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Cell proliferation, cell death and total granule cell-number in the dentate gyrus in the hippocampus of the four-striped mouse (Rhabdomys pumilio) and common mole-rat (Cryptomys hottentotus)

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CELL PROLIFERATION, CELL DEATH AND TOTAL GRANULE CELL-NUMBER IN THE DENTATE GYRUS IN THE HIPPOCAMPUS OF THE FOUR-STRIPED MOUSE *(RHABDOMYS PUMILIO)* AND COMMON MOLE-RAT *(CRYPTOMYS HOTTENTOTUS)*

Olatunbosun Oriyomi Olaleye



WITS
UNIVERSITY

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, 2016

DECLARATION

I, Olatunbosun Oriyomi Olaleye, declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

…… Signature of Candidate

……day of ……2016

DEDICATION

TO THE GENTLE VOICE THAT ASSURES ME.

THE THICK CLOUD THAT SHIELDS ME

MY DAYSPRING AND COMPANION IN LONLELY NIGHTS

MY WISDOM, STRENGTH AND STAFF

BLESSED JESUS,

UNTO YOU I SUBMIT THIS CROWN

AND IN LOVING MEMORY OF MY LATE FATHER, SAMUEL ABIODUN OLALEYE

AND MY GREAT UNCLE, CHIEF EBENEZER SOLANKE SOTIMIRIN.

PUBLICATION ARISING FROM THE STUDY

Olaleye O.O. and Ihunwo A.O. Adult neurogenesis in the four-striped mouse (*Rhabdomys pumilio*). *Neural Regeneration Research*. 2014; 9 (21):1907-1911.

PRESENTATIONS ARISING FROM THIS STUDY

Postal presentation at the Faculty of Health Sciences Research day Expo 2014 titled:

Correlation of cell death and total granule cell number in the four-striped mouse *(Rhadomys pumilio)* and common mole-rat *(Cryptomys hottentotus).*

ABSTRACT

This study investigated a wild and a captive-bred wild animal models to provide evidence that adult neurogenesis occurs in the brain of the four-striped mouse (FSM) *(Rhabdomys pumilio)* and common mole-rat (CMR) *(Cryptomys hottentotus)* and also estimate the total granule cell number in the dentate gyrus (DG) of the hippocampus as a baseline for comparing cell proliferation and cell death in the two species. Adult male four-striped mouse (n=6) and common mole-rat (n=7) were used to investigate for cell proliferation, cell death and immature neurons. The animals were anaesthetized and transcardially perfused with saline followed by paraformaldehyde fixative (4% paraformaldehyde in 0.1 M phosphate buffer), pH 7.4. Brains were removed and post fixed in the same fixative overnight. Following equilibration in 30% sucrose in PB, the left hemisphere was cut at 50 µm thickness, frozen serial sagittal sections. Ki-67 immunohistochemistry was carried out to identify proliferative cells and Doublecortin (DCX) staining for immature neurons. The right hemispheres of the brains were plastic embedded and sections cut at 20 µm, were subjected to Giemsa staining for the estimation of total granule and pyknotic cell numbers in the dentate gyrus. Ki-67 immunostaining confirmed adult cell proliferation in the dentate gyrus (DG) of the hippocampus of the four-striped mouse and common mole-rat. DCX immunopositive cells confirmed presence of immature neurons in the DG of the hippocampus of four-striped mouse and common mole-rat. There was no difference in the category of DCX positive cells observed in the DG of four-striped mouse and common mole-rat as both were predominantly in the postmitotic stage. The mean Ki-67 immunopositive cell number, an indication of cell proliferation was 993.33 ± 683.4 in the four-striped mouse and 190.83 ± 209.51 in the common mole-rat. The mean pyknotic cell number, an indication of cell death was 199.17 ± 92.8 in the four-striped mouse and 227.5 ± 108.77 in the common mole-rat. The estimated total granule cell number in the dentate gyrus was between 1.3×10^6 and 2.0×10^6 in the four-

striped mouse and 0.6 x 10^6 and 1.1 x 10^6 in the common mole-rat. The Gundersen coefficient of error was between 0.05 - 0.06 in the four-striped mouse and 0.05 and 0.078 in the common mole-rat. The estimated total granule cell number in the four-striped mouse and common mole-rat represents over a 50 times increase compared to the values reported from laboratory rodents. Despite the larger brain size and body weight in the common mole-rat, the four-striped mouse had about five times the number of cell proliferation rate. A statistically significant higher cell proliferation rates and granule cell numbers were seen in the four-striped mouse compared to common mole-rat ($p < 0.03$ and 0.00). There was no significant difference in the immature neuron and pyknotic cell ($p=0.43$ and 0.70) respectively. The rate of immature neurons and cell death was higher in the common mole-rat than in the four-striped mouse. There was no significant correlation between cell proliferation, cell death and Total granule cell number in both animals. Regression analysis did not yield any significant relationship between these variables except between cell proliferation and cell death in common mole-rat.

In conclusion, despite the complex burrowing system in the common mole-rat with associated higher cognitive, learning and memory function, similar adult neurogenesis was found in both four-striped mouse and common mole-rat. The cell proliferation and cell death does not have any effect on the total granule cell number in the four-striped mouse and common mole-rat.

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1. CHAPTER ONE

1.0. INTRODUCTION

We have believed for generations that the adult brain does not create new brain cells but that "a neuron lost is lost forever". However, new neurons are continuously added throughout life (Akers *et al.*, 2014). Researches over the last four decades have produced growing evidence that the adult human brain creates new neurons, a process known as neurogenesis. Neurogenesis has been defined as the ability of brain cells to regenerate themselves. Majority of these neurons tend to survive and incorporates into the working brain, which suggests the potentiality for a self-healing brain. Brain cells that possess regenerative abilities are called neural stem cells (Okano, 2002); these being self-renewing, multipotent cells that normally generate the main phenotypic cells of the nervous system namely neurons, astrocytes and oligodendrocytes (Taupin, 2006).

Through years of experimentation and observation, researchers have learned and described how the diverse cells of the brain are produced. It is now well established that the adult mammalian brain has the capacity to produce new neurons. Adult neurogenesis occurs predominantly in two regions of the brain, the rostral subventricular zone (SVZ) of the lateral ventricle and the sub-granular zone of the dentate gyrus (DG) of the hippocampus (Kaplan and Hinds, 1977; Kaplan and Bell, 1984; Kempermann *et al.*, 1997; Schauwecker, 2006). The new neurons produced in the SVZ migrate to the olfactory bulb (OB) via the rostral migratory stream (Ming and Song, 2011; Bergmann *et al.*, 2015). However, there is a continuous addition of the new neurons in the striatum of humans but these new neurons are not evident in humans' OB compared to other mammals (Bergmann *et al.*, 2015). These cells are believed to participate in odorant identification, memory formation and

social interactions (Ming and Song, 2011; Bergmann *et al.,* 2015). In contrast to other mammals, adult neurogenesis in the OB is absent in humans (Rochefort *et al.,* 2002; Bergmann *et al.,* 2015). The new neuronal cells in the hippocampus are usually produced in the subgranular region and gets incorporated in the granular layer (Kempermann *et al.,* 2002; Bergmann *et al.,* 2015). These new cells are believed to be very important for brain function and disease (Spalding *et al.,* 2013; Bergmann *et al.,* 2015).

The concept of adult neurogenesis began as early as 1912 when scientists discovered that mitotically active cells reside in the mammalian central nervous system throughout life (Watts *et al.,* 2005). In the 1930s and 1940s, cytological investigations revealed the presence of these cells in the postnatal and adult rodent brain (Alvarez-Buylla and Garcia-Verdugo, 2002; Lennington *et al.,* 2003). In 1965, Joseph Altman and colleague reported that adult neurogenesis occurs in discrete areas such as the dentate gyrus (DG) of the hippocampus and lateral wall of the subventricular zone (SVZ) of the adult brain in rodents (Watts *et al.,* 2005). These discrete areas, DG and SVZ, were referred to as the potential sites (Ihunwo and Pillay, 2007). Other neurogenic or non-neurogenic regions with neurogenic potentials are striatum (Bergmann *et al.,* 2015), substantia nigra (Zhao *et al.,* 2003), third ventricle (Xu *et al.,* 2005), spinal cord (Temple and Alvarez-Buylla, 1999), amygdala (Bernier *et al.,* 2002), cerebral cortex (Takemura, 2005; Olaleye, 2011), olfactory bulb (Gritti *et al.,* 2002; Bergmann *et al.,* 2015) and dorsal vagal complex (Moyse *et al.,* 2006). With the advent of new methods for labelling and identifying dividing cells, (e.g. Bromodeoxyuridine (BrdU) labelling, retroviral labelling and confocal microscopy), investigators have since confirmed the findings of Joseph Altman and the fact that adult neurogenesis also occurs in all primates, including humans (Watts *et al.,* 2005). Several studies have shed light on adult neurogenesis in both laboratory and wild animals (Amrein *et al.,* 2011). The concept of adult neurogenesis is now widely accepted

by the scientific community (Kaplan and Hinds, 1977; Kaplan and Bell, 1984; Kempermann *et al.,* 1997; Alvarez-Buylla and Garcia-Verdugo, 2002; Lennington *et al.,* 2003; Zhao *et al.,* 2003; Takemura, 2005; Watts *et al.,* 2005; Luzzati *et al.,* 2006; Schauwecker, 2006). Amrein *et al.,* (2004a) compared the rate of adult cell proliferation in the DG of different species of wild and laboratory rodents and reported a high level of proliferation rate in wild animals than laboratory animals. Hence, adult cell proliferation is believed to play a major role in spatial memory and learning (Amrein *et al.*, 2007). Hence, the need for the study of adult hippocampal neurogenesis in both captive bred four-striped mouse and wild caught common mole-rat. This is necessary to have a broad understanding of the newly proliferated cells in the dentate gyrus (DG) in proportion to cell death using total granular cell number of the DG as a reference point to enable the understanding of correlative assessment of cell survival in relation to cell death.

1.1. Objectives of the study

1.1.1. Main objective

To compare cell proliferation, cell death and total granule cell number in the dentate gyrus in the hippocampus of the four-striped mouse *(Rhabdomys pumilio)* and common mole-rat *(Cryptomys hottentotus).*

1.1.2. Specific objectives

I. To estimate the number of Ki-67 positive cells in the dentate gyrus of the hippocampus in the four-striped mouse and common mole-rat.

II. To estimate the pyknotic cell number in the dentate gyrus of the hippocampus in the four-striped mouse and common mole-rat.

III. To categorise the DCX positive neurons in the DG of the hippocampus in the four-striped mouse and common mole-rat.

IV. To estimate the total granule cell numbers in the dentate gyrus of the hippocampus in four-striped mouse and common mole-rat using stereological principle of the optical fractionator.

V. To correlate cell proliferation with cell death and the estimate of total granule cell number using Ki-67 positive cell and pyknotic cell numbers.

VI. Compare the results between four-striped mouse and common mole-rat with reports in other laboratory and wild rodents.

1.2. Limitation of the study

- Plastic embedding technique was quite tenacious hence reduced the sample size by cracking upon sectioning on the microtome.
- The number of four-striped mouse and common mole-rat (n±6) was a limiting factor in the analysis of the stereological values of estimated total granule cell and statistical numbers obtained for cell proliferation and cell death.

1.3. Literature review

Adult neurogenesis is a complex process involving four stages; proliferation, survival, differentiation and functional integration of new cells into the hippocampus (Plumpe *et al.*, 2006; Bordiuk *et al.*, 2014). Neurogenesis has been reported in the mammalian brain of rats (Merrill *et al.*, 2003; Amrein *et al.*, 2004b), mice (Amrein *et al.*, 2004a; Hauser *et al.*, 2009; Olaleye, 2011; Olaleye and Ihunwo, 2014), tree shrews (Gould *et al.*, 1997), guinea pigs (Altman and Das, 1967), rabbits (Gueneau *et al.*, 1982), cats (Wyss and Sripanidkulchai, 1985), monkeys (Rakic and Nowakowski, 1981; E. Gould *et al.*, 1999a; Kornack and Rakic, 1999; Rakic, 2002) and humans (Eriksson *et al.*, 1998; Spalding *et al.*, 2013; Bergmann *et al.*, 2015). It is low or absent in bats (Amrein *et al.*, 2007).

The cellular and molecular mechanisms that regulate adult neurogenesis remain unclear (Schauwecker, 2006). However, the process is believed to be modulated by a variety of factors (Grote and Hannan, 2007) including glutamate receptor activation (Cameron *et al.*, 1995; Gould *et al.*, 1997; Cameron *et al.*, 1998; Bernabeu and Sharp, 2000), dietary restriction (Lee *et al.*, 2000; Lee *et al.*, 2002a; 2002b), growth factors (Palmer *et al.*, 1995; Scharfman *et al.*, 2005), stress (Brunson *et al.*, 2005; Nichols *et al.*, 2005) and neuronal injury (Parent, 2003; Cooper-Kuhn *et al.*, 2004). Enriched environments (Kempermann *et al.*, 1997), running wheel exercise (van Praag *et al.*, 1999), hippocampal-dependent learning (Gould *et al.*, 1999a), and dietary restriction (Lee *et al.*, 2002b), all increase neurogenesis in the adult hippocampus, while Stress (Gould *et al.*, 1997, 1998) and social isolation (Lu *et al.*, 2003; Lievajová *et al.*, 2011) reduces neurogenesis. Albeit, Hauser *et al.*, (2009) reported that running does not have an effect on adult hippocampal cell proliferation. Even though estradiol alter hippocampal neurogenesis in the adult female rodents, a decrease in hippocampal neurogenesis do not always correlate with the development of learned helplessness in male rats (Vollmayr *et al.*, 2003; Pawluski and

Galea, 2006). Learned helplessness which is the maladaptive refraining displayed by animals and people resulting from skill with irrepressible actions (Seligman and Peterson, 2001).

Likewise adult neurogenesis occur in primates (Gould *et al.*, 1999b; Bernier *et al.*, 2002; Rakic, 2002) and humans (Eriksson *et al.*, 1998; Spalding *et al.*, 2013; Bergmann *et al.*, 2015). The concept of adult neurogenesis is now widely accepted in the scientific community (Kaplan and Hinds, 1977; Kaplan and Bell, 1984; Alvarez-Buylla and Garcia-Verdugo, 2002; Lennington *et al.*, 2003; Zhao *et al.*, 2003; Takemura, 2005; Watts *et al.*, 2005; Luzzati *et al.*, 2006). Recently the presence of adult hippocampal neurogenesis has been reported in over seventy mammalian species from thirteen different taxonomy (Patzke *et al.*, 2015). Table 1 below shows a list of some strains of rats and mice investigated to date. All species differ significantly in the rate at which new cells are continuously added to the DG (Amrein *et al.*, 2004b).

Table 1.1: List of some strains of rats and mice investigated to date.

