A TRANSLATIONAL ANIMAL MODEL OF RADIATION-INDUCED EFFECTS ON COGNITIVE FUNCTIONS: A FEASIBILITY STUDY

A Thesis

Presented to

The Faculty of the Department of Psychology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

By

Melissa Trevino

May, 2013

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ABSTRACT

Neurocognitive late-effects are commonly reported among survivors of pediatric brain cancer. Radiation therapy has been linked to these neurocognitive deficits in attention, working memory, and processing speed. The current feasibility study was conducted in order to establish whether it is possible to model these deficits in the rodent. Using the 5-Choice Serial Reaction Time Task (5-CSRTT), prefrontal cognitive processes, which are reported to be among the most affected in the pediatric cancer population, can be assessed in the rodent. Irradiated and control animals were trained to perform the 5-CSRTT and tested at four separate time points. Irradiated animals showed significant impairments compared with control animals at 5, 7, and 12 months post-irradiation. These results show that deficits in prefrontal-mediated cognitive processes induced by radiation therapy can be successfully modeled in the rodent.

Keywords: radiation therapy, pediatric cancer, cognitive deficits, attention, 5-choice serial reaction time task

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In 2012, approximately 12,060 cases of childhood cancer will be diagnosed among children younger than 14 years of age (Siegel et al., 2012). Cancer is the second highest leading cause of death among U.S. children, with approximately 1,340 childhood cancer deaths predicted in 2012 (American Cancer Society, 2012). Historically, the prognosis of pediatric cancers has been poor with high mortality rates compounded with low 5-year relative survival rates. Today, there has been considerable improvement in both mortality rates and the 5-year relative survival rates for the majority of pediatric cancers. In 2010, the 5-year relative survival rates for all childhood cancers combined were 80% (Costa, 2010). These improvements have been attributed to advanced treatment regimens that have been created to treat cancer. Patients are now living longer but there has been increasing concern over various adverse effects arising in patients, specifically following radiation therapy.

Multimodality of Treatment

Treatment regimens for pediatric cancer use a multimodal approach. Treatment regimens include: surgery, radiation therapy, and chemotherapy, which all have been progressively evolving since their initial use as a treatment for cancer. Since the 1930s, surgery in combination with radiation therapy has been used as treatment for pediatric cancers (Costa, 2010). In the 1960s, chemotherapy was introduced as a curative measure for pediatric cancer, initially for acute childhood leukemia (Devita & Chu, 2008). Today, exact treatment protocols are dependent on several factors, such as type, stage, location of the cancer, and age of the child. However, radiation therapy remains an integral part of cancer treatments, in which approximately half of patients diagnosed with all types of cancer will be

treated with radiation therapy (Ringborg, 2003). The use of radiation therapy has been reduced or eliminated with children who are less than 5 years old due to concerns of its effects on the developing brain (Costa, 2010). These changes in treatment protocols for radiation therapy are due to the adverse effects being increasingly reported.

Classification of Effects

The effects of radiation on children treated for pediatric cancer have been investigated since the 1970s and have long been acknowledged as a primary cause of neurological complications and neurocognitive decline (Danoff et al., 1982). As reviewed by Donahue (1992), radiation effects can be grouped into three categories: acute, early, and late effects. Acute effects appear within days of treatment, early effects occur within the first six months after treatment, and late effects become apparent years after treatment. The different side effects are observed because cells and tissue are differentially affected by radiation. Radiation destroys cells and tissue by impairing their DNA and consequently when they begin to divide they die. Acute reacting tissue and cells are rapidly trying to divide and they are the first affected by radiation, which is called the alpha effect. The alpha effect is associated with damage to the skin, hair follicles, and mucous membranes. Late reacting tissues and cells do not attempt to divide for months or years after radiation. When they do divide they then begin to die; this effect is called the beta effect. The beta effect is associated with CNS necrosis, diffuse white matter degeneration, bone and teeth abnormalities, and ocular toxicity.

Neurocognitive deficits are classified as late effects and children who have been treated for pediatric cancer are at risk to develop them (Costa, 2010). These neurocognitive deficits are apparent 2 to 3 years after the start of radiation therapy and there is a further

progressive decline (Cousens et al., 1988; Mulhern, Fairclough, & Ochs, 1991; Rubenstein, Varni, & Katz, 1990; Jankovic et al., 1994; Schatz et al., 2000). Historically, IQ scores have been the standard method to evaluate cognitive impairments after treatment. There have been a number of studies reporting a significant decrease in IQ scores in association with the usage of cranial radiation (Fletcher et al., 1988; Packer et al., 1989; Mulhern et al., 1998; Kieffer-Renaux et al., 2000; Grill et al., 2004; Mulhern et al., 2004; Butler et al., 2006; Watanabe et al., 2011). Additionally, studies investigating age at time of radiation therapy have reported a decrease in IQ scores associated with younger age. Packer et al. (1989), found that children who were irradiated before the age of 7 had a decrease in IQ scores of an average of 25 points a year post-irradiation. Lowering the dose of radiation has been associated with an increase in IQ scores (Mulhern et al., 1998; Kieffer-Renaux et al., 2000; Grill et al., 2004). Decreasing craniospinal irradiation (CSI) from 35Gy to 25Gy increased full scale IQ by 10 points (Mulhern et al., 1998; Kieffer-Renaux et al., 2000).

Additional late-effects that have been commonly reported among survivors of childhood cancer include both psychological and social deficits. Survivors are more likely to be diagnosed with depression and have a higher chance of suicidal ideation (Recklitis et al., 2009). As well, survivors are less likely to attend college and get married (Gurney et al., 2009). Pang et al. (2008), reported survivors of childhood cancer are less likely to be employed and there is a higher risk of unemployment associated with survivors who had received cranial radiation therapy.

Neurocognitive Deficits in Pediatric Cancer Population

Neurocognitive deficits can be classified as either core or secondary deficits. Core deficits include impairments seen in processing speed, attention, memory, and executive functions, such as failure in planning and organizing behavior. Secondary deficits include low IQ scores and poor academic achievement, which are speculated to be the outcome of impairments in core abilities (Mulhern et al., 2001). These core and secondary deficits define the general neurocognitive sequelae that results from pediatric cancer and their treatment regimens.

A variety of deficits in attentional processes have been well-documented following radiation therapy. Lockwood et al. (1999), evaluated survivors of acute lymphoblastic leukemia (ALL) that were treated either with chemotherapy alone or cranial radiation therapy (CRT) and chemotherapy. Participants were given a standard neuropsychological test battery. Survivors who were treated with cranial radiation therapy early in childhood (<54 months) had an increase in impairments in focusing, tracking, shifting, and sustaining attention. Survivors who were treated with cranial radiation therapy later in childhood had milder impairments in shifting and sustain attention.

Butler and colleagues (1999), investigated the effects of radiation therapy on attentional skills, in children who were diagnosed with ALL who were either treated with or without CRT. Participants were given a standard neuropsychological test battery that included the Continuous Performance Test (CPT), the Brief Test of Attention, and the Wisconsin Card Sorting Test. Children treated with CRT had significantly greater deficits in vigilance attention, working memory, and cognitive flexibility.

Attentional and information processing were examined in children diagnosed with ALL by Anderson et al. (2004). Participants were either treated with chemotherapy alone or CRT and chemotherapy. The CRT and chemotherapy group showed selective and shifting deficits compared to the chemotherapy only group in attention and processing speeds for complex tasks, such as the CPT. The chemotherapy only group had generally intact attentional skills. Reeves et al. (2006), evaluated attention functioning in childhood survivors of pediatric brain tumors. Participants were survivors of pediatric medulloblastoma and all were treated with surgical resection, chemotherapy, and CRT. Participants were administered a neuropsychological battery that included the California Verbal Learning Test, Child Version (CVLT-C) and the CPT. Attention was showed to be significantly impaired, specifically selective attention.

Subsequently, deficits in attentional processes can lead to difficulties within the school, such as with learning-related processes of encoding and recalling information (Wolfe et al., 2012). Impairments in attention have also been found to predict a decline in performance in other important cognitive domains, such as reading and mathematical reasoning (Reeves et al., 2006). Similar to other neurocognitive deficits following radiation therapy, attention processes have been reported well into adulthood. Maddrey et al. (2005), investigated 10-year survivors of pediatric brain cancer and found more than half of participants were impaired with sustained attention; as well 90% had deficits in motor-based attention. In sum, attentional impairments encompass a multitude of processes that impact survivors throughout their life.

Review of Animal Literature

Further investigations on the adverse effects of radiation therapy have been conducted through the use of animal models. In the rodent literature, the majority of studies have focused exclusively on the effects of radiation on the hippocampus. Specifically, studies have investigated effects on hippocampal neurogenesis, and hippocampal-mediated cognitive functions (Fan et al., 2007; Naylor et al., 2008; Ramanan et al., 2009; Fike et al., 2009; Wong-Goodrich et al., 2010; Rao et al., 2011). Impairments in hippocampal-mediated cognitive functions due to radiation have been reported using tasks such as the Radial Arm Task, Novel Location Task, and The Morris Water Maze (Rola et al., 2004; Brown et al., 2007; Rao et al., 2011). Cognitive impairments that have been found include: spatial learning, spatial working memory, and memory retention.

