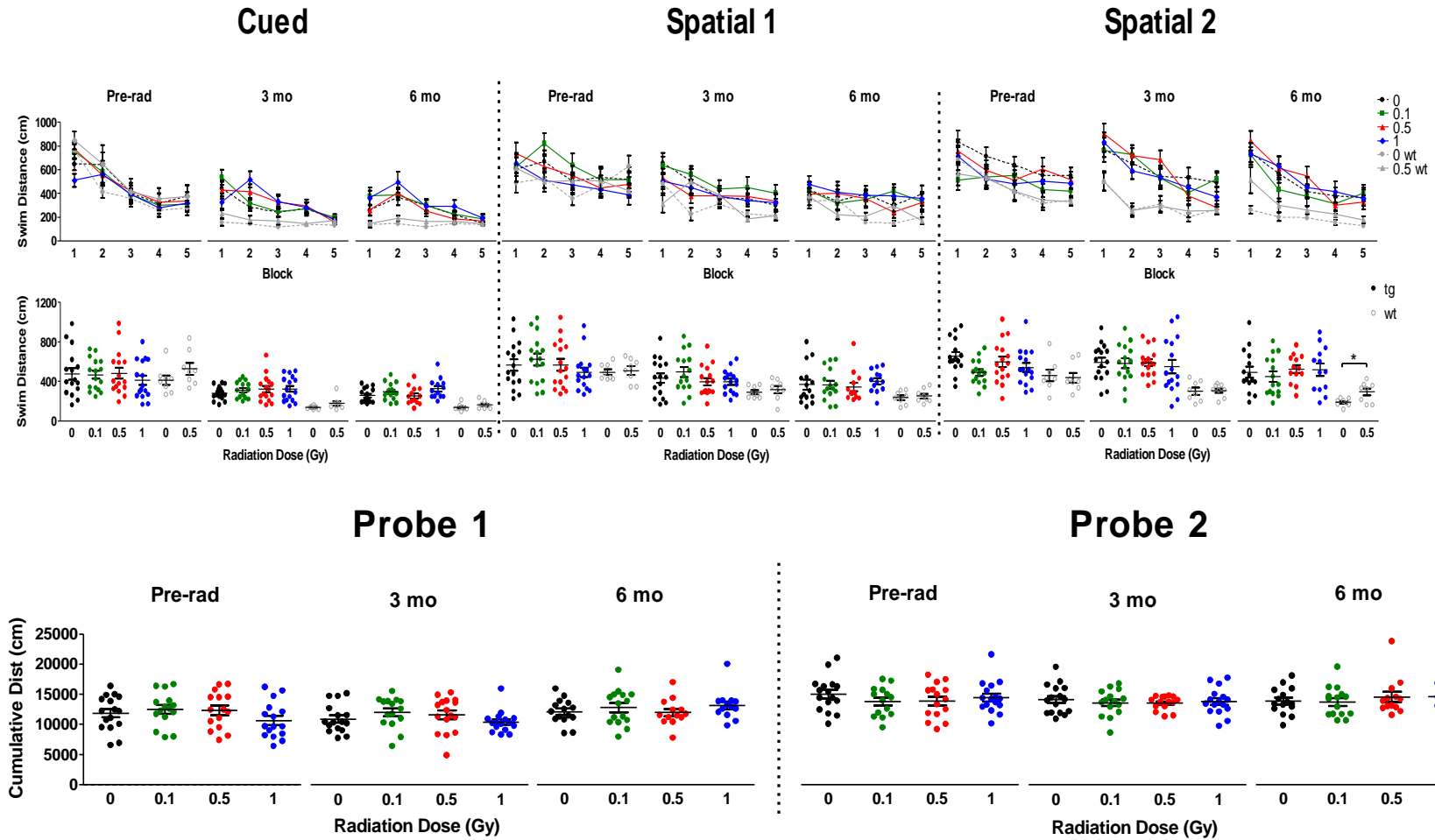


Figure 19. WM tg and wt behavior across all time-points. All groups showed significant improvement from the pre-irradiation to 3 month post-irradiation time-points for the cued and spatial 1 tests, but only wt animals showed significant improvement on the spatial 2 test. This improvement over time is likely due to practice effects. No significant improvement was seen across time-points on the probe tests.