Rodent	**Site**	**Reference**
Rats		
Sprague- Dawley	III VEN, SN	Frielingsdorf *et al.*, 2004; Xu *et al.*, 2005
Wistar	DG, CTX	Seaberg and van der Kooy, 2002; Takemura, 2005
Fisher 344	SEP/STR	Palmer *et al.*, 1995
Mice		
CD1	DG, SC	Weiss *et al.*, 1996; Kempermann *et al.*, 1997; Seaberg and van der Kooy, 2002
C57BL/ 6	SN, DG, SGZ	Kempermann *et al.*, 1997; Zhao *et al.*, 2003; Schauwecker, 2006
A/J	SGZ	Kempermann and Gage, 2002
BALB/ c	SGZ	Kempermann *et al.*, 1997
C3H/ H3J	SGZ	Kempermann *et al.*, 1997
DBA/ 2J	SGZ	Kempermann *et al.*, 1997
129/ SVJ	DG	Kempermann *et al.*, 1997
Albino	OB	Gritti *et al.*, 2002
FVB/NJ	DG	Schauwecker, 2006
Wood mouse	DG	Hauser *et al.*, 2009
Four-striped mouse	DG, SVZ, OB, SN	Olaleye, 2011; Olaleye and Ihunwo, 2014
Common mole-rat	DG, SVZ, OB, CTX, SN	Olaleye, 2011

Key: OB, olfactory bulb; DG, Dentate gyrus; SN, Substantia nigra; SC, Spinal cord; SEP\STR, Septum and Striatum; CTX, Cerebral Cortex; III VEN, Third ventricle; SGZ, Subgranular zone.

1.4. Stem Cells and their characteristics

Stem cells are cells that can divide to give rise to a new copy of itself and at least on other specialized cell type (Mummery *et al.*, 2011). Stem cells can self-renew and differentiate or specialize (Ihunwo and Pillay, 2007; Mummery *et al.*, 2011). Stem cell characteristics such as undifferentiating, proliferation, self-maintenance and differentiation vary in different physiological systems and it is still debated as to whether stem cells in the CNS adhere to all these criteria (Ihunwo and Pillay, 2007). A stem cell differs from progenitor cells in two essential ways: they are multipotent and self- renewing. There are embryonic, foetal, and adult types of stem cells that can also be grouped into three categories: totipotent, pluripotent, and multipotent cells (Hou and Hong, 2008). The totipotent cells are stem cells that have the ability to give rise to a whole organism, pluripotent stem cells can give rise to all body cell types (e.g. embryonic stem cells) and multipotent stem cells give rise to only some specialized cells (e.g. adult neural stem cells).

1.5. Adult neural stem cells

Adult neural stem cells are found in the adult mammalian brain including humans. They are predominantly found in the rostral subventricular zone lining the lateral ventricle and part of the dentate gyrus of the hippocampus called the sub-granular zone (Lennington *et al.*, 2003; Taupin, 2006). New neurons are continually born in these two places throughout adulthood (Lennington *et al.*, 2003); but adult neurogenesis also occurs in other areas of the brain such as the septum and striatum, walls of the third ventricle, spinal cord, amygdala, cerebral cortex, olfactory bulb and the subcortical white matter (Reynolds and Weiss, 1992; Zhao *et al.*, 2003; Luzzati *et al.*, 2006). The discovery of neurogenesis and neuronal replacement in the adult brain is likely to affect the ways in which we think about neurological diseases such as Parkinson's and Huntington's diseases and neuronal repairs

(Schauwecker, 2006; Abdipranoto *et al.*, 2008; Bordiuk *et al.*, 2014; Bergmann *et al.*, 2015). The discovery of adult neuronal stem cells strengthens the hope that there will be therapeutic benefits (Jun *et al.*, 2012; Latchney and Eisch, 2012). Glutamate receptor activation (Cameron *et al.*, 1995; Gould *et al.*, 1997; Cameron *et al.*, 1998; Bernabeu and Sharp, 2000), dietary restriction (Lee *et al.*, 2000; Lee *et al.*, 2002a & 2002b), growth factors (Palmer *et al.*, 1995; Kempermann *et al.*, 1997; Scharfman *et al.*, 2005), stress (Brunson *et al.*, 2005; Nichols *et al.*, 2005) and neuronal injury (Parent, 2003; Cooper-Kuhn *et al.*, 2004) are believed to modify adult neurogenesis. However, the cellular and molecular mechanisms that control adult neurogenesis remain uncertain (Schauwecker, 2006).

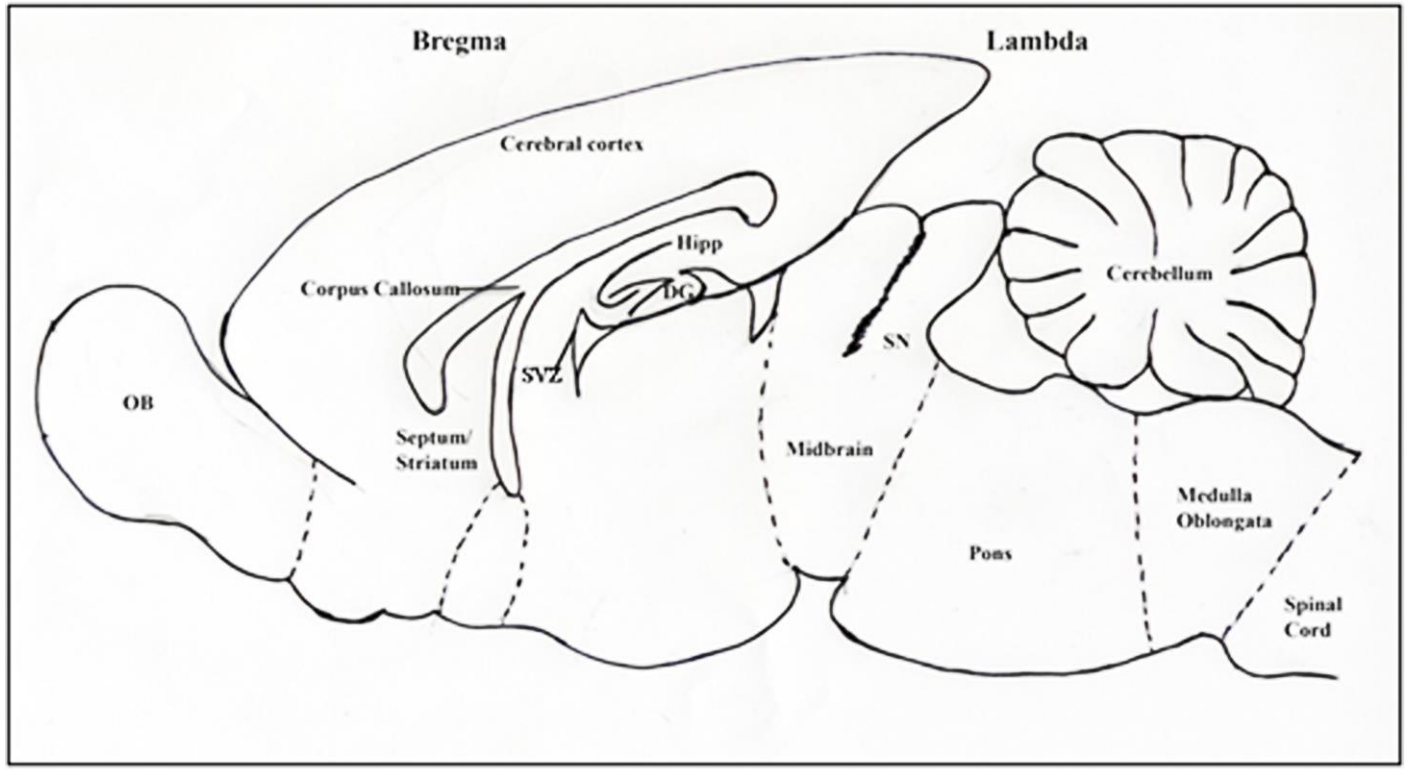
1.6. Established neurogenic sites

The subventricular zone (SVZ) (Fig 1) is considered the largest active neurogenic site in the brain (Schauwecker, 2006). Studies have shown the SVZ to be a source for cortical and subcortical neurons (Alvarez-Buylla and Garcia-Verdugo, 2002; Watts *et al.*, 2005). The SVZ has two precise layers of cells: the first is a monolayer of multiciliated cells lining the lateral ventricle called the ependymal layer; and the second layer is a 2-3 cell layer thick area adjacent to the ependymal layer called the subependymal layer. The SVZ neuroblast cells migrate a long distance to the olfactory bulb through a network of interconnecting pathways that become confluent at the rostral margin of the lateral ventricular wall to form the rostral migratory stream (RMS) (Watts *et al.*, 2005).

Neurogenesis persists in the adult subgranular zone in the dentate gyrus (DG) of the hippocampus, which generates neural precursor cells that exhibit stem cell properties (Eriksson *et al.*, 1998; Taupin, 2006; Bordiuk *et al.*, 2014; Bergmann *et al.*, 2015). In the rodent DG, as many as 9000 neuronal cells are produced everyday, contributing 0.01% of

the granule cell population per day (Cameron and Mckay, 2001). Most of these cells undergo apoptosis with only a restricted number of cells that go on to mature (Biebl *et al.*, 2000). The matured cells can survive for an extended amount of time, and this may lead to a permanent replacement of cells (Taupin, 2006). Hippocampal neurogenesis occurs over the lifetime of a mammal and it appears to maintain normal hippocampal function of brain tissues (Eriksson *et al.*, 1998; Cameron and Mckay, 2001). There is now evidence of adult neurogenesis in the subventricular zone and dentate gyrus of the four-striped mouse (FSM) and common mole-rat (CMR) (Olaleye, 2011; Olaleye and Ihunwo, 2014).

Figure 1.1 A sagittal section of an adult rodent brain showing the different regions of the brain.



A schematic diagram of the saggital section of the rat brain showing the different regions of the brain. Drawing modified from Paxinos and Watsons, (2006) 5th ed. **DG-** dentate gyrus, SVZ-subventricular zone, **SN**-substantia nigra, **Hipp-** hippocampus, **OB-** olfactory bulb.

1.7. Other Adult Neurogenic Sites

Adult neurogenesis does not only occur in the SVZ and the DG of the brain but also in other areas of the mammalian central nervous system (Reynolds and Weiss, 1992; Ihunwo and Pillay, 2007; Olaleye, 2011; Olaleye and Ihunwo, 2014). Fibroblast growth factor (FGF) was reported to stimulate proliferation of neuronal progenitors in the septum and striatum in rodents (Palmer *et al.*, 1995). The hippocampus and SVZ yield more established colonies and a larger number of progenitors than the septum and striatum. The substantia nigra pars compacta (SNPC) is another region where adult neurogenesis is said to be present as evidenced by the slow turnover of dopaminergic projection neurons in the adult rodent brain (Zhao *et al.*, 2003). However, no new dopaminergic neurons in the adult mammalian SNPC was found (Frielingsdorf *et al.*, 2004).

Evidence provided by (Bernier *et al.*, 2002) indicates that neurogenesis is present in the amygdala and surrounding cortex of adult monkeys with the occurrence of a temporal migratory stream (TMS), in addition to the RMS. From reports of multipotent stem cells in more caudal regions of the neuroaxis such as the spinal cord (SC), it has become clear that stem cells are present in all parts of the central nervous system (CNS) (Weiss *et al.*, 1996; Temple and Alvarez-Buylla, 1999).

1.8. Non- Neurogenic Regions with Neurogenic Potential

Adult neural stem cells reside in regions considered to be non-neurogenic, such as the cerebral cortex (Pagano *et al.*, 2000; Gritti *et al.*, 2002). Takemura, (2005) provided evidence that active neurogenesis persists within the white matter beneath the temporal neocortex (a previously overlooked neurogenic region) in the adult rat brain and detected the evidence of neuron production within the subcortical white matter. Olaleye, (2010) also

confirm the presence of immature neurons in the cortical regions of the common mole-rat. From these results, cell genesis, death, and migration persist in a restricted sub-region of the adult white matter. Arsenijevic *et al*.(2001), showed that multipotent precursor cells exist in the adult human cerebral cortex and Moyse *et al.* (2006) confirmed neurogenesis in the dorsal vagal complex of the brain stem of rat (a major center for autonomic reflexes). Ihunwo and Pillay (2007) provided a detail review of active and potential neurogenic sites in the adult mammalian brain (Table 1).

Table 1.2: A summary of sites of neurogenesis in adult mammalian brains (from Ihunwo and Pillay, 2007).

Site	SVZ	DG	SEP\ STR	SN	III VEN	SC	AMG	CTX	OB\ RE	DVC
Rats	*	*	*		*			*	*	*
Mice	*	*	*	*	*	*				
Rabbits	*	*	*							
Human	*	*						*	*	
Squirrel Monkeys	*	*					*			
Macaque monkey	*	*					*	*		

Key: SVZ, subventricular zone; **DG**, Dentate gyrus; **SEP/ STR**, Septum and Striatum; **SN**, Substantia nigra; **III VEN**, Third Ventricle; **SC,** Spinal Cord; **AMG**, Amygdala; **CTX**, Cerebral cortex; **OB/ RE**, Olfactory bulb, **DVC**, Dorsal Vagal Complex.

1.9. Correlation of cell proliferation, cell death and total granule cell number in the dentate gyrus of hippocampus

Kempermann et al. (1997) investigated four different strains of laboratory mice (Table 1.2) and concluded that adult hippocampal neurogenesis is differentially influenced by the genetic background of the species. Amongst these species, proliferation was highest in C57BL/6 and survival rate of newborn cells was highest in CD1. However, no significant differences in the relative ratio of neurogenesis and gliogenesis were observed in the dentate gyrus of the FVB/ NJ and C57L/ 6J strains of mice (Schauwecker, 2006). Olaleye (2010), provided evidence that in the four-striped mouse the other potential sites observed were striatum, substantia nigra, olfactory bulb and in the common mole-rat, striatum, substantia nigra, olfactory bulb cerebral cortex depending on the cell proliferation markers that were used. However, the correlation of the rate of cell proliferation, cell death and total granule cell number is the focus of this study. The same correlation was applied to common mole-rat.

Adult cell proliferation is believed to be balanced by cell death to maintain a constant number of cells within the dentate gyrus (Amrein *et al.,* 2004a; Bordiuk *et al.*, 2014), though it was reported that granule cell numbers does not increase under a laboratory setting (Rapp and Gallagher, 1996; Rasmussen *et al.,* 1996; Merrill *et al.,* 2003). It was reported that there is a down regulation of cell proliferation in different strains of laboratory rodents (Kuhn *et al.,* 1996; Gould *et al.*, 1999a &1999b; Lichtenwalner *et al.*, 2001), primates (Gould *et al.,* 1999b) and bats (Amrein *et al.*, 2007) providing the need to quantify the number of pyknotic cells (Amrein *et al.,* 2004a). Accordingly, there is need to quantify granule cell in the adult mammalian hippocampus (Amrein *et al.,* 2004a & 2004b; Hauser *et al.*, 2009). It is believed that granule cell quantity does not increase with age

(Rapp and Gallagher, 1996; Rasmussen *et al.*, 1996; Merrill *et al.*, 2003) which causes adult cell proliferation to be regulated by cell death (Amrein *et al.*, 2004a). Conclusively, there is down-regulation of cell proliferation and cell death in laboratory rats (Kuhn *et al.*, 1996; Gould *et al.*, 1999b; Lichtenwalner *et al.*, 2001) and bats (Amrein *et al.*, 2007) concurrently. Arguably, cell proliferation can lead to an increase in granule cell number (Amrein *et al.*, 2004a; Bordiuk *et al.*, 2014; Bergmann *et al.*, 2015) which is species and age specific (Kempermann *et al.*, 1997).

