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Behaviorally-Induced Structural Remodeling of the Hippocampus

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Behaviorally-Induced Structural Remodeling of the Hippocampus

by

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

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

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ABBREVIATIONS

NASA	National Aeronautics and Space Adminstration
CA3	Cornu Ammonus Region 3
ANOVA	Analysis of Variance
Gy	Gray
WM	Water Maze
Co-γ	Cobalt γ
γ	Gamma Radiation
Timm’s	Timm’s Sulphide Silver
BDNF	Brain Dervied Nuerotrophic Factor

ABSTRACT OF THE THESIS

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by

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Doctor of Philosophy, Graduate Program in Clinical Psychology
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Dr. Richard E. Hartman, Chairperson

NASA is planning to send a manned spacecraft to Mars by 2030. Interplanetary space travel puts astronauts at a high risk for exposure to several types of radiation exposure. This exposure has the potential to disrupt neurogenesis in the hippocampus, which has long been associated with learning and memory. This research proposes to study the effect of γ radiation on mossy fiber growth in the CA3 region of the hippocampus. Research has demonstrated that rats exhibit mossy fiber growth from the stratum lucidum to the stratum oriens of the hippocampus after completion of spatial learning. Water maze training is a spatial learning task that requires the animal to use distal cues to locate a platform hidden two centimeters below the water. Rats with a higher density of mossy fiber growth were expected to spend more time in the correct quadrant. This study investigated the effects of high dose single exposure radiation on presynaptic organization in the stratum oriens region of the hippocampus. Spatial learning was conducted using a water maze at a rate of ten trials per day for a period of four days. Five days after completion of spatial learning task, a probe test was completed. The delayed period was to allow for mossy fiber sprouting. The brain was then to extracted and sectioned at 30μm then stained with Timm's staining. Timm's stain allows visualization of metal such as zinc in newly formed axon terminals. A repeated measures

ANOVA was conducted to determine if there was a significant difference between radiation and water maze performance. There were no other significant differences in spatial or probe performance. Hippocampal tissue was analyzed by measuring the area of stratum oriens with Timm's staining present using grid point counting. There was no significant difference the in the area of Timm's staining. These findings are consistent with current research, which suggests that there are no significant differences in behavioral learning or axon density immediately after exposure.

CHAPTER ONE

INTRODUCTION

NASA is currently funding research to determine the risk and effects of prolonged exposure to low doses of radiation with the aim of sending astronauts to Mars by 2030. Depending on the specific protocol followed, estimated travel time could range from 6 months to a year one way. Earth's electromagnetic field deflects harmful cosmic radiation (rays from this point forward) away from the atmosphere. The electromagnetic field provides protection to astronauts during Earth orbiting mission (Cucinotta & Durante, 2006). Mars lacks an electromagnetic field allowing for cosmic rays from solar flares to reach the surface of the planet. Missions beyond the protection of the Earth's electromagnetic field require additional shielding from cosmic rays and for passage through radiation belts (Nelson, 2016). Current shielding is not effective at deflecting ionizing radiation for long periods of time. During low earth orbit missions, astronauts are exposed to proton and electron radiation trapped in the Van Allen belt. This results in increased radiation levels outside the spacecraft and suit as well as exposure due to the interactions between the radiation and shielding material (Nelson, 2016). Several organizations are attempting to create shielding that will provide adequate protection for humans without significantly increasing the weight and size of the spacecraft. An extremely large or heavy spacecraft would create additional problems leaving the gravitation pull of the Earth's atmosphere.

The cosmic environment is a complex field of radiation. The space environment contains the full range of electromagnetic radiation, high energy particle radiation, solar emissions and trapped radiation in the radiation belts (Nelson, 2016). Researchers have found that astronauts would be exposed to small particle radiation daily and large particle radiation weekly. (Cucinotta & Durante, 2006; Yasuda, Komiyama, & Fujitaka, 2001, Nelson, 2016). Charged particles contacting DNA can lead to premature cell death or point mutations. Large particle ions such as Fe, poses a significant risk to astronauts both directly and indirectly through the Compton Effect. Astronauts orbiting Earth are exposed to less cosmic rays because they are still within the electromagnetic field (Yasuda, Komiyama, & Fujitaka, 2001). The hippocampus plays an important role in learning and memory formation. Neuronal growth in the hippocampus is suspected to play a role in long-term memory formation. The dorsal pole of the hippocampus is associated with spatial learning and the ventral pole is associated with anxiety-type behaviors (Strange, Witt, Lein, & Moser, 2014). Spatial learning is associated with an anterior to posterior gradient with cellular changes occurring in the anterior to intermediate sections with fewer changes in the posterior sections (Strange et al., 2014). Cancer treatments have demonstrated that the brain, and especially the hippocampus, is particularly susceptible to the effects of radiation (Tofilon & Fike, 2000). Radiation can reduce the density of neurons and their dendritic spines in the hippocampus (Chakraborti, Allen, Allen, Rosi, & Fike, 2012). There is very little research on the specific effects of high dose radiation for the treatment of cancer. Researchers have focused on the efficacy of the treatment and the number of physical complaints from patients. Researchers have not investigated the link between areas irradiated and specific dysfunction.

It is essential to understand the risks and effects of long-term space travel. This study investigated whether high-dose γ radiation exposure will inhibit presynaptic reorganization in the hippocampus and the ability to learn. This study also investigated whether radiation exposure inhibits the ability to learn, form long-term memories, and inhibit mossy fiber growth. Majority of researchers have focused on post-synaptic organization. Post-synaptic research includes changes in spine density, resting cellular voltage, action potential threshold, and neurogenesis. Presynaptic research is an area that is lacking in radiation studies. Research on whole brain radiation for the treatment of brain cancer suggests that neuropsychological functioning show impairment within five years (Baschnagel, Wolters, & Camphausen, 2008).

γ radiation is a photon created from decaying radioactive material. It is accelerated using funneled mirrors to target a specific region. Γis a form of radiation referred to as indirectly ionizing radiation. γ radiation causes damage to cells by striking molecules in strands of DNA or by striking electrons and causing free radicals to be released into the body (Cucinotta & Durante, 2006). The radiation spectrum in space consists of directly ionizing radiation that can cause changes by striking molecules or through Coloumbic forces. The absorbed amount or dose of radiation referred to as a Gray (Gy). Gray can be increased by the length of exposure to radiation making the length of travel to Mars particularly dangerous. While this type of radiation is not naturally occurring in space, it provides a basic framework for understanding the effects of radiation on presynaptic organization. Future research could include types of radiation that are likely to be encountered during space travel.