Barnes Maze Figures

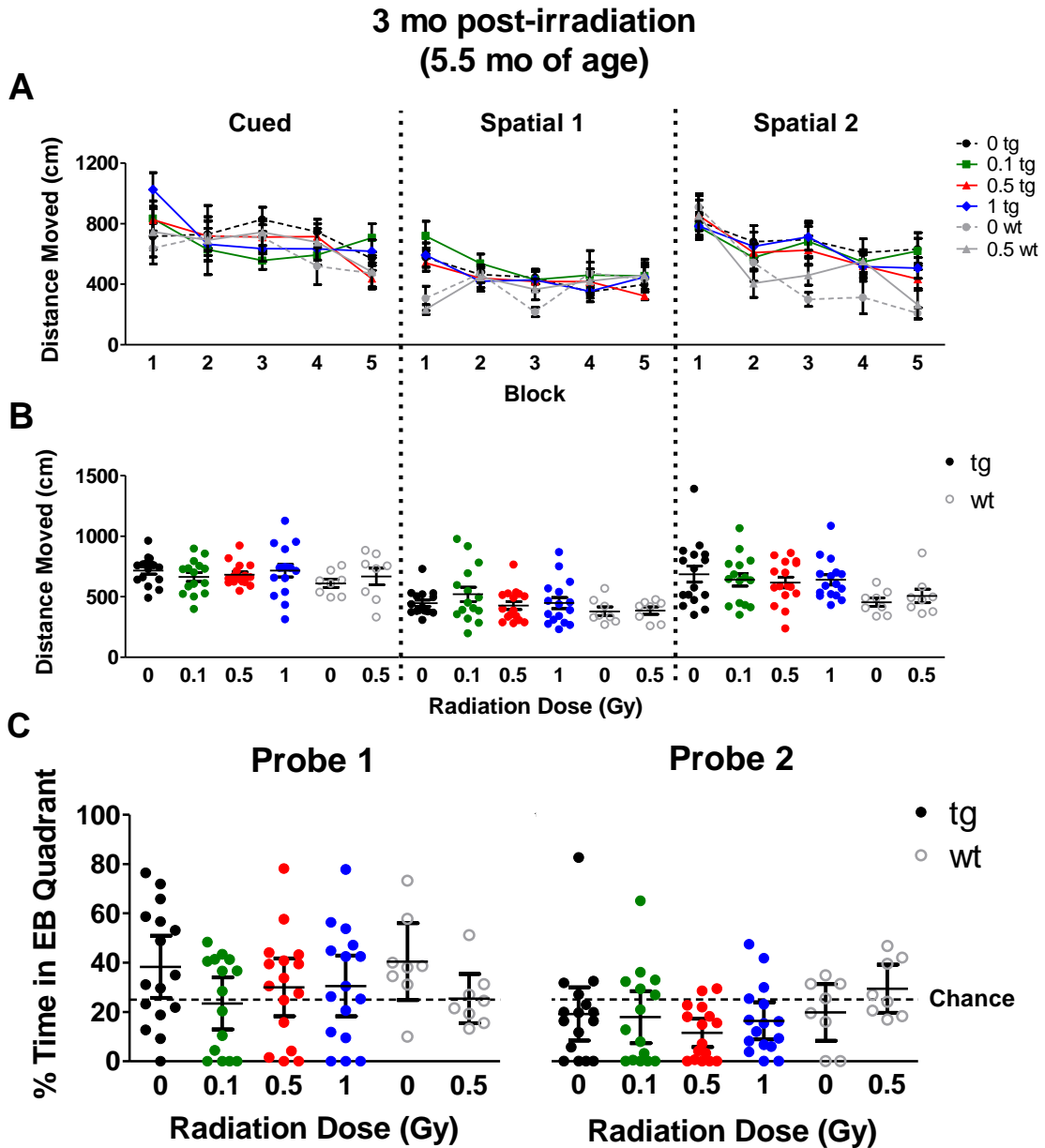


Figure 20. BM tg and wt 3-month post-irradiation behavior. Panel A shows performance on each of the 5 blocks, while panel B shows performance averaged over the blocks. Genotype effects were observed on the spatial 2 test. Panel C shows percent time spent in the quadrant where the EB was previously located. No significant differences were observed among the tg mice on any test during the 3-month post-irradiation time period. However, tg controls were the only group that spent a greater-than-chance percentage of time in the platform quadrant during probe 1. Error bars represent \pm SEM for A and B, 95% confidence interval for C.