Presently, there is no correlation between cell proliferation, cell death and total granule cell number in the four-striped mouse *(Rhabdomys pumilio)* and the common mole-rat *(Cryptomys hottentotus)*. The granule cell layer is the final destination of the surviving proliferating cells (Ki-67) from the subgranular layer. In the process of migration and maturation, some cells undergo cell death (pyknotic cells). Therefore, the estimated granule cell number becomes a baseline to compare the relationship between cell proliferation and cell death in the four-striped mouse and common mole-rat. Herein lies the significance of this study with confirmation of presence of adult hippocampal neurogenesis in the four-striped mouse already established (Olaleye and Ihunwo, 2014) and in the common mole-rat (Olaleye, 2011).

2. CHAPTER TWO

2.0. Materials and Methodologies

2.1. Experimental Animals

Six adult four-striped mouse and seven male adult common mole-rat were used for the study. The animals were treated and used according to the University of the Witwatersrand Animal Ethics and Screening Committee (AESC Clearance No: 2007/45/03) guidelines. The four-striped mice were captive reared at the Central Animal Service (CAS) Unit of the University of the Witwatersrand, Johannesburg, South Africa but had wild caught ancestors. The common mole-rats were wild caught from a golf course in Pretoria, South Africa and transported to the CAS at the medical school of the University of the Witwatersrand. All animals used were classified as adults based on their body weight, dentition and sexual maturity. They were kept under standard laboratory conditions with a 14:10 hourly light-dark cycle with lights on at 6 am in the morning for the four-striped mouse and 12 hourly light and dark for the common mole-rat. Room temperature was between 20-24 ^{0}C and 30 %- 60 % relative humidity. A 40 X 12 X 25 cm (length, height and width) Labotec cages (Labotec, Halfway House, South Africa) with saw dust or wood waste shavings as litter and hay as nesting material was used to house these animals. Experimental animals were maintained under standard laboratory conditions and fed laboratory pellets/ chow and water *ad libitum*. The animals were allowed to acclimatize for three weeks before they were sacrificed.

2.1.1. Four-striped mouse (*Rhabdomys pumilio*)

The four-striped mouse belongs to the kingdom Animalia; phylum is Chordata in the class of Mammalia and in the order of the Rodentia. Its family is Muridae and genus is Rhabdomys. They are widely distributed in Southern Africa, occurring in different habitats, such as grassland, marsh, forests, semi- deserts and deserts (Schradin and Pillay, 2004). The four-striped mice, *Rhabdomys pumilio*, are small diurnal murid rodent that are usually found in colonies. It is easily identified by the four distinct dark longitudinal stripes running the length of the back (Figure 2.1).



Figure 2.1 Photograph of the four-striped mouse (Rhabdomys pumilio) by Selvakumar. Internet accessed 12 June 2010.

Unlike most rodents, the four-striped mouse displays a diurnal bimodal activity pattern with its activities mainly in the mornings and evenings. It has a reduced activity in the afternoon or midday period. It is an omnivorous animal and has the ability to survive without water provided its diet contains a minimum of 15% water in it (Willan and Meester, 1989). It has an extreme plasticity in habitat preference which gives the reason for

it widespread distribution throughout Southern Africa (Rambau *et al.*, 2003). Colour of the stripe varies from dark brown to grey-white. The four-striped mouse have a body mass ranging from 40-80 g (Maini, 2003; Schradin and Pillay, 2004) and a small brain with an average mass of about 0.64 g (Schradin and Pillay, 2004).

It breeds seasonally usually from spring to autumn (Schradin and Pillay, 2003). Their gestation period is 22-23 days. Their females, that are free-living, give birth to approximately five pups: captive females have slightly larger litter 6-7 (Pillay, 2000). Their pups begin to consume solid food at ten days after birth and leaving their nest from twelfth day after gestation. Weaning starts at around the sixteenth day after birth. Sexual maturity is reached at around fifth to sixth week of life (Schradin and Pillay, 2004; Brooks, 2009) which depends on environmental and social factors as well as its development status (Pillay, 2000).

The four-striped mouse has a flexible social organization and mating system which is controlled mainly by resource availability and population density. In the arid habitat, they can be described as territorial, group-living and solitary foragers that display bi-parental care (Schradin and Pillay, 2005). In mesic, grassland habitats and semi-succulent thorny scrub, four-striped mouse are solitary, with their females raring their offspring on their own. Both sexes maintain their territory which overlaps their opponent's (Schradin and Pillay, 2005). In captivity, males from both mesic and xeric populations display parental care. Four-striped mouse from the southwestern regions of southern Africa are slightly larger than the northern regions.

2.1.2. Common mole-rat (*Cryptomys hottentotus*)

The common mole-rat belongs to the kingdom Animalia; phylum is Chordata in the class of Mammalia and in the order of the Rodentia. Its family is Bathyergidae and genus is Cryptomys. The common mole-rat, *Cryptomys hottentotus*, (Figure 2.2) is a burrowing rodent that is found in Africa, mainly in southwest Cape Province in South Africa. Also found in other parts of Africa like Lesotho, Malawi, Mozambique, Swaziland, Tanzania, Zambia and Zimbabwe. They have a reduced visual function. They have an average body length of 10.5-16.5 cm with tail length of 1.2-3.8 cm. It has a thick fur with many different colours. Their body shape is cylindrical with short limbs. They have a chisel-like incisor that is used for digging (Bennett and Faulkes, 2000; Bruening and Bruening, 2001). They have an average body mass of 120.5 g, and a brain mass on average of 1.26 g (Sims *et al.*, 1980). They have a unique characteristic of having one reproductive pair, consisting of the largest female and male in one group. Mating occurs between the months of September and October They breed seasonally (October–January), the gestation period is about 81 days with 2-5 litters. They dwell in small colonies (up to 14 individuals) that are comprised of breeding female, her consorts, and their non-breeding offspring (the workers). Common mole-rat reaches sexuality at about 450 days. Females maintain reproductive function during non-reproductive months.

Common mole-rats are fossorial mammals that can live in wide range of substrates. They are herbivorous. They are wide spread and they show a sign of localization due to soil requirements. Their pattern of borrowing optimizes their access to food whereas it has a negative economic impact to man in that it damages properties but also improves soil drainage and turnover as a positive view.

Common mole-rats have the ability to generate their own heat and keep their body temperature above ambient temperature, which gives them an added advantage to survive any weather. They have lower individual body masses in arid environment that helps with energy conservation. They also have long sensory hairs, vibrissae, which stand out from their fur covering their body. Common mole-rats are social creatures that live in family units of up to 14 per group. They exhibit specialized behaviour and cooperative care of the young. The younger ones are like to-be workers and older ones may be casual workers. As casual workers, they burrow and store forages most of the time. The oldest are breeders.

Even though the exact age of the four-striped mouse is known, nothing about the age of common mole-rat was known because they were caught from the wild. Weight and wear in the dentition of the common mole-rat are criteria used to classify it as an adult.



Figure 2.2 Photograph of the common mole-rat (CMR) Klaus Rudloff (2011). Internet accessed on 19tth of June, 2014.

2.2. Markers for cell Proliferation

There are two main classes of markers used to label proliferating cells: exogenous and endogenous markers. Endogenous markers are molecules that the cell expresses during the progression of the cell cycle, which correlates with the duplication of its DNA or with the mitotic division while exogenous markers were injected, they bind to DNA in vivo and may produce DNA mutations. These types of marker have been widely used in the study of adult neurogenesis. One of the endogenous markers used in this study is Ki-67.

Doublecortin (DCX) is a protein that is linked with neuronal differentiation that functionally has been associated with immature cell relocation which includes synaptogenesis. DCX is a microtubule that is associated protein expressed by neuronal precursor cells and immature neurons in embryonic and adult cortical structures (Plumpe *et al.,* 2006). DCX is used as a confirmatory staining for new neurons in the dentate gyrus of the hippocampus in this study.

2.2.1. Ki-67 marker

Ki-67 is an endogenous proliferation marker, which reacts with a nuclear antigen. Ki-67 is expressed in all proliferating cells in all the phases of the cell cycle except in the resting phase (G_0) (Gil-Perotin et al., 2009). In G_1, Ki-67 is predominantly localized in the perinucleolar region and also found in nuclear matrix in the later phases (Gerdes, 1990). Ki-67 is thought to be involved in the maintenance of cell proliferation however its exact mechanism for function is unknown. Due to these facts, it is considered an important marker for evaluation of tumour diagnosis and prognosis. Ki-67 labels the nuclei of proliferating cells.

2.2.2. Doublecortin (DCX) marker

Doublecortin (DCX) is expressed in migrating neurons throughout the central and peripheral nervous system during embryonic and postnatal development (Gleeson *et al.*, 1999). DCX co-assembles with brain microtubules, and recombinant DCX stimulates the polymerization of purified tubulin. Over expression of DCX in heterologous cells leads to a dramatic microtubule phenotype that is resistant to depolymerization. Therefore, DCX, likely directs neuronal migration by regulating the organization and stability of microtubules (Gleeson *et al.*, 1999). Doublecortin is a phenotypic marker which stains the cytoplasm and processes of immature neuronal cells (Lu *et al.*, 2003).

2.3. Tissue processing

Animals were euthanased with sodium pentobarbital (Euthanaze, i.p. 80 mg/kg) and transcardially perfused with 0.9 % cold saline (4 °C) followed by 4 % paraformaldehyde in 0.1 M Phosphate buffer (PB). Brains were then carefully removed from the skull, weighed and post-fixed in 4 % paraformaldehyde in 0.1 M PB, then allowed to equilibrate in 30 % sucrose in 0.1 M PBS. The left hemisphere of the brains were then kept frozen in dry ice and sectioned frozen using a sliding microtome in the sagittal plane at 50 µm section thickness covering the complete brain. Subsequently, sections were placed in vials containing cryoprotectant solution (CPS). A one in five series of sections were stained for Ki-67 (Proliferating) and DCX (Immature neurons).

2.3.1. Ki-67 immunohistochemical staining

Sections were washed 2 times for 10 minutes in PBS and then rinsed with tris-buffer saline triton-X (TBST) once for 5 minutes under gentle shaking at room temperature. The sections were then treated for 40 minutes at 94 ºC (in water bath) in citrate buffer pH 6.1

diluted with distilled water (1:10) for anti-gene retrieval. Sections were then allowed to cool down on the bench to room temperature for 20 minutes.

Sections were then washed in TBST 2 times for 5 minutes under gentle shaking and transferred into blocking solution, 5 % normal goat serum (NGS) in TBST for 30 minutes. Tissues were then transferred into primary antibody, NCL-Ki-67 (Novocastra, Wetzlar, Germany; 1:5000) in TBST supplemented with 2 % bovine serum albumin (BSA) and 2 % NGS overnight under gentle shaking at 4º C.

On the following day, tissues were brought out and left on the bench for 30 minutes to equilibrate to room temperature under gentle shaking. The tissues were then washed 3 times for 10 minutes in TBST under gentle shaking at room temperature. Secondary antibody was then applied, biotinylated goat- anti- rabbit (Vector lab, CA, USA; 1:250) in TBST supplemented with 2 % NGS for 60 minutes at room temperature. Tissues were then washed 3 times for 10 minutes in TBST. AB Complex (ABC) reagent (Vector lab, CA, USA; 1:100, A and B) was then applied for 40 minutes at room temperature under gentle shaking. After that, sections were then washed in TBS 2 times for 15 minutes under gentle shaking at room temperature. Tissues were then washed in TB pH 7.6 two times for 10 minutes under gentle shaking at room temperature. They were then pre-incubated in the dark for 30 minutes with 3, 3'-diaminobenzidine (DAB) solution, 0.5 mg/ml, in TB 7.6 under gentle shaking at room temperature using 2 ml per vial. 35 µl of 0.5 % H_2O_2 was then added to each vial and mixed very well to develop under visual guidance until a strong nuclear staining was observed. Reaction was then stopped by washing sections in TB pH 7.6 3 times for 10 minutes under gentle shaking at room temperature. Tissues were then washed in PB and mounted onto 0.5 % gelatinized slides and air-dried overnight.

They were then dehydrated in a graded series of alcohols, cleared in xylene, and coverslipped with Entelan.

2.3.2. Doublecortin (DCX) immunohistochemical staining

Sections were washed 2 times for 10 minutes in PBS and then rinsed with TBST once for 5 minutes under gentle shaking at room temperature. Sections were then treated with blocking solution, 5 % normal rabbit serum (NRbS) in TBST for 30 minutes.

Tissues were then transferred into primary antibody DCX (Santa Cruz biotech, CA, USA; 1:400) in TBST supplemented with 2 % BSA and 2%NRbS overnight at 4 ºC under gentle shaking.

On the following day, tissues were brought out and left on the shaker to equilibrate at room temperature followed by a 3 times 10 minutes wash in TBST under gentle shaking at room temperature. Secondary antibody, biotinylated rabbit-anti-goat (Vector lab, CA, USA; 1:250) in TBS supplemented with 2% NRbS for 60 minutes at room temperature under gentle shaking was then applied. After that, sections were washed 3 times for 10 minutes each under gentle shaking at room temperature. Thirty minutes prior to the time of use, ABC reagent in TBS (1:100, A plus B) was applied to the tissues for 40 minutes at room temperature under gentle shaking. Sections were then washed in TBS twice at 15 minutes each under gentle shaking at room temperature followed by washing in TB (pH 7.6) two times for 10 minutes each.

Section were then pre-incubated in DAB solution, 0.5 mg/ml in TB (pH 7.6) by using 2 ml per vial for 30 minutes in the dark under gentle shaking at room temperature. 35 µl of 0.5 % H_2O_2 was added to each vial and mixed very well and allowed to develop under visual guidance until strong nuclear staining appears within the rostral migratory stream. The

reaction was stopped by washing sections in TB pH 7.6, 3 times for 10 minutes each under gentle shaking at room temperature. Tissues were then washed in PB and mounted onto 0.5% gelatinized slides and air dried overnight. They were then dehydrated in a graded series of alcohols, cleared in xylene and cover slipped with Entelan.

2.3.3. Plastic Embedding Technique

The right hemisphere of the brains of the four-striped mouse *(Rhabdomys pumilio)* and common mole-rat *(Cryptomys hottentotus)* was embedded in glycolmethacrylate (Technovit 7100, Kulzer GmbH & Co, Wertheim, Germany) in accordance with the manufacturer instruction and as described (Ajao *et al.,* 2010; Ihunwo and Schliebs, 2010).

1. Fixation of the brain in 4% paraformaldehyde
2. The brain was then washed in PBS 4 times for 10 minutes.
3. Serial dehydrations in graded alcohol as described below were followed:
    a. Ethanol 70% 4 hours
    b. Ethanol 96% 4 hours
    c. Ethanol 100% 26 hours

The long-time duration of dehydration was employed because of the size of the brains (NB: This can vary depending on the size of the brain).

4. Brains were pre-infiltrated in Technovit base solution (100 ml Technovit 7100 + 1g hardener 1) in 100 ml of 100% ethanol for 2 hours (the mixture ratio 1:1).
5. Brains were infiltrated in Technovit base solution (100 ml Technovit 7100 + 1g hardener 1) for 48 hours
6. Brains were then embedded in Technovit base solution + 1g hardener II (15 ml Technovit base solution + 1 ml hardener II). Only a small portion of the solution

was made up at a time as polymerization goes rather fast and shaking the solution gently for 1 minute, followed by these processes:

i. A quarter of the embedding solution was poured into the mould forms, with the bottom filled up 1 – 2 mm. The form was protected from light while waiting for the solution to get sticky.

ii. The tissue was put in the form in the desired orientation for cutting (medial surface to face the predetermined cutting surface) and was not allowed to sink to the bottom with enough space around the tissue.

iii. The form was filled up with the embedding solution, covered and incubated for 1 hour at 37^0C and allowed to harden at room temperature overnight.

iv. Labels were stuck to the plastic blocks for identification. The forms were subsequently removed and the blocks allowed to dry at room temperature over a couple of days (minimum of three days).