This study looked to identify the relationship between radiation exposure after learning and mossy fiber growth. Mossy fiber growth in stratum oriens of animals exposed to radiation after four days of WM training was compared to the same region in sham animals to determine the effects of radiation. The results could provide a platform for future studies to develop ways of preventing the structural and/or functional effects of radiation exposure.

Specific Aims and Hypotheses

The purpose of the study was to determine whether exposure to 5 Gy of γ radiation exposure prior to spatial learning causes deficits and/or affects the growth of mossy fibers into the stratum oriens of the hippocampus. The study also investigated whether radiation exposure after spatial learning will affect mossy fiber. The radiation will penetrate through the brain and likely expose hippocampus cells. The dose given is consistent with doses used in cancer treatment. While this level of γ radiation is not an exact replica of the dose and type of radiation in space, it is a representative model of a single high dose exposure.

The first aim of the study was to determine whether a spatial learning task induces the growth of mossy fibers within the hippocampus; the main effect of the task. We hypothesized that animals exposed to a learning task will have more mossy fiber growth than animals that only swam. The second aim of the study was to determine whether γ radiation inhibits the growth of mossy fibers within the hippocampus; the main effect of treatment. We hypothesized that animals exposed to 5 Gy of γ radiation will have less mossy fiber growth than sham-exposed animals. We also hypothesized that animals exposed to radiation after a spatial learning task will have less mossy fiber growth than animals exposed to γ radiation prior to a spatial learning task. The third aim of the study was to determine whether mossy fiber growth in the hippocampus is correlated with spatial learning performance. We hypothesized that animals that perform well on the spatial learning task will have more mossy fiber growth than animals that perform poorly. We also hypothesized that animals that spend more time in the correct quadrant during the probe test will exhibit a higher density of mossy fibers in the stratum oriens.

CHAPTER TWO

LITERATURE REVIEW

Brain Structures of Interest

Learning and memory are the result of changes in the way that neurons communicate (e.g., neuroplasticity). This can result from structural changes, chemical changes or the number of neurons activated. These plastic events are thought to underlie processes of memory formation (Holahan, Rekart, Sandoval, & Routtenberg, 2006; Ramirez-Amaya, Balderas, Sandoval, Escobar, & Bermudez-Rattoni, 2001; Rekart, Sandoval, Bermundez-Rattoni, & Routenberg, 2007). Most research has focused on postsynaptic plasticity or changes in downstream neurons. Recent research has focused on pre-synaptic changes, or changes in how many neurons are activated (Holahan et al., 2006; Ramirez-Amaya et al., 2001; Rekart et al., 2007).

The hippocampus is one of the only areas of the adult brain that continues to develop new neurons (Ben-Ari & Represa, 1990; Derrick, York, & Martinez, 2000). The hippocampus plays an important role in learning and memory. Children that had a temporal lobectomy including hippocampalectomy to reduce seizures experienced losses in working, verbal, and episodic memory (Jambaque et al., 2007). H.M. is a famous case study that is often used to infer the role of the hippocampus in learning (Squire, 2009). H.M. had his hippocampus removed to eliminate a severe seizure disorder. After the procedure, H.M. developed anterograde amnesia, or the inability to create new memories (Scoville & Milner, 1957). H.M was able to recall memories of events that occurred several years prior to his surgery and was able to learn procedural tasks, however, he was unable to consolidate new declarative memories. The case study of H.M. and similar studies suggest that the hippocampus is required to create long-term declarative memories.

Animal Research in Memory and Learning

Animal models currently provide the best modality to research interactions between plasticity in the hippocampus and learning. Case studies such as H.M. allow researchers to speculate about the mechanisms of memory and learning. However, they do not elucidate the specific mechanisms and structures of learning or memory.

Research has shown that the hippocampus plays an important role in spatial learning in rats. Animals with abnormal neuron projections in the hippocampus perform poorly on spatial tests (Holahan, Honegger, & Routenberg, 2009). Rats with lesions in the hippocampus also perform poorly on tests for spatial memory. Recent studies focusing on pre-synaptic plasticity in the hippocampus of animals have found a change in axon density in the dentate gyrus granule cells referred to as mossy fiber. Rekart et al. (2007), Holahan et al. (2006), and Ramirez-Amaya et al. (2001) found that rats completing a week of spatial training in a water maze (WM) displayed a significant growth of hippocampal mossy fibers. The WM measures the ability for animals to use distal cues to learn the location of a platform submerged approximately two centimeters below the surface of the water. The research found that there are a large number of mossy fiber projections to the CA3 region of the rat hippocampus. Figure 1 displays the structural anatomy of the rat hippocampus.

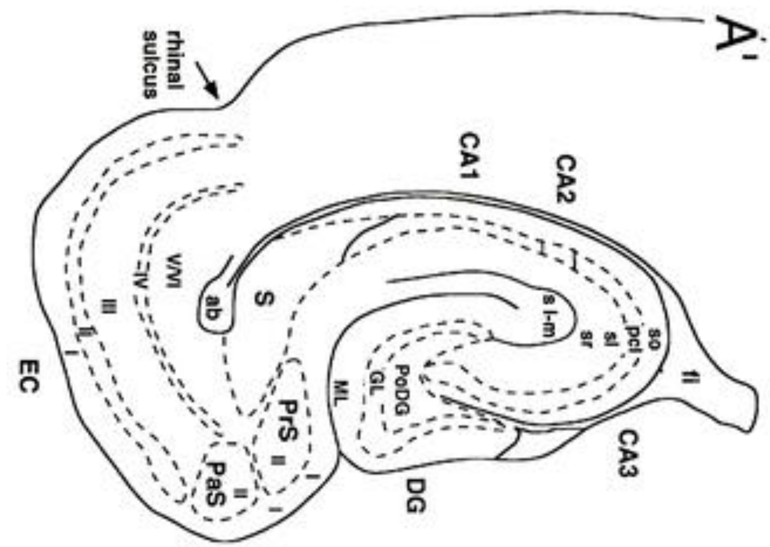

Figure 1 Cross section of the rat hippocampus. Abbreviations: EC, entorhinal cortex; DG, dentate gyrus; CA, cornu ammonus; S, subiculum; PrS, presubiculum; PaS, parasubiculum; ab, angular bundle; so, striatum oriens; pcl, pyramidal cell layer; sl, strum lucidum; sr, stratum radiatum; s l-m, stratum lacunosum moleculare; PoDG, polymorphic layer of the DG; GL, granule layer of the DG; ML, molecular layer of the DG. From Amaral and Witter, 1995