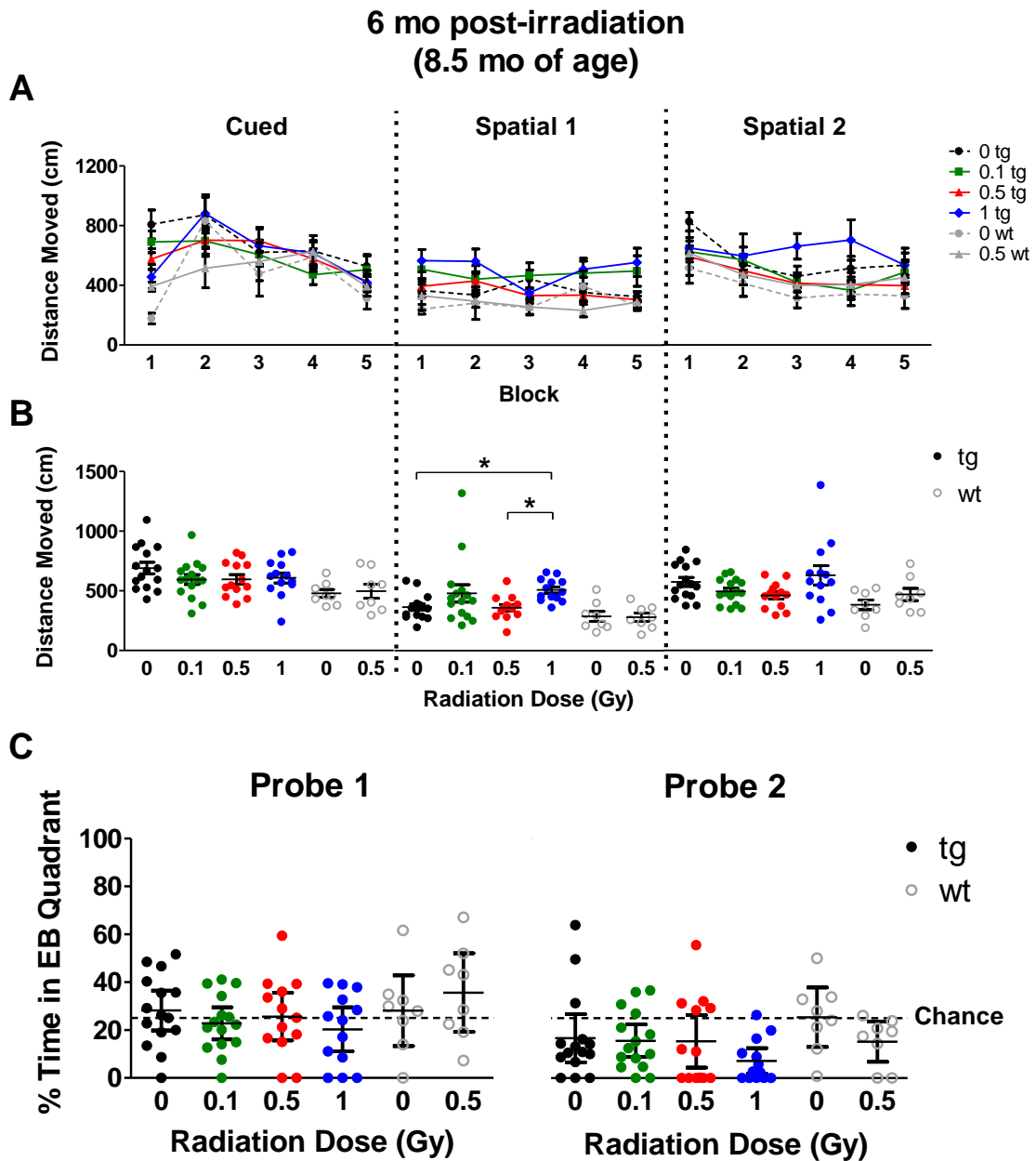


Figure 21. BM tg and wt 6-month post-irradiation behavior. Panel A shows performance on each of the 5 blocks, while panel B shows performance averaged over the blocks. Genotype effects were observed on all three tests. Panel C shows percent time spent in the quadrant where the EB was previously located. No significant differences were observed among the tg mice on any test during the 6-month post-irradiation time period. Error bars represent \pm SEM for A and B, 95% confidence interval for C. * represents $p < .05$.