2.3.4. Sectioning

The embedded block were mounted on the microtome and sectioned at 20 μm. A one in five serial section was obtained and mounted on frosted non-gelatinized slides. The mounted sections were placed in the incubator overnight at 80 ^{0}C.

2.3.5. Giemsa staining

The sections were stained according to Iniguez *et al.,* (1985). Incubation in Giemsa staining solution (Giemsa stock solution, Merck, Darmastadt, Germany) diluted in buffer (67 mmol Potassium dihydrogenophosphate, K_2HPO_4) at room temperature for 40 minutes. Sections were rinsed in 1 % acetic acid for 10 seconds and differentiated in 99 % alcohol three times for 10 minutes. Sections were then cleared in Xylene and cover-slipped with

dippex. The neurons appeared blue, and the white matter appeared red-violet under the microscope.

2.4. Light microscope analysis

Sections of the brains were analysed with a light microscope using the Zeiss Axioskop 2 plus microscope, Germany. The immunostained sections were compared with previous report studies (Lu *et al.*, 2003; Amrein *et al.*, 2004a & 2004b; Plumpe *et al.*, 2006; Hauser *et al.*, 2009; Lagace *et al.*, 2010). Photomicrographs were taken at different magnification with the aid of the Zeiss Axioskop 2 plus microscope with a fitted Axiocam HRc camera.

2.5. Camera Lucida Drawing

The sections of the Ki-67 immunohistochemistry staining were examined under a stereomicroscope (Leica MZ 75) and the architectural borders were traced using a Camera Lucida. After completing the tracing, the sites where adult neurogenesis was observed were marked on the drawings. Drawings were scaled down and redrawn using the Canvas drawing program.

2.6. Proliferating cell count

The proliferating cells were counted exhaustively in every fifth section on an axiovision light microscope using 100X oil-immersion lens and multiplied by the section sampling fraction *(ssf)* to obtain the estimated total proliferating cell number *(Etgcn)* in this study. Cells in the top focal plane of the section were not counted. All the Ki-67 positive cells in the subgranular layer (SGL) and granule cell layer (GCL) of the selected hemisphere were counted (Ajao *et al.,* 2010).

2.7. Pyknotic cell count

The pyknotic cells were identified in Giemsa stained sections by their intensely stained nuclei with condensed chromatin peripherally into a C or doughnut shape, solid and sometimes with numerous cell bodies. The counting method used was same as for the proliferating cell count above (Ajao *et al.*, 2010).

2.8. DCX cell count

The DCX cells were identified in DCX immunopositive stained sections by their strongly stained nuclei along with their processes. Using the optical fractionator of the Stereo investigator (MicroBrightField, Inc., Williston, USA) (West *et al.*, 1991). Briefly, the outlines of granule cell layer of each section of the series were drawn using a 5X objective lens of the Zeiss Axioskop Imager M2. A one in five serial section was used (section sampling fraction *(ssf)* = 0.2) giving rise to an average of 10 and 11 sections for DCX immunohistochemical sections analysed in four-striped mouse and common mole-rat per animal respectively. A uniform counting frame of 40 µm X 40 µm in a sampling grid size of 130 µm X 130 µm was used giving an area sampling fraction *(asf)* of 0.095. These counting frame and grid sizes were used in order to achieve an acceptable co-efficient of error (that is CE < 0.1). The optical dissector of 50 µm in height was centrally placed in the z-axis and the thickness of the tissue was measured by fine focus of the top of the tissue and then the bottom of the tissue before counting. Counting was done using the 100X oil immersion lens (NA = 1.4). The counting of DCX positive cells was based on the cell body only. The mean weighted estimate of the total number of DCX positive cells in the subgranular and granule cell layer of the DG of the hippocampus *(N)* was obtained using the following algorithm which takes into account the ssf, asf and thickness sampling factor (tsf- the fraction of the dissector height *(h)* to the mean measured thickness *(t)*:

$$N = \sum Q \times \frac{t}{h} \times \frac{1}{asf} \times \frac{1}{ssf}$$

Where $\boldsymbol{\Sigma Q}$ is the total number of neurons actually counted (West *et al.,* 1991).

$$asf = B/D^2$$

Where $\boldsymbol{B}$ is the area of counting frame and $\boldsymbol{D}$ is the area of sampling grid (West *et al.,* 1991).

$$ssf = 1/serial\ section$$

2.9. Estimation of total granule cell numbers

The total granule cell numbers in the granule cell layer of the dentate gyrus were estimated by applying unbiased stereology using the optical fractionator of the Stereo investigator (MicroBrightField, Inc., Williston, USA) (West *et al.,* 1991). Briefly, the outlines of granule cell layer of each section of the series were drawn using a 5X objective lens of the Zeiss Axioskop Imager M2. A one in five serial section was used (section sampling fraction *(ssf)* = 0.2) giving rise to an average of 28 and 25 sections for plastic embedded sections analysed in four-striped mouse and common mole-rat per animal respectively. A uniform counting frame of 100 µm X 100 µm in a sampling grid size of 110 µm X 110 µm was used giving an area sampling fraction *(asf)* of 0.827. These counting frame and grid sizes were used in order to achieve an acceptable co-efficient of error (that is CE < 0.1). The optical dissector of 10 µm in height was centrally placed in the z-axis and the thickness of the tissue was measured by fine focus of the top of the tissue and then the bottom of the tissue before counting. Counting was done using the 100X oil immersion

lens (NA = 1.4). The mean weighted estimate of the total number of cells in the granule cell layer of the DG of the hippocampus *(N)* was obtained using the following algorithm which takes into account the ssf, asf and thickness sampling factor (tsf- the fraction of the dissector height *(h)* to the mean measured thickness *(t)*:

$$\boldsymbol{N = \sum Q \times \frac{t}{h} \times \frac{1}{asf} \times \frac{1}{ssf}}$$

Where $\boldsymbol{\Sigma\, Q}$ is the total number of neurons actually counted (West *et al.*, 1991).

$$\boldsymbol{asf = B/D^2}$$

Where $\boldsymbol{B}$ is the area of counting frame and $\boldsymbol{D}$ is the area of sampling grid (West *et al.*, 1991).

$$\boldsymbol{ssf = 1/serial\ section}$$

2.10. Statistical Analyses

All numerical analyses were performed using both STATA version 13 and IBM SPSS statistics 22. Numerical values were expressed in means and standard deviations. Paired t test was performed to determine the differences in the mean values obtained for cell proliferation, cell death and total granule cell number in four-striped mouse and common mole-rat. Also Independent student t test was done to compare the values of cell proliferation from Ki-67, immature neuron from DCX positive cells, cell death from pyknotic cells and total granule cell number between four-striped mouse and common mole-rat. Sampling was considered optimal when the coefficient of error (CE) is half or less than half of coefficient of variation (CV) (Gundersen and Osterby, 1981) especially in the estimation of the total granule cell number of the dentate gyrus. Correlation analysis

was performed to assess the relationship of cell proliferation, cell death and total granule cell numbers in four-striped mouse and common mole-rat using Spearman Rho correlation analysis. Since the data was not normally distributed and appeared to be non-linear, log transformation of the values obtained for the variables was done. Regression analysis of the log transformed values was done to obtain the coefficient of variability (R^2). Differences were considered statistically significant at p-value ≤ 0.05.

3. CHAPTER THREE

3.0. RESULTS

The main objective of this study was to provide quantitative data on cell proliferation, cell death and total granule cell number in the four-striped-mouse (FSM) *(Rhabdomys pumilio)* and common mole-rat (CMR) *(Cryptomys hottentotus)* and compare the possible relationship that exists between cell proliferation and cell death in the dentate gyrus of hippocampus of four-striped mouse and common mole-rat. This was achieved in each species by comparing;

- Ratio of proliferating cells to total granule cell counts
- Ratio of pyknotic cells to total granule cell counts
- Ratio of proliferation to pyknotic cell count
- Ratio of DCX positive cells to pyknotic cells
- Ratio of cell proliferation to pyknotic cell to total granule cell counts.
- Ratio of body weight to brain weight

All these were investigated in the dentate gyrus of the hippocampus of the four-striped mouse and common mole-rat. The distribution of the cell proliferative marker Ki-67 and immature neuronal marker DCX were found to be different between the four-striped mouse and the common mole-rat. Normal structures of the dentate gyrus and hippocampus proper (CA regions) were observed in the two experimental animals.

Ki-67, labelled the nuclei of the proliferating cells which appeared dark and in clusters in the dentate gyrus of the hippocampus of the two experimental animals.

Doublecortin labelled the immature cells along with their processes. They appeared mostly in clusters in the dentate gyrus. The DCX positive cells were categorized according to the shape and presence of apical dendrites as described earlier by Plumpe *et al.,* (2006) (Figure 3-1).

- Category A and B: DCX positive cells with very short or no processes respectively. The processes were less than one nucleus wide in the DCX positive cells of category B. These categories were described as being in the proliferative stage.

- Category C and D: DCX positive cells with processes of intermediate length and immature morphology. The processes were longer in DCX positive cells in category C compared to processes of DCX positive cells in category B whereby the processes reached the granule cell layer but did not reach the molecular layer in the dentate gyrus of the hippocampus. In DCX positive cells in category D, the processes reached the molecular layer. These categories were described as being in the intermediate stage.

- Category E and F: DCX positive cells with a more matured appearance. For category E, they had a one thick dendrite that reached into the molecular layer and displayed a comparatively sparse branching in the molecular layer. The DCX positive cells of category F had a dendritic tree which showed delicate branching and few major branches close to the soma or within the granule cell layer. These categories were described as being in the postmitotic stage.

The three stages the DCX positive cells were grouped was according to Plumpe *et al.*, (2006) (Figure 3-1).

DCX positive cells were observed in the dentate gyrus of the hippocampus of the four-striped mouse and common mole-rat in the above categories.

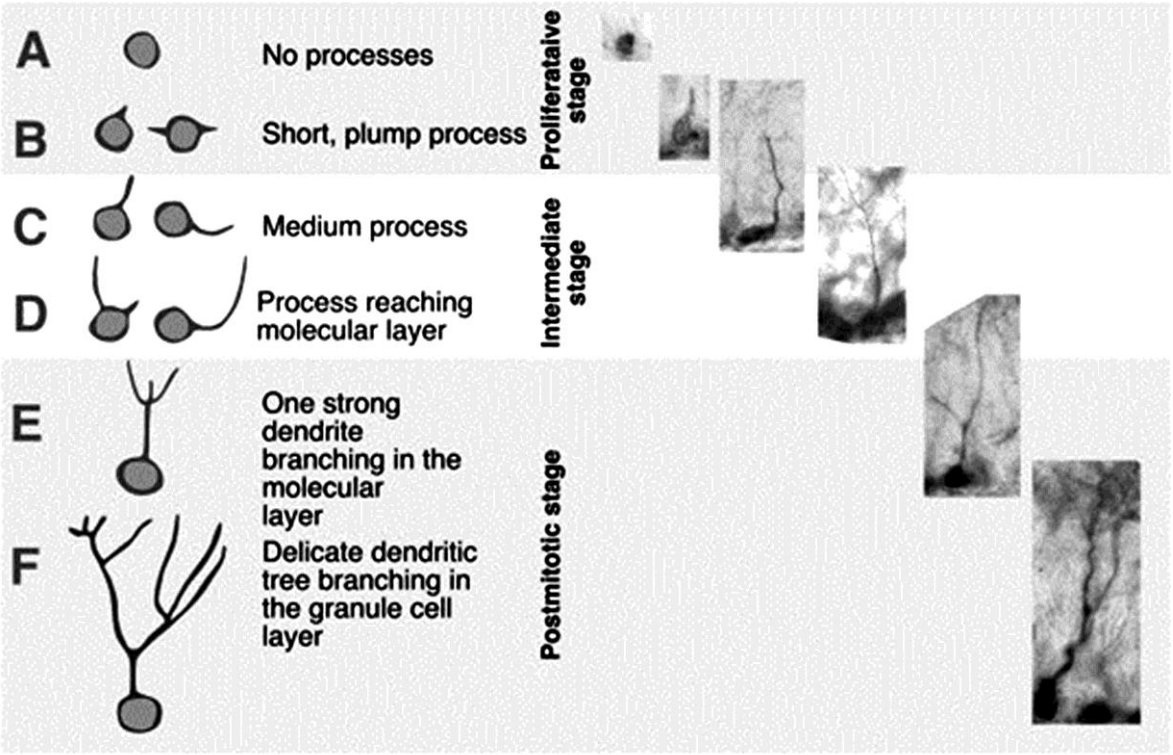


Figure 3.1 Categorization of dendritic morphology by Plumpe et al., (2006).

3.1. Qualitative results in the four-striped mouse

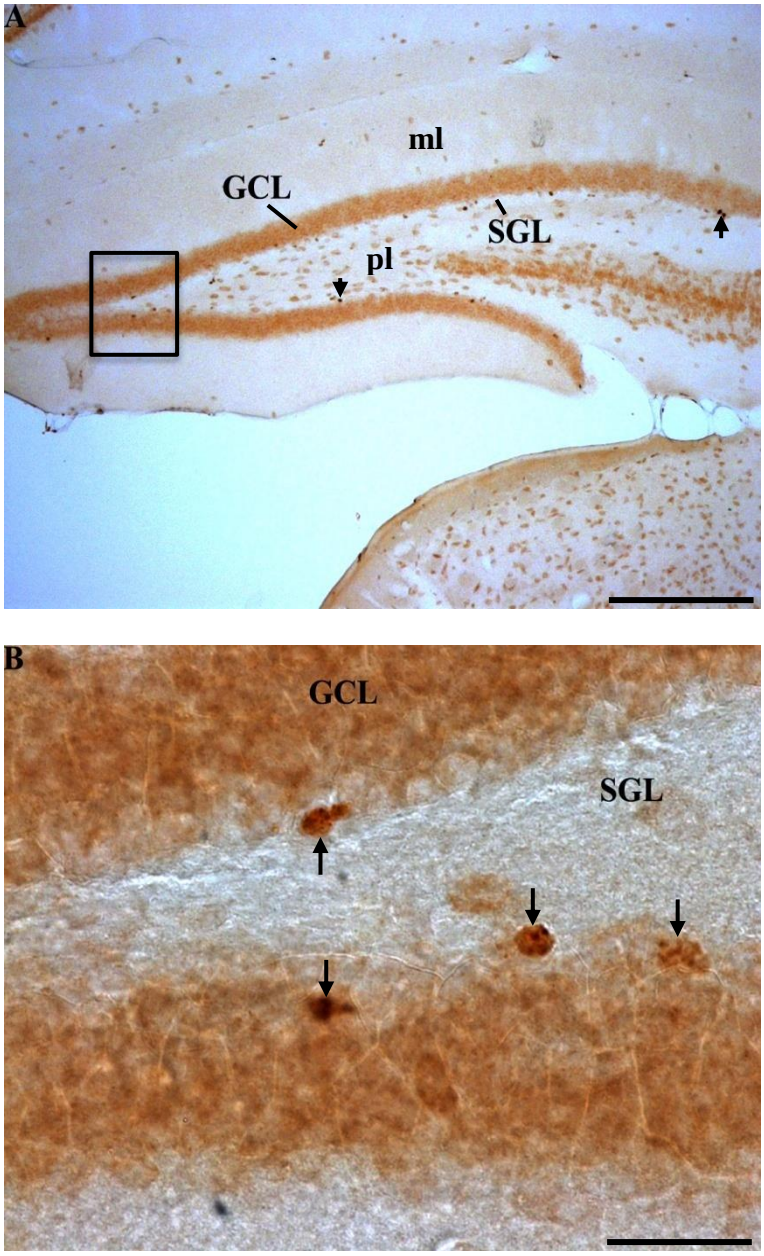
This section presents the results of the immunostaining for Ki-67 and DCX for cell proliferation and immature neurons in the dentate gyrus of the hippocampus of four-striped mouse respectively. Furthermore, the classification of DCX positive cells into proliferative, intermediate and postmitotic stages of immature neuron was also done. In addition, the result of the Giemsa staining for pyknotic cells is presented.

