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Modeling Impaired Hippocampal Neurogenesis after Radiation Exposure

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MODELING IMPAIRED HIPPOCAMPAL NEUROGENESIS AFTER RADIATION EXPOSURE

By

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Bachelor of Science -- Nuclear Medical Science
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2012

A thesis submitted in partial fulfillment of
the requirements for the

Master of Science – Medical Physics

Department of Health Physics and Diagnostic Sciences
School of Allied Health Sciences
Division of Health Sciences
The Graduate College

University of Nevada, Las Vegas
May 2018
This thesis prepared by

Sidath Kapukotuwa

entitled

Modeling Impaired Hippocampal Neurogenesis after Radiation Exposure

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Department of Health Physics and Diagnostic Sciences

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Abstract

The birth of neuronal cells from neuronal stem cells is known as neurogenesis, and the granular cell layer of the dentate gyrus of hippocampus is one of the two regions in the brain this process occurs. Cognitive damages following radiation therapy for brain cancers in both children and adults have been linked to impairment of neurogenesis in the hippocampus. Studies followed using mice and rats as model animals have shown impairment in neurogenesis process following exposure to radiation. Obtaining experimental data for radiation-induced changes in neurogenesis in humans is very difficult. Model was developed and applied to mouse data previously; this study aims to apply the model to rat data. The patterns of neurogenesis impairment following radiation exposure can then provide insights for extrapolations with relevance to human physiology. A mathematical model was designed to represent the time, age and dose dependent changes occurring to several cell populations that participate in neurogenesis using nonlinear differential equations (ODE). To model the alterations in hippocampal neurogenesis following radiation exposure, four neuronal stem cell populations were considered: neural stem cells, neuroblasts, immature neurons and glioblasts. Matlab Simulink was used to solve nonlinear ODEs. With this model we were able to successfully produce data matching the experimental data for the dynamics of the rat hippocampal cell population under unirradiated and irradiated conditions. Development of these mathematical models may lead to help optimizing radiation therapy for cancer patients in the future.
I would sincerely like to express my gratitude to my major professor and advisor, Prof. Francis Cucinotta for giving me the opportunity to work on this research project and for the encouragement along the way. I appreciate my committee members: Dr. Steen Madsen, Dr. Yu Kuang and Dr. Ronald Gary for their constructive criticism and advice on the project.

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Chapter 1: Introduction

1.1 Neurogenesis

The ability to produce new neurons is called neurogenesis. The concept of neurogenesis was first introduced during the 1960s; however, it was proven during the 1990s (Curtis, Kam, & Faull, 2011; Kennea & Mehmet, 2002). The discovery of neural stem cells (NSC) made it easy to prove this concept of dividing neurons (Kornblum, 2007). NSC can be seen throughout the life span of mammals. These NSC, once activated differentiates into neuroblasts (NB) and glioblasts (GB). Neuroblasts then differentiate into immature neurons (ImN), whereas GB differentiates into astrocytes and oligodendrocytes (Encinas et al., 2011; Gage, 2002). Two regions of the mammalian brain have been identified to be producing new neurons. First region is the subventricular zone (SVZ) of the anterior lateral ventricles and the second region is the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus (Andres-Mach, Rola, & Fike, 2008). A recent study on mice has provided evidence for a third region (basolateral amygdala) that exhibits adult neurogenesis (Jhaveri et al., 2017).

1.2 Hippocampus

In this study we are interested on the hippocampal region due to its functions and complications after cranial radiation therapy. The hippocampus can be found in the medial temporal lobe (Fig. 1). The two halves of the hippocampus lie in the right and left sides of the brain. The function of the hippocampus is to deal with declarative memories, spatial relationships and turn short term memories into long term memories.*

* https://www.medicalnewstoday.com/articles/313295.php
Hippocampus can be divided into three major subfields, the dentate gyrus, the CA3 region and the CA1 region (Fig. 2) (Freund & Buzski, 1996).

Figure 1. Location of the Hippocampus (OpenStax College, 2013)

Figure 2. Major subfields of the hippocampus (Kino, 2015)
1.3 Hippocampal neurogenesis in adults

Neural stem cells in the SGZ of the DG (Fig. 2) are continuously generating new granular cells. These stem cells have an appearance of radial glia cells and show properties of astrocytes (Klempin & Kempermann, 2007). New born neurons ultimately integrate into the existing network of neurons in the CA3 region (Fig. 2) (Kennea & Mehmet, 2002). Hippocampal neurogenesis is decreased with age as a result of depletion of NSC due to their continuous division, while reaching a steady-state for adults (Fig. 3) (Encinas et al., 2011; Gage, 2002).

![Figure 3. Depletion of NSC with the age in mice](image)

1.4 Types of the cells in the hippocampus

Major cell types of the hippocampus are neural stem cells, neuronal progenitor cells or neuroblasts, immature neurons, glioblasts, astrocytes, oligodendrocytes and microglia (Encinas et al., 2011; Gage, 2002) (Fig. 4).
1.4.1 Neural stem cells (NSC)

Several groups of researchers were able to identify a subset of stem cells that can be found in the central nervous system (CNS) during early 1990s. Their capability of differentiation is limited to CNS cell types; thus they were named as neural stem cells (Kornblum, 2007).

![Differentiation of NSC](image)

**Figure 4. Differentiation of NSC (Casarosa, Bozzi, & Conti, 2014)**

1.4.2 Neuroblasts (NB)

Neuroblasts are arising from NSC. Later they are differentiated into immature neurons. Some may undergo apoptosis.
1.4.3 Microglia

Microglia are the “resident” macrophage of the CNS. About 12% of CNS cells are microglia, however with some variation for different brain regions. Microglia has two phases; “Resting” and “Activated”. Once activated, microglia can migrate into the site of injury and have the capability of releasing substances that can act as both beneficial and harmful to the surroundings (Kettenmann, Hanisch, Noda, & Verkhratsky, 2011).
1.5 Cell Markers

Cell markers, also named as cell surface antigens can be used to identify a specific type of cells. Most of them are either molecules or antigens. Double labeling with a cell proliferation marker and a marker for specific cell lineages is a powerful technique used in neurogenesis experimentation.

1.5.1 5-Bromo-2-deoxyuridine

5-Bromo-2-deoxyuridine, also known as BrdU is a synthetic nucleoside that can be used to substitute thymidine during the S phase of the cell cycle. BrdU has a long term retention period over 2 years and they can be incorporated into daughter cells (Kee, Sivalingam, Boonstra, & Wojtowicz, 2002).

BrdU is administered via intraperitoneal cavity as a single injection or a series of injections, depending on the experiment. After a certain period of time, subjects are sacrificed and the tissues are fixed with a standard paraformaldehyde based fixative. Primary antibodies specific for BrdU can be used to detect the BrdU labeled cells in the tissue. These primary antibodies need to be labeled with a secondary antibody tagged with a fluorescent compound or with a substrate for diaminobenzidine (DAB) (Kee, Sivalingam, Boonstra, & Wojtowicz, 2002).

1.5.2 Ki-67

Ki-67 is a protein that can be used to detect diving cells. Unlike BrdU, Ki-67 is present during all active phases of the cell cycle; G1, S, G2 and M (Gerdes et al., 1984; Kee, Sivalingam, Boonstra, & Wojtowicz, 2002). Ki-67 is used in neurogenesis studies as the marker for neuroblasts
(Cacao & Cucinotta, 2016). Similar to BrdU, immunohistochemistry can be used to detect Ki-67 (Kee, Sivalingam, Boonstra, & Wojtowicz, 2002).