Rao et al. (2011), investigated the effects of whole brain irradiation (WBI) in young male C57BL/6 mice. Animals at one month of age were given a fractionated scheme of 4Gy over five days for a total dose of 20Gy. A decrease in hippocampal dependent cognitive functions was found using a non-spatial learning task, the Novel Location Task. The Novel Location Task measures the ability for animals to tell the difference between a novel and familiar spatial location of an object. Compared to controls (n=11), irradiated animals (n=11) performed poorly on this task at 1 month and 5 months post-irradiation. Brown et al. (2007), evaluated the effects of fractionated 40Gy of WBI in adult Fischer 344 rats. Animals were irradiated twice a week for four weeks with a fractionated scheme of 5Gy for a total dose of 40Gy. Animals were assessed using the Radial Arm Maze, which is intended to measure spatial working memory in rodents. Brown et al. (2007), reported irradiated animals (n=9) made significantly more working memory errors than control animals (n=9) at 6 and 9

months post-irradiation. Rola et al. (2004), reported deficits in the hippocampal-mediated cognitive function of spatial memory using The Morris Water Maze. C57BL/J6 male mice were irradiated with a single dose of 5Gy at 21-days old and tested at 3 months of age. Irradiated animals (n=12) were found to have spatial memory retention deficits compared to control animals (n=12).

As outlined above, previous animal studies investigating the effects of radiation on cognition have almost exclusively focused on hippocampal-mediated functions. However, many neurocognitive deficits that have been reported following radiation therapy are considered to be heavily dependent upon the pre-frontal cortex. Such deficits are seen in attention, working memory, and executive functions, such as allocating attentional resources (Mulhern & Palmer, 2003; Armstrong et al., 2009). Therefore, an animal model of neurocognitive effects that are prefrontal-mediated is necessary. Robbins et al. (2011), conducted a pilot study investigating radiation and its effects on higher-order cognitive functions in non-human primates (NHP). Adult male rhesus monkeys that received WBI were evaluated using the Delayed Match to Sample Task. Animals were 6-9 years old, which are considered a young adult, were irradiated with a total dose of 40Gy with two fractions of 5Gy a week for four weeks. Cognitive tests were done 4 months prior to radiation and up to 11 months post-irradiation. Performance in low cognitive load trials was significantly lower at 7 months post-irradiation. Performance in high cognitive load trials was significantly lower at 1 month post-irradiation followed by a recovery in performance for 1-2 months then a progressive decrease in performance through 11 months post-irradiation. Cerebral glucose metabolism was also measured using blood samples and PET scans. Scans were taken when the animals performed the Delayed Match to Sample Task and when they were not

performing the task. Scans were taken one week prior to radiation and nine months post-irradiation. Scans taken at the nine month post-irradiation indicated a reduction in glucose uptake during the task in the cuneate and prefrontal cortex and an increase in the cerebellum and thalamus when compared to the one week prior to radiation scans. These results suggest irradiation-induced changes in cognition and brain metabolism. This pilot for a NHP model of irradiation-induced deficits in higher-order cognitive function indicates that not only hippocampal-mediated functions can be investigated in an animal model. However, the present pilot does not investigate deficits in a young sample that received radiation therapy and thus does not help elucidate cognitive deficits reported in survivors of pediatric cancer. NHP research is also particularly expensive compared to rodent studies. Thus, a rodent model of higher-order cognitive deficits is needed.

5-Choice Serial Reaction Time Task

Up until now, there have been no investigations into radiation-induced prefrontal impairments in the rodent. With the use of the 5-Choice Serial Reaction Time Task (5-CSRTT) our aim is to measure prefrontal cognitive processes in the rodent. The 5-CSRTT is traditionally utilized in the rodent drug addiction literature in order to assess different neurocognitive processes. Neurocognitive processes assessed by the 5-CSRTT are visual attention, including sustained, divided, and selective attention. As well, processing speed and impulsivity can be assessed. The task obtains different measures based on the various processes through latency times, accuracies, and amount of different errors made during the task. Using the 5-CSRTT, neurocognitive processes that have been reported to be among the most affected in the pediatric cancer population can be assessed in the rodent. An animal model of pediatric radiation allows for further intricate and precise investigations in cognitive

deficits to improve empirical knowledge and lay the foundation to help elucidate these neuropsychological vulnerabilities.

The 5-CSRTT has been utilized in a variety of different animal models to assess attentional processes. Hahn et al. (2002), investigated the effects of nicotine on attention using the 5-CSRTT. Specifically, nicotine increased accuracy, decreased errors (omission errors), and decreased response latencies. As well, nicotine was found to affect one of the measures of 5-CSRTT, selection attention. Nicotine contributed to animals focusing attention on appropriate stimuli compared to irrelevant stimuli, such as noise distractions. This investigation illustrated that the task was sensitive to detect attention-enhancing effects of nicotine on certain 5-CSRTT measures. Dalley et al. (2007), utilized the 5-CSRTT to investigate impulsivity in rodents and whether high impulsivity predicted the use of intravenous cocaine self-administration. The 5-CSRTT was used to characterize animals either as high-impulsive or low-impulsive. One of the measures of the task is determining impulsivity based on the number of premature responses made. High-impulsive rats compared to low-impulsive rats showed a significant increase in cocaine intake and tendency to develop compulsive cocaine self-administration.

A feasibility study was conducted that utilized the 5-CSRTT to assess radiation therapy effects on neurocognitive processes in rodents. Rodents have an accelerated aging process compared to humans, and are therefore fundamental in investigating effects that are deemed long-term in humans. It will be the first time the 5-CSRTT will be used to assess radiation induced cognitive impairments. In this feasibility study, we used the 5-CSRTT to assess impulsivity, selective, and sustained attention following radiation.

Methods

Subjects

Six male Wistar rats (Harlan Laboratories Inc., Indianapolis, IN) served as subjects. Subjects were housed individually. They were kept in an environmentally-controlled room with temperature kept at approximately 21˚C and relative humidity at 60%. Animals were under a light-dark cycle (light from 6:00 a.m. – 6 p.m.). Diet was controlled to maintain the subjects at approximately 90% of their free-feeding weight. Animals were placed on a restricted diet in order to be motivated to participate in the 5-CSRTT. Water was available *ad libitum* except in the 5-CSRTT operant chamber. All animal handling, experiments, and other care were in accordance with the National Institutes of Health (NIH) Public Health Service Policy on Humane Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee at Baylor College of Medicine. Male rats were chosen because the incidence of tumors is reportedly higher in males than females, specifically in pediatric medulloblastomas (Palmer, 2007). Wistar rats were chosen in order to extend current 5-CSRTT research to future investigations involving a pediatric tumor model.

Procedure

Animals were received at 6-8 weeks of age (n=6). Young animals were chosen in order to better generalize to the age at diagnosis reported in the pediatric cancer population. Pediatric medulloblastomas are generally diagnosed up until the age of 10 and with peak incidence at 5 years of age (Gottardo & Gajjar, 2006). Three of the animals received partial-brain irradiation, focusing on the pre-frontal cortex. A lead shield was used to protect the

eyes of the animals. The rats received a total dose of 20Gy given as 4Gy fractions for 5 days starting one day after arrival. Following the five days of irradiation, animals were given a week for recovery. After recovery, animals were handled for 5 minutes each for three days and then diet restriction began. The diet of all animals was restricted in order to maintain animals at 90% of their free-feeding weight. Animals were weighed each day throughout the entire training session and during the four test sessions. After the third day of handling and diet restriction, animals began the habituation process to the 5-CSRTT operant chamber. Animals completed the habituation process at different days, ranging from 1 day to 3 days, with the majority of animals completing the habituation process in 1 day. One control and one irradiated animal took two days to complete the habituation process. Approximately 2 weeks post-irradiation, after completing the habituation process, animals began training in the 5-CSRTT (See Appendix A & B). Ethovision software malfunctioned for four days and animals were unable to train on those days. Training lasted a total of 73 daily training sessions and was stopped due to animals not achieving target criteria. Target criteria were for animals to successfully complete all training stages within the appropriate amount of daily sessions (30-35 daily sessions). Target criteria were based on previous 5-CSRTT publications (Hahn et al., 2002; Dalley et al., 2007; Bari et al., 2008). Animals failed to attain criteria, thus modifications were implemented (i.e. stopping training after 73 training sessions).