3.1.1. Immunohistochemical staining for Ki-67 positive cells in the four-striped mouse

The Ki-67 immunostaining was used as a neuronal marker for the proliferating cells in the subgranular and granule cell layers in dentate gyrus of the hippocampus. Ki-67 positive cells were distributed along the length of the subgranular cell layer of the dentate gyrus of the hippocampus (Figure 3.2). The nuclei of the Ki-67 positive cells were centrally located, darkly stained and distributed specifically in the subgranular layer of dentate gyrus of the hippocampus (Figure 3.2B). This was further illustrated on the architectonic border tracing that was done using the Ki-67 immunohistochemical slides to illustrate the location of the proliferative cells and the different layers in the dentate gyrus (Figure 3-3 A&B). The different layers observed were the molecular, granule cell and the polymorphic layers. In describing the feature of the dentate gyrus, it is easier to refer to a particular transverse portion of the "V"- or "U"- shaped structure. The portion of granule cell that is located between the CA3 and the CA1 regions is call the suprapyramidal region and the portion opposite to this is called the infrapyramidal region (Amral *et al.*, 2007). The region connecting the suprapyramidal and infrapyramidal is called the crest. Majority of the Ki-67 positive cells were located in the suprapyramidal region of the DG while relative amounts were evenly distributed in the crest and infrapyramidal regions (Figure 3.2B). The suprapyramidal and infrapyramidal layers were all located in the granule cell layer of the dentate gyrus. The Ki-67 positive cells were identified by their labelled nuclei and irregular shaped cluster. The cells appear in clusters of 2-5 cells (Figure 3.2B)

Figure 3.2 Representative photomicrograph showing Ki-67 positive cells in the dentate gyrus of the hippocampus in the four-striped mouse.

Majority of the cells are located in the subgranular layer (arrows) in the suprapyramidal region of the DG. Marked area in A (insert) is shown in B, Ki-67 positive cells appear darkly stained and in clusters. GCL-granule cell layer, ml- molecular layer, pl-polymorphic layer and SGL-subgranular layer. Scale bar; A =10 µm, B=1 µm.


A
ml
GCL
SGL
pl
B
GCL
SGL

3.2. Architectonic border tracing of the dentate gyrus in the four-striped mouse

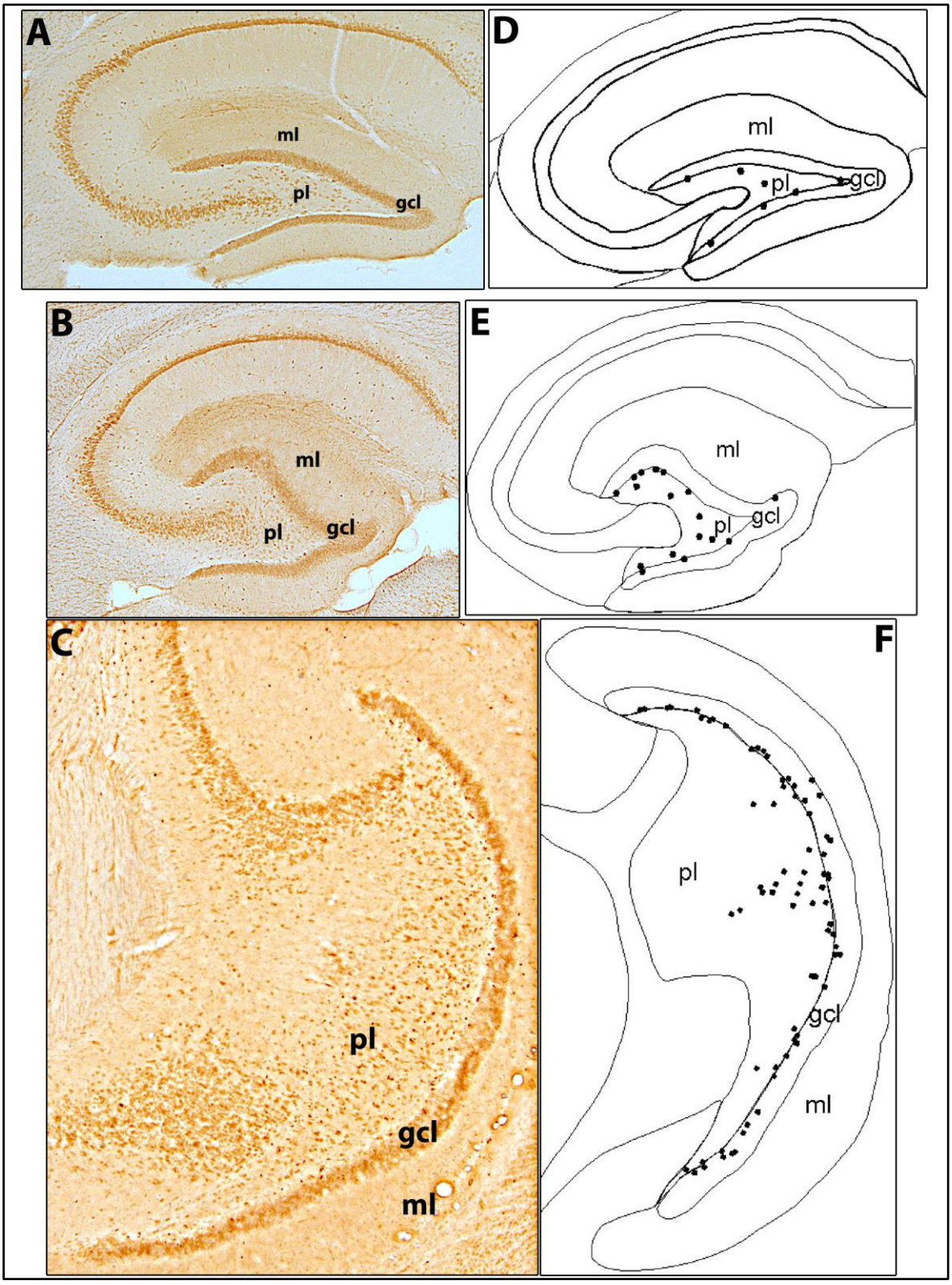
The comparative neuroanatomy of the dentate gyrus in the four-striped mouse is similar to the brain of other rodents. The trilaminar structure of the dentate gyrus was observed in the four-striped mouse (Figure 3.3). The granule cell layer of the dentate gyrus consisted of granule cells that were compactly arranged in 3-4 layers. The subgranular layer of the granule cell layer consisted of single cell layer which was closest to the polymorphic cell layer. The polymorphic cell layer was in the region of the overlap of CA3 region of the hippocampus by the two limbs of the dentate gyrus. The molecular layer contained sparsely arranged cell bodies and dendritic fibres from granule cell. The CA3 field inserts into the dentate gyrus between the suprapyramidal and infrapyramidal limbs of the dentate gyrus to share a common boundary and adjoin with the cells of the polymorphic region of the dentate gyrus in the four-striped mouse (Figure 3.3).

Figure 3.3 Photomicrograph (A-C) and diagrammatic reconstruction (D-E) of a sagittal section through the brain of four-striped mouse (Rhabdomys pumilio) illustrating the location of Ki-67 positive cells in the DG of the hippocampus.

A-C The photomicrograph represents most medial (A), approximately middle area of the hemisphere (B) and most lateral (C) regions of the DG showing the different layers in four-striped mouse. Scale bar; A =50 µm, B=50 µm and C = 40 µm.

D-E The drawing represents the illustration of the location of Ki-67 positive cells in the DG of the hippocampus showing most medial (D), approximately middle area of the hemisphere (E) and most lateral (F) regions of the DG showing the different layers in four-striped mouse.

ml- molecular layer; **gcl**- granule cell layer; **pl-** polymorphic layer.


A
ml
pl
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D
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E
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C
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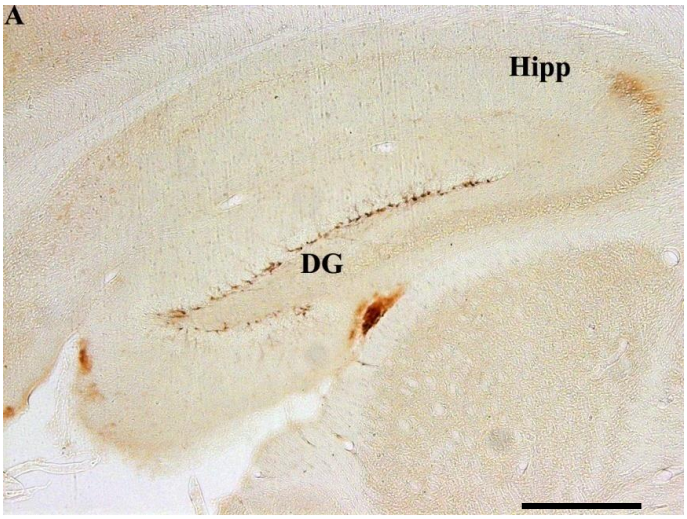
ml
F
pl
gcl
ml

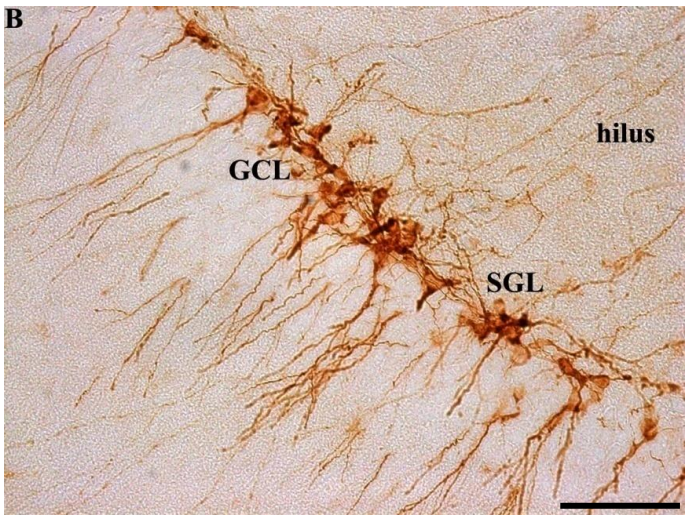
3.2.1. Doublecortin (DCX) positive cells in the four-striped mouse

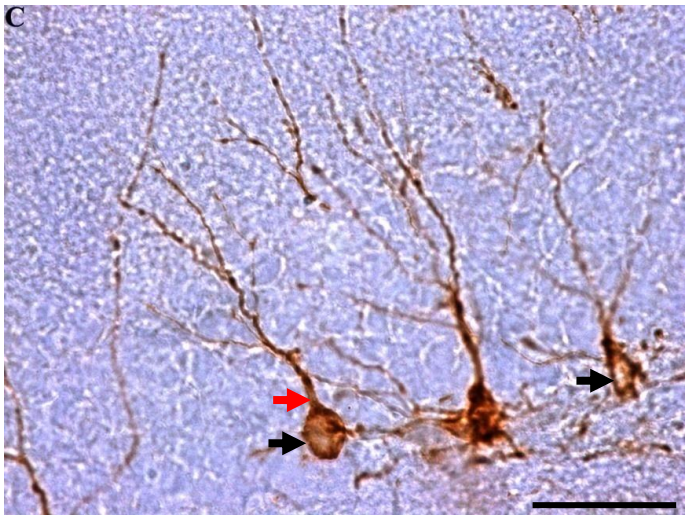
DCX positive cells were observed in the dentate gyrus of the hippocampus as immature neurons along with their processes (Figure 3.4). The cell bodies lined the subgranular layer of the dentate gyrus of the hippocampus (Figure 3.4B). Majority of the DCX positive cells in the four-striped mouse were observed to be in their post-mitotic stage of development. Hence, the cells are bipolar with an ovoid soma and fall under categories E and F. In category E, a singular thick dendrite extends into the molecular layer and then displays a comparatively sparse branching in this layer (Figure 3.4). In the category F, the dendritic tree expresses a more complex branching with relatively few branching near the soma or within the granule cell layer (Figure 3.4). Their processes extended into the granular and molecular layers (Figure 3.4 B and C). No DCX positive cell was found in the polymorphic layer. The DCX positive cells in the four-striped mouse represent immature neurons in the dentate gyrus of the hippocampus in the postmitotic phase of cell proliferation. However, the DCX positive cells were not observed in the polymorphic layer of the dentate gyrus but some dendrites/ axons can be seen extending into the polymorphic hjnulayer or hilar region particularly from DCX positive cells that are located in the crest and infrapyramidal regions of the DG of the hippocampus of four-striped mouse (Figure 3.4B).

Figure 3.4 Representative photomicrograph showing DCX positive cells in the dentate gyrus (DG) of the four-striped mouse.

DCX positive cells are in cluster (B and C) with their soma (black arrow) and processes (red arrow). The soma lies in the subgranular layer with their processes (red arrow) projecting as far as the molecular layer. Hipp- hippocampus, DG- dentate gyrus, SGL subgranular layer and GCL- granule cell layer. Scale bar; A= 20 µm, B=2.5 µm, C=1 µm.