![Figure 7. Ki-67 vs BrdU. Ki-67 and BrdU labeling in representative tissue sections from the rat dentate gyrus. (A) Alexa-568 labeling of Ki-67 positive nuclei. (B) High power view of selected Ki-67 positive nuclei (indicated by arrow). (C) Alexa-488 labeling of BrdU incorporated in nuclei (injected 24 h prior to the assay). (D) High power view of selected BrdU positive nuclei (indicated by arrow). GCL—granule cell layer (Kee, Sivalingam, Boonstra, & Wojtowicz, 2002)]

### 1.5.3 Doublecortin (DCX)

Doublecortin is a microtubule-associated protein that is expressed in immature neurons. Hence it is used in neurogenesis studies to label immature neurons. DCX is also expressed in the daughter cells for 2 to 3 weeks. Immunohistochemistry can be used to detect DCX labeled cells.
1.5.4 Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling

Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) was introduced by Gavrieli et al. can be used to detect apoptotic cells by detecting the naturally occurring chromatin DNA strand breaks during apoptosis (Kasagi, Gomyo, Shirai, Tsujitani, & Ito, 1994).

1.5.5 CD68 (Cluster of Differentiation 68)

CD68 (Cluster of Differentiation 68) is a protein expressed at high levels in some cells of the immune system such as monocytes, circulating or tissue macrophages (e.g. microglia) (Holness & Simmons, 1993). Therefore, CD68 can be used to identify activated microglia. CD68 positive cells can be detected using immunohistochemistry.

1.5.6 Nestin

Nestin is an intermediate filament protein that is used as a cell marker for staining neural stem cells. Expression of nestin has been detected in undifferentiated neural stem cells both in the
developing stage as well as in adult stage. Furthermore, it has also been detected in tumorous neuronal cells.

1.6 Hippocampal Neurogenesis after Radiation Exposure

The use of cranial radiation therapy is often employed in the treatment of various types of brain tumors. However, complications such as neurocognitive detriments have been reported in children as well as adults. These include progressive deficits in short and long term memory loss, spatial relations, visual motor processing, quantitative skills and attention (Cacao & Cucinotta, 2016; Dietrich, Monje, Wefel, & Meyers, 2008; Monje & Palmer, 2003). The reason behind this phenomenon has not yet been well understood. However, observations have suggested that the severity of cognitive deterioration is linked to the exposed dose on the medial temporal lobes. This provides evidence that changes to the proliferation and apoptosis of neuronal precursor cells in the dentate gyrus of the hippocampus may lead to the impairments mentioned above (Cacao & Cucinotta, 2016; Monje, Mizumatsu, Fike, & Palmer, 2002; Monje, Toda, & Palmer, 2003; Monje & Palmer, 2003; Naylor et al., 2008; Raber et al., 2004; Rola et al., 2004). Neuronal stem cells (NSCs) in hippocampus are active throughout the lifespan (Altman & Das, 1965; Spalding et al., 2013; Zhao, Deng, & Gage, 2008). As a result, changes in neurogenesis during cranial radiation therapy may lead to hippocampal related cognitive detriments in both children and adults. Studies followed using mice and rats as model animals have shown that after being exposed to low linear energy transfer (LET) radiation at doses as low as 1 Gy of X rays, electrons or medium or high energy photons, the neurogenesis process was changed or impaired. Furthermore, behavioral changes were observed in these animals, which has led to the hypothesis that the changes caused
in neurogenesis by exposure to radiation has a direct relationship with cognitive impairments (Cacao & Cucinotta, 2016).

Although it is attempted to avoid a dose to the hippocampus during treatment planning, due to out of field effects it is unavoidable. During the period of radiation therapy, hippocampus may be subjected to a total dose of 1-10 Gy. By looking at the radio sensitivity of the cell types in the hippocampus, it can be seen that neuroblasts are more sensitive to radiation than other types of cells. However, reduction in cell count of immature neurons can be seen at higher doses (>5 Gy) (Cacao & Cucinotta, 2016).

Radiation damages the cells and surroundings of the hippocampus causing neuroinflammation, including causes the activation of microglia. Activation of microglia inhibit the proliferation of NSC and shift neurogenesis to gliogenesis at a dose of 10 Gy (Cacao & Cucinotta, 2016; Mizumatsu et al., 2003; Monje, Mizumatsu, Fike, & Palmer, 2002; Monje, Toda, & Palmer, 2003; Rola et al., 2004; Tada, Parent, Lowenstein, & Fike, 2000). Also negative feedback regulation on proliferation can be seen at late post irradiation times (1–3 months) (Cacao & Cucinotta, 2016).

1.7 Model of the hippocampal neurogenesis after irradiation

Animal experiments are useful for understanding the relationships between radiation impairment of neurogenesis and cognitive impairments, but these studies have practical limitations, as the number of doses or numbers of dose fractions that can be administered to these animal models are small. Furthermore, there are differences in the cell kinetics and radiation sensitivity between animal species and humans. Mathematical approaches have been developed to predict radiation responses of the blood system in different species (Hu & Cucinotta, 2010;
Smirnova, Hu, & Cucinotta, 2014a; Smirnova, Hu, & Cucinotta, 2014b) that can provide a framework to consider inter-species variations in radiation impairments of neurogenesis. Developing a mathematical model for different species will support approaches to interpolate and extrapolate the observed experimental data. This would be useful to predict the radiation-induced conditions at varying doses. (Cacao & Cucinotta, 2016).

Figure 9. Schematic diagram of hippocampal neurogenesis model after irradiation. Neuronal cell population $n_1, n_2, n_3, n_4$ and $n_5$ represent neural stem cell (NSC), neuroblast (NB), immature neurons (ImN), glioblast (GB) and apoptotic cells after irradiation, respectively. Radiation induced damages are described by rate constants $k_1, k_2, k_3$ and $k_4$ while damage repair rates are depicted by $\alpha_1, \alpha_2, \alpha_3$ and $\alpha_4$. The number of apoptotic cells damaged by irradiation are defined from rate of apoptosis of heavily damaged cells ($\nu_1, \nu_2, \nu_3$ and $\nu_4$) and misrepaired weakly damaged cells ($\alpha_{1m}, \alpha_{2m}, \alpha_{3m}$ and $\alpha_{4m}$). Subscript 1-4 denotes the four neuronal cell populations being considered in this model (Cacao & Cucinotta, 2016).
In our model, hippocampal neurogenesis was modeled into four compartments. The four compartments (Fig. 9) are: 1) NSC, 2) NB, 3) ImN and 4) GB. This model considers age dependence of neurogenesis and the output will be varying according to the age. The model also considers the negative feedback regulation on proliferation for late post-irradiation times (1-3 months). During late post-irradiation time, an increased number of activated microglial cells can be observed as a result of inflammatory response (Cacao & Cucinotta, 2016; Monje, Mizumatsu, Fike, & Palmer, 2002; Monje, Toda, & Palmer, 2003). We have included the inflammatory responses in the model. The model has been successfully applied for the mouse (Cacao & Cucinotta, 2016). Nonlinear differential equations are being used in the mathematical model.