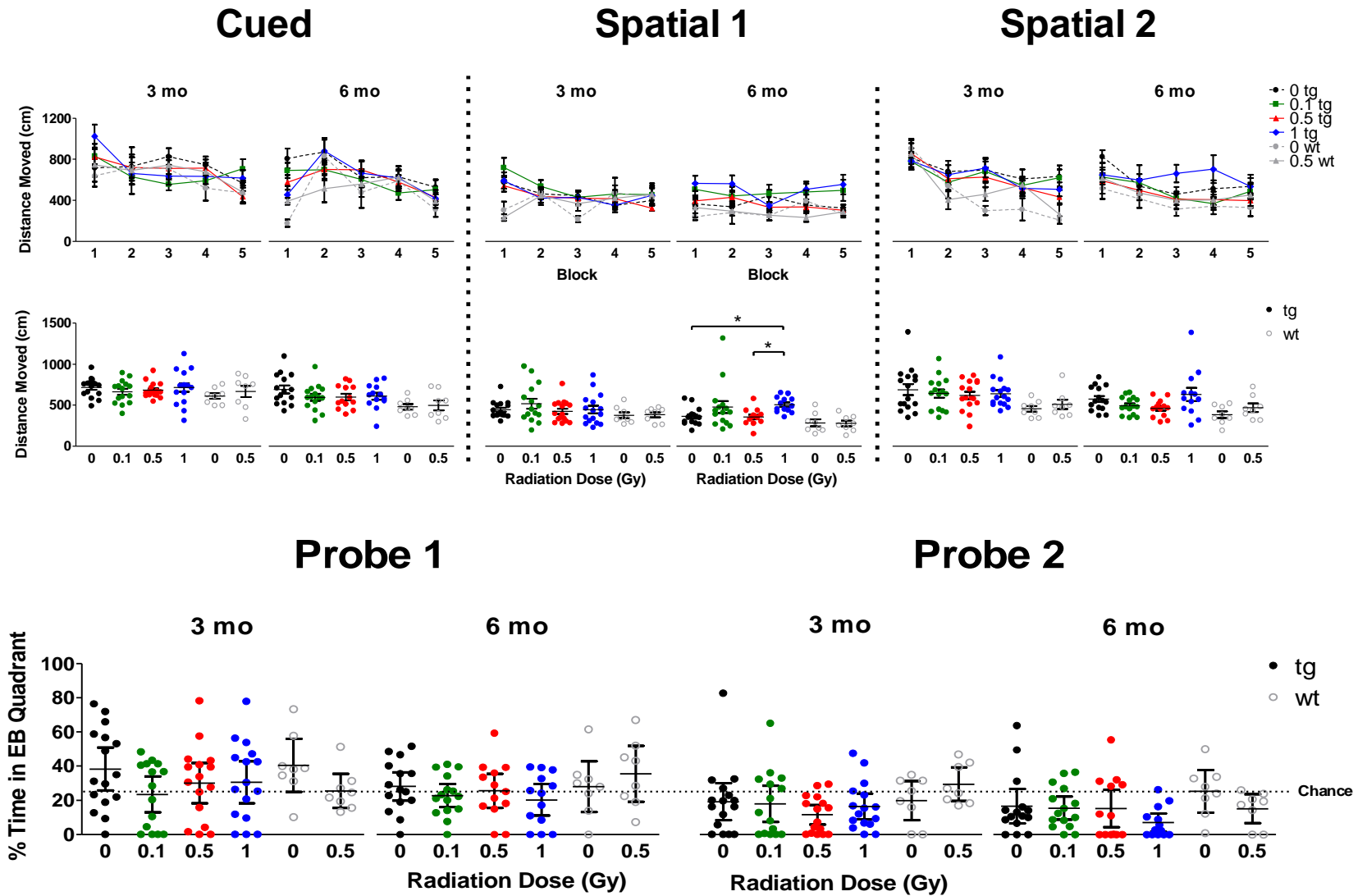


Figure 22. BM tg and wt behavior across all time-points. No significant improvement was seen across time-points on any test for the tg mice. Wild-type mice showed significant improvement across time on the cued test only.

Zero Maze Figure

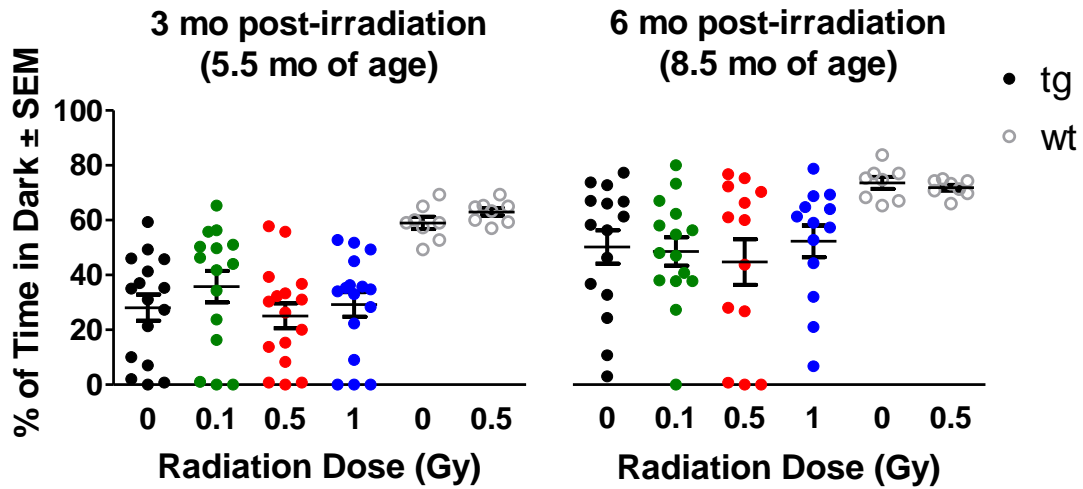


Figure 23. ZM tg and wt behavior. No significant differences were observed among the tg mice at either the 3 or 6 month post-irradiation time point. However, a significant change over time was observed in all groups. Transgenic mice demonstrated reduced anxiety-like behavior compared to wt mice. Error bars represent \pm SEM.

Weights Figure

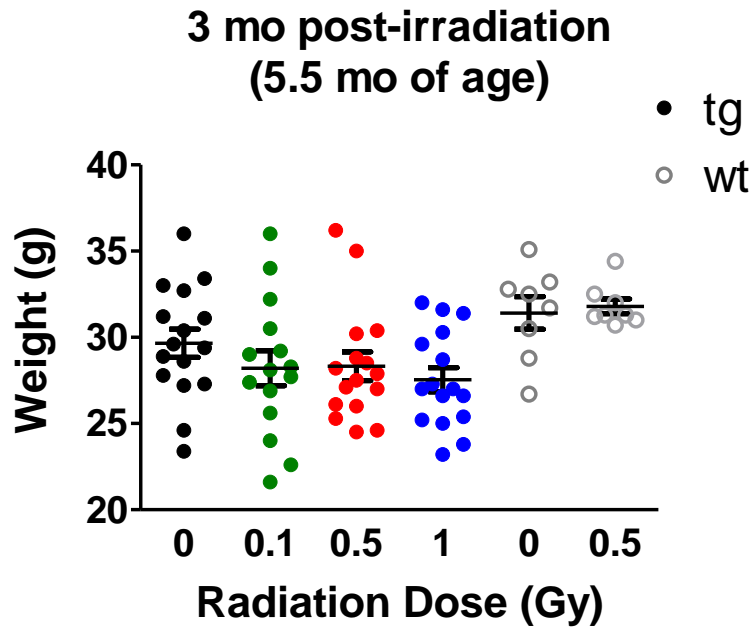


Figure 24. Weights for tg and wt mice at 3 months post-irradiation. Results show that wt mice were significantly heavier than tg mice.