A
Hipp
DG


B
hilus
GCL
SGL

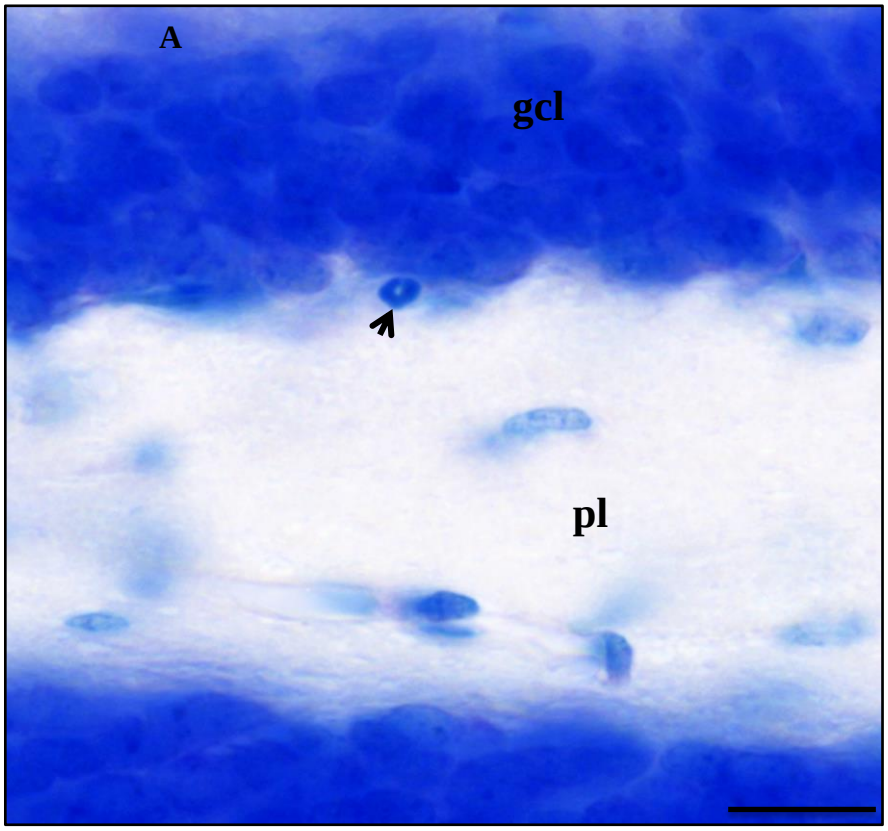

C

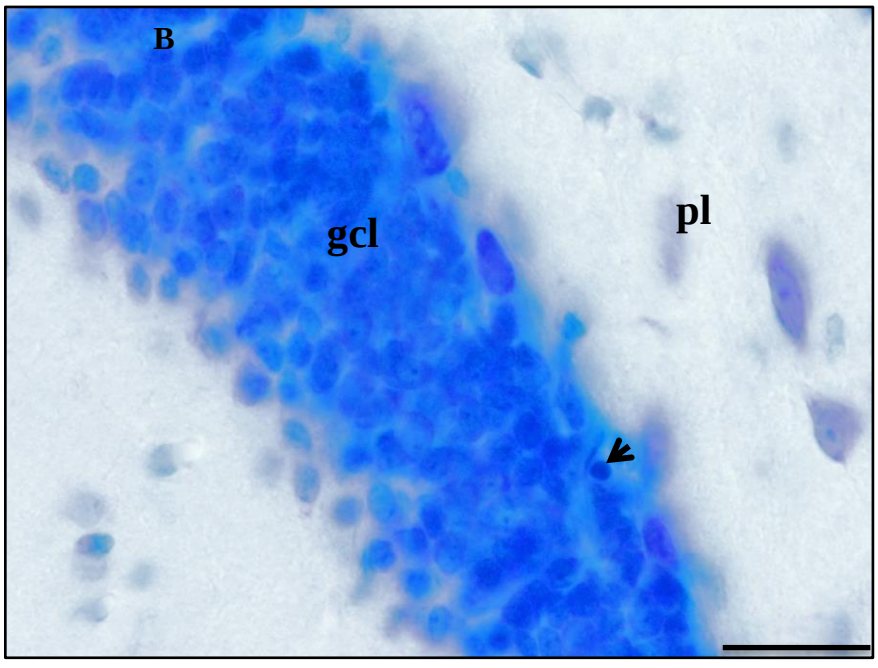
3.2.2. Giemsa staining for pyknotic cells in four-striped mouse

The pyknotic cells present in the dentate gyrus of the four-striped mouse (Figure 3.5) were identified by their strongly stained nuclei with condense chromatin that is peripheral in location and formed a C- or doughnut shape (Figure 3.5A). The pyknotic cells are solid or multiple darkly stained bodies which are between two to five and in clusters. The pyknotic cells represent cell death in the DG of the hippocampus. The majority of pyknotic cells are located in the granule cell layer and fewer numbers in the subgranular layer. No pyknotic cell was identified in the polymorphic layer of the DG.

Figure 3.5 Representative photomicrograph showing pyknotic cells in the dentate gyrus (DG) of the four-striped mouse.

Pyknotic cells located along the margin of the subgranular layer (arrows) with a doughnut-shaped peripheral condensation. Hipp- hippocampus, DG- dentate gyrus, SGL subgranular layer and GCL- granule cell layer. Scale bar; A= 200 µm; B=100 µm.


A
gcl
pl


B
gcl
pl

3.3. Qualitative results in the common mole-rat

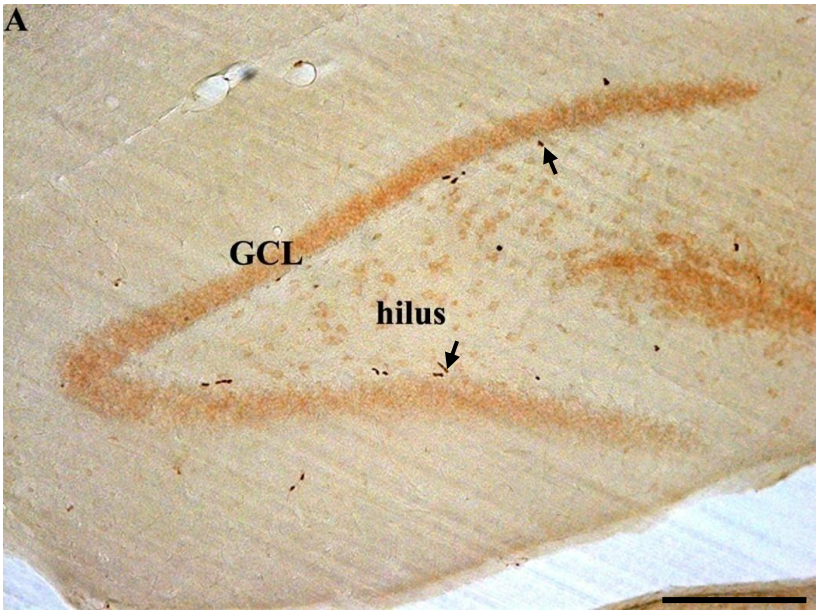
This section presents the results of the immunostaining for Ki-67 and DCX for cell proliferation and immature neurons in the dentate gyrus of the hippocampus of common mole-rat respectively. The DCX positive cells were classified into proliferative, intermediate and postmitotic stages of immature neurons. In addition, the result of the Giemsa staining for pyknotic cells is also presented.

3.3.1. Immunohistochemical staining of Ki-67positive cells in the Common mole-rat

Ki-67 positive cells were distributed along the length of the subgranular cell layer of the dentate gyrus of the hippocampus (Figure 3.6, A&B) in the common mole-rat. The nuclei of the Ki-67 positive cells were darkly stained and distributed specifically in the subgranular layer of dentate gyrus of the hippocampus (Figure 3.6, B). This was further illustrated on the architectonic border tracing that was done using the Ki-67 immunohistochemical sections to illustrate the location of the proliferative cells and the different layers in the dentate gyrus (Figure 3.6 A&B) of the common mole-rat. The different layers observed in the common mole-rat were the molecular, granule cell and the polymorphic layers. Majority of the Ki-67 positive cells were located in the suprapyramidal region of the DG while few amounts were evenly distributed in the crest and infrapyramidal regions (Figure 3.6, B) in the common mole-rat.

Figure 3.6 A & B- Representative photomicrograph showing Ki-67 positive cells in the dentate gyrus of the hippocampus of common mole-rat.

Ki-67 positive cells appear in cluster and more rounded with darkly stained nuclei (Figure 3.12 B). SGL- subgranular layer and GCL- granule cell layer. Scale bar; A= 10 µm and B=1 µm.


A
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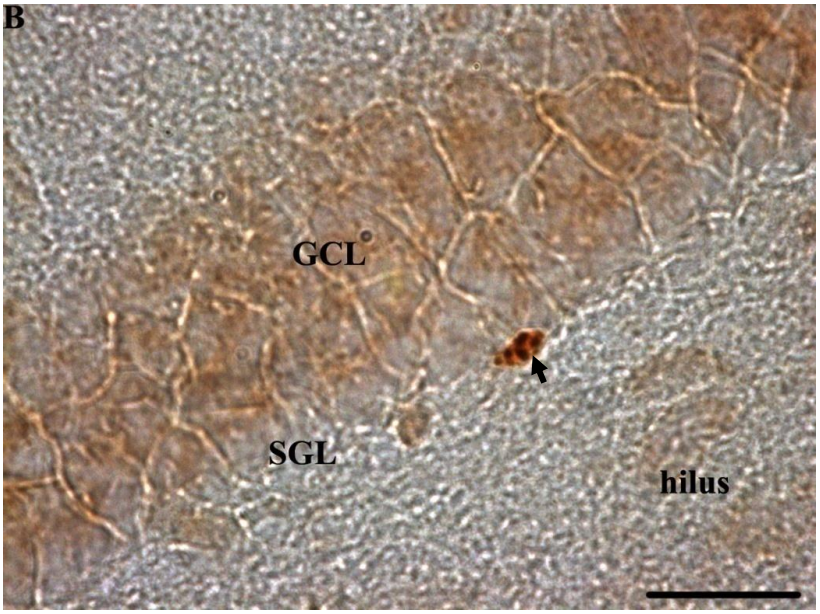

B
GCL
SGL
hilus

3.4. Architectonic border tracing of the dentate gyrus in the common mole-rat

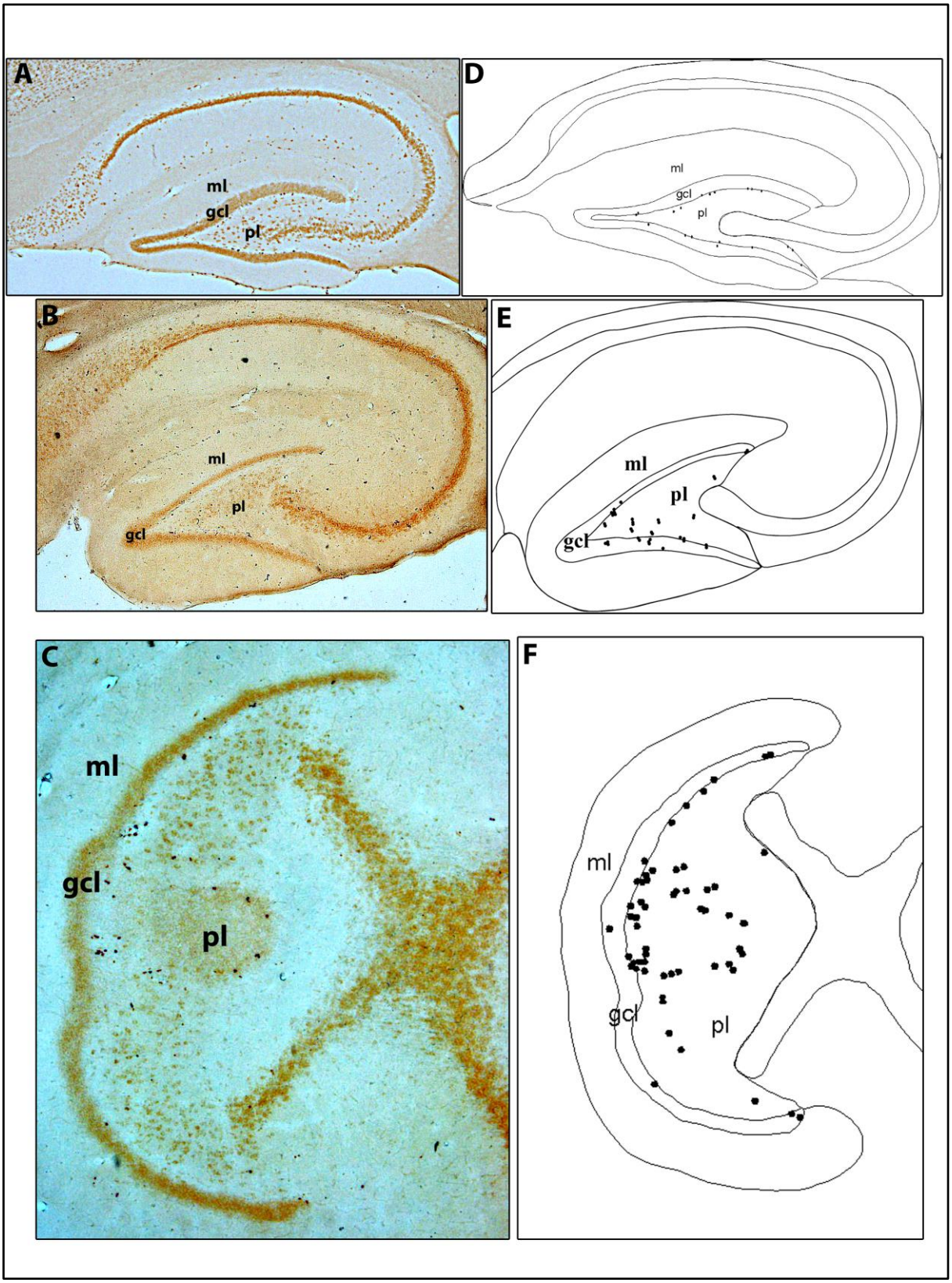
The comparative neuroanatomy of the dentate gyrus in the common mole-rat is similar to the brain of other rodents. The trilaminar structure of the dentate gyrus was observed in the common mole-rat (Figure 3.7, A-C). The granule cell layer of the dentate gyrus contained granule cells that were compactly arranged in 3-4 layers. The subgranular layer of the granule cell layer consisted of single cell layer which was closest to the polymorphic cell layer (Figure 3.7, D-F). The polymorphic cell layer was in the region of the overlap of CA3 region of the hippocampus by the two limbs of the dentate gyrus. The molecular layer contained sparsely arranged cell bodies and dendritic fibres from granule cell (Figure 3.7, D-F). The CA3 field inserts into the dentate gyrus between the suprapyramidal and infrapyramidal limbs of the dentate gyrus to share a common boundary and adjoin with the cells of the polymorphic region of the dentate gyrus in the common mole-rat (Figure 3.7, A-C).

Figure 3.7 Photomicrograph (A-C) and diagrammatic reconstruction (D-F) of a sagittal section through the brain of common mole-rat (Cryptomys hottentotus) illustrating the location of Ki-67 positive cells in the DG of the hippocampus.

A-C The photomicrograph represents most medial (A), approximately middle area of the hemisphere (B) and most lateral (C) regions of the DG showing the different layers in common mole-rat. Scale bar; A =50 µm, B=50 µm and C = 40 µm

D-E The drawing represents the illustration of the location of Ki-67 positive cells in the DG of the hippocampus showing most medial (D), approximately middle area of the hemisphere (E) and most lateral (F) regions of the DG showing the different layers in common mole-rat.

ml- molecular layer; **gcl**- granule cell layer; **pl**- polymorphic layer.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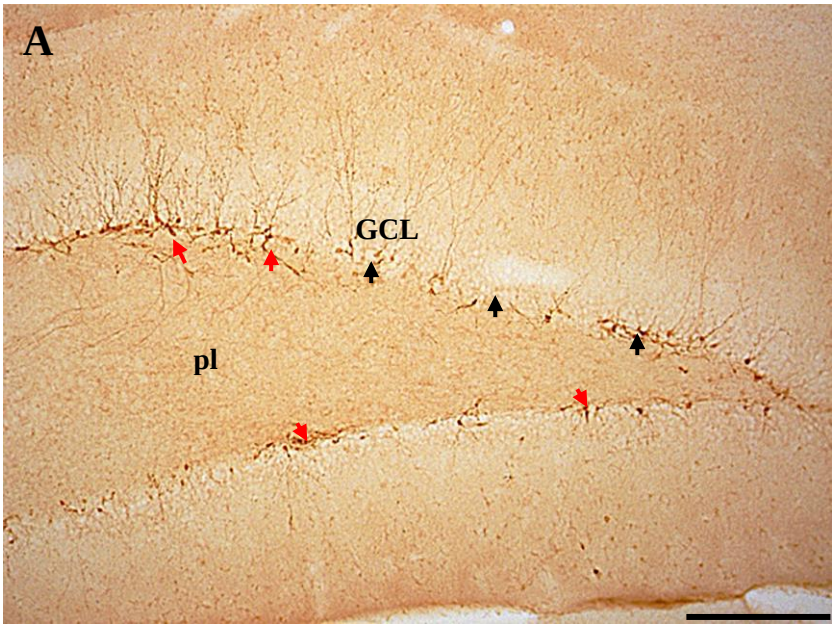
3.4.1. Doublecortin (DCX) positive cells in the common mole-rat

DCX positive cells were observed in the dentate gyrus of the hippocampus of the common mole-rat as immature neurons along with their processes (Figure 3.8, A&B). The cell bodies lined the subgranular layer of the dentate gyrus of the hippocampus (Figure 3.8B). Majority of the DCX positive cells in the common mole-rat were observed to be in their post-mitotic stage of development which is similar to the four-striped mouse (Figure 3.4, C). Hence, the cells are bipolar with an ovoid soma and fall under categories E and F. In category E, a singular thick dendrite extends into the molecular layer and then displays a comparatively sparse branching in this layer. In the category F, the dendritic tree expresses a more complex branching with relatively few branching near the soma or within the subgranular zone and granule cell layer. Their processes extended into the granular and molecular layers (Figure 3.8, A&B). No DCX positive cells were found in the polymorphic layer. The DCX positive cells in the common mole-rat represent immature neurons in the dentate gyrus of the hippocampus in the postmitotic phase of cell proliferation. However, the DCX positive cells were not observed in the polymorphic layer of the dentate gyrus but some processes can be seen extending into the polymorphic layer particularly from DCX positive cells that are located in the crest and infrapyramidal regions of the DG of the hippocampus of the common mole-rat (Figure 3.8, B).