1.8 Nonlinear Differential Equations

Differential equations can be divided into several different types such as ordinary or partial, linear or nonlinear. Also they can be classified as 1st order, 2nd order and so on.

Assuming \( x \) and \( y \) are functions of time \( t \), we can write,

\[
x = x(t)
\]

\[
y = y(t)
\]

Thus the derivative with respect to “\( t \)” is as followed,

\[
\frac{dy}{dx} = \dot{x}(t)
\]

A differential equation with a finite set of variables, is classified as an ordinary differential equation (ODE). Whereas a differential equation with an infinite set of variables is known as a partial differential equation (PDE).
Order of a differential equation is classified by the highest derivative in the equation. To indicate the derivative, we use quotes. For example, 1st derivative can be written as $\dot{x}$. Therefore, the 2nd derivative is $\ddot{x}$.

When the variable in an equation appears only with a power of one, those are linear. Therefore, $x^2$ is non-linear. Other functions for example, such as $\sin(x)$ are non-linear.

Solving of non-linear equations is not as direct or well established compared to linear equations. Normally the non-linear equations are difficult to solve*. In biochemical kinetic approaches the use of non-linear ODE’s allows for a diverse range of solutions to be achieved including simple exponential responses, damped and undamped oscillations, and chaotic responses. The type of behavior that occur will depend on the order and structure of the ODEs and the values of the model parameters. For the model of neurogenesis established here the important feature of the ODE system to be considered is the allowance for positive and negative feedback controls on cell proliferation.

1.9 Goal of study

The objective of the proposed study is to apply a mathematical model developed for mouse, to rat; in order to predict alterations to neurogenesis following acute and fractionated exposure to radiation in rats. The mathematical model is developed using a system of nonlinear ordinary differential equations (ODEs) that exemplify the time, age and dose dependent changes observed in the neural stem cells, neuronal progenitor cells, immature neurons and glioblast cell populations in rats treated with low-LET radiation. The developed model will also be able to predict the negative regulation over cell proliferation following radiation treatments at early stages (<3 days)
and late stages (1 – 3 months). Furthermore, we are going to compare data with the previous study for mouse. This might give us insight on how to predict the outcome for Humans. A challenge for the modeling efforts will be the range of rat strains used in the published experimentally data which will introduce variations in determination of model parameters to be discussed below.

Chapter 2: Methods and Materials

2.1 Materials

This section includes the materials that were used for this study. Materials are subcategorized into three major groups; Research articles that were used to estimate parameters for unirradiated conditions, Research articles that were used to estimate parameters for irradiated conditions and Software used. Also extracted data from articles are presented. Data for each of the conditions tested in the model were extracted from results and graphs reported in the cited publications. When raw data were not presented on the article, extrapolations were used to extract data.

2.1.1 Research articles used to estimate parameters for unirradiated condition and data extracted


These articles were used to estimate initial values of cell populations (Rat age = 0). Data used are given below (Table 1).
Table 1. Initial values of cell population in mouse (Cacao & Cucinotta, 2016).

<table>
<thead>
<tr>
<th>Cell Population</th>
<th>Initial value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSC</td>
<td>47680</td>
</tr>
<tr>
<td>NB</td>
<td>40318</td>
</tr>
<tr>
<td>ImN</td>
<td>209654</td>
</tr>
<tr>
<td>GB</td>
<td>69885</td>
</tr>
</tbody>
</table>


This article was used to estimate parameters for unirradiated conditions. Age related changes in neuroblasts (Table 2) and immature neurons (Table 3) were extracted from this article.

Table 2. Changes in Neuroblast population with Age.

<table>
<thead>
<tr>
<th>Age (Days)</th>
<th>Number of Ki-67 positive cells/DG</th>
</tr>
</thead>
<tbody>
<tr>
<td>72</td>
<td>7875</td>
</tr>
<tr>
<td>100</td>
<td>5375</td>
</tr>
<tr>
<td>245</td>
<td>1875</td>
</tr>
<tr>
<td>335</td>
<td>750</td>
</tr>
</tbody>
</table>
Table 3. Changes in Immature neurons with Age.

<table>
<thead>
<tr>
<th>Age (Days)</th>
<th>Number of DCX positive cells/DG</th>
</tr>
</thead>
<tbody>
<tr>
<td>72</td>
<td>30714</td>
</tr>
<tr>
<td>100</td>
<td>22857</td>
</tr>
<tr>
<td>245</td>
<td>9286</td>
</tr>
<tr>
<td>335</td>
<td>2143</td>
</tr>
</tbody>
</table>

2.1.2 Research articles used to estimate parameters for irradiated condition and data extracted

2.1.2.1 Tada, E., Parent, J. M., Lowenstein, D. H., & Fike, J. R. (2000). X-irradiation causes a prolonged reduction in cell proliferation in the dentate gyrus of adult rats. *Neuroscience, 99*(1), 33-41. This article was used to estimate parameters for neuroblast cell population after irradiation. Apoptotic data were also extracted. Young adults (Age 8-10 weeks) male “Fisher 344” rats were used.

Table 4. Total number of BrdU-positive cells in three sections (5µm thick) of SGZ of rats after 24h after irradiation.

<table>
<thead>
<tr>
<th>Radiation Dose (Gy)</th>
<th>Number of BrdU positive cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>23.7</td>
</tr>
<tr>
<td>2</td>
<td>5.2</td>
</tr>
<tr>
<td>5</td>
<td>3.4</td>
</tr>
<tr>
<td>10</td>
<td>1.9</td>
</tr>
<tr>
<td>15</td>
<td>1.5</td>
</tr>
</tbody>
</table>
Table 5. Time-course for apoptotic changes in SGZ of rats after single dose of 15 Gy.

<table>
<thead>
<tr>
<th>Time (Hr)</th>
<th>Number of Apoptotic cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>115</td>
</tr>
<tr>
<td>6</td>
<td>149</td>
</tr>
<tr>
<td>12</td>
<td>78</td>
</tr>
<tr>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>48</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 6. Total number of Apoptotic cells in SGZ of rats at different radiation doses.

<table>
<thead>
<tr>
<th>Radiation Dose (Gy)</th>
<th>Number of Apoptotic cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>67</td>
</tr>
<tr>
<td>3</td>
<td>137</td>
</tr>
<tr>
<td>5</td>
<td>144</td>
</tr>
<tr>
<td>15</td>
<td>149</td>
</tr>
</tbody>
</table>


This article was used to estimate parameters for immature neurons after radiation exposure to rat hippocampus. S-D rats (Charles River) rats with age of 12 weeks were used.
Table 7. Absolute numbers of DCX positive and Ki67 positive cells at different doses.

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>DCX positive cells</th>
<th>Ki-67 positive cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>21316</td>
<td>3290</td>
</tr>
<tr>
<td>1</td>
<td>9605</td>
<td>3618</td>
</tr>
<tr>
<td>2</td>
<td>5000</td>
<td>6513</td>
</tr>
<tr>
<td>3</td>
<td>2368</td>
<td>6908</td>
</tr>
<tr>
<td>4</td>
<td>3421</td>
<td>7895</td>
</tr>
<tr>
<td>5</td>
<td>2303</td>
<td>3421</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>3290</td>
</tr>
</tbody>
</table>

Table 8. Depletion of immature neurons with radiation.