Comparison to Electrophysiology

Upon completion of behavioral testing, the mice were sent to a collaborator's lab (Roman Vlkolinský) where synaptic transmission and plasticity in hippocampal slices was evaluated electrophysiologically in order to help determine the cellular mechanisms underlying the behavioral decrements. This procedure was conducted 9 months post-irradiation. Hippocampal slices were placed in magnesium-free artificial cerebrospinal fluid to evoke spontaneous activity, which was recorded in the CA3 and CA1 regions. Incidence of spontaneous activity was expressed as the inter-event interval between spontaneous oscillations in 5 min recordings.

The reason for including electrophysiological data in the present study is because the incidence of certain types of spontaneous activity in the hippocampus, such as sharp-waves/ripple complexes, is associated with memory consolidation processes (Behrens, van den Boom, de Hoz, Friedman, & Heinemann, 2005; Girardeau, Benchenane, Wiener, Buzsáki, & Zugaro, 2009). The memory consolidation process is dependent on the spread of these oscillations from the hippocampus to the cortical mantle, so any activity that impedes this process can impair recall ability. Thus, it was hypothesized that radiation would interfere with this process and lead to memory impairment.

There was found to be a significant increase in the inter-event interval (i.e., less sharp waves per unit of time) after 0.5 Gy proton radiation in wt mice at 9 months post-irradiation, pointing to decreased hippocampal activity (see Figure 25). However, no differences in activity were observed among tg mice (see Figure 26). The slightly smaller sample size relative to behavioral data is due to the inability to assess the waveforms in several mice during the complex electrophysiological recordings.

We used a Pearson product-moment correlation to compare behavioral and electrophysiological outcomes. The 6-month WM reversal learning data (i.e., Spatial 2) positively correlated with the spontaneous activity data (Pearson's $r_{10} = -0.72, p < .01$). Specifically, as the distance moved increased (meaning poorer performance) the interval between waves of activity increased (see Figure 27). However, no significant correlation was found among the tg mice (see Figure 28).

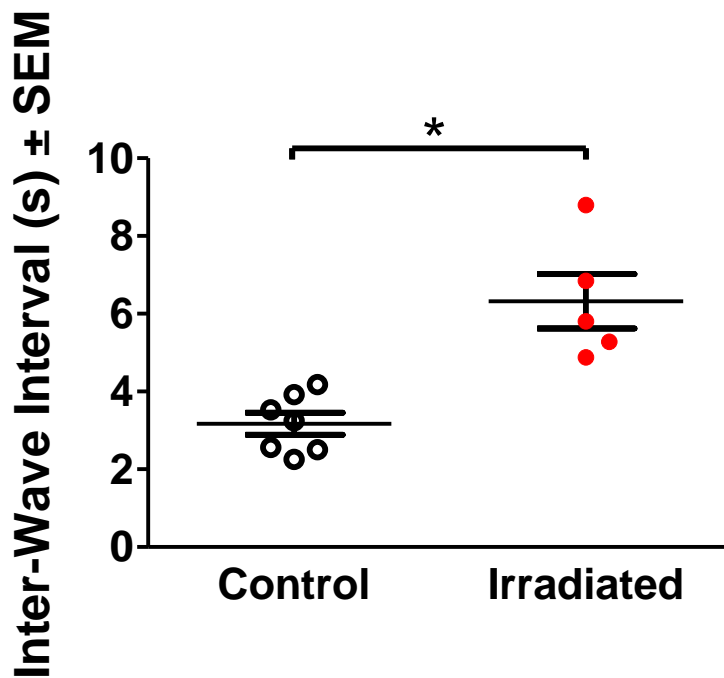


Figure 25. Spontaneous activity in CA1 region of hippocampus in wt mice. Results showed that irradiated mice exhibited a greater interval between waves, meaning less spontaneous activity compared to controls.

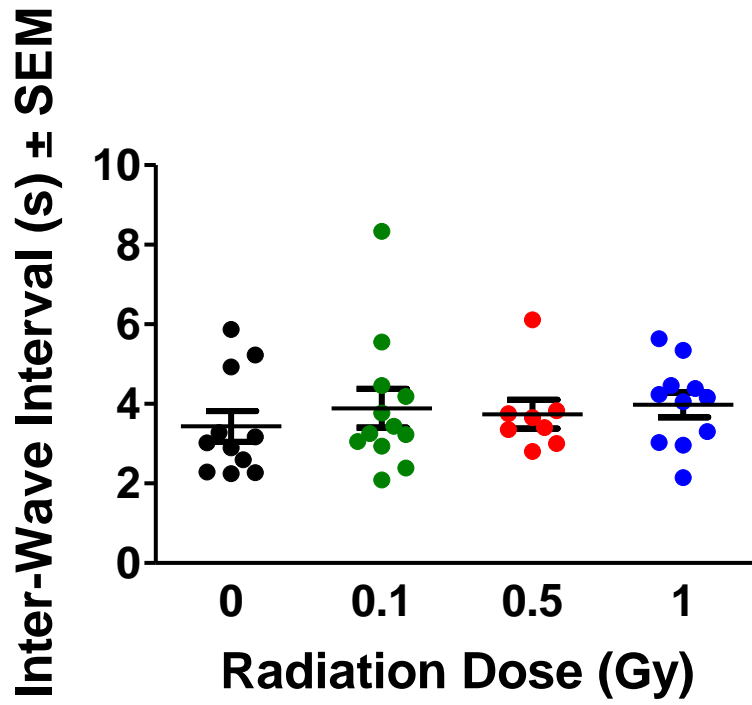


Figure 26. Spontaneous activity in CA1 region of hippocampus in tg mice. Results showed that there were no radiation-induced differences in spontaneous activity, or the average interval between waves, among tg mice.