Figure 3.8 Representative photomicrograph showing DCX positive cells in the dentate gyrus of the common mole-rat.

DCX positive cells appear in cluster (A lower magnification) with their soma and definite processes which falls under categories E and F respectively (B higher magnification). In category E, the thick dendrites extends into the molecular layer and displayed a wide range of branching in the molecular layer while in the category F, the dendritic tree showed a more complex branching in the molecular layer and very few branches around the soma or close to the granule cell layer. There was no difference in the morphology of DCX positive cells located in the subgranular zone (red arrows) and close to the molecular layer (black arrows). **SGL**- subgranular layer and **GCL**- granule cell layer. Scale bar; A=10 μm and B=1 μm.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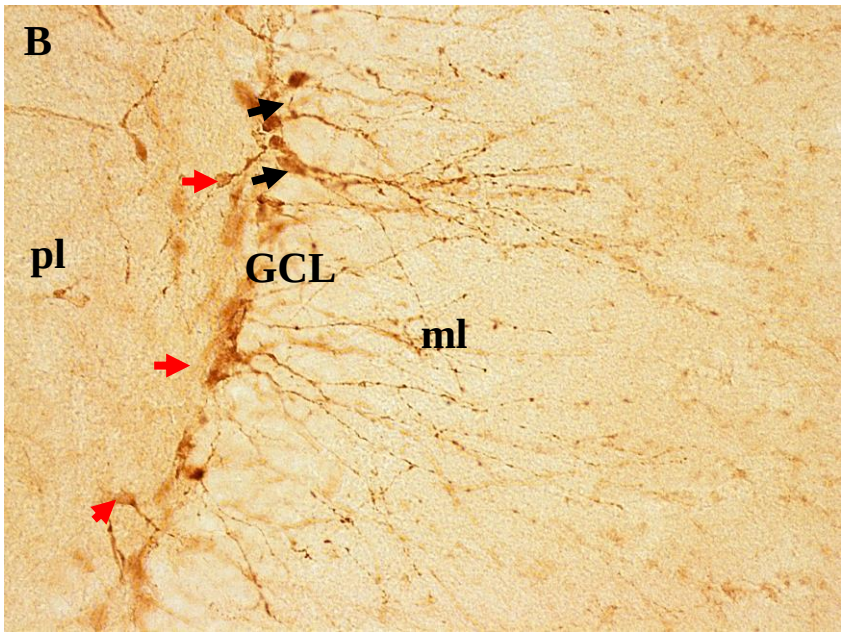
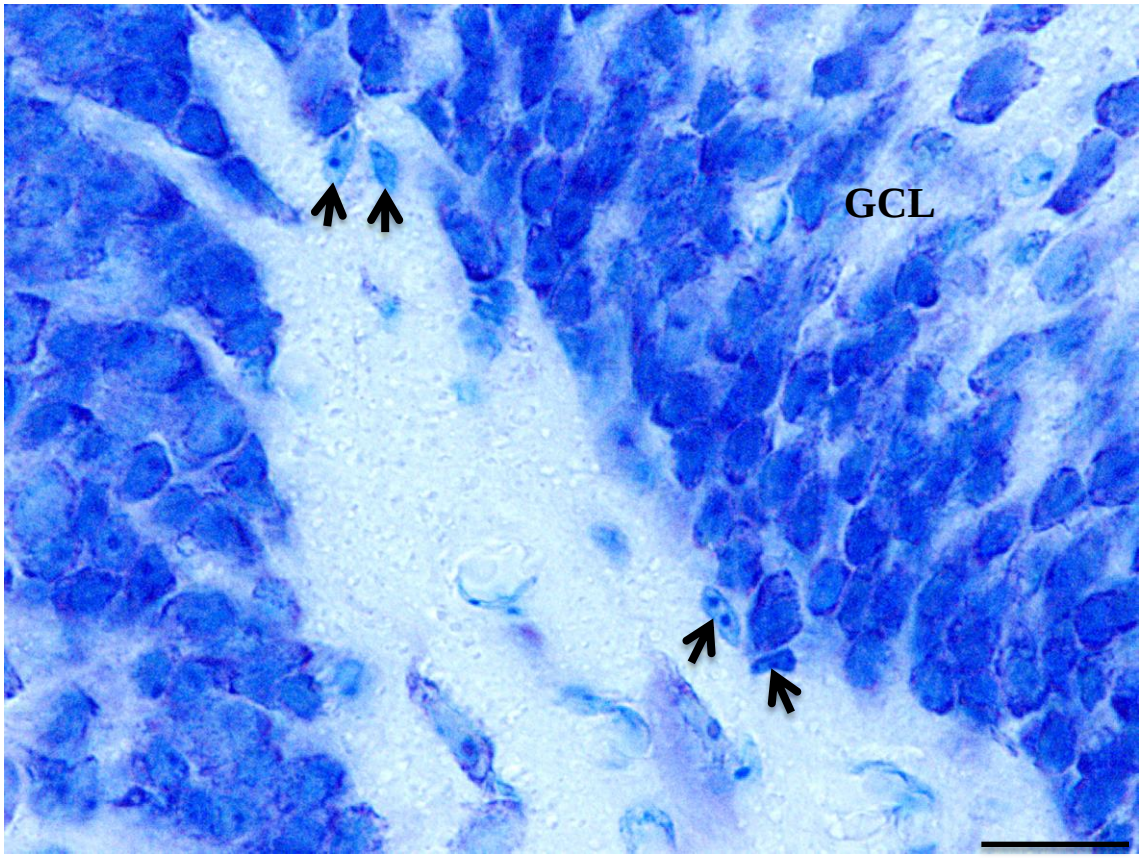

B
pl
GCL
ml

3.4.2. Pyknotic cells in common mole-rat

The pyknotic cells present in the dentate gyrus of the common mole-rat were identified following Giemsa staining procedure. The pyknotic cells in the dentate gyrus presented strongly stained nuclei with condense chromatin that is peripheral in location and formed a C- or doughnut shape. The pyknotic cells were solid or multiple darkly stained bodies which are between two to five and in clusters (Figure 3.9). The pyknotic cells represent cell death in the DG of the hippocampus. The majority of pyknotic cells are located in the granule cell layer and fewer numbers were observed in the subgranular layer. No pyknotic cell was identified in the polymorphic layer of the DG.

Figure 3.9 Representative photomicrograph showing pyknotic cells in the dentate gyrus (DG) of the common mole-rat.

Pyknotic cells located along the margin of the subgranular layer (arrows) with a doughnut shaped peripheral condensation. Hipp- hippocampus, **DG** - dentate gyrus, **SGL**- subgranular layer and **GCL**- granule cell layer. Scale bar; = 10 µm.


GCL

3.5. Quantitative Results for cell proliferation, cell death and total granule cell number.

3.5.1. Quantitative results for four-striped mouse *(Rhabdomys pumilio)*

This section presents the quantitative results for the proliferating cell count, pyknotic cell count and estimates of the total granule cell number in the six four-striped mice. Three out of the six four-striped mice analysed had brain weight above the total mean value while three four-striped mice had values below the mean value. The four-striped mouse with the highest brain weight had the lowest cell proliferation and one of the highest pyknotic and granule cell numbers (Table 3.1). Only two out of the six four-striped mice had body weight below the total mean value while four had body weight above the mean value.

3.5.1.1. Total number of proliferating and DCX positive cells in the four-striped mouse

The four-striped mouse (FSM4) with the highest number of cell proliferation had the lowest number of pyknotic cells and lowest number of DCX positive cells. It also had one of the lowest number of granule cells (Table 3.1). Furthermore, the animal with the highest number of immature neuron had the highest number of pyknotic cell. Whereas, the animal with the lowest number of immature neuron had the lowest number of pyknotic but highest number of cell proliferation despite almost similar brain and body weights (Table 3.1). Following Ki-67 immunohistochemistry, the minimum and maximum values of Ki-67 positive cells obtained in the dentate gyrus of the four-striped mouse was 455-2460 respectively. The mean total number of Ki-67 positive cells in the four-striped mouse was 993.3±683.4 (Table 3-1). Four out of the six four-striped mice analysed were below the mean value of Ki-67 positive cells. Also, three out of the six four-striped mice analysed

had DCX positive cell values below the mean value while three four-striped mice had values above the mean value.

Table 3.1: Illustration of body weight, brain weight, Ki-67, DCX, pyknotic cells and total granule cell number in individual adult four-striped mouse

Animals	**BW**	**Br W**	**Ki-67**	**DCX**	**Pyknotic**	**Total Granule cell number**
FSM3	82.3	0.8	590	3430	105	2.00×10^6
FSM4	81.7	0.7	2460	2068	90	1.52×10^6
FSM5	80.6	0.7	690	7186	180	1.73×10^6
FSM6	85.1	0.7	1085	4355	225	1.44×10^6
FSM7	75.5	1	455	7785	225	1.87×10^6
FSM8	78	0.8	680	10235	370	1.38×10^6
Mean ± SD	**80.53 ± 3.38**	**0.78 ± 0.12**	**993.33 ± 683.4**	**5843.17 ± 3070.90**	**199.17 ± 92.8**	**$1.66 \times 10^6 \pm 2.5 \times 10^5$**

Key: FSM, Four-striped mouse; **BW**, Body weight; **BrW**, Brain weight; **DCX**, Doublecortin.

3.5.1.2. Total number of pyknotic cell

The pyknotic cell population represents the number of cell death in the dentate gyrus of the hippocampus. The four-striped mouse (FSM8) with the highest pyknotic value had the highest DCX positive cell value and one the lowest body weight. The minimum and maximum values of pyknotic cells obtained for four-striped mouse was 90-370. The mean total number of pyknotic cells in four-striped mouse was 199.17 ± 92.8 (Table 3-1). Three out of the six four-striped mice analysed had pyknotic cell values below the mean value while three four-striped mice had values above the mean value.

3.5.1.3. Estimation of total number of granule cells

The total granule cell numbers in the dentate gyrus was estimated using the Optical Fractionator principle. The four-striped mouse (FSM3) with the highest granule cell number had one of the lowest pyknotic, DCX positive cell and proliferating cell values. The estimate of total granule cells in the dentate gyrus of four-striped mouse was 1.65 x 10^6 (Table 3-1). Three out of the six four-striped mice analysed had granule cell numbers below the mean value while three four-striped mice had values above the mean value.

3.5.2. Quantitative results for common mole-rat *(Cryptomys hottentotus)*

This section presents the quantitative results for the proliferating cell count, pyknotic cell count and estimates of the total granule cell number for common mole-rat. There appeared to be no discernible relationship between these parameters and the body or brain weight of the common mole-rat (Table 3.2). Four out of the seven common mole-rats analysed had brain weight values above the total mean value while three common mole-rats had values below the mean value. Only four out of the seven common mole-rats had body weight below the total mean value while three had body weight above the mean value.

3.5.2.1. Total number of proliferating and DCX positive cells

The common mole-rat (CMR9) with the highest cell proliferation also had the highest numbers of immature neuron and cell death. On the other hand, common mole-rat (CMR5) with the lowest number of cell proliferation had the lowest number of immature neuron. Furthermore, common mole-rat (CMR6) with the highest number of granule cell had one

of the lowest value of Ki-67 positive cells. Following Ki-67 immunohistochemistry, the minimum and maximum values of Ki-67 positive cells obtained for the common mole-rat was 40-605 in the hemispheres. The mean total number of Ki-67 positive cells in the common mole-rat from seven rats was 190.8 ± 209.5 (Table 3.2). Four out of six common mole-rat analyse had mean values below the total mean value of Ki-67 positive cells. Four out of seven common mole-rats had their DCX positive cells above the total mean DCX positive cells.

Table 3.2: Body weight, brain weight, Ki-67, DCX, pyknotic cells and total granule cell number in individual adult common mole-rats

No	BW	Br W	Est. Ki-67	DCX	Pyknotic	Total Granule cell number
CMR5	116	1.4	40	950	NA	NA
CMR6	154	1.3	65	8344	285	0.71×10^6
CMR7	134.5	1.4	125	8238	150	0.65×10^6
CMR8	124	1.5	120	5915	100	0.11×10^6
CMR9	105	1.3	605	17005	375	0.12×10^6
CMR10	160	1.4	190	3908	NA	NA
CMR11	102	1.3	NA	10456	NA	NA
Mean ± SD	**127.93 ± 22.76**	**1.38 ± 0.08**	**190.83 ± 209.51**	**7830.86 ± 5129.83**	**227.5 ± 108.77**	**$0.9 \times 10^6 \pm 0.3 \times 10^6$**

Key: FSM, Four-striped mouse; **BW**, Body weight; **BrW**, Brain weight; **DCX**, Doublecortin; **NA**, Not available.

3.5.2.2. Total number of pyknotic cell

The pyknotic cells were observed and counted in the Giemsa stained sections. Counting method that was used was the same for proliferating cell count. The common mole-rat (CMR9) with the highest pyknotic cell had the highest DCX positive and proliferative cell. The minimum and maximum values of pyknotic cells obtained for common mole-rat in each hemisphere was 100-375. The mean total number of pyknotic cells in common mole-rat from four rats was 227.5 ± 108.77 (Table 3.2). Two out of four common mole-rats had their mean pyknotic cell value below the total mean value.

3.5.2.3. Estimation of total number of granule cells

The total granule cell number in the dentate gyrus was estimated using the Optical Fractionator principle. The mean estimates of total granule cells obtained in the dentate gyrus of the adult common mole-rat from four rats were 0.9×10^6 (Table 3.2). The common mole-rat (CMR8) had the lowest pyknotic cell. Three out of four common mole-rats had their mean granule cell number below the total mean granule cell number.

3.5.3. Comparison of mean values of cell proliferation, cell death and total granule cell numbers in four-striped mouse

Paired sample T-test was done to compare the mean value of cell proliferation and cell death, cell proliferation and total granule cell numbers and cell death and total granule cell numbers in the four-striped mouse. There was no significant difference in cell proliferation and cell death in the four-striped mouse (p= 0.06) (Table 3.3). However, there were significantly higher total granule cell numbers compared to cell proliferation in four-striped mouse (p= 0.00). Also, there were significantly higher granule cell numbers compared to cell death in the four-striped mouse (p= 0.00) (Table 3.3).