Baseline data was collected for all 6 animals. The last six days of training were used as baseline data, which was 4 months post-irradiation. Two irradiated and all three controls reached stage 12 for baseline collection. One irradiated reached stage 11 for baseline collection. Animals were tested in the 5-CSRTT at four later time points. All four testing periods were done over 6 days. Test 1 was 5 months post-irradiation. A total of 31 days

elapsed between Test 1 and Test 2. Test 2 was 7 months post-irradiation. A total of 31 days elapsed between Test 2 and Test 3. Test 3 was 8 months post-irradiation. A total of 103 days elapsed between Test 3 and Test 4. Test 4 was approximately one year post-irradiation.

Apparatus. Neurocognitive functioning of animals were assessed using the 5-CSRTT (Med-Associates Inc., St. Albans, VT, USA). The version of 5-CSRTT that was used is specifically adapted for rats (25x25x25cm) (See Figure 1). Computer software to program the 5-CSRTT was purchased from Noldus Information Technology, Leesburg, VA. The 5-CSRTT's programming was done at our laboratory. The operant chamber is composed of 4 walls. Two walls that face opposite of each other are composed of aluminum. One of the aluminum walls of the operant chamber is slightly curved and contains five square shaped apertures that each hold one LED light and have IR beams within each aperture that are being projected across the aperture. The second aluminum wall of the chamber that is not curved houses the food magazine. The food magazine is square shaped and contains IR beams that are being projected across the food magazine. The food magazine is where a reward pellet (45mg) will be kept when it is released from a pellet dispenser that is located outside of the chamber. The pellet dispenser is connected to the food magazine through a clear plastic cylinder tube. The 5-CSRTT is housed within a larger chamber to attenuate outside sound. The two sidewalls are composed of clear polycarbonate. One of the clear polycarbonate walls is attached to the operant chamber by hinges and is used as the door of the 5-CSRTT. The floor of the chamber is composed of a metal grid and beneath the entire metal grid is a plastic tray that is removable. The roof of the camber is composed of clear polycarbonate.

Neural Substrates

One year post-irradiation, underlying impairments in the prefrontal cortex and hippocampus were investigated among all animals. Specific areas investigated were the dorsomedial prefrontal cortex (dmPFC) and the dentate gyrus (DG) of the hippocampus. The prefrontal cortex has long been associated with cognitive processes that include: attention, working memory, and inhibitory control (Diamond, 1988; Levy & Goldman-Rakic, 1999; Fuster, 2001). The rodent medial prefrontal cortex (mPFC) is comparable to the primate dorsolateral prefrontal cortex, as well has been associated with working memory, planning, and problem solving (Gabbott et al., 2005; Seamans et al., 2008). The dmPFC has been indicated with spatial working memory, the ability to inhibit inappropriate responses, attentional processes, and decision-making (Small et al., 2003; Rodgers et al., 2004; Narayanan et al., 2006; Horst et al., 2009). The DG of the hippocampus is one site in the adult brain where neurogensis occurs (Zhao et al., 2008). Neurogensis is essential for cognitive processes such as learning and long-term spatial memory (Deng et al., 2009; Shors et al., 2012). Both the dmPFC and DG of the hippocampus were examined in all animals.

BrdU Administration. After the completion of testing 4, Bromodeoxyuridine (BrdU) was injected in all animals to detect proliferating progenitor cells in the dmPFC and the DG of the hippocampus. Animals were given a single injection two hours prior to perfusion.

Histological Procedures. Each animal was perfused 2 hours after BrdU injection. They were lethally anesthetized and transcardially perfused with cold saline, followed by 4% paraformaldehyde until the upper body was stiff. Brains were removed from the skull and were post-fixed for 24 hours, and then refrigerated in 30% sucrose. Brains were cut in 50-µm

coronal sections on a microtome from a random start point at the level of the prefrontal cortex through the hippocampus. Sections were stored in cryoprotectant in 24-well microtiter plates at -20 ˚C.

Immunohistochemistry for BrdU. For BrdU detection, sections from each animal were processed separately. Every sixth serial section was rinsed with 0.1 M tris-buffered saline (TBS) three times at room temperature for 10 minutes each. Sections were then quenched for 30 minutes at room temperature in 0.6% hydrogen peroxide followed by three 10 minute washes in TBS. Sections were then pretreated for 2 hours at 65˚C in 50% formamide and then rinsed twice for 15 minutes each at room temperature in 2X SSC. Sections were incubated in 2N HCl at 37°C for 30 minutes each. Next, sections were washed in 0.1 M borate buffer for 10 minutes and then rinsed six times for 10 minutes each in TBS. These sections were then be blocked for 60 min in 3% normal donkey serum (Sigma-Aldrich, St. Louis, MO), followed by incubation at 4°C for 72 hours in primary antibody (sheep anti-BrdU, Exalpha Biologicals, Watertown, MA; 1:400). After two TBS rinses for 15 minutes each and 15 minutes blocking in 3% normal donkey serum, sections were incubated overnight at room temperature in secondary antibody (biotinylated donkey anti-sheep, Jackson ImmunoResearch Laboratories, West Grove, PA; 1:250). Next, sections were rinsed three times in TBS for 10 minutes each, then treated for 90 minutes in avidin-biotin complex (ABC, Vector Labs, Burlingame, CA) and then rinsed three times in TBS for 10 minutes each. Sections were reacted and visualized with diaminobenzidine (DAB, Vector Labs, Burlingame, CA) and then rinsed four times in TBS for 10 minutes each, before being mounted onto gelatinized slides and allowed to dry overnight. Once dried, all brain sections were counterstained with cresyl violet, cleared in xylene and coverslipped using Protexx. All

slides were coded so that when the tissue was viewed under the microscope, the investigator was blind to the experimental condition.

Immunohistochemistry for Doublecortin (DCX). Doublecortin (DCX) was used as a marker of migrating neuroblasts. Doublecortin-labeled cells allowed for quantification of new neurons that are generated in the DG of the hippocampus. For DCX detection, sections from each animal were processed separately. Every sixth serial section was rinsed with 0.1 M tris-buffered saline (TBS) three times at room temperature for 10 minutes each. Sections were then quenched for 30 minutes at room temperature in 0.6% hydrogen peroxide followed by three10 minute washes in TBS. These sections were then be blocked for 60 min in 3% normal donkey serum (Sigma-Aldrich), followed by incubation at 4°C for 72 hours in primary antibody (goat anti-DCX, Santa Cruz Biotechnology Inc., Santa Cruz, CA; 1:100). After two TBS rinses for 15 minutes each and 15 minutes blocking in 3% normal donkey serum, sections were incubated overnight at room temperature in secondary antibody (biotinylated donkey anti-goat, Jackson ImmunoResearch Laboratories, West Grove, PA; 1:250). Next, sections were rinsed three times in TBS for 10 minutes each, then treated for 90 minutes in avidin-biotin complex (ABC, Vector Labs, Burlingame, CA) and then rinsed three times in TBS for 10 minutes each. Sections were reacted and visualized with diaminobenzidine (DAB, Vector Labs, Burlingame, CA) and then rinsed four times in TBS for 10 minutes each, before being mounted onto gelatinized slides and allowed to dry overnight. Once dried, all brain sections were counterstained with cresyl violet, cleared in xylene and coverslipped using Protexx. All slides were coded so that when the tissue was viewed under the microscope, the investigator was blind to the experimental condition.

Quantification. Estimating the total population of cells in the dmPFC and DG of the hippocampus using stereology was not possible due to the limited number of cells within all animals. In control animals, there were too few cells to count due to the animals' advanced age. It has been reported that aging decreases neuronal proliferation, as well as neurogenesis in rodents (Kuhn et al., 1996). As well, the low cell counts of irradiated animals could be attributed to an age-related decrease, as well as from the radiation. Software-assisted cell counting was performed (StereoInvestigator, MicroBrightField, Williston, VT) on a Nikon Eclipse 80i upright microscope. The software was used in order to delineate areas (dmPFC and DG) in the brain and subsequently quantify the total number of proliferating progenitor cells (BrdU+ cells) as well as the number of new neurons generated in the DG of the hippocampus. The region of interest was traced, using the 10× objective, and cells were counted within two-dimensional counting frames using a 100× oil-immersion objective for DCX+ cells and a 40× oil-immersion objective for BrdU+ cells. Based on the average mounted section thickness, (approximately 37 µm), top and bottom guard zones were set at 5 µm each in order to eliminate problems of uneven section surface and cell stripping.

For the dmPFC, the subregions of the anterior cingulate cortex (Cg), prelimbic cortex (PrL), and infralimbic cortex (IL) were counted for total number of BrdU+ cells in bregma regions 4.20–2.10 (Kodama et al, 2004; Stewart & Plenz, 2006; George et al., 2008). The hippocampal subregion of the DG was quantified for BrdU+ cells and DCX+ cells in bregma regions -1.80– -6.30. As well, dorsal and ventral regions of the DG were quantified for the total number of BrdU+ cells and DCX+ cells in bregma regions -1.88– -4.30 for dorsal and in bregma regions -4.52– -6.04 for ventral regions (Wolf et al., 2002). The counting frame for the dmPFC and the DG of the hippocampus was 60 x 60 µm and the grid size was 60 x 60

μm.