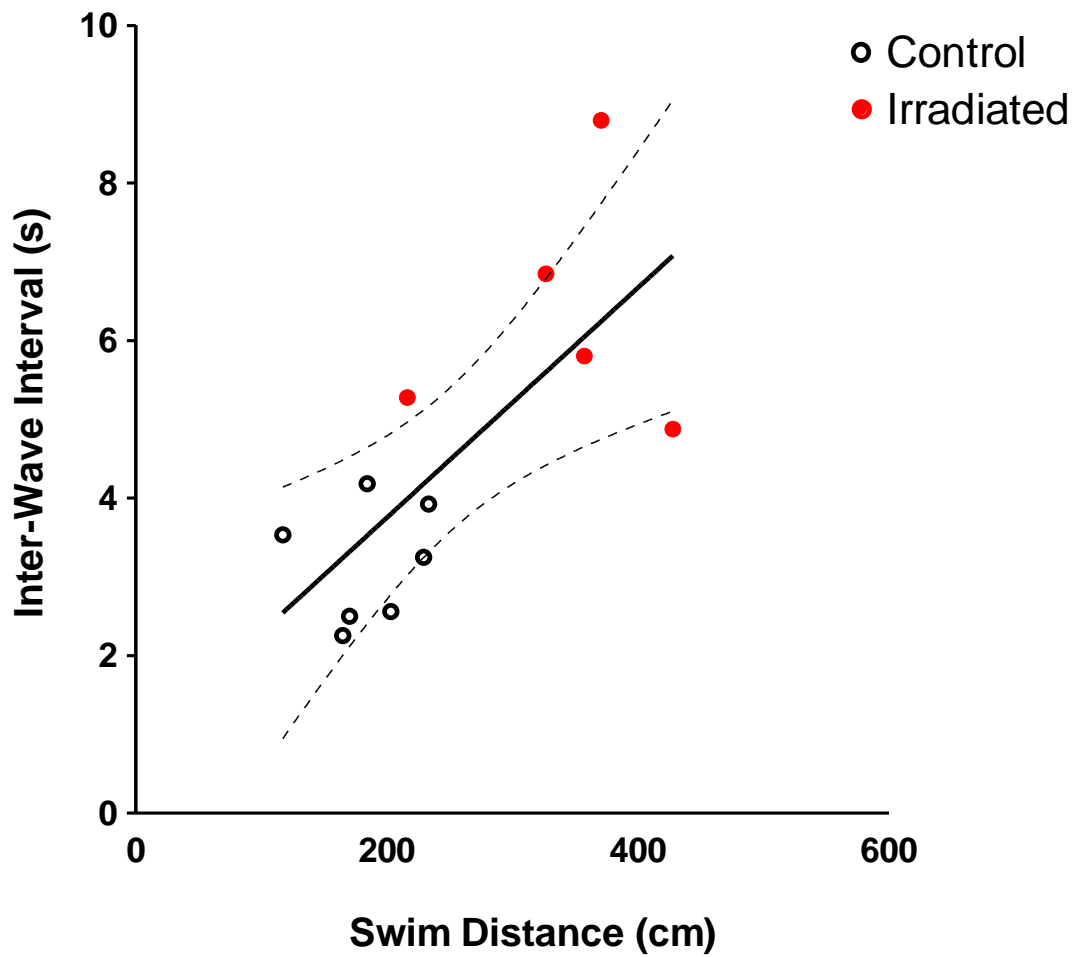


Figure 27. Correlation between spontaneous activity and 6-month WM reversal learning data in wt mice. The inter-wave interval significantly correlated with swim distance in the WM, meaning that mice that performed better (less swim distance) showed more hippocampal activity.