Table 3.3: Comparison of cell proliferation, cell death and total granule cell numbers in four-striped mouse

Variables compared	Mean diff.	Std. Deviation	95 % confidence interval of the difference Lower	Upper	t	p
Proliferation FSM- Cell death	794.17	802.08	-47.56	1635.89	2.43	0.06
Proliferation FSM- Granule cell number	-1655373.33	252406.62	-1920257.83	-1390488.84	-16.07	.00
Cell death FSM- Granule cell number	-1656167.5	252135.03	-1920766.98	-1391568.02	-16.09	.00

3.5.4. Correlation and regression of variables in the Four-striped mouse

Due to data skewedness, log transformation of the variables was done for ease of data interpretation for correlation analysis.

3.5.4.1. Correlation and regression analysis of cell proliferation and Cell death in the four-striped mouse

There was no significant correlation between cell death and cell proliferation in the dentate gyrus of the hippocampus (r= -0.38; p>0.05) (Table 3.4). This implies that cell proliferation was not directly proportional to cell death in the dentate gyrus. However, to determine the contribution of number of cell death to cell proliferation, a log transformed correlation coefficient (R^2= 0.25) was obtained which suggested that, about 25 % of newly formed cells can be accounted for by the indirect influence of the number of cell death recorded. However, this was not statistically significant (p= 0.3). Regression log cell proliferation= (-0.4362 + 3.5247) log cell death, (R^2= 0.25) (Figure 3.10).

Table 3.4: Nonparametric correlation of cell death against cell proliferation in four-striped mouse

			Pyknotic	**Proliferation**
Spearman's rho	Pyknotic	Correlation Coefficient	1.000	-0.377
		Sig. (2-tailed)	0.0	0.461
	Proliferation	Correlation Coefficient	-0.377	1.000
		Sig. (2-tailed)	0.461	0.0

Table 3.5: Log transformed values of cell proliferation and cell death in four-striped mouse

Log cell proliferation	**Log cell death**
2.770852	2.021189
3.390935	1.954243
2.838849	2.255273
3.03543	2.352183
2.658011	2.352183
2.832509	2.568202

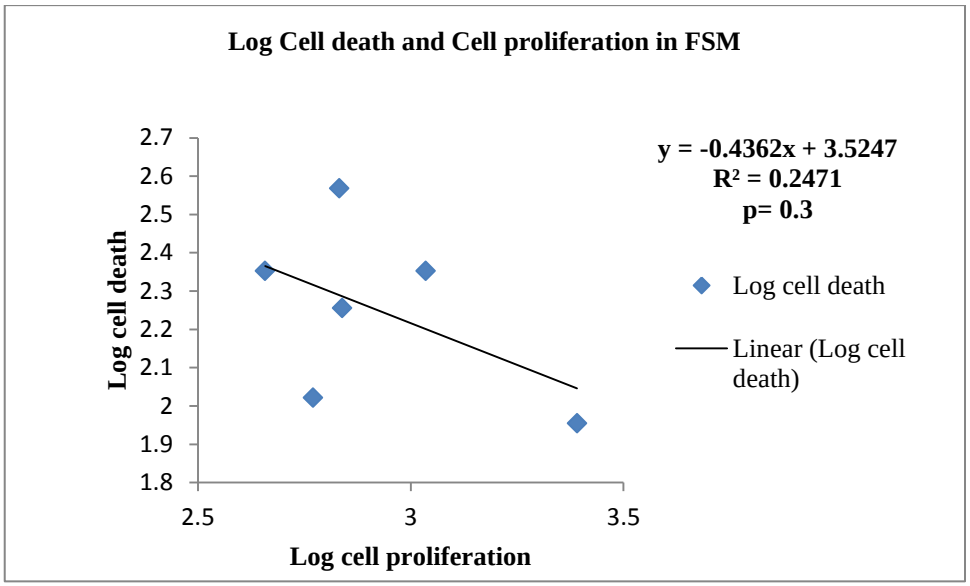


Figure 3.10 Regression analysis of log transformed values of cell proliferation against cell death in the four-striped mouse

3.5.4.2. Correlation and regression analysis of Cell death and total granule cell number in the four-striped mouse

There was no significant correlation between cell death and total granule cell number in the dentate gyrus of the hippocampus (r= -0.52; p>0.05) (Table 3.6). This implies that, in the four-striped mouse, total granule cell number is not directly proportional to cell death in the dentate gyrus. However, to determine the contribution of the number of cell death to total granule cell numbers, a log transformed correlation coefficient (R^2=0.21) was obtained which suggested that, 21 % of cell death could be accounted for by the indirect influence of the number of total granule cell numbers recorded. However, it was not statistically significant (p=0.3). Regression log cell proliferation= (-0.1321 + 6.5122) log cell death, (R^2=0.21) (Figure 3.11).

Table 3.6: Nonparametric correlation of cell death against total granule cell number in four-striped mouse

			Pyknotic	Granule cell
Spearman's rho	Pyknotic	Correlation Coefficient	1.000	-.522
		Sig. (2-tailed)	.	.288
	Granule cell	Correlation Coefficient	-.522	1.000
		Sig. (2-tailed)	.288	.

Table 3.7: Log transformed values of cell death and total granule cell number in the four-striped mouse

Log cell death	Log total granule cell number
2.021189	6.302321
1.954243	6.181984
2.255273	6.239131
2.352183	6.157085
2.352183	6.270809
2.568202	6.13865

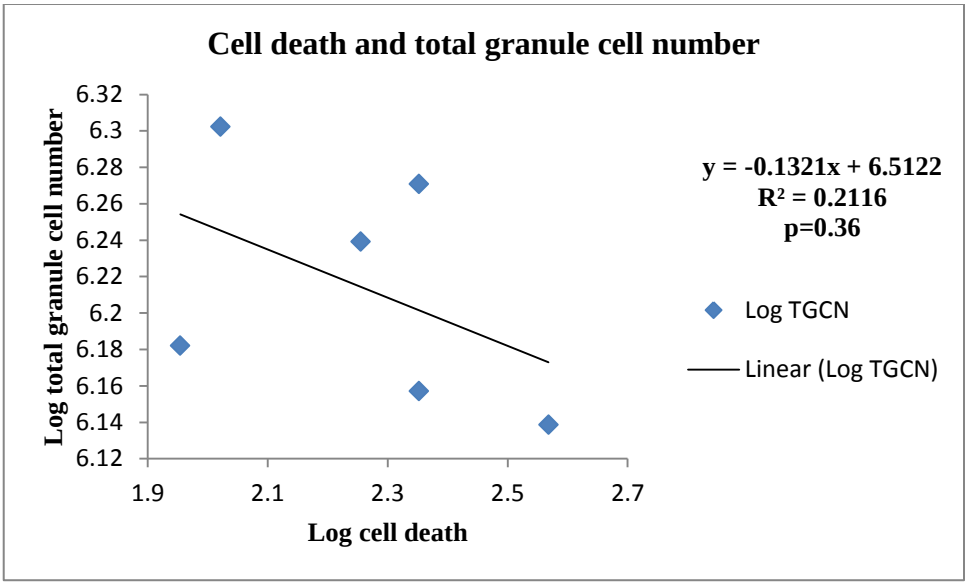


Figure 3.11 Regression analysis of log transformed values of cell death and total granule cell number in the four-striped mouse

3.5.4.3. Correlation and regression analysis of cell proliferation and total granule cell number in the four-striped mouse

There was no significant correlation between cell proliferation and total granule cell number in the dentate gyrus of the hippocampus (r=-0.5; p>0.05) (Table 3.8). This implies that the total granule cell number is not directly proportional to cell proliferation in the dentate gyrus. However, to determine the contribution of number of cell death to cell proliferation, a log transformed correlation coefficient (R^2= 0.28) was obtained which suggested that, about 28 % of newly formed cells can be accounted for by the indirect influence of the total granule cell numbers. However, it was not statistically significant (p=0.28). Regression log cell proliferation= (-2.091 + 15.917) log cell death, (R^2 =0.28) (Figure 3.12).

Table 3.8: Nonparametric correlation of cell proliferation against total granule cell numbers in the four-striped mouse

			Total granule cell	**Cell proliferation**
Spearman's rho	Total granule cell	Correlation Coefficient	1.000	-0.543
		Sig. (2-tailed)	0.0	0.266
	Cell proliferation	Correlation Coefficient	-0.543	1.000
		Sig. (2-tailed)	0.266	0.0

Table 3.9: Log transformed values of cell proliferation and total granule cell numbers in the four-striped mouse

Log total granule cell numbers	**Log cell proliferation**
6.302321	2.770852
6.181984	3.390935
6.239131	2.838849
6.157085	3.03543
6.270809	2.658011
6.13865	2.832509

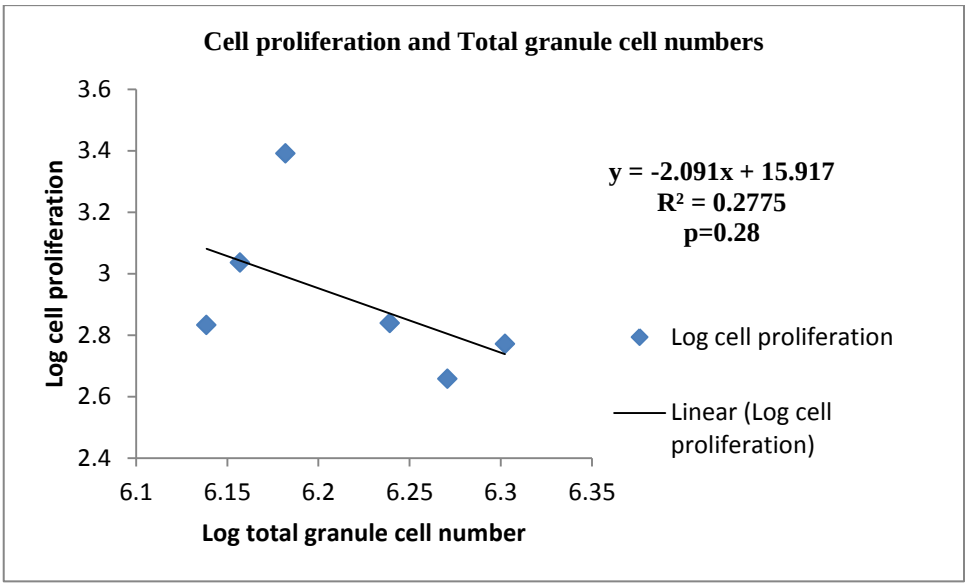


Figure 3.12 Regression analysis of log transformed values of cell proliferation and total granule cell numbers in the four-striped mouse

3.5.5. Comparison of cell proliferation, cell death and total granule cell numbers in the common mole-rat.

Paired sample T-test was done to compare the mean value of cell proliferation and cell death, cell proliferation and total granule cell numbers and cell death and total granule cell numbers (Table 3.10). There was no significant difference in cell proliferation and cell death in the common mole-rat ($p= 0.99$). However, there were significantly higher total granule cell numbers compared to cell proliferation in common mole-rat ($p= 0.006$). Also, there were significantly higher granule cell numbers compared to cell death in the common mole-rat ($p= 0.006$).

Table 3.10: Comparison of cell proliferation, cell death and total granule cell numbers in common mole-rat

Variables compared	Mean diff	Standard Deviation	95 % confidence interval of the difference Lower	Upper	t	p
Proliferation CMR- Cell death	1.25	184.68	-292.62	295.12	0.014	0.99
Proliferation CMR- Granule cell number	-894224.25	253181.02	-1297091.75	-491356.75	-7.06	0.006
Cell death CMR- Granule cell number	-894225.5	253329.71	-1297329.6	-491121.4	-7.06	0.006

3.5.6. Correlation and regression of variables in the common mole-rat

Due to data skewedness, log transformation of the variables was done for ease of data interpretation for correlation analysis.

3.5.6.1. Correlation and regression of total granule cell number and cell proliferation in the common mole-rat

There was no significant correlation between cell proliferation and total granule cell number in the dentate gyrus of the hippocampus ($r= 0.38$; $p>0.05$) (Table 3-11; Figure 3.13). This implies that the total granule cell number is not proportional to cell proliferation in the dentate gyrus. There was no significant correlation between cell proliferation and total granule cell number in the dentate gyrus of the hippocampus ($r=0.38$; $p>0.05$) (Table 3.11). This implies that the total granule cell number is not directly proportional to cell proliferation in the dentate gyrus. However, to determine the contribution of number of cell death to cell proliferation, a log transformed correlation

coefficient (R^2= 0.6245) was obtained which suggested that, about 62 % of newly formed cells can be accounted for by the indirect influence of the total granule cell numbers. However, it was not statistically significant (p=0.06). Regression log cell proliferation= (-5.9612 + 16.529) total granule cell number, (R^2= 0.6245) (Figure 3.13).

Table 3.11: Nonparametric correlation of cell proliferation against total granule cell number in common mole-rat

			Total granule cell	**Cell proliferation**
Spearman's rho	Total granule cell	Correlation Coefficient	1.000	0.377
		Sig. (2-tailed)	0.0	0.461
	Cell proliferation	Correlation Coefficient	0.377	1.000
		Sig. (2-tailed)	0.461	0.0

Table 3.12: Log transformed values of cell proliferation and total granule cell numbers in the common mole-rat

Log total granule cell numbers	**Log cell proliferation**
5.847988	1.60206
5.8154	1.812913
6.023308	2.09691
6.066053	2.079181
	2.781755
	2.278754

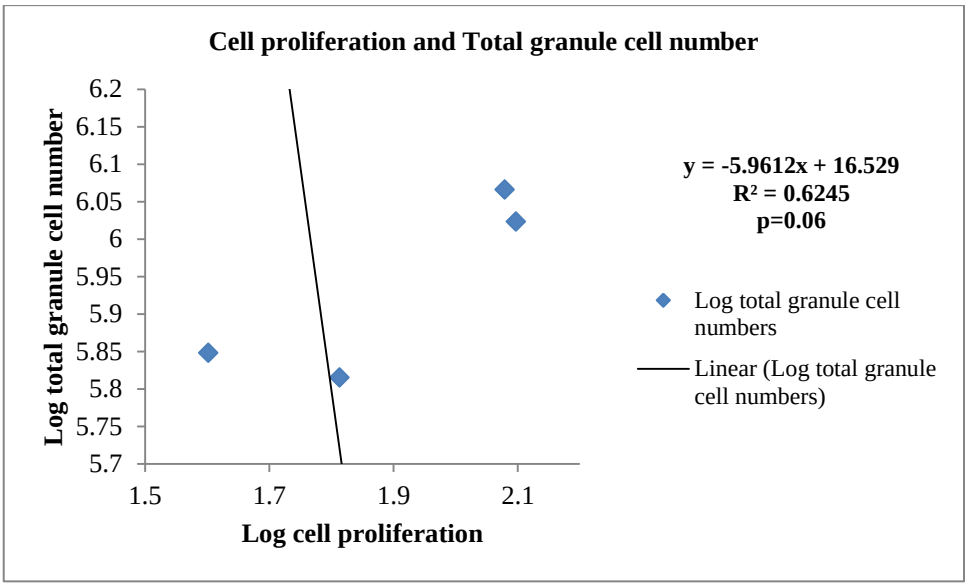


Figure 3.