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>Percentage loss immature neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>42.75</td>
</tr>
<tr>
<td>1</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>77</td>
</tr>
<tr>
<td>3</td>
<td>88.75</td>
</tr>
<tr>
<td>4</td>
<td>83.75</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>
2.1.3 Other useful articles and extracted data.


Table 9. Number of nestin-positive progenitor cells in the granular cell layer and SGZ from unirradiated rats.

<table>
<thead>
<tr>
<th>Age of the rat (Days)</th>
<th>Cell count</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>98</td>
</tr>
<tr>
<td>16</td>
<td>42</td>
</tr>
<tr>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>94</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 10. Dose dependent decrease in total NB cells.

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>NB Cell count (Age 21 days)</th>
<th>NB Cell count (Age 50 days)</th>
<th>NB cell count (Age 70 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9333</td>
<td>8000</td>
<td>6111</td>
</tr>
<tr>
<td>0.3</td>
<td>7600</td>
<td>6400</td>
<td>5333</td>
</tr>
<tr>
<td>3</td>
<td>6167</td>
<td>5867</td>
<td>5000</td>
</tr>
<tr>
<td>10</td>
<td>2933</td>
<td>2480</td>
<td>2000</td>
</tr>
</tbody>
</table>


Table 11. NB and Apoptotic cell counts after irradiation in 10µm section.

<table>
<thead>
<tr>
<th>Hours after irradiation</th>
<th>NB cell count</th>
<th>Apoptotic cell count</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>21.1</td>
<td>0.17</td>
</tr>
<tr>
<td>1</td>
<td>21.6</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>3.8</td>
<td>23.4</td>
</tr>
</tbody>
</table>

2.1.4 Software and tools

For data analysis and mathematical modeling Matlab 2017b (Mathworks, Ins) was used. Matlab Simulink was used to solve differential equations. The built-in ODE solvers ode45 and ode15s were used to solve ordinary differential equations for unirradiated and irradiated conditions
respectively. Built-in curve filling tool was used to fitting of the experimental data extracted from research articles.

2.2 Methods

2.2.1 Initial values (Age = 0) of cell populations

Age related hippocampal neurogenesis studies with neural stem cell populations for rats were not found in a literature search. Therefore, an assumption was made based on the observations reported in Snyder et al., where they detected that the granular cell population of rat is three times higher than mice. Therefore, assuming rats have three times higher initial value of NSC population, initial value for NSC population of rats was estimated using the initial value of NSC population of mice (Cacao & Cucinotta, 2016).

For mouse the ratio of NSC over amplifying neuroprogenitor (ANP) at the age of zero days is 2.7 and average divisions of ANP is 2.3 (Encinas et al., 2011). Assuming this hold for rats as well, the initial value of NB cell population was estimated.

Initial value of ImN was estimated using the ratio (5.3) of ImN over NB at age zero days (Andres-Mach, Rola, & Fike, 2008). Similarly, initial value of GB was estimated using the ratio (0.3) of GB over ImN at age zero days (Verkhratsky & Butt, 2013; Ziebell, Martin-Villalba, & Marciniak-Czochra, 2014).

2.2.2 Parameters for rat hippocampal neurogenesis

Initially, parameters for mouse hippocampal neurogenesis were used (Cacao & Cucinotta, 2016). Changes to the parameters was made to fit the model with the experimental data.
2.2.3 Dynamics of neuronal cell population for unirradiated condition

The following assumptions were made during the mice study (Cacao & Cucinotta, 2016) using previously reported experimental data. Same assumptions were used in this study.

1. Neural stem cells (n1) are regulated by their proliferation differentiation into neuroblasts (n2) and glioblasts (n4). We further assume that NSC proliferation is a function of other cell populations to describe possible feedback mechanisms.

2. The population of neuroblasts (n2) that arise via NSC differentiation has two fates. They will either undergo normal cell division once and then differentiate into immature neurons (n3) or undergo cell death by apoptosis.

3. The amount of immature neuronal (n3) population is determined by the amount that differentiated from NB and the amount that was lost due to apoptosis.

4. The population of glioblasts (n4) are also derived starting from NSC and some of it will undergo apoptosis. The fraction of the glioblasts that differentiate into astrocytes and oligodendrocytes are not accounted for in this proposed model.

Using estimated parameters and taking into account the above assumptions, differential equations can be written to propose or predict the fate of each cell population in the hippocampus. The following set of differential equations have been proposed for the fate of the four cell populations in the hippocampus at unirradiated conditions (Cacao & Cucinotta, 2016).

\[
\frac{dn_1(t)}{dt} = p_1 n_1(t) - d_1 n_1(t) \tag{1}
\]

\[
\frac{dn_2(t)}{dt} = 2x_a d_1 n_1(t) - d_2 n_2(t) - a_2 n_n(t) \tag{2}
\]
\[
\frac{dn_3(t)}{dt} = d_2n_2(t) - a_3n_3(t) \tag{3}
\]

\[
\frac{dn_4(t)}{dt} = x_n d_1 n_1(t) - a_4n_4(t) \tag{4}
\]

The parameters used for this are described as followed;

\[d_1 = \text{rate of differentiation from NSC to NB}\]

\[d_2 = \text{rate of differentiation from NB to ImN}\]

\[a_2 = \text{rate of apoptosis for NB}\]

\[a_3 = \text{rate of apoptosis for ImN}\]

\[a_4 = \text{rate of apoptosis for GB}\]

\[x_n = \text{fraction of NSC that differentiate into NB}\]

\[x_b = \text{fraction of NSC that differentiate into GB}\]

\[p_1 = \text{proliferation rate of NSC}\]

\[p_1 \text{ can be calculated using following equation.}\]

\[
p_1 = \frac{\Psi}{1+(\theta_1 n_1+\theta_2 n_2+\theta_3 n_3)} \tag{5}
\]

Where,

\[\Psi = \text{maximum proliferation rate on NSC proliferation.}\]

\[\theta_1 = \text{dissimilar contributions of NSC in the negative feedback on NSC proliferation.}\]

\[\theta_2 = \text{dissimilar contributions of NB in the negative feedback on NSC proliferation.}\]

\[\theta_3 = \text{dissimilar contributions of ImN in the negative feedback on NSC proliferation.}\]
2.2.4 Dynamics of neuronal cell population after irradiation

For the prediction of the dynamics of neuronal cell population after irradiation, the model shown in Fig (1) will be used. In this model, radiosensitive cells are classified as; undamaged \( n_j \), weakly damaged \( n_{jw} \) and heavily damaged \( n_{jh} \). Where \( j = 1-4 \), which represent the four cell populations considered in this model. Weakly damaged cells have two paths that is either repair or apoptosis. Heavily damaged cells only undergo apoptosis. This leads to the following system of coupled ordinary differential equations to describe the dynamics of neuronal cell populations after irradiation (Equation (6) through (21) from Cacao & Cucinotta, 2016).