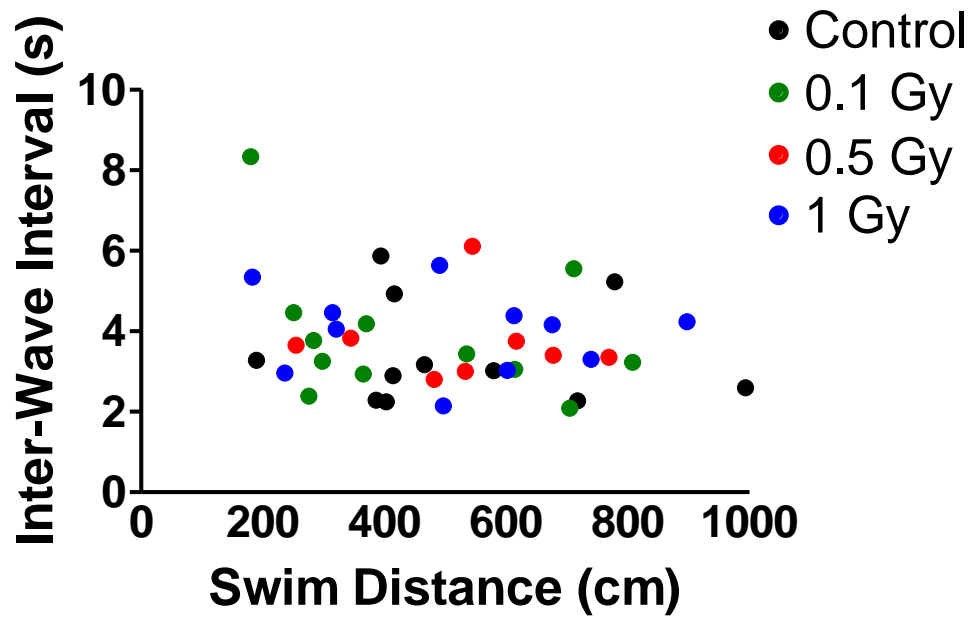


Figure 28. Correlation between spontaneous activity and 6-month WM reversal learning data in tg mice. Results showed that there was no significant correlation between inter-wave interval and swim distance.

CHAPTER FIVE

CONCLUSION

Discussion/Implications of the Findings

In the present study, we exposed young male wt and tg mice to 0.5 Gy of proton radiation and measured the effects on behavior up to 6 months after irradiation. Few studies have assessed the effects of a low dose of proton radiation on learning and memory function despite evidence that this is the major type of radiation astronauts are likely to receive on extended missions planned for the near future (Cucinotta et al., 2012; Cucinotta et al., 2013). Similarly, no known study has looked at the effects of such exposure past 3.5 months post-irradiation or assessed the effects on mice with AD-like neuropathology. Here we show evidence that such exposure induces behavioral impairments in wild-type mice, but may not exacerbate or accelerate deficits in mice with a predisposition to developing age-related, AD-like pathology.

Aim 1

Although the behavioral testing was assessed repeatedly before irradiation and 3 and 6 months post-irradiation, significant behavioral decrements were identified only at 6 months post-irradiation, where we observed impaired reversal learning in the WM. Such decrement is suggestive of an inability to remain cognitively flexible in novel situations and has been shown to be particularly indicative of dysfunction in the prefrontal cortex and hippocampal memory system (Altafaj et al., 2001). Cognitive flexibility involves the ability to inhibit a previously learned platform-finding strategy in order to acquire a new navigation approach (Clapcote & Roder, 2004; Nasir et al., 1995). Animals with

hippocampal damage can often learn an initial task, but are unable to adapt to changing conditions, such as platform relocation (e.g., day 2 of spatial testing; Wishaw et al., 1995; Wishaw & Jarrard, 1996; Wishaw & Tomie, 1997). In the present study, the irradiated mice were unable to make a strategic switch, and often perseverated on the previous platform location (see Figure 12), demonstrating their cognitive inflexibility and reduced problem-solving ability.

Finding behavioral deficits at 6 months, but not at 3 months, after irradiation suggests that the effects of a low dose of proton radiation may have a relatively late onset. One potential explanation for this delay is that the radiation exposure decreased neurogenesis. Since new neurons can take weeks to months to come to full maturation (Praag et al., 2002; Raber et al., 2004), reduced neurogenesis may have caused a gradual depletion of new neurons in the dentate gyrus (and/or other mitotically active brain regions), which would have given the irradiated mice a disadvantage in a task of learning and memory at 6, but not 3, months after irradiation. Although other rodent studies using proton radiation have not found radiation-induced behavioral effects on learning and memory (Dulcich & Hartman, 2013; Rabin et al., 2002; Shukitt-Hale et al., 2004), none of them assessed behavior past 2.5 months after exposure.

Data from the BM trended to the same patterns as the results from the WM, but did not show significant differences. Although some authors suggest that mice may perform worse in the WM than the BM due to its aquatic nature (which may be more suitable for rats; Wishaw & Tomie, 1996), others have indicated that neither test is superior to the other, each using a different motivational technique that may produce varying results (Gerlai & Clayton, 1999; Patil, Sunyer, Höger, & Lubec, 2009). For this

reason, it is often recommended that the tests be used as parallel measures of spatial learning and memory ability. Our group, however, has found the WM to be quite sensitive for determining behavioral deficits in a number of mouse models (Fukuda et al., 2013; Hartman et al., 2012; Lekic et al., 2011; Pop et al., 2013).

Regarding the comparison to electrophysiological data, the inter-wave interval was measured to assess hippocampal activity levels. The increased inter-wave interval among the irradiated mice suggests that radiation inhibits the memory consolidation process associated with the spread of oscillations from the hippocampus to the cortical mantle, as was hypothesized. Such a reduction has been shown to be associated with memory impairments (Behrens et al., 2005; Girardeau et al., 2009), and offers a mechanism to explain our behavioral findings. Indeed, the electrophysiological data significantly correlated with our behavioral data from the Spatial 2 test, demonstrating that as the frequency of spontaneous activity increased the performance on a spatial learning task improved (see Figure 27).