Statistical Analysis

Behavioral measures of accuracy, correct responses, wrong responses, premature responses, omissions, perseverations made in a correct aperture, perseverations made in a wrong aperture, perseverations made in multiple apertures, the latency in response to a correct aperture, and the latency in response to a wrong aperture were analyzed. Accuracy was calculated as the number of correct responses divided by the total number of correct and incorrect responses expressed as a percentage. Correct responses were classified as nose-pokes made in the aperture where the light stimulus was presented. Wrong responses were nose-pokes made in all apertures other than the one where the light flashed. Premature responses were nose-pokes made before the light stimulus in any of the apertures. Omissions occurred when the animal did not nose-poke in any apertures during the trial. Perseverations made in a correct aperture were defined as a nose-poke made more than once in the correct aperture. Perseverations made in a wrong aperture were defined as a nose-poked made more than once in one wrong aperture. Perseverations made in multiple apertures were nose-pokes made more than once in two or more wrong apertures. Latency to a correct response was the time it took an animal to nose-poke in a correct aperture after the stimulus light was presented. Latency to a wrong response was the time it took an animal to nose-poke in a wrong aperture after the stimulus light was presented. Behavioral measures were analyzed using a two-way mix repeated measures analysis of variance (ANOVA), with group (irradiated, control) as the between-subject factor and time point (baseline, test 1, test 2, test 3, test 4) as a repeated measure factor. Post hoc comparisons were conducted after a significant interaction or significant main effect of group. Simple effects investigated only

the between-subject factor of groups in order to see if there was a difference between groups at each time-point. Simple effects on the within-subject factor of time were not conducted. Post hoc comparisons were conducted with Bonferroni adjustments.

Independent *t*-tests were used to analyze the total number of BrdU+ cells and DCX+ cells in the DG of the hippocampus, as well as BrdU+ cells in the dmPFC between groups. To analyze differences between groups in the number of BrdU+ cells and DXC+ cells among the regions of the hippocampus, a factorial ANOVA was used with group (irradiated, control) as the between-subjects factor and region (dorsal, ventral) as the within-subjects factor. Post hoc comparisons were conducted using an independent *t*-test.

A factorial ANOVA, was used to compare the number of days spent on each training stage between irradiated and control groups, with group (irradiated, control) as the between-subject factor and stages as the within-subjects factor. Additionally, looking at each group individually, a factorial ANOVA was used within the irradiated group with stages (habituation to apparatus and stages 1-12) as the within-subjects factor. Post hoc comparisons between stages were conducted using an independent *t*-test. A factorial ANOVA was used within the control group with stages (habituation to apparatus and stages 1-12) as the within-subjects factor. Post hoc comparisons between stages were conducted using an independent *t*-test.

A two-way MANOVA was conducted to investigate the effect of group membership and its influence on accuracy performance on the overall twelve training stages and on the overall five testing time-points. As well, the observed power was computed. The level of significance for all analyses was set at $p<0.05$. Results were analyzed with SPSS Statistics

20.0 (IBM SPSS Statistics, IBM, Chicago, IL).

Results

Irradiation-Induced Behavioral Changes

Average accuracy had a non-significant main effect of group ($F[1,4]=7.515$, $P=.052$), a significant main effect of time point ($F[4,16]=5.514$, $P<0.05$), and a significant time point by group interaction ($F[4,16]=4.856$, $P<0.05$). Post hoc comparisons demonstrated that there was not a difference between irradiated animals and control animals at baseline, ($F[1,4]=.220$, $P>0.05$); a significant difference at time point one, ($F[1,4]=11.939$, $P<0.05$); a significant difference at time point two, ($F[1,4]=13.269$, $P<0.05$); no difference at time point three, ($F[1,4]=.008$, $P>0.05$); and no difference at time point four, ($F[1,4]=2.624$, $P>0.05$) (See Figure 2).

For correct responses, there was not a significant main effect of group ($F[1,4]=1.488$, $P>0.05$). There was a significant main effect of time point ($F[4,16]=24.980$, $P<0.05$) and time point by group interaction ($F[4,16]=4.913$, $P<0.05$). Post hoc comparisons demonstrated that there was no difference between irradiated animals and control animals at baseline, ($F[1,4]=.22$, $P>0.05$); at time point one, ($F[1,4]=4.615$, $P>0.05$); at time point two, ($F[1,4]=1.674$, $P>0.05$); at time point three, ($F[1,4]=.059$, $P>0.05$); and no difference at time point four, ($F[1,4]=3.98$, $P>0.05$)

For wrong responses, there was no significant main effect of group ($F[1,4]=.677$, $P>0.05$), or time ($F[4,16]=.937$, $P>0.05$), and the time point by group interaction ($F[4,16]=2.447$ $P>0.05$) was not significant. For omission responses, there was no significant main effect of group ($F[1,4]=.299$, $P>0.05$). There was a significant main effect of time point

(F[4,16]=5.514, $P<0.05$). The time point by group interaction (F[4,16]=1.275, $P>0.05$) was not significant.

For premature responses, there was no significant main effect of group (F[1,4]=.037, $P>0.05$) or time (F[4,16]=.763, $P>0.05$). There was a significant time point by group interaction (F[4,16]=3.373, $P<0.05$). Post hoc comparisons demonstrated that there was no difference between irradiated animals and control animals at baseline, (F[1,4]=2.124, P>0.05); there was a significant difference at time point one, (F[1,4]=13.778, P<0.05); no difference at time point two, (F[1,4]=.617, P>0.05); there was a significant difference at time point three, (F[1,4]=10.350, P<0.05); no difference at time point four, (F[1,4]=.218, P>0.05).

For perseverative responses made in a correct aperture, there was no significant main effect of group (F[1,4]=2.324, $P>0.05$), or time (F[4,16]=2.112, $P>0.05$), and the time point by group interaction (F[4,16]=.874 $P>0.05$) was not significant. Similarly, for perseverative responses made in a single wrong aperture, there was no significant main effect of group (F[1,4]=1.483, $P>0.05$), time (F[4,16]=1.119, $P>0.05$), and the time point by group interaction (F[4,16]=.786 $P>0.05$) was not significant.

For perseverative responses made in apertures other than the correct aperture and other than a single wrong aperture, there was no significant main effect of group (F[1,4]=.014, $P>0.05$) and time (F[4,16]=2.299, $P>0.05$). There was a significant time point by group interaction (F[4,16]=3.218, $P<0.05$). Post hoc comparisons demonstrated no difference between irradiated animals and control animals at baseline, (F[1,4]=3.286, P>0.05); no difference at time point one, (F[1,4]=1.460, P>0.05); no difference at time point two, (F[1,4]=3.255, P>0.05); no difference at time point three, (F[1,4]=3.114, P>0.05); no difference at time point four, (F[1,4]=.483, P>0.05).

For latency to a wrong aperture, there was no significant main effect of group ($F[1,4]=.307$, $P>0.05$). There was a significant main effect of time point ($F[4,16]=3.538$, $P<0.05$). Also, there was no significant time point by group interaction ($F[4,16]=1.337$, $P>0.05$). For latency to a correct aperture, there was no significant main effect of group ($F[1,4]=.932$, $P>0.05$). There was a significant main effect of time point ($F[4,16]=3.183$, $P<0.05$). Also, there was no significant time point by group interaction ($F[4,16]=.779$, $P>0.05$).

Irradiation-Induced Effects on the Hippocampus & Prefrontal Cortex

Comparing the total number of DCX+ cells in the DG of the hippocampus among groups showed that the control animals had a higher number of DCX+ cells (M=88, SD=20) than irradiated animals (M=16, SD=18). This difference was statistically significant, $t(4)=4.547$, $P<0.05$), (See Figure 3). The total number of BrdU+ cells in DG of the hippocampus among groups found that the control animals had a higher number of BrdU+ cells (M=20, SD=1.7) than irradiated animals (M=7, SD=7). This difference was statistically significant, $t(2.244)=3.122$, $P<0.05$) (See Figure 4). The total number of BrdU+ cells in the dmPFC of irradiated and control animals showed that on average, control animals had a higher number of BrdU+ cells (M=9.3, SD=4) than irradiated animals (M=1.3, SD=1.5). This difference was statistically significant, $t(4)=3.207$, $P<0.05$) (See Figure 5).

It was also determined whether there was a difference in the number of BrdU+ cells between the dorsal and ventral regions of the DG. The main effect of group was not significant ($F[1,4]=5.765$, $P>0.05$). There was a significant main effect of region ($F[1,4]=25$, $P<0.05$) and region by group interaction ($F[1,4]=12.250$, $P<0.05$). Post hoc comparisons

demonstrated that control animals and irradiated animals did not significantly differ in the number of BrdU+ cells in the granule cell layer of the ventral hippocampus t(4)=1.200, $P>0.05$), while there was a significant difference between groups in the dorsal subregion t(4)=3.240, $P<0.05$) (See Figure 6).