\[
\frac{dn_1(t)}{dt} = p_1 n_1(t) - d_1 n_1(t) - k_1 n_1(t) + \alpha_1 n_{1w} \tag{6}
\]

\[
\frac{dn_{1w}(t)}{dt} = k_{1w} n_1(t) - \alpha_1 n_{1w}(t) \tag{6a}
\]

\[
\frac{dn_{1h}(t)}{dt} = k_{1h} n_1(t) - \nu_1 n_{1h}(t) \tag{6b}
\]

\[
\frac{dn_2(t)}{dt} = 2x \alpha d_1 n_1(t) - d_2 n_2(t) - a_2 n_n(t) - k_2 n_2(t) + \alpha_2 n_{2w} \tag{7}
\]

\[
\frac{dn_{2w}(t)}{dt} = k_{2w} n_2(t) - \alpha_2 n_{2w}(t) \tag{7a}
\]

\[
\frac{dn_{2h}(t)}{dt} = k_{2h} n_2(t) - \nu_2 n_{2h}(t) \tag{7b}
\]

\[
\frac{dn_3(t)}{dt} = d_2 n_2(t) - a_3 n_3(t) - k_3 n_3(t) + \alpha_3 n_{3w} \tag{8}
\]

\[
\frac{dn_{3w}(t)}{dt} = k_{3w} n_3(t) - \alpha_3 n_{3w}(t) \tag{8a}
\]

\[
\frac{dn_{3h}(t)}{dt} = k_{3h} n_3(t) - \nu_3 n_{3h}(t) \tag{8b}
\]

\[
\frac{dn_4(t)}{dt} = x \beta d_1 n_1(t) - a_4 n_4(t) - k_4 n_4(t) + \alpha_4 n_{4w} \tag{9}
\]

\[
\frac{dn_{4w}(t)}{dt} = k_{4w} n_4(t) - \alpha_4 n_{4w}(t) \tag{9a}
\]

\[
\frac{dn_{4h}(t)}{dt} = k_{4h} n_4(t) - \nu_4 n_{4h}(t) \tag{9b}
\]
\[
\frac{dn_{sh}(t)}{dt} = \alpha_2 m n_{2w}(t) + \nu_2 n_{2h}(t) + \alpha_3 m n_{3w}(t) + \nu_3 n_{3h}(t) - \nu_5 n_5(t)
\]  

(10)

Where,

\[k_j = \text{rate of radiation induced damage}\]
\[\alpha_j = \text{rate of damage repair}\]
\[\nu_j = \text{rate of apoptosis of heavily damaged cells}\]

We assume that repair rate is a fraction of the overall damage rate defined by equation (11).

\[\alpha_j = \omega k_j\]  

(11)

Rate of radiation induced damage is divided into two components. Rate of repair also divided into two components

\[k_{jw} = \gamma_j k_j\]  

(12a)
\[k_{jh} = (1 - \gamma_j) k_j\]  

(12b)
\[\alpha_{jr} = \xi_j \alpha_j\]  

(13a)
\[\alpha_{jm} = (1 - \xi_j) \alpha_j\]  

(13b)

Where,

\[k_{jw} = \text{rate of radiation induced weakly damaged cells}\]
\[k_{jh} = \text{rate of radiation induced heavily damaged cells}\]
\[\alpha_{jr} = \text{rate of repair of weakly damaged cells}\]
\[\alpha_{jm} = \text{rate of apoptosis of misrepaired cells}\]
\[\gamma_j = \text{fraction of weakly damaged cells}\]
We assume that heavily damaged cells undergo apoptosis without a time delay while misrepaired weakly damaged cells undergo apoptosis with a time delay. Therefore, $v_j > \alpha_{jm}$. This give rise to the equation (14).

\[
\frac{\Gamma}{\Phi} = \frac{v_j}{\alpha_{jm}} \tag{14}
\]

Where, $\Gamma$ and $\Phi$ are dimensionless multipliers.

Equation (5) can be modified to account for radiation induced damage as follows;

\[
p_{1,IR} = \frac{\psi}{1 + \theta_1 n_1 + \theta_2 (n_2 + \phi n_{2w} + \Gamma n_{2h}) + \theta_3 (n_3 + \phi n_{3w} + \Gamma n_{3h})} \tag{15}
\]

Because data on the time course and dose dependence of microglia cell activation is sparse only a parametric model is used based on limited experimental data. To describe effects of activated microglial cells on proliferation and neurogenic cell fate of hippocampal neurogenesis, following parametric equations (equation (16) and (17)) were used.

\[
\frac{d\mu(\tau)}{dt} = \left[A_0 \left(\frac{dose}{dose + A_1}\right) + B\tau + C\tau^2\right]e^{-\lambda\tau} \tag{16}
\]

Where, $\tau = t - t_d$ ($t_d = 30$)

This equation goes to zero when, $t < t_d$

\[
\frac{d\Delta(\tau)}{dt} = \left[A_0 \left(\frac{dose}{dose + A_1}\right) + B_0 \mu + B_1 \mu \tau + C\tau^2\right]e^{-\lambda\tau} \tag{17}
\]

Where, $\Delta = x_{a,IR}/x_a$

This equation goes to one when, $t < t_d$
Therefore, equation can be written with this negative feedback as follows,

\[ p_{1,IR} = \frac{\psi}{1 + \theta_1 n_1 + \theta_2 (n_2 + \phi n_{2w} + \gamma n_{2h}) + \theta_3 (n_3 + \phi n_{3w} + \gamma n_{3h}) + \theta_{mg} \mu} \]  

(18)

Where, \( \theta_{mg} \) is a constant added to represent the contribution of increased number of activated microglia on proliferation.

When acute irradiation is considered, we can safely assume that rates corresponding to proliferation and differentiation, as well as damage repair are negligible compared to the rates for damage induction during irradiation period. Hence, we can use following simplified version of the model to solve the differential equations.

\[ n_j(t_{IR}) = n_j(0) e^{(-k_j t_{IR})} = n_j(0) e^{(-\frac{D}{D_{0j}})} \]  

(19)

\[ n_{jw}(t_{IR}) = \gamma_j n_j(0) \left[ 1 - e^{(-\frac{D}{D_{0j}})} \right] \]  

(20)

\[ n_{jh}(t_{IR}) = (1 - \gamma_j) n_j(0) \left[ 1 - e^{(-\frac{D}{D_{0j}})} \right] \]  

(21)

Where, \( D = \) absorbed dose in Gy

\( D_{0j} = \) characteristic dose where 37% of the cells are undamaged

Equations (19) - (21) can be used as the initial conditions to solve equations (6) - (10).