Prior to training, dense projections of mossy fibers are found in the stratum lucidum, but very few projections are found in the stratum oriens. This suggests that a significant change in the number of mossy fiber projections in the stratum oriens would constitute synaptic plasticity, possibly related to memory formation (Ben-Ari & Represa, 1990). Rats that completed one week of spatial training in a WM with a hidden platform had more mossy fiber projections in the stratum oriens (Holahan et al., 2006; Ramirez-Amaya et al., 2001; Rekart et al., 2007). The CA3 region of the rats in the spatial learning condition was compared to the CA3 region of controls and rats that only swam (with no platform). The results suggest that tasks requiring spatial learning induce an increase in mossy fiber projections into the stratum oriens. Rekart et al. (2007) added a “visible platform with a cue” condition to their study. They found that rats in the visible platform condition had no increase in mossy fiber growth. The results of these studies imply that learning a spatial task requires synaptic plasticity within the hippocampus.

Most of the research on mossy fiber growth has been conducted on Wistar rats. Wistar rats are one of the first rats breed for scientific research. They are one of the most popular in scientific research today and have a large amount of baseline data in the literature. Other strains of rats that are common in research include the Lewis and Sprague-Dawley. Wistar rats have a low density of mossy fibers in the stratum oriens prior to training and perform relatively poorly in spatial learning tasks. As Wistar rats improve on such tasks, the density of mossy fibers in the stratum oriens increases (Ramirez-Amaya et al., 2001).

Radiation can affect hippocampal plasticity in Wistar rats. After 10 Gy of radiation, there are significantly fewer hippocampal pyramidal cells in Wistar rats (Khoshbin-Khoshnazar, Jahanshahi, & Azami, 2012).Both the CA1 and the CA3 regions of the hippocampus were sensitive to radiation. This suggests that radiation at 10 Gy is a sufficient dose enough to reach the CA3 region of the hippocampus in Wistar rats.

CHAPTER THREE

METHODS

Materials

36 Male Wistar rats (~225-275 g; Charles River Laboratories) were group-housed, two per cage, fed *ad libitum* and maintained on a 12-h light-dark cycle with lights on between 0700-1900 in accordance with Loma Linda University Animal Care Facility guidelines. Training took place during the light cycle. Rats were randomly assigned to Radiation (pre-training), radiation (post-training), or no radiation treatment groups (control), and the hidden platform (spatial learning) or no platform (swim-only control) tasks. Table 1 shows the sample size for each condition.

Table 1. Conditions and number of rats used in the current study.

Treatment	Task	Sample Size
Radiation (pre)	No platform	n=6
Radiation (pre)	Hidden platform	n=6
Radiation (post)	No platform	n=6
Radiation (post)	Hidden platform	n=6
No radiation	No platform	n=6
No radiation	Hidden platform	n=6

Within each treatment group, the rats in the hidden platform task were yoked to the rats in the swim control task, with pairings based on random assignment. The experiment was performed in 2 phases, with 18 rats in each phase. The first phase contained the radiation prior to spatial learning conditions and half of the control animals. The second phase contained the radiation after spatial learning conditions and the second half of the control condition.

Irradiation

All animals were anesthetized using 2% isoflurane and placed on a platform for radiation. Sham animals remained in the holding chamber for approximately the time amount time required to administer radiation to the rats in the experimental conditions. The rats were anesthetized for approximately 10-12 minutes to perform the procedure. The heads of the rats in the irradiation condition will be irradiated with Cobalt γ (Co-γ) at a rate of approximately 0.5Gy/minute to a dose of 5 Gy. The radiation field was focused to only to the head of the rat, the rest of the body was not exposed to radiation. The field size will be set to approximately $5x5cm^{2,}$ covering the entire brain. The same procedure will be followed for each set of animals. The rats in the *radiation (pre)* group were irradiated one day prior to beginning behavioral training. The rats in the *radiation (post)* group were irradiated one day following the completion of the behavioral training.

Behavioral Training

The Morris water maze (WM) is a learning paradigm that requires the rat to use distal spatial cues to learn the location of a hidden platform (10 cm in diameter) submerged 2 cm below the surface of the water in a circular metal pool (110 cm in diameter × 50 cm in height), filled to a depth of 30 cm with water mixed with black non-toxic paint and maintained at 18 °C. Accurate navigation is rewarded with escape from the water onto the platform.

Water maze testing was performed daily for 4 consecutive days, 5 blocks of 2 trials per day. At the beginning of each trial, the rat was immersed in the water at a randomized start location facing the pool wall. Rats in the hidden platform group were allowed 60 s to find the platform. If the platform was not found within 60 s, the rat was guided to it. Once the rat reached the platform, it remained there for 30 s. At the end of each trial, the rat was placed in an acrylic waiting cage away from the pool for 30 s before being returned to the maze for its next trial. The platform was removed from the tank for rats in the swim control group, and each swim control rat swam for the same period of time in each trial as its yoked hidden platform rat.

Performance on each trial was recorded by an overhead camera and analyzed with image tracking software (Ethovision). This software provides dependent variable measures such as escape latency, swim distance, and swim speed to find the hidden platform, as well as information on the swim control trials, such as time spent in the area where the platform had been previously located.

Following behavioral training, the rats were left in their cages for five days to allow for mossy fiber sprouting. Rats receiving radiation after the completion of the behavioral training had five days to allow for mossy fiber growth, which included the day they were irradiated. During this period, the rats were group housed at the animal control facility. A probe trial was completed on the fifth day of sprouting. Each animal performed one probe trial. The animal was placed in the water at a randomized start location facing the pool wall. The rat swam for 60 s and then was placed back into the holding cage. The test was used to determine if the rats in the hidden platform condition consolidated the location of the platform and distal cues into long-term memory.

Histology

Following the sprouting period, the rats were weighed and euthanized by injection with euthanyl at 100mg/kg and perfused through the heart with 0.9% physiological saline. Rats weighed between 300-400 grams at the time of euthanasia.Once perfused, the brains were fixed in 3% glutaraldehyde and 4% paraformaldehyde and then stored in 30% sucrose until they sunk. They were shipped to NeuroDigiTech in San Diego for sectioning at 30µm and staining with Timm’s Sulphide Silver (referred to as Timm’s from here on) on every section. Figure 2 shows mossy fibers stained with Timm’s.

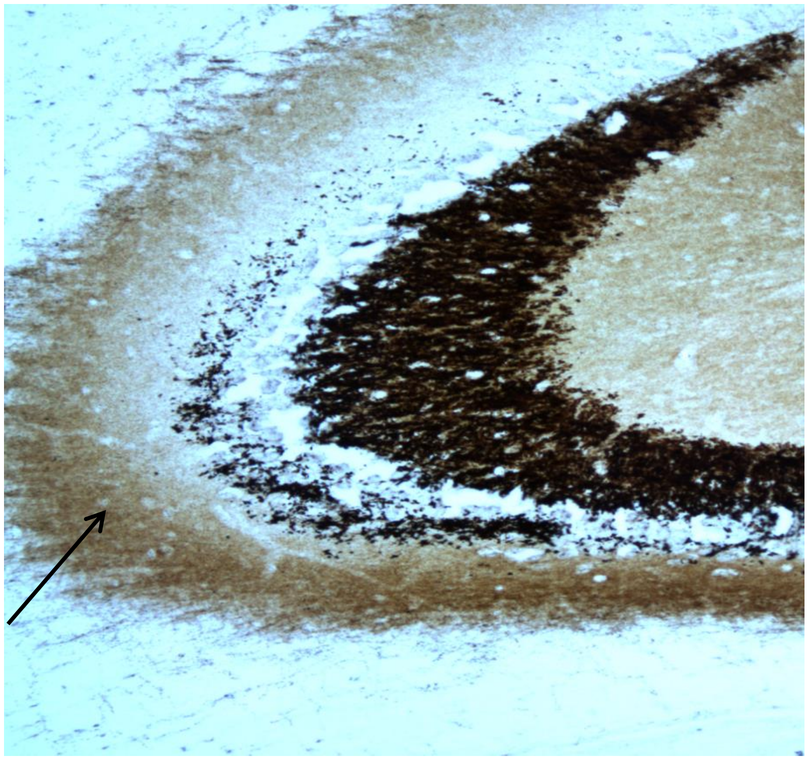

Figure 2 This image displays mossy fiber axon terminals stained with Timm's in the stratum oriens. The region identified as the stratum orien is the blue space next to the dentate. This space typically does not have axon terminal unless there is sprouting to the CA3 region.

Timm's staining is a very sensitive technique for identifying metal ions in the central nervous system. It is particularly sensitive to the presences of zinc. Mossy fiber terminals contain high concentrations of zinc. This makes Timm's staining the most appropriate method for identifying mossy fiber growth. Stained mossy fiber terminals can be identified under a light microscope to determine whether they are in the stratum lucidum or the stratum oriens. The stratum lucidum and stratum oriens were identified by locating the areas outside of the dentate gyrus without any staining present. Grid point counting was used to identify cells in the target area as shown in figure 3.

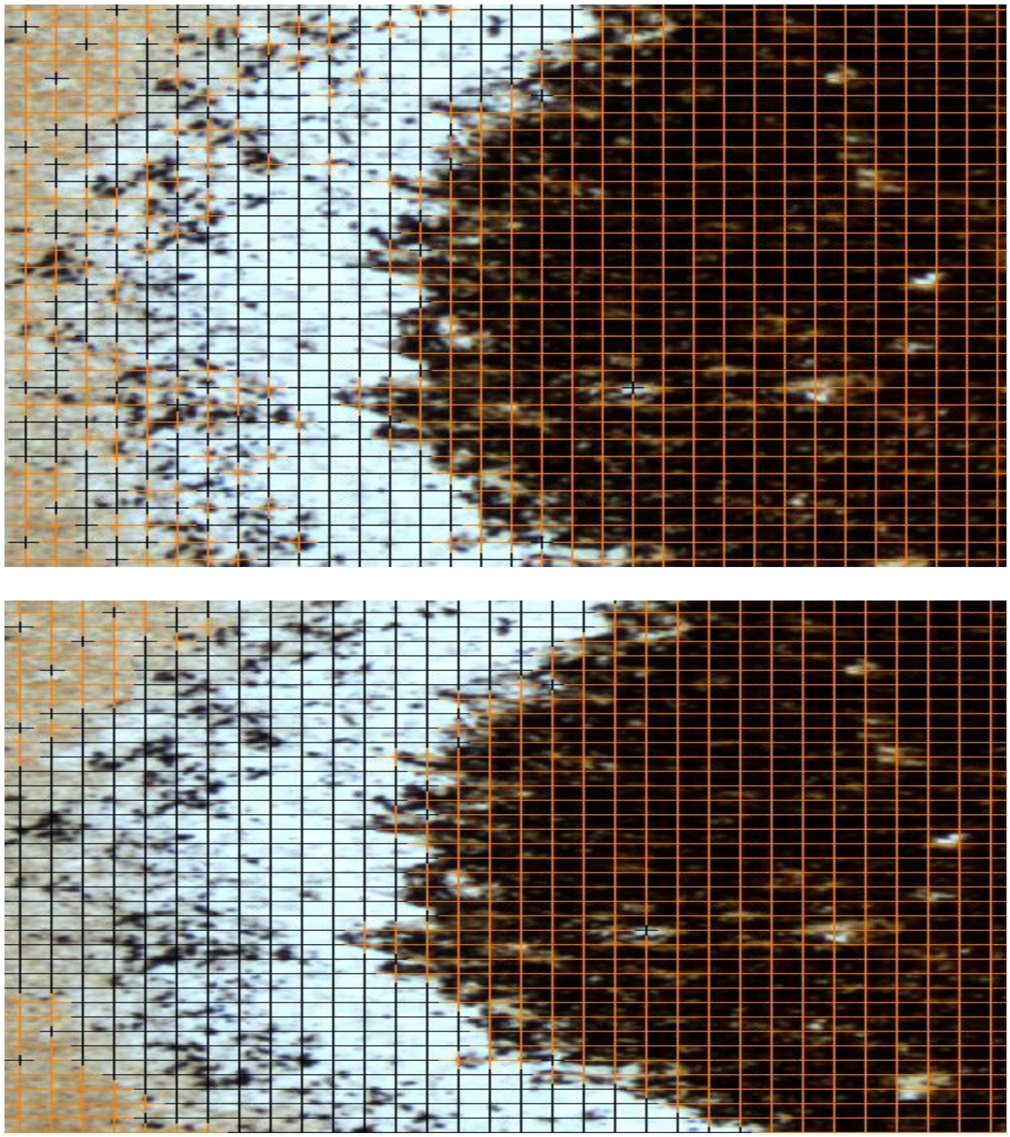

Figure 3. The figure displays how the areas of mossy fiber terminals were calculated. The image on top is a tissue section with a point counting grid overlay. The intersection points that covered an area that contained Timm's staining were identified by a red intersection. Each intersecting point in the stratum orien was unselected to determine the number of hits in the region of interest. The bottom image represents the resulting image after the hits were deselected from the region of interest.