Examining a possible difference in the number of DCX+ cells among the dorsal and ventral regions of the DG yielded a significant main effect of group (F[1,4]=9.424, $P<0.05$). There was not a significant main effect of region (F[1,4]=.662, $P>0.05$) or region by group interaction (F[1,4]=1.231, $P>0.05$). Post hoc comparisons demonstrated that control animals had significantly more DCX+ cells in the granule cell layer of the ventral hippocampus then did irradiated animals t(4)=2.180, $P<0.05$). In addition, there was a significant difference between groups in the dorsal subregion (t(4)=3.559, $P<0.05$) (See Figure 7).

5-CSRTT: Stages

Examination of the number of days at each stage between groups showed a significant main effect of stages (F[12,48]=6.396, $P<0.05$). There was not a significant main effect of group (F[1,4]=.500, $P>0.05$) (See Figure 8) and the stages by group interaction was not significant (F[12,48]=.463, $P>0.05$).

Investigating only the irradiated group within each of the twelve stages we find a significant main effect of stages (F[12,24]=2.737, $P<0.05$). Post hoc comparisons demonstrated that there was no difference in the number of days between habitation to the apparatus stage and stage 1 (t(4)=.603, $P>0.05$); stage 1 and stage 2 (t(4)=1.061, $P>0.05$); stage 2 and stage 3 (t(2.)=1, $P>0.05$); stage 3 and stage 4 (t(4)=1.606, $P>0.05$); stage 4 and stage 5 (t(4)=.424, $P>0.05$). There was a significant difference between stage 5 and stage 6

(t(4)=4.070, P<0.05). No difference between stage 6 and stage 7 (t(2.160)=1.035, P>0.05); stage 7 and stage 8 (t(4)=.533, P>0.05); stage 8 and stage 9 (t(4)=.000, P>0.05); stage 9 and stage 10 (t(4)=.557, P>0.05). There was a significant difference between stage 10 and stage 11 (t(4)=3.149, P<0.05). No difference between stage 11 and stage 12 (t(4)=.088, P>0.05) (See Figure 9).

Looking at only the control group at each of the twelve stages there was a significant main effect of stages (F[12,24]=4.215, P<0.05). Post hoc comparisons demonstrated that there was no difference in the number of days between habitation to the apparatus and stage 1 (t(4)=2.00, P>0.05). There was a significant difference between stage 1 and stage 2 (t(4)=.000, P<0.05). No difference between stage 2 and stage 3 (t(4)=1.512, P>0.05); stage 3 and stage 4 (t(4)=.224, P>0.05); stage 4 and stage 5 (t(4)=.200, P>0.05). There was a significant difference between stage 5 and stage 6 (t(4)=2.846, P<0.05). No difference between stage 6 and stage 7 (t(4)=2.429, P>0.05); stage 7 and stage 8 (t(4)=.171, P>0.05); stage 8 and stage 9 (t(4)=.895, P>0.05); stage 9 and stage 10 (t(4)=.676, P>0.05); stage 10 and stage 11 (t(4)=1.134, P>0.05); stage 11 and stage 12 (t(4)=.524, P>0.05) (Figure 10).

In order to compare group membership and its influence on accuracy performance across the twelve training stages and across the five testing time-points a two-way MANOVA revealed a non-significant multivariate main effect for group membership, Wilks' $\lambda = .102$, F (4, 1) = 2.201, P >. 05. Observed power was .092. Thus, there was not enough power to detect an effect.

Discussion

Prefrontal-mediated cognitive deficits are commonly reported among survivors of pediatric cancer. Although the use of radiation therapy results in improved survival rates, it has also been a contributing factor in these emerging deficits (Crossen et al., 1994; Ris et al., 2001; Costa, 2010). The objective of the present study was to determine whether it is feasible to detect prefrontal-mediated cognitive deficits after irradiation in rats. Previous research has examined deficits post-irradiation in hippocampal-mediated cognitive functions (Rola et al., 2004; Brown et al., 2007; Rao et al., 2011). The current study introduced the use of the 5-CSRTT to measure attentional processing and impulsivity post-irradiation, cognitive processes that are prefrontal-mediated.

Accuracy Performance in 5-CSRTT

Irradiated and control animals were trained to perform the task and tested at four different time points. Out of the ten behavioral results obtained from the task, average accuracy yielded the most valuable information regarding irradiation and its effects on performance. Baseline performance was taken after the completion of training, 4 months post-irradiation. At baseline, there was not a significant difference in accuracy between the groups. At Test 1 (five months post-irradiation) and at Test 2 (7 months post-irradiation), the control group had significantly higher accuracy than the irradiated group. For Test 3 (8 months post-irradiation) and Test 4 (one year post-irradiation) there was no difference in accuracy between the groups (See Figure 2). The results indicate attentional deficits induced by irradiation can be found in the rat.

Accuracies across the different time-points are similar with the outcomes obtained from Robbins and colleagues (2011) pilot study with non-human primates. They investigated

radiation and its effects on higher-order cognitive functions and found performance on demanding cognitive trials of the Delayed Match to Sample Task was significantly lower at 1 month post-irradiation followed by a recovery in performance for 1-2 months then a progressive decrease in performance through 11 months. They did not attribute or make further reference to the recovery in performance after the initial decrease. However, in the current feasibility study, not finding a significant difference between groups at Test 3 could be attributed to practice effects due to the limited number of days between test points. There were 31 days between Test 1 and Test 2, as well as between Test 2 and Test 3. Testing at close intervals may have influenced performance and subsequently improved the performance of irradiated animals.

Studies show when survivors of pediatric cancer are placed in intensive cognitive remediation programs they are able to acquire effective learning techniques in order to cope with attentional and working memory deficits (Costa, 2010; Butler & Mulhern, 2005). Our findings suggest giving irradiated animals the opportunity to further practice in the task improves performance, instead of demonstrating a progressive decline when no such opportunity is given. Poor performance at Test 1 & Test 2 illustrates behavioral impairments after irradiation. Improved performance at Test 3 and Test 4 may be attributed to subsequent training. However, when a longer interval between testing days was implemented, as between Test 3 and Test 4 of 103 days, irradiated performance began to drop, though not significantly.

Neural Substrates 1-Year Post-Irradiation

One year post-irradiation animals had significantly fewer proliferating progenitor cells in dentate gyrus (DG) of the hippocampus, as well as the dorsomedial prefrontal cortex

(dmPFC). These findings are in line with previous evidence indicating impairment of hippocampal neurogenesis in the rat. Specifically, it has been reported precursor cells in the dentate are radiosensitive and undergo apoptosis immediately after treatment (Monje et al., 2002; Naylor et al., 2008; Fike et al. 2009). Compared to previous studies, which irradiated the whole brain, the present feasibility study irradiated only the prefrontal cortex. As well, unlike previous investigations, the current study investigated both the hippocampus and prefrontal cortex.

Irradiated animals had significantly fewer migrating neuroblasts compared to controls in the DG of the hippocampus. This finding is similar to a previous report showing a decrease in neuronal proliferation in the adult rat following radiation (Madsen et al., 2003). However, radiation was focused only on the hippocampal formation compared to the prefrontal cortex of the current study. A decrease in both proliferating progenitor cells and migrating neuroblasts is evidence of long-term neural dysfunction induced by irradiation. This lack of plasticity after irradiation may be linked indirectly to poor performances on the 5-CSRTT, as well to irradiated animals not completing all training stages.

Within the hippocampus, the dorsal and ventral subregions have been reported to be functionally dissociable. The dorsal subregion is associated with spatial working and spatial reference memory (Bannerman et al., 1999, 2002; Richmond et al., 1999; Pothuizen et al., 2004). The ventral subregion is most notably associated with and affected by anxiety (Richmond et al., 1999; Bannerman et al., 2003). The current study found control animals had significantly more proliferating precursor cells in the dorsal subregion and more migrating neuroblasts in both ventral and dorsal subregions of the hippocampus. Significantly, more new neurons and proliferating progenitor cells in control animals might

indirectly explain why they had stable performances across all time points in the 5-CSRTT.

Training Stages of the 5-CSRTT

Analysis among the different 5-CSRTT training stages indicated control and irradiated groups did not differ in the number of days spent on each stage. However, with irradiated animals there was a significant difference in the number of days on stage 5 compared to stage 6, as well as between stages 10 and 11. For control animals, there was a significant difference between stage 1 and 2, as well as stage 5 and 6. These findings indicate that for both groups advancing to stage 6 was difficult, which may be due to the stimulus duration being reduced from 2.5 to 1.20 seconds.

Investigating whether group membership had an effect on accuracy performance across training stages and across the five time-points resulted in non-significant effects. Not being able to find an effect can be attributed to a small sample size (n=6) and consequently low power. Subsequently, low power indicated that the use of further time series analysis on accuracy performance would be incapable in detecting differences between groups.