To solve the ordinary differential equations for unirradiated conditions, mouse parameter values were used as initial conditions (Table 12).
Table 12. Mouse parameter values for unirradiated conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (Unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Psi$</td>
<td>0.5 (day$^{-1}$)</td>
</tr>
<tr>
<td>$\theta_1$</td>
<td>0.005</td>
</tr>
<tr>
<td>$\theta_2$</td>
<td>0.05</td>
</tr>
<tr>
<td>$\theta_3$</td>
<td>0.005</td>
</tr>
<tr>
<td>$d_1$</td>
<td>0.015 (day$^{-1}$)</td>
</tr>
<tr>
<td>$d_2$</td>
<td>0.060 (day$^{-1}$)</td>
</tr>
<tr>
<td>$a_2$</td>
<td>0.008 (day$^{-1}$)</td>
</tr>
<tr>
<td>$a_3$</td>
<td>0.022 (day$^{-1}$)</td>
</tr>
<tr>
<td>$a_4$</td>
<td>0.008 (day$^{-1}$)</td>
</tr>
<tr>
<td>$x_a$</td>
<td>0.85</td>
</tr>
<tr>
<td>$x_b$</td>
<td>0.15</td>
</tr>
</tbody>
</table>

To solve the ordinary differential equations for irradiated conditions following mouse parameter values were used as initial conditions.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (Unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{02}$</td>
<td>0.75</td>
</tr>
<tr>
<td>$D_{03}$</td>
<td>7.5</td>
</tr>
<tr>
<td>$\gamma_2$</td>
<td>0.04</td>
</tr>
<tr>
<td>$\gamma_3$</td>
<td>0.20</td>
</tr>
<tr>
<td>$\xi$</td>
<td>0.99</td>
</tr>
<tr>
<td>$\nu_2$ (day$^{-1}$)</td>
<td>14</td>
</tr>
<tr>
<td>$\nu_3$ (day$^{-1}$)</td>
<td>1.4</td>
</tr>
<tr>
<td>$\nu_5$ (day$^{-1}$)</td>
<td>2.1</td>
</tr>
<tr>
<td>$\theta_{mg}$</td>
<td>50</td>
</tr>
<tr>
<td>$\Phi$</td>
<td>1</td>
</tr>
<tr>
<td>$\Gamma$</td>
<td>1.05</td>
</tr>
</tbody>
</table>
Chapter 3: Results

3.1 Initial values (Age = 0) of cell populations

As discussed in the Methods (Page 22), previously estimated mouse cell populations (Cacao & Cucinotta, 2016), assumptions are made for the model when data are not available, and assumptions made using Snyder et al. were used to estimate the initial values of the rat cell populations (Table 14).

Table 14. Initial values of cell population in rat.

<table>
<thead>
<tr>
<th>Cell Population</th>
<th>Initial value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSC</td>
<td>143040</td>
</tr>
<tr>
<td>NB</td>
<td>120954</td>
</tr>
<tr>
<td>ImN</td>
<td>628962</td>
</tr>
<tr>
<td>GB</td>
<td>209655</td>
</tr>
</tbody>
</table>

3.2 Age dependence of NB cell population in unirradiated rat

Parameters $d_1$ (rate of differentiation from NSC to NB) and $a_2$ (rate of apoptosis for NB) were estimated using the fitting the experimental data (Merkley, Jian, Mosa, Tan, & Wojtowicz, 2014) with the model (Fig. 10). Parameter $d_1$ was estimated to 0.018 day$^{-1}$ and estimated value for parameter $a_2$ was 0.001 day$^{-1}$. 
3.3 Age dependence of ImN cell population in unirradiated rat

Parameters $d_2$ (rate of differentiation from NB to ImN) and $a_3$ (rate of apoptosis for ImN) were estimated using fitting of the experimental data (Merkley, Jian, Mosa, Tan, & Wojtowicz, 2014) with the model (Fig. 11). Parameter $d_2$ was estimated to 0.16 day$^{-1}$ and estimated value for parameter $a_3$ was 0.07 day$^{-1}$.

Figure 10. Age dependence of NB cell population in unirradiated rat
3.4 Age dependence of NSC and GB cell populations in unirradiated rat

Modeling dynamics of the NSC and GB cell populations are shown below (Fig. 12 and 13).

3.5 Parameters for rat hippocampal neurogenesis

Parameters $d_1$, $d_2$, $a_2$ and $a_3$ are different from the estimated mouse parameters. Other parameters for rat are similar in value with mouse parameters (Table 15).
Figure 12. Age dependence of NSC cell population in unirradiated rat

Figure 13. Age dependence of GB cell population in unirradiated rat
Table 15. Parameters for rat hippocampal neurogenesis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (Unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ψ</td>
<td>0.5 (day⁻¹)</td>
</tr>
<tr>
<td>θ₁</td>
<td>0.005</td>
</tr>
<tr>
<td>θ₂</td>
<td>0.05</td>
</tr>
<tr>
<td>θ₃</td>
<td>0.005</td>
</tr>
<tr>
<td>d₁</td>
<td>0.018 (day⁻¹)</td>
</tr>
<tr>
<td>d₂</td>
<td>0.16 (day⁻¹)</td>
</tr>
<tr>
<td>a₂</td>
<td>0.001 (day⁻¹)</td>
</tr>
<tr>
<td>a₃</td>
<td>0.07 (day⁻¹)</td>
</tr>
<tr>
<td>a₄</td>
<td>0.008 (day⁻¹)</td>
</tr>
<tr>
<td>xₐ</td>
<td>0.85</td>
</tr>
<tr>
<td>xₐ</td>
<td>0.15</td>
</tr>
</tbody>
</table>

3.6 Initial values for the irradiated cell populations

Initial values of neuronal cell populations of irradiated rats were estimated by interpolating Fig. 10 – 13. Data from Tada et al. for average age of 9 weeks (63 days) and data from Tan et al. for age of 12 weeks (84 days) were used. Estimated data are showed in the table 16.
Table 16. Initial values for the irradiated cell populations

<table>
<thead>
<tr>
<th>Cell population</th>
<th>Number of cell before irradiation (Age = 63 days)</th>
<th>Number of cell before irradiation (Age = 84 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSC</td>
<td>46950</td>
<td>32590</td>
</tr>
<tr>
<td>NB</td>
<td>10020</td>
<td>6941</td>
</tr>
<tr>
<td>ImN</td>
<td>39510</td>
<td>23170</td>
</tr>
<tr>
<td>GB</td>
<td>137700</td>
<td>118400</td>
</tr>
</tbody>
</table>

3.7 Fraction of weakly damaged NB cells ($\gamma_2$)

Data extracted from Tada et al. were used to estimate $\gamma_2$. Fraction of weakly damaged ImN cells ($\gamma_3$) and fraction of repairable weakly damaged cells ($\xi$) were kept at 0.2 and 0.9 respectively and variation of the fraction of NB cell population for different $\gamma_2$ values with radiation dose (Gy) were observed and compared with experimental data (Fig. 14). The value for $\gamma_2$ was estimated to be 0.02.

Figure 14. Dose dependent fraction of BrdU with varying fraction of weakly damaged NB cells.
3.8 Fraction of weakly damaged ImN cells ($\gamma_3$)

Data extracted from Tada et al. and Tan et al. were used to estimate $\gamma_3$. Fraction of weakly damaged NB cells ($\gamma_2$) and fraction of repairable weakly damaged cells ($\xi$) were kept at 0.02 and 0.9 respectively and variation of the fraction of ImN cell population for different $\gamma_3$ values with radiation dose (Gy) were observed and compared with experimental data (Fig. 15). The value for $\gamma_2$ was estimated to be 0.2.

![Figure 15. Dose dependent fraction of DCX with varying fraction of weakly damaged ImN cells.](image)

Furthermore, dose dependent fold change in number of apoptotic cells were plotted against dose (Gy) for different $\gamma_3$ values, while keep $\gamma_2$ and $\xi$ at 0.02 and 0.9 respectively (Fig. 16).
3.9 Fraction of repairable weakly damaged cells ($\xi$)

Dose dependent fraction of BrdU (Fig. 17), DCX (Fig. 18) and fold change in number of apoptotic cells (Fig 19) with varying fraction of repairable weakly damaged cells were plotted and the value of $\xi$ was estimated to be 0.8-0.99. For the model, the value was chosen as 0.9.
Figure 17. Dose dependent fraction of BrdU with varying $\zeta$.