Aim 2

Transgenic mice performed significantly worse than wt mice on most behavioral measures and at most time-points, confirming that tg mice developed AD-like pathology observed in other studies using this model (Cherry et al., 2012; Harrison et al., 2009; Jankowsky et al, 2004). Among tg mice, the only difference found was at 6 months post-irradiation on the spatial 1 test of the BM, where the 0 and 0.5 Gy group performed significantly better than the 1 Gy group (see Figure 21). However, due to the lack of a learning curve, large degree of variance, and no differences on the more difficult spatial 2

test, these results are likely spurious. No other differences were observed among tg mice that were exposed to radiation at doses up to 1 Gy as compared to non-irradiated tg controls. The findings suggest that exposure to low doses of proton radiation do not accelerate or exacerbate AD-like pathology.

It is likely that the learning and memory deficits from the AD-like pathology were overshadowing any potential radiation effects. Though higher doses may have resulted in a noticeable effect, doses above 1 Gy may not be applicable to space travel under normal circumstances (Cucinotta et al., 2012; Cucinotta et al., 2013). The data indicate that individuals with an AD predisposition may not be further affected by low doses of proton radiation. Such findings infer that it may not be necessary to screen astronauts for an AD predisposition and potentially preclude them from service based on such a discovery.

Regarding the comparison to electrophysiological data, the inter-wave interval was measured to assess hippocampal activity levels. Unlike the wt results, there were no differences among tg mice and the data did not correlate with our behavioral data. These findings add further support to the behavioral results, suggesting that radiation does not exacerbate impairment in tg mice.

Conclusions

Understanding the potential risks of exposure to low doses of proton particle radiation on cognitive functioning is imperative to the success of future space missions, as well as to ensure quality of life for our astronauts. In the present study, we used behavioral measures to demonstrate that low doses of 150 MeV proton radiation impaired learning and memory in wt mice but did not exacerbate deficits in tg mice. The data

suggest that astronauts traveling outside the magnetosphere are at risk of developing long-term, radiation-induced learning and memory deficits. However, radiation exposure is not likely to interact with an AD predisposition in a deleterious manner. These findings highlight the need for improved shielding and/or compensatory strategies to decrease radiation-induced risks.

Limitations

We acknowledge several limitations in the present study:

1. Proton particle radiation doses up to 1 Gy may not have been large enough to result in detectable effects. However, this amount of radiation was the most appropriate to use since it most accurately models the space environment.
2. Repeated behavioral testing resulted in practice effects that could have attenuated the detection of subtle deficits. However, all groups received the same number and sequence of behavioral tests to avoid any differences in practice effects by group.
3. It may have been beneficial to include wt groups exposed to 0.1 and 1 Gy to be able to directly compare to the tg mice given those doses. Only the 0 and 0.5 Gy wt groups were originally included since NASA was mainly interested in the tg mice and resources were limited.
4. It is possible that using a single tg mouse model of AD, rather than the double tg model we used, may have led to less AD pathology, allowing the effects of radiation exposure to be observable. However, results from other labs using the double tg model have shown that iron radiation exacerbates AD pathology

(Cherry et al., 2013), which led us to hypothesize that this model would be appropriate for the present study.

5. Repeated, fractionated radiation exposure (e.g., exposing the mice to four separate doses of 0.25 Gy) may have elicited different results than giving mice a dose of 1 Gy all at once. However, the logistics of repeatedly exposing the mice (e.g., the time, money, and radiation beam access involved in doing so) precluded us from pursuing this option. Additionally, recent findings suggest that a fractionated dose does not affect the outcome (Rivera et al., 2013).
6. Immunohistochemical analysis of various biological markers of AD (e.g., plaque load) would have aided in the determination of a mechanism of the effects. This data is forthcoming.

Future Directions

The present findings lead way to many more questions regarding the effects of low doses of radiation. Since this study was the first to look at low doses of proton radiation on mice with a genetic predisposition for developing AD-like pathology, replication studies are needed to ensure the validity of the present findings. Additionally, subsequent studies could use other measures to test different behavioral constructs (e.g., activity level, depression, fine and gross motor ability, etc.), as well as different time-points. It may also be informative to assess various biomarkers to better understand potential mechanisms, such as ROS, inflammation, or reduced neurogenesis.

Though protons are the most abundant particles future astronauts are likely to be exposed to, other particles may be present as a result of galactic cosmic rays and solar

activity. Our lab has already begun testing the effects of these heavier, larger particles on behavior in APP/PSEN1 mice. The literature suggests that such particles (e.g., iron or silicon) may cause more damage than less massive ones, such as protons (Cherry et al., 2012). Preliminary data from our lab's follow-up studies suggests that both iron and silicon particle radiation cause significant cognitive changes.

Once an adequate understanding of radiation effects is achieved, the next step is to construct interventions to ameliorate such deficits. One study from our lab has already demonstrated that pomegranate juice rescued some detrimental effects of proton radiation (Dulcich & Hartman, 2013). Other studies are needed to assess the effects of antioxidants on different types of radiation, or to discover other kinds of potential interventions.

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