Limitations and Future Directions

The current feasibility study allowed us to identify limitations and possible solutions in order to improve future investigations. The use of Wistar rats is one limitation because they are an albino strain. It has been reported albino rat strains generally do poorly on the task (Dalley et al, 2007) compared to non-albino strains, such as the Lister hooded rat. The Lister hooded strain has been known to acquire the task in a substantially less time (Bari et al., 2008). Using the Lister hooded strain and thus acquiring the task faster may lessen the influence of extraneous factors that occurred in the present study, malocclusion and accelerated growth of the front teeth (Nager, 2001). Another limitation was the use of target

criteria that were extremely difficult for the Wistar strain. Target criteria were not adjusted, however future studies can manipulate the various time intervals used in the 5-CSRTT and subsequently reduce the number of training days. For example, changing the stimulus duration to 1 second instead of the present study's duration of .05 second can dramatically reduce training days. Additionally, the n used in the current preliminary feasibility study was not sufficient to detect subtle effects.

Future investigations can also incorporate recent research that has indicated white matter dysfunction among survivors of pediatric cancer, especially among the frontal lobes (Wolfe et al., 2012). Research investigations using a rodent model can incorporate the use of diffusion tensor imaging (DTI) to examine the integrity of white matter tracts and the interconnections among various regions after radiation therapy. As well, in order to closely mimic radiation protocols of pediatric cancer patients, the radiation scheme should incorporate whole-brain radiation with an additional boost of radiation to the posterior fossa to replicate treatment protocols for medulloblastomas, the most common diagnosed brain tumor among children (Crawford et al., 2007).

Conclusions

The current feasibility study has a laid a solid foundation for future investigations. It demonstrates that a rodent model of radiation-induced frontal lobe dysfunction is feasible and can accurately model cognitive deficits reported among survivors of pediatric cancer. Using the 5-CSRTT, the prefrontal-mediated processes of attention and impulsivity were examined post-irradiation. Attentional processes were found to be severely impaired. Additionally, the results show that the prefrontal and hippocampus have enduring deficits in cell genesis

following irradiation. In summary, this feasibility study yielded valuable information to aid future rodent studies of cognitive late-effects in patients treated for pediatric cancer.

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Appendix A

5-CSRTT Training Stages & Criteria

Training Stage	Stimulus Duration (s)	ITI (s)	LH (s)	Criterion to move to next stage
1	30	2	30	≥30 Correct trials
2	20	2	20	≥30 Correct trials
3	10	5	10	≥50 Correct trials
4	5	5	5	≥50 Correct trials > 80% Accuracy
5	2.5	5	5	≥50 Correct trials > 80% Accuracy < 20% Omissions
6	1.2	5	5	≥50 Correct trials > 80% Accuracy < 20% Omissions
7	1	5	5	≥50 Correct trials > 80% Accuracy < 20% Omissions
8	0.9	5	5	≥50 Correct trials > 80% Accuracy < 20% Omissions
9	0.8	5	5	≥50 Correct trials > 80% Accuracy < 20% Omissions
10	0.7	5	5	≥50 Correct trials > 80% Accuracy < 20% Omissions
11	0.6	5	5	≥50 Correct trials > 80% Accuracy < 20% Omissions
12 (Target Parameters)	**0.5**	**5**	**5**	**≥50 Correct trials > 80% Accuracy < 20% Omissions**

Appendix B

Procedure for 5-CSRTT

Each animal needs to be trained in order to perform in the 5-CSRTT. The animal goes through 12 stages that increase in difficulty throughout the stages in order to achieve and perform at the target parameters, which are set at stage 12. Target parameters include a limited hold of 5 seconds, stimulus light presenting for .5 second, and a limited hold of 5 seconds. These target parameters are set at these specific values because they are attainable parameters in which to yield measures that can indicate deficits when the animal can no longer perform the 5-CSRTT.

Each session at target criteria, stage 12, begins when the animal is placed in the 5-CSRTT. At the start of the session the houselight is turned on. A "free" reward pellet is dispensed in the food magazine. This "free" reward pellet is only dispensed at the beginning of each session not the beginning of each trial. Once, the animal retrieves the reward pellet then Ethovision begins counting each trial that is completed as well as timing how long the session is taking. One session lasts either 100 trials or 30 minutes, whichever condition comes first. After the animal retrieves the food pellet then the Inter-Trial-Interval (ITI) period begins that lasts 5 seconds. ITI is a period of time that the animal has to wait from responding until the stimulus light turns on. Thus, the animal has to hold from nose poking in any of the five apertures to successfully complete the ITI. If the animal nose-pokes in any of the five apertures during the ITI period then he will follow the first trajectory of behavior, which is labeled as a premature response. If the animal prematurely responds then a time-out period is presented. The time-out period is defined as a period that the animal will associate as resulting from an inappropriate response. A time-out period last 5 seconds and is characterized by the house-light turning off during the 5 seconds. If

the ITI period is completed successfully then one of the five stimulus lights that are located within the five apertures is turned on. The presentation of the stimulus lights is chosen pseudorandomly. The selection of stimulus lights is pseudorandom because there are 100 trials for each session and each of the five lights will be turned on for 20 times each. The stimulus light is turned on for .5 second. Once, the stimulus light is turned on the animal has only a short period of time to nose-poke in the correct aperture. The period of time the animal has to respond is called the Limited Hold. The target limited hold lasts for 5 seconds. After the stimulus light is presented the animal has three additional trajectories that he can follow. First, the animal can correctly nose-poke in the aperture where the stimulus light was presented and within the limited hold period, this is labeled as a correct response. If the animal correctly response then automatically a reward pellet is dispensed from the pellet dispenser into the food magazine. Second, the animal can nose-poke in an aperture where the stimulus light was not presented, this is labeled as a wrong response. If the animal gives a wrong response then a time-out period is presented. The time-out period last 5 seconds and is characterized by the house-light turning off for 5 seconds. Third, the animal does not nose-poke in any of the five apertures and the limited hold period expires, this response is labeled as an omission. If the animal response in an omission then a time-out period is presented. After each of the four trajectories, following a premature response, correct response, wrong response, and an omission, are completed then a new trial begins.

Throughout the twelve stages the difficulty increases and this is evident in the increasing criteria that the animal has to achieve in order to advance. As well, the three parameters that increase with difficulty are the ITI, time the stimulus light is presented, and the limited hold. Increasing the ITI indicates the animal must inhibit for a longer period of time from responding

prematurely until the stimulus light is presented. The duration of the stimulus light is decreased throughout the twelve stages. Decreasing the duration indicates the animal must allocate its attentional resources toward the apertures because the light is not presented for a long period of time and in order to not miss the stimulus. The limited hold decreases during the twelve stages. A shorter limited hold indicates the animal has to adapt to responding in a short period of time or if he does not respond within the appropriate time the response will be counted as an error (omission error).

Figure 1. Image of 5-Choice Serial Reaction Time Task ("5-CSRTT", 1998)

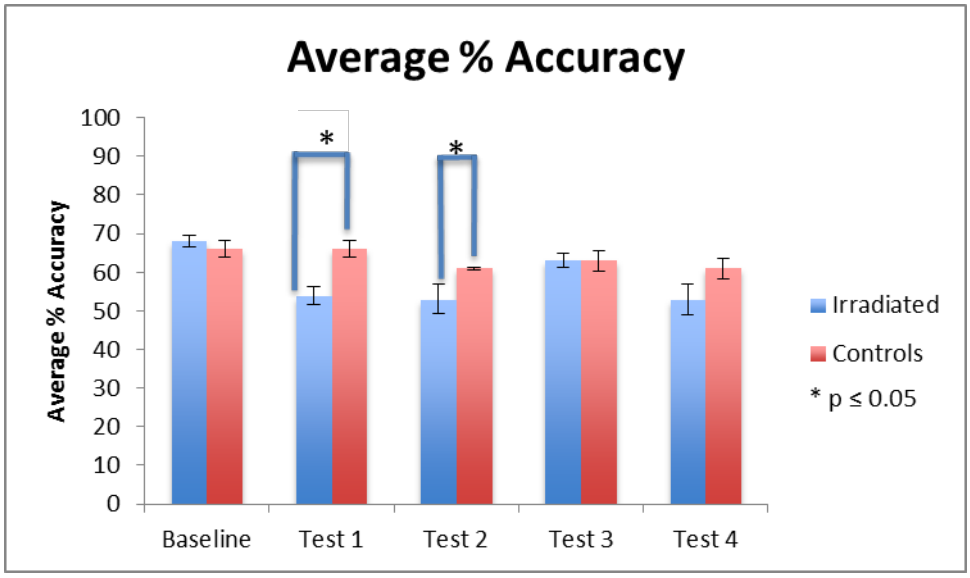

Figure 2. Performance in the 5-CSRTT measured in average accuracy for both groups.