Figure 18. Dose dependent fraction of DCX with varying $\zeta$. 
3.10 Apoptotic rates of heavily damaged cells ($v_2$, $v_3$ and $v_5$)

Apoptotic data from Tada et al. were used to estimate $v_2$, $v_3$ and $v_5$. By fitting experimental data with the Simulink model; rate of apoptosis of heavily damaged NB cells ($v_2$), rate of apoptosis of heavily damaged ImN cells ($v_3$) and disappearance rate of apoptotic cells from hippocampus ($v_5$) were successfully estimated (Table 17).

Table 17. Rates of apoptosis of heavily damaged cells

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (Day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$v_2$</td>
<td>3</td>
</tr>
<tr>
<td>$v_3$</td>
<td>5</td>
</tr>
<tr>
<td>$v_5$</td>
<td>5</td>
</tr>
</tbody>
</table>
3.11 $D_0$ of NB and ImN cells

Data obtained from Tada et al. were used to estimate $D_{02}$ and $D_{03}$ values for 63 days old rats (Table 16). However, for 84 days old rat $D_{02}$ could not be estimated due to lack of experimental data. Therefore, $D_{02}$ value for 84 days old rats were assumed to be similar to 63 days old rats. Data obtained from Tan et al. were used to estimate the value for $D_{03}$ of 84 days old rats (Table 18).

Table 18. $D_0$ values of neuroblasts and immature neurons

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tada (Age = 63 days)</th>
<th>Tan (Age = 84 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{02}$ (Gy)</td>
<td>1.09</td>
<td>1.09</td>
</tr>
<tr>
<td>$D_{03}$ (Gy)</td>
<td>2</td>
<td>0.48</td>
</tr>
</tbody>
</table>

3.12 Parameters for Rat Hippocampal Neurogenesis after Irradiation.

Parameter $\Phi$ and $\Gamma$ were similar to previously estimated mouse parameter values (Cacao & Cucinotta, 2016). Parameter value for the $\theta_{mg}$ could not be estimated. Therefore, assumed to be similar to mouse parameter value (Cacao & Cucinotta, 2016). Parameters for rat hippocampal neurogenesis after irradiation are given in Table. 19.
Table 19. Parameters for Rat Hippocampal Neurogenesis after Irradiation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tada</th>
<th>Tan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of the Rat in days</td>
<td>63</td>
<td>84</td>
</tr>
<tr>
<td>$D_{02}$ (Gy)</td>
<td>1.09</td>
<td>1.09</td>
</tr>
<tr>
<td>$D_{03}$ (Gy)</td>
<td>2</td>
<td>0.48</td>
</tr>
<tr>
<td>$\gamma_2$</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>$\gamma_3$</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>$\xi$</td>
<td>0.8-0.99</td>
<td>0.8-0.99</td>
</tr>
<tr>
<td>$v_2$ (day$^{-1}$)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>$v_3$ (day$^{-1}$)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>$v_5$ (day$^{-1}$)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>$\Phi$</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$\Gamma$</td>
<td>1.05</td>
<td>1.05</td>
</tr>
<tr>
<td>$\theta_{mg}$</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

3.13 Parameters for Effects of Activated Microglia

Data obtained from Greene-Schloesser et al. were used to estimate the parameters given in Table 20.
Table 20. Parameters for Effects of Activated Microglia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_0$</td>
<td>0.034</td>
</tr>
<tr>
<td>$A_1$</td>
<td>9.5 (Gy)</td>
</tr>
<tr>
<td>$B$</td>
<td>$-7 \times 10^{-5}$ (day$^{-1}$)</td>
</tr>
<tr>
<td>$C$</td>
<td>$-0.75 \times 10^{-6}$ (day$^{-2}$)</td>
</tr>
<tr>
<td>$\Lambda$</td>
<td>0.05 (day$^{-1}$)</td>
</tr>
</tbody>
</table>

3.14 Dose dependent response of hippocampal neurogenesis to acute radiation exposure

Dose dependent response of NB cells after 24 hours are plotted in Figure 20. Experimental data were extracted from Tada et al.

Figure 20. Dose dependent response of NB cells after 24 hours.
Dose dependent response of ImN cells after a week are plotted in Figure 21. Experimental data were extracted from Tan et al.

![Figure 21. Dose dependent response of ImN cells after a week](image1)

Dose dependent increase in apoptotic cells after 6 hours are plotted in Figure 22. Experimental data were extracted from Tada et al.

![Figure 22. Dose dependent increase in apoptotic cells after 6 hours.](image2)
3.15 Modeling dynamics of hippocampal neurogenesis after different doses of acute radiation: Long term effects

Simulink program generated, BrdU Fraction (Fig. 23) and DCX Fraction (Fig 24) of rats (Tada, Parent, Lowenstein, & Fike, 2000) from 0-240 days after acute exposure to different doses of radiation.

Figure 23. BrdU fraction of 63 days old rats from 0-240 days after acute exposure to different doses of radiation.
Figure 24. DCX fraction of 63 days old rats from 0-240 days after acute exposure to different doses of radiation.

3.16 Role of gliogenesis and activated microglia on hippocampal neurogenesis

Figure 25-28 represent the dose dependent neurogenesis of NSC, NB, ImN and GB after acute radiation exposure in the presence (Left side) and absence (Right side) of both activated...
microglia and shift to gliogenesis respectively. Parameters used are based on experimental data from Tada et al.

Figure 25. Dose dependent neurogenesis: Effect of gliogenesis on NSC after acute radiation exposure

Figure 26. Dose dependent neurogenesis: Effect of gliogenesis on NB after acute radiation exposure
Figure 27. Dose dependent neurogenesis: Effect of gliogenesis on ImN after acute radiation exposure

Figure 28. Dose dependent neurogenesis: Effect of gliogenesis on GB after acute radiation exposure.
Chapter 4: Discussion and Future Directions

This research follows the mathematical model of radiation induced changes to neurogenesis in young and adult mice (Cacao & Cucinotta, 2016) and applies it for young and adult rats. As mentioned in the “Introduction” neurogenesis occurs in subventricular zone (SVZ) and subgranular zone (SGZ) of all mammalian species throughout their lifespan (Altman & Das, 1965; Cucinotta, Alp, Sulzman, & Wang, 2014; Spalding et al., 2013; Taupin & Gage, 2002; Wei et al., 2012; Zhao, Deng, & Gage, 2008). Several studies in the past decades related to neurogenesis (Dietrich, Monje, Wefel, & Meyers, 2008; Greene-Schloesser et al., 2012; Mizumatsu et al., 2003; Monje, Mizumatsu, Fike, & Palmer, 2002; Monje, Toda, & Palmer, 2003; Monje & Palmer, 2003; Naylor et al., 2008; Raber et al., 2004; Rola et al., 2004; Sweet et al., 2014; Tada, Parent, Lowenstein, & Fike, 2000), have shown a strong association with cognitive changes after cranial radiotherapy. However there has not been a mathematical model to describe these observations.

The number of doses, fractionation schemes, age of the subjects, etc. that can be used in experiments are limited. However, by using a mathematical model, data can be extrapolated for other conditions.