Statistical Analysis

To test the hypotheses that radiation effects behaviorally induced synaptogenesis a repeated measures ANOVA was conducted. The independent variable was radiation (pre-training, post-training, and control). The dependent variables were the percentage of Timm's staining present in the stratum oriens/lucidum. Four serial sections were used from the rostral region of the hippocampus. Tissue from one rat was damaged during the staining process, normal imputation was conducted to impute the values. To test the hypothesis that radiation effects behavioral performance, a repeated measures ANOVA was conducted. The independent variable was radiation at the time of water maze training (irradiated or non-irradiated). The dependent variables were the total cumulative distance to the platform on each day. A one-way ANOVA was conducted to determine if radiation (pre-training, post-training, and control) affected probe performance (time in target zone). A correlational analysis was used to test the hypotheses that there is a relationship between swim performance and mossy fiber sprouting.

CHAPTER FOUR

RESULTS

A repeated measures ANOVA was conducted to determine if radiation and behavioral training were associated with changes in synaptogenesis. Assumptions of ANOVA were tested and the assumption of sphericity was violated when analyzing behavioral condition and radiation dose on mossy fiber sprouting. The assumptions of sphericity ensures equal variance between groups and violation cause increase type I error. To correct the violation, researchers used the Greenhouse-Geisser method. This method does not change the F statistic but instead changed the degrees of freedom to reduce type I error. The result is a significance level that is considered a conservative estimate. All other assumptions of a repeated measures ANOVA were met. There was no significant effect for the interaction between radiation and behavioral training on synaptogenesis ($p > .15$). Animals irradiated after water maze training had a slightly higher level of mossy fiber sprouting than control and animals irradiate before water maze training; however, there was not a significant difference.

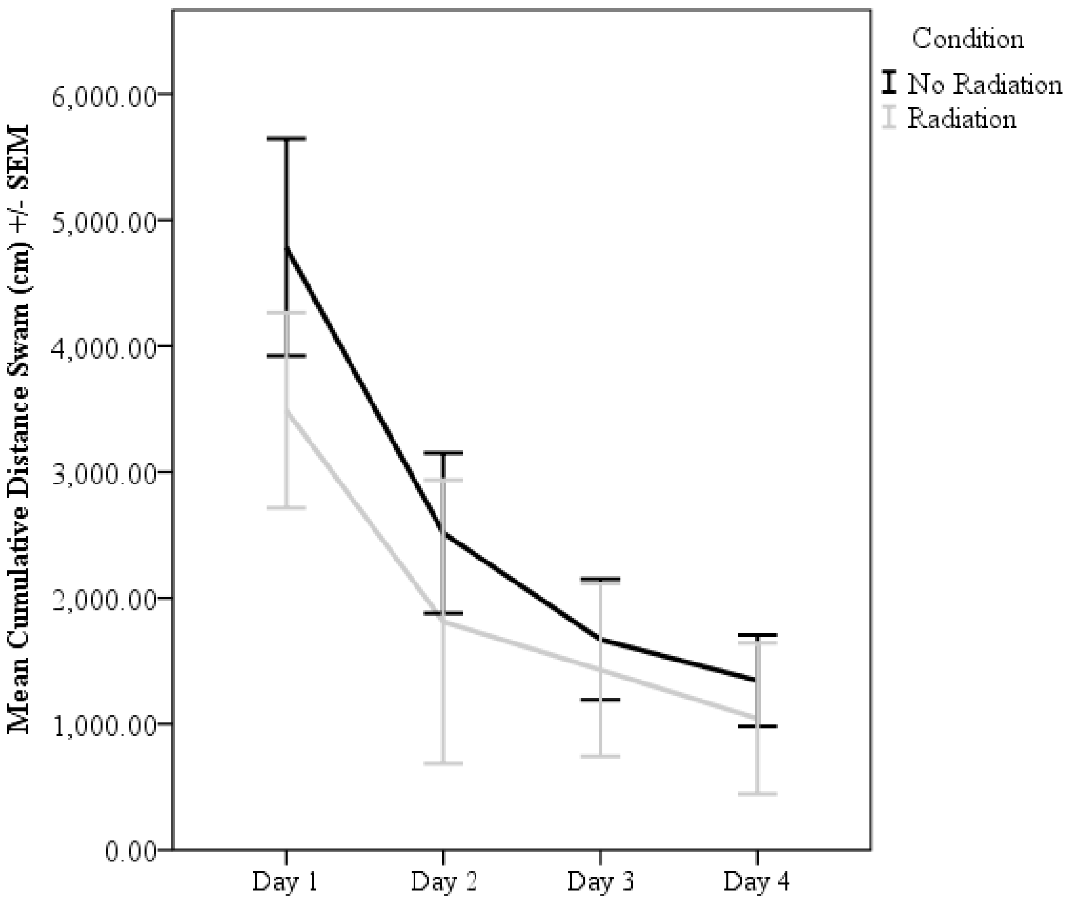

Figure 4. The figure displays mean performance by block on the water maze. Performance was measured by distance swam. There was a significant difference in performance by time, which controls showing a stronger learning curve. There was no significant difference between groups by day. Error bars represent a 95% CI.

A repeated measures ANOVA was conducted to determine the effects of radiation on behavioral training. All the assumptions of a repeated measures ANOVA were checked and met. Overall, a significant large main effect of time on behavior was found in the repeated measures ANOVA, $F(3, 35) = 59.87$, $p < .001$, with irradiated animals performing slightly better. This result confirms that all the animals learned over time which is displayed in figure 4. There was no significant difference between performances by day shown in figure 5. Additionally, there was no significant main effect for radiation or interaction between radiation and time (*p* > .1).

Table 2. Results of repeated measures ANOVA Measuring Effects of Radiation and Condition on Mossy Fiber Growth

	F	*df*	*p*-value
Mossy Fiber x Condition	.467	[2.09, 35]	> .60
Mossy Fiber x Radiation	.721	[4.3, 35]	> .50

Table 3.Results of repeated measures ANOVA Measuring Effects of Radiation and Condition on Behavior

	F	*df*	*p*-value
Behavior	132.10	[3, 35]	>.01
Behavior x Radiation	2.10	[3, 36]	> .12

Table 2 and 3 shows the results for the main effect and interactions for radiation and behavior by condition. There was no significant main effect for mossy fiber sprouting and behavioral condition (*p* > .6). Figure 6 displays mossy fiber sprouting by condition from rostral to caudal pole.

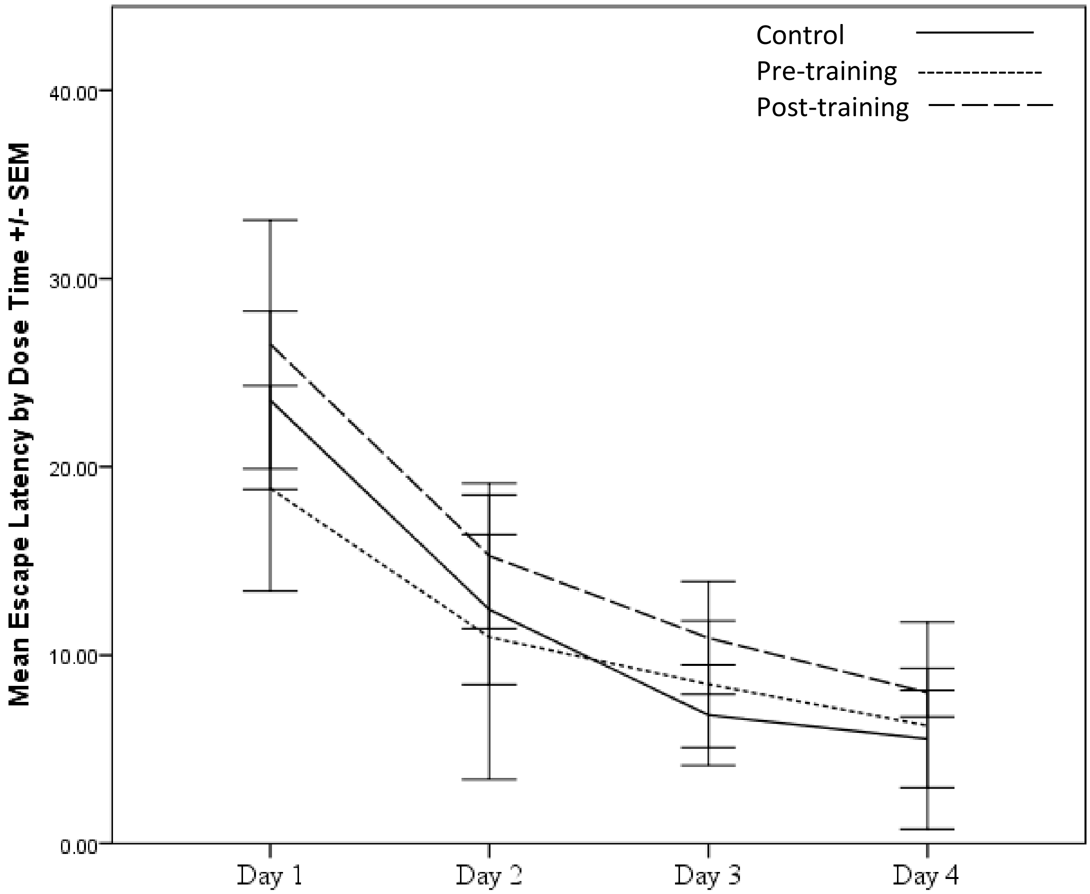

Figure 5. The figure displays mean performance by day on the water maze. Performance was measured by mean escape latency from each day. The curve of the line suggests learning in all three conditions. There was no significant difference between groups. Error bars represent a 95% CI.

A univariate ANOVA was conducted to determine if radiation dose affected probe performance. All assumptions of an ANOVA were analyzed and met. There was no significant difference on probe trail performance by radiation dose ($p > .8$).

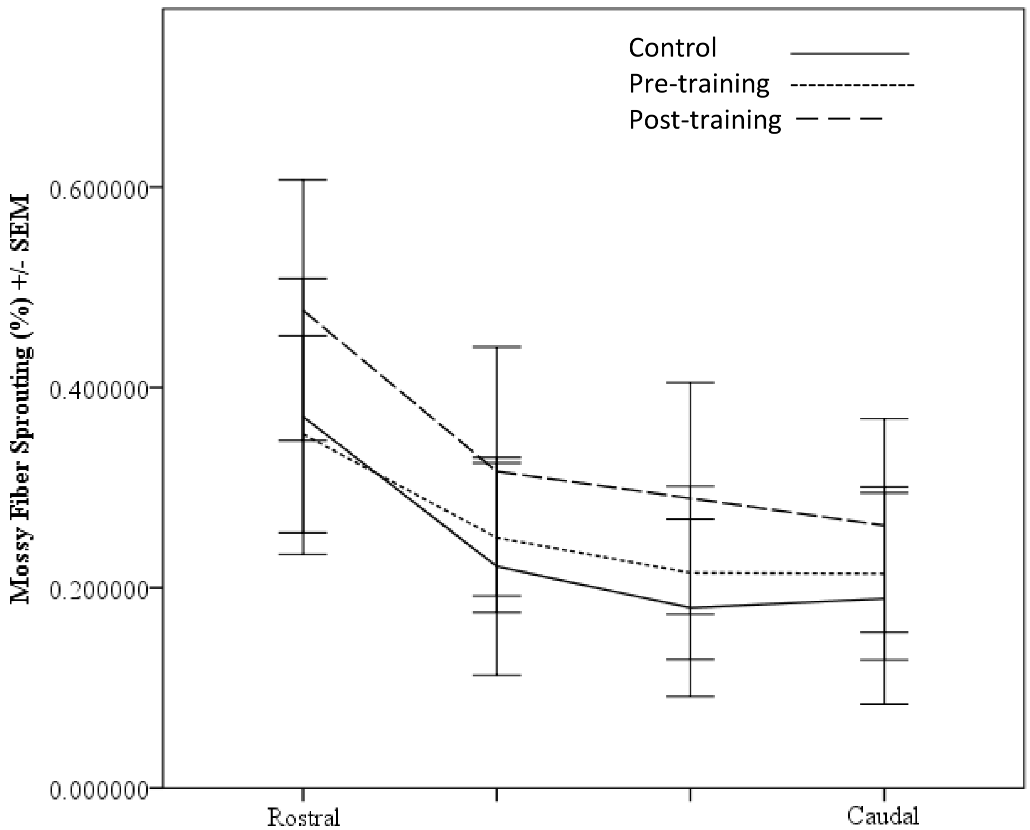

Figure 6. The samples represent area of mossy fiber in the stratum oriens from rostral to caudal in the hippocampus. There was no significant different between groups. The curvature of the line represents the decreasing number of mossy fiber terminal in the stratum oriens in the caudal region of the hippocampus.

Figure 7 displays the amount of time spent in the target quadrant for each condition. A correlational analysis was conducted to determine performance on the water maze or probe predicted changes in mossy fiber density. There were no significant relationships between water maze or probe performance and mossy fiber density ($p > .05$).

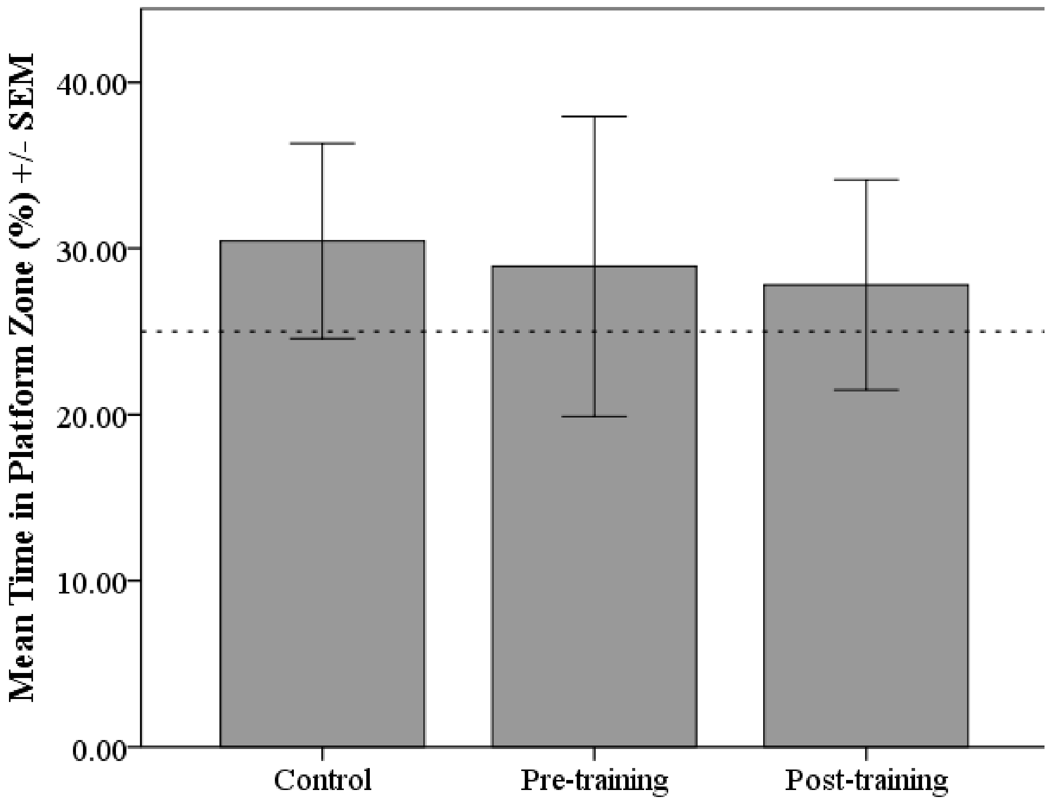

Figure 7. The bars represent the percentage of time spent in the correct quadrant during the probe trial. The reference line is set at 25% to represent the likelihood that the time spent in the quadrant was by chance. The results indicate that none of the groups were able to successfully encode the location of the platform and recall it a week later.

Table 4 contains the correlations between mossy fiber density and probe performance. Figure 8 shows a scatterplot of the relationship between spatial learning, probe performance, and mossy fiber density. Water maze performance was assessed by the mean distance from the platform across all trials. Probe performance was assessed by mean time in the target zone during the probe trial. Figures 4 and 5 display the learning curves exhibited by the rats by condition using mean distance to the platform and escape latency respectively.

Table 4.Correlation and Descriptive Mossy Fiber Growth and WM Performance

				Mean	SD
Mossy Fiber	1			.24	.11
WM Perform.	.014	1		124.39	57.24
Probe Perform.	.263	.154	1	29.06	6.55

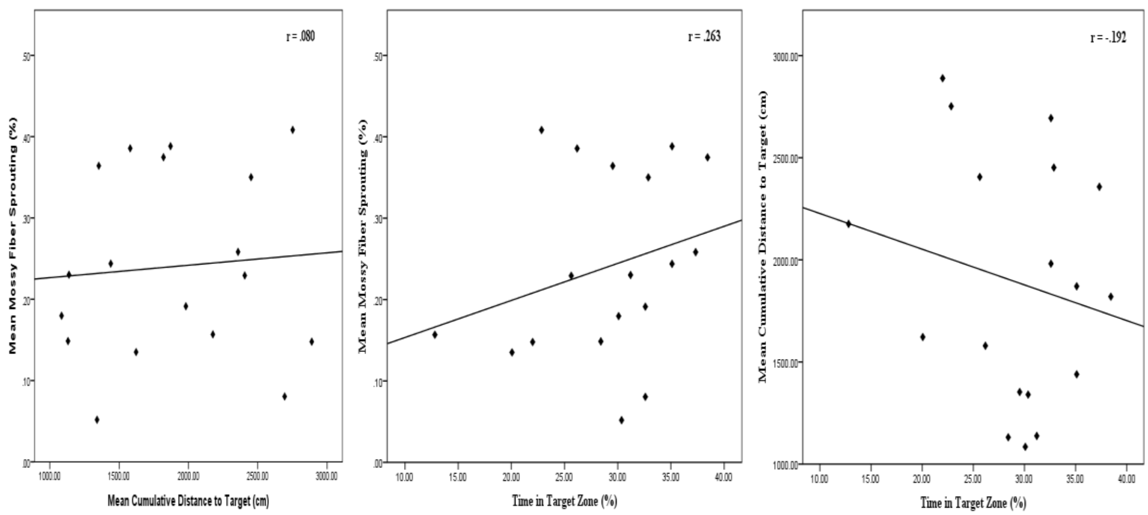

Figure 8. The scatterplots represent the relationships between spatial performance and mossy fibers, mossy fibers, and probe performance, and spatial performance and probe performance respectively. There were no significant correlations between the measures

CHAPTER FIVE

DISCUSSION

We hypothesized that rats in the hidden platform condition and rats in the control condition would present with a higher density of mossy fiber growth. There was no significant difference between any of the groups in mossy fiber density. We also hypothesized a relationship between the water maze and probe performance and mossy fiber density. There was no relationship between better performance during behavioral training and mossy fiber density. We failed to reject the null hypotheses on all of the proposed hypotheses. The observed changes in mossy fiber terminal density are consistent with the changes reported by Holahan et al (2007). This suggests that behavioral training is sufficient to produce changes in mossy fiber terminal density. The gradient of change in mossy fiber density from rostral to caudal pole is consistent with spatial learning occurring. Additionally, the learning slopes suggest that during the WM trials, the rats were able to use spatial clues to learn the location of the platform. There appears to be an acute stabilization period after exposure to a high dose of radiation. Table 5 summarizes a list of research focusing on changes to mossy fibers and the outcomes from each study.

Table 5. Comparison of studies evaluating hippocampal changes in rodents.

Study	Animal	Behavioral Task	Dependent Variable	Sample Size	Result
Current Study	Wistar Rats	Water Maze	5 Gy of Cobalt	n=36	No difference in cell density
Khoshbin khoshnazar et.al	Wistar Rats	None	10 Gy of Cobalt	n=24	Reduction of pyramidal cells
Toscano-Silva et. al	Wistar Rats	Treadmill	Exercise	n=86	Mossy fiber difference at 12 days, not 7 days
Jahanshahi et.al	Wistar Rats	Avoidance Memory Apparatus	2 Gy/10 Gy of Cobalt	n=42	10 Gy reduced neurogenesis and avoidance response
Raber et. al	C57BL/6J mice	Water Maze Barnes Maze	10 Gy of X-ray	n=28	No difference in water maze, reduction in Barnes maze
Rola et. al	C57BL/6J mice	Water Maze	2/5/10 Gy of X-ray	n=16	Reduction of neurogenesis and spatial learning at 3 months

Researchers have found that whole brain radiation in humans' results in neurocognitive deficits that are detectable approximately one to two years after exposure to the radiation (Baschnagel, Wolters, & Camphausen, 2008). Chakraborti et al. (2012) found that dendrites were affected within two weeks of radiation. Researchers studying membrane potentiality have found that neurons exposed to radiation polarize further at resting state approximately 90 days after exposure to radiation. These timelines suggest that dendritic spines may be the first structure that is affected by radiation, followed by the increase polarization of the resting membrane potential. With fewer dendritic spines and a larger action potential required for the neuron to fire, it is likely that long-term depression will occur resulting in the pruning of axon terminals. It would then be feasible to suspect that the changes in connectivity could result in neurocognitive deficits. This proposed mechanism would explain the difference in time between exposure and behavioral symptomology.

Researchers have found that immature granule cells in the subgranular zone are more susceptible to irradiation (Rola et al., 2004) Researchers found that changes in brain-derived neurotrophic factor (BDNF) was reduced after exposure to radiation. BDNF signals differentiation of immature granule. Researchers have found behavioral disturbances in animals that have lower levels of BDNF and increased immature granular cells (Ji et al., 2014, Rola et al., 2004). Mossy fibers are not directly affected by BDNF; however, increased granule cells would likely lead to additional synaptogenesis in mossy fibers. Researchers have also found that behavioral changes related to mossy fiber sprouting are better measured with the Barnes Maze (Raber et.al, 2004).

Researchers have found that exercise, forced or voluntary, can be sufficient to cause changes mossy fiber density (Toscano-Silva et al., 2010). They found that five days of exercise lead to increases of mossy fiber terminals in the stratum oriens seven days later. This study included four days of exercise and mossy fiber terminals were measured seven days post exercise, similar to the study performed by Toscano-Silva et al. (2010). It would be feasible to suspect that changes in the mossy fiber terminals at seven days post training are due to exercise and not directly caused by learning.

When considering the health of astronauts during interplanetary travel, the evidence-based time is very important to consider. Immediately following exposure to a high dose of radiation, such as during a solar flare, there are likely to be few if any behavioral or cognitive symptoms. The danger would occur approximately 2 years later when cognitive deficits would be measurable. The Mars mission would take approximately two and a half to three years to complete. If astronauts were exposed to radiation during the flight to Mars, they could potentially experience neurocognitive deficits during the return phase of the mission. This could be potentially dangerous because it is unlikely that neuropsychological testing would be completed during the mission or prior to attempting to navigate a dangerous situation.

Patients that receive radiation therapy could also be at risk for undetected neurocognitive declines. During treatment, patients are monitored on a frequent basis. If the treatment were successful, the frequency of visits would be significantly fewer two years post-treatment. It is unlikely that a patient would relate cognitive decline with a treatment that occurred two years prior given that there were no other confounding events. Researchers are currently investigating methods for ameliorating the long-term effects of radiation. Consistent neuropsychological evaluations could provide insight into the timeline of changes in the major areas of neurocognitive functioning.

Future studies could focus on long-term changes to synaptogenesis and changes in BDNF. As previously stated, BDNF contributes to the maturation of granule cells and may contribute to synaptic reorganization between mossy fibers and granule cells.

Additionally, behavior changes such as, fear reduction and behavioral strategy selection could be analyzed to determine if exposure to radiation causes behavioral changes that may not be detected by traditional water maze analysis.

Limitations

A limitation of this research is that each condition has a small number of rats (*n*=6). A larger number of rats would increase the power of the study. G*Power 3.1.5 suggests a sample size of at least 60 animals. The current study was limited to 36 animals in total. This would result in a power of approximately .87 with a large effect size. The research resulted in a small effect size reducing the power to approximately .51. The availability of radiation equipment and room forced the rats to be transported the same day that behavior testing began for the pre-training condition. This may have affected their activity level or swim speed during the first few trials. However, Figures 4 and 6 demonstrate that the rats in this study were able to learn over time and that the mossy fiber density changes occurred in the rostral pole. These findings suggests that the conditions were sufficient to produce learning and sprouting in the rats.

This study yoked swim control animals with spatial learning animals based on time. The researchers observed that the swim control animals had a tendency to float for longer periods as the study progressed. Swim control animals may have learned that swimming behavior did not result in removal from the water maze. Future research could yoke the animals based on swim distance to determine if physical activity increases mossy fiber growth.

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