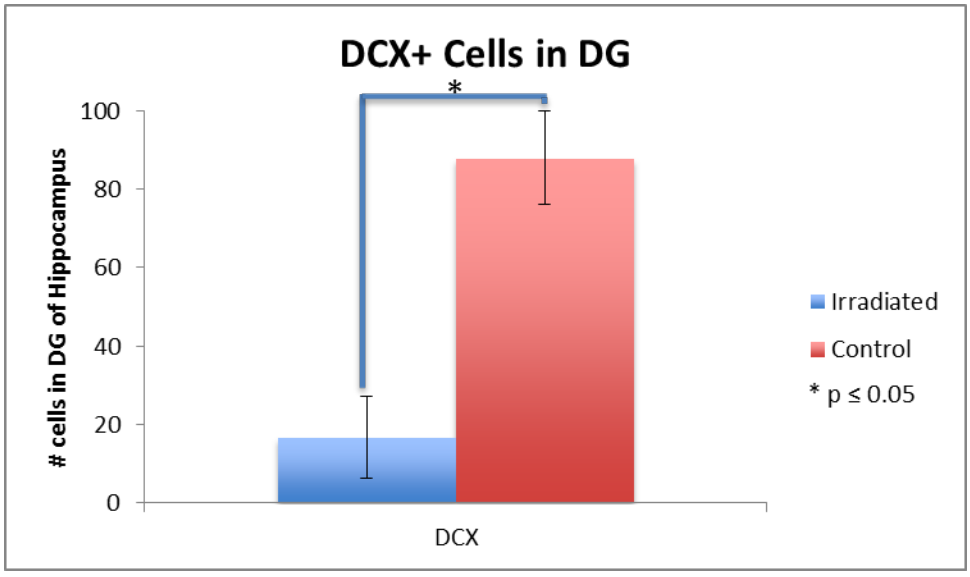

Figure 3. Total number of DCX+ cells counted in dentate gyrus (DG) of hippocampus.

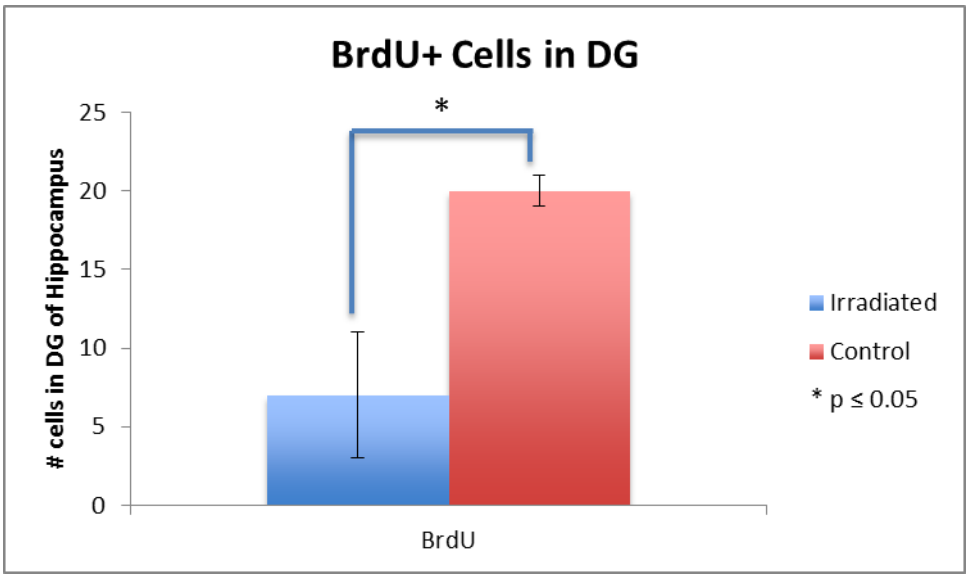

Figure 4. Total number of BrdU + cells counted in dentate gyrus (DG) of hippocampus.

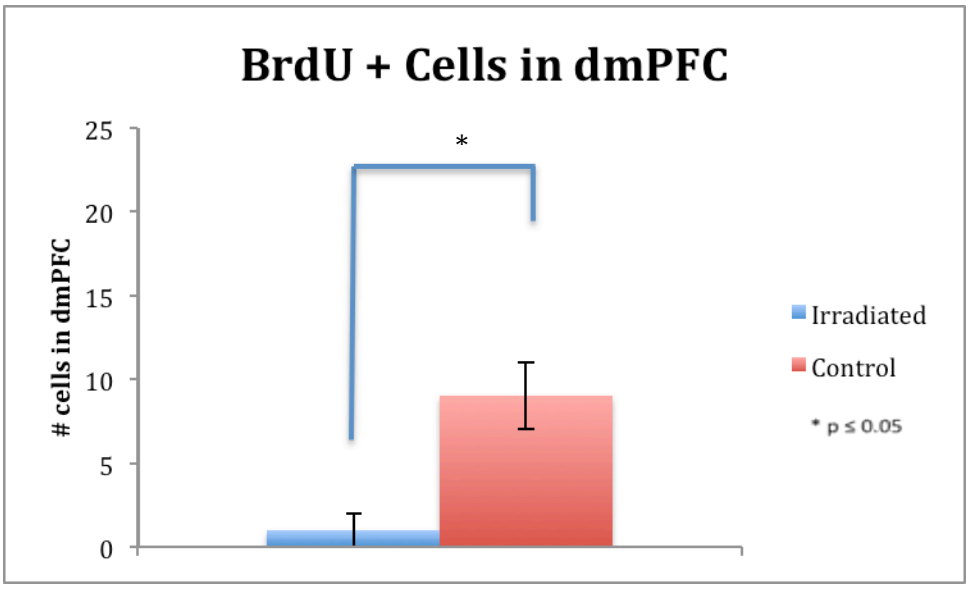

Figure 5. Total number of BrdU+ cells in the dorsomedial prefrontal cortex(dmPFC).

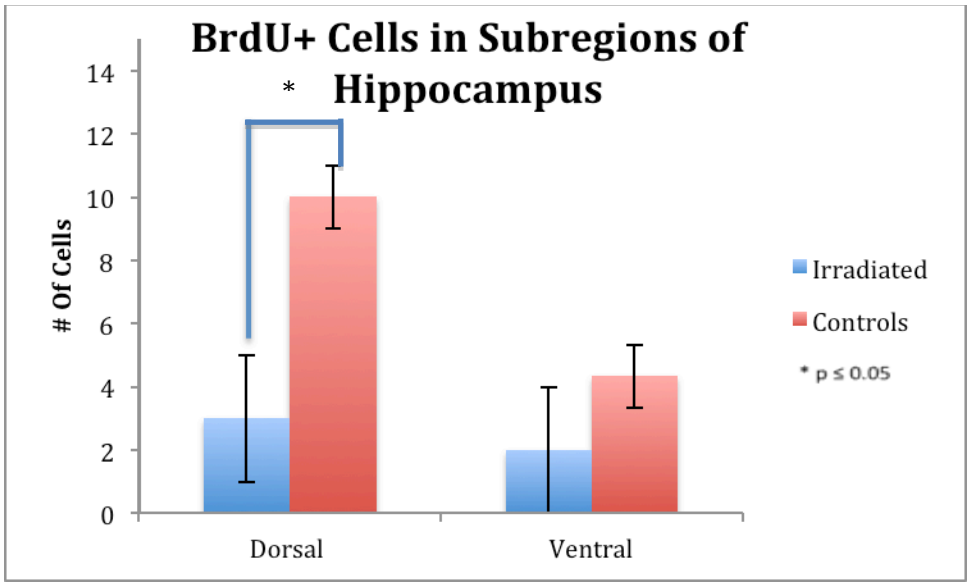

Figure 6. Total number of BrdU+ cells in dorsal and ventral subregions of Hippocampus.

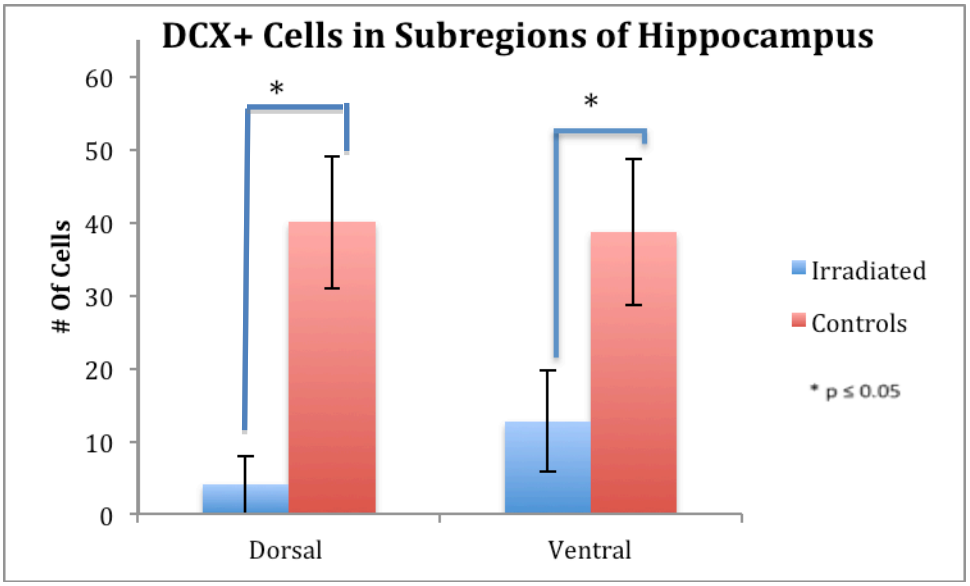

Figure 7. Total number of DCX+ cells in dorsal and ventral subregions of Hippocampus.