A set of ordinary differential equations were used to describe the outcome of the main neuronal cell populations including neuronal stem cells, neuroblasts, glioblasts and immature neurons following exposure to radiation. However, we did not consider in this model the outcome of radiation exposure specifically on the migration of cells following neurogenesis. New neurons are believed to migrate only a very short distance into the granule layer in the SGZ. To consider the effects of radiation on the migration patterns we can develop partial differential equations or Monte Carlo methods. These can be used to extend the current model.
The age dependence of neuroblasts and immature neurons of unirradiated rats were observed using the data obtained from Merkley et al. Model data were well fitted (Fig. 10 and 11) with the experimental data and parameters for unirradiated conditions were successfully estimated (Table 15). Differentiation rate of NSC to NB ($d_1 = 0.018$ day$^{-1}$) and differentiation rate of NB to ImN ($d_2 = 0.16$ day$^{-1}$) of rats were observed to be higher than that of mice ($d_1 = 0.015$ day$^{-1}$ and $d_2 = 0.06$ day$^{-1}$). This observation is supported by the observation of Snyder et al. Rate of apoptosis of NB ($a_2 = 0.001$ day$^{-1}$) for rats were observed to be lower than mice ($a_2 = 0.008$ day$^{-1}$). This observation also agrees with the observations made by Snyder et al. However, the rate of apoptosis of ImN ($a_3 = 0.07$ day$^{-1}$) for rats was higher than mice ($a_3 = 0.022$ day$^{-1}$). This may be due to the limitation of our mathematical model, such as the exclusion of migration of new neurons. Migration of new neurons also contribute to the disappearance of ImN. However, in our model we only consider the apoptosis of ImN. Therefore, the value of $a_3$ is higher than the actual value. Other parameters for unirradiated conditions were assumed to be similar to mice parameters.

For irradiated conditions, cell damaged parameters such as fraction of weakly damaged cells ($\gamma$), fraction of repairable weakly damaged cells ($\xi$) and rate of apoptosis of heavily damaged cells ($\upsilon$) of NB and ImN were estimated from the experiments considered (Table 19). Fraction of weakly damaged NB cells ($\gamma_2$) for rat was estimated to be 0.02, whereas the value for mouse was 0.04. However, fraction of weakly damaged ImN cells ($\gamma_3$) for rat was found out be similar to the mouse. It was estimated to be 0.2. The “$\xi$” value was not well defined. By using dose fractionation variable dose rate experiments, we can improve the estimated value for $\xi$. However, such experiments could not be found. Rate of apoptosis of heavily damaged cells ($\upsilon_2 = 3$ day$^{-1}$, $\upsilon_3 = 5$ day$^{-1}$ and $\upsilon_5 = 5$ day$^{-1}$) of rats were found out to be different than mice ($\upsilon_2 = 14$ day$^{-1}$, $\upsilon_3 = 1.4$ day$^{-1}$ and $\upsilon_5 = 2.1$ day$^{-1}$). This may be due to the difference of their maturation (Snyder et al.,
As mentioned earlier, all four compartments of the model are age dependent. In the model we included equations to derive effects of radiation on neurogenesis depending on age. It was observed in the results that the age of both mice and rats was an important parameter in determining the effects of radiation on neurogenesis. Especially 1-2 months following radiation treatments in rats and 1-3 months following radiation treatments in mice. Figure 23 shows the dynamics of neuroblast cell population after irradiation in 63 days old rats. Just after irradiation the NB cell population is significantly reduced and survivability is dose dependent. Recovery of the cell population starts within a day after irradiation and somewhat recover within 12-15 days. Dynamics of immature neuron cell population was observed to be similar to NB cell population. However, ImN cell population takes more days to recover (Fig. 24).

Unlike the mouse study (Cacao & Cucinotta, 2016), immature neurons were observed to be radiosensitive (Table 18) for the experiments considered (Tada, Parent, Lowenstein, & Fike, 2000; Tan, Rosenzweig, Jaffray, & Wojtowicz, 2011). This maybe due to the fact that immature neurons in rats are maturing faster than that of mice. This assumption is supported by the observation of Snyder et al. However, the radio sensitivity of neuroblasts could not be observed due to the lack of experimental data. From the experiments used to extract data, no observation of radio sensitivity on proliferation or apoptotic rates were made. A noticeable difference in D03 values of rat (D03 for 63 days old rat is 2 Gy and 0.48 Gy for 84 days old rats) and mouse (D03 = 7.5 Gy) was observed. The maturation of ImN in mouse occur with a 3 week delay according to the observation made by Snyder et al., whereas in rats’ maturation of ImN occur almost immediately. Therefore, fast dividing (more radio sensitive than non-dividing cells) ImN cells in rats has a lower D03. D03 for rats was determined to be age dependent.
A similar approach as followed in the mouse study for negative feedback on NSC proliferation was made for the rat study (Cacao & Cucinotta, 2016). Proliferation of NSC depends on normal, as well as radiation damaged NSC, NB and ImN cell populations (Equation 18). Equations 11-14 describes the contributions of undamaged cells, weakly damaged cells and heavily damaged cells on the feedback. In the study of mouse, some parameters were found to be similar for NB and ImN cell populations ($\Phi_2 = \Phi_3 = \Phi$ and $\Gamma_2 = \Gamma_3 = \Gamma$) and therefore do not play a significant role. We thus assumed that similar conditions will hold for the rat study. The assumption was found to be true and those parameters did not differ from the mouse study.

Inflammatory response and neurogenic fate were described by equations 16 and 17 respectively. From the mouse study, it was found that the inflammatory response and neurogenic fate were more prominent after 1 month post irradiation. Same equations were used for the rat study and parameters for inflammatory response were successfully estimated from the data obtained from Greene-Schloesser et al. All the parameters were observed to be different than that of the mouse study. However, parameters for neurogenic fate were not estimated due to lack of experimental data. Therefore, those parameters were assumed to be similar to mice parameters. Effects of gliogenesis on the four cell populations are shown in Figure 25-28. When gliogenesis is absent, hippocampal neurogenesis fully recover within 1-2 months in rats.

The main goal of this study was to apply the mathematical model used on mice for rats and compare the differences in two species. The model was successfully developed using Matlab Simulink. The program has the capability of predicting alterations to neurogenesis following exposure to radiation in rats. Initially, parameters of mice were used to build the Simulink model. Changes in parameters were made to fit the experimental data extracted for rats. In the end parameters for unirradiated and irradiated conditions were successfully estimated. Some
parameters could not be estimated due to the lack of experimental data and were assumed to be similar in values to mice. Also, unlike the mouse study, data for the same strain of rats could not be obtained. Therefore, an assumption was made that all strains of rats exhibit similar dynamics in age related neurogenesis.

For future studies we are expecting to study the differences in rat strains as well as differences in male and female rats. This study was only focused on alterations in neurogenesis after acute exposure. In future studies we hope to focus on studying the alterations in neurogenesis after fractionated exposures. Furthermore, the study can be extended to consider high-LET induced modifications of neurogenesis (Cacao & Cucinotta, 2016), provided that such data can be obtained. Also the experiments use different protocols for labeling (how many times, which days, etc.). This could be modeled in future by making study of how labeling last relative to cell times for different cell types. Modeling of mechanisms of activation of microglia is also needed and what sustain it for many months.
References


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