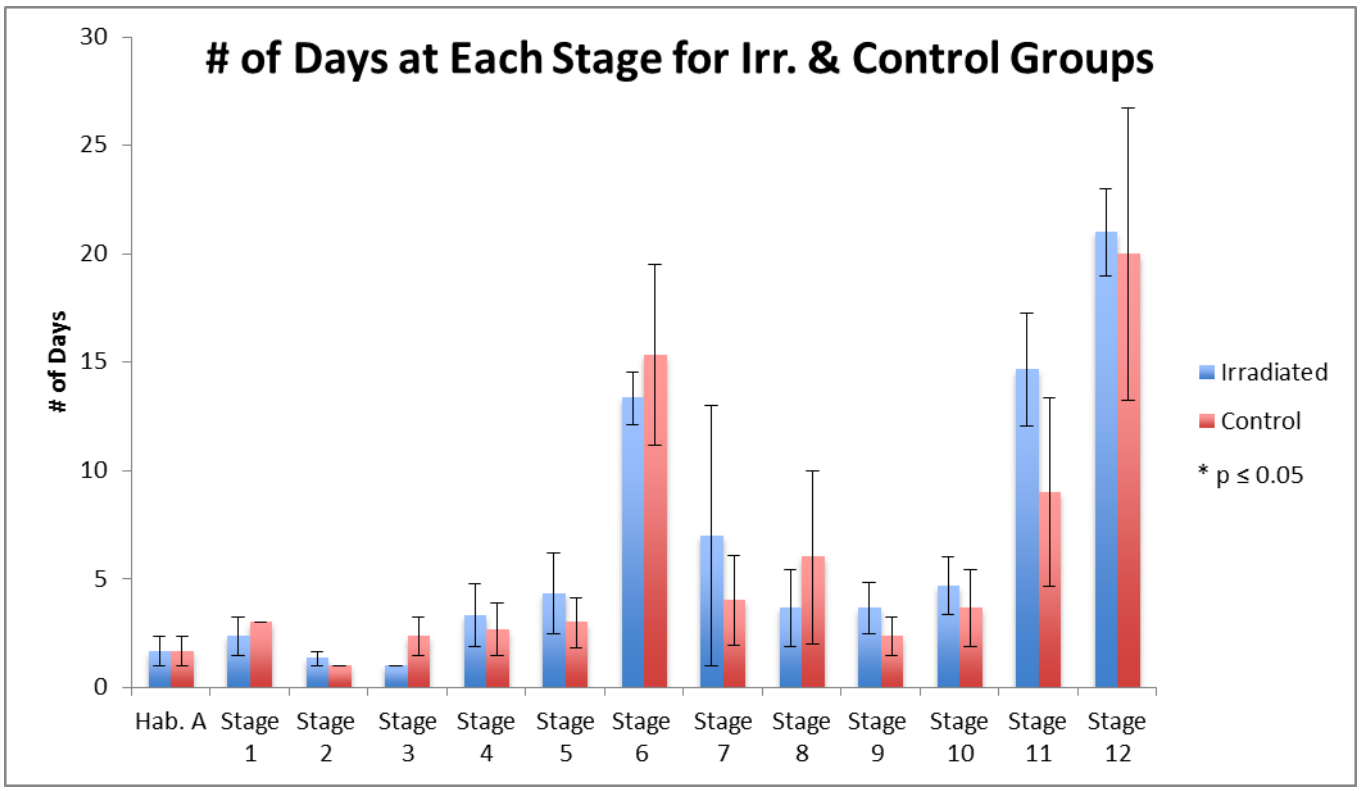

Figure 8. Number of days at each stage for irradiated and control groups.

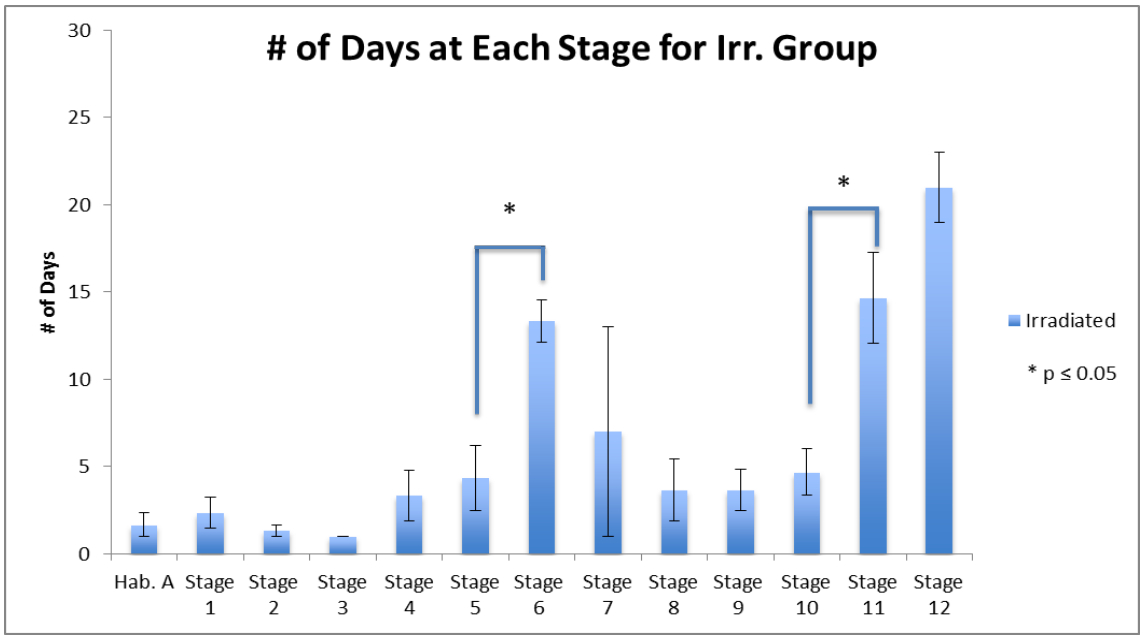

Figure 9. Number of days at each stage for irradiated group.

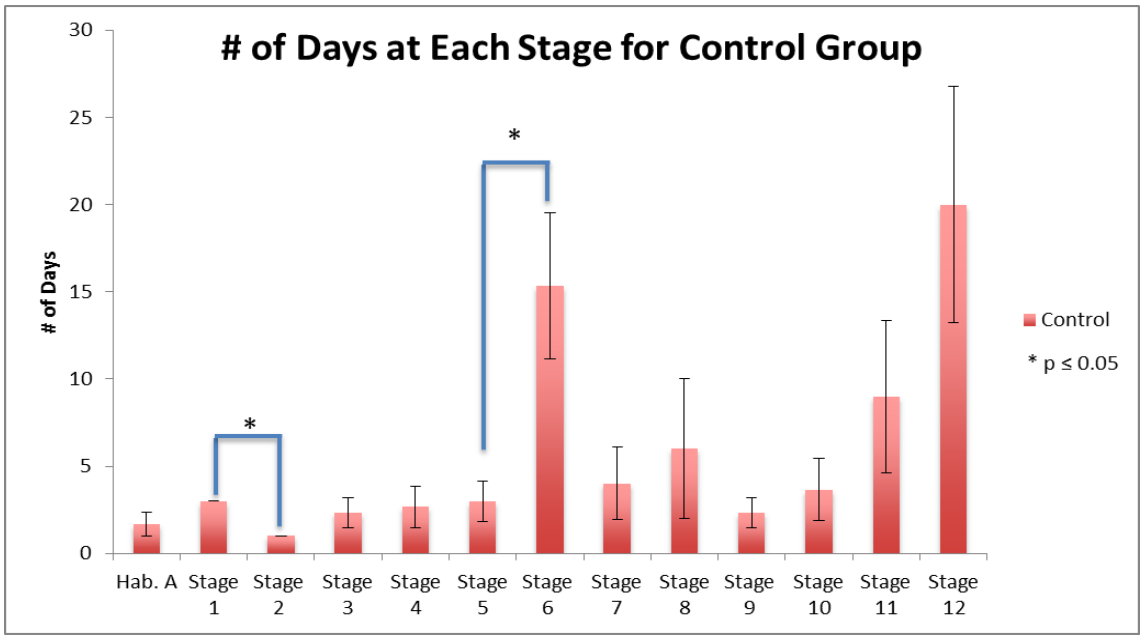

Figure 10. Number of days at each stage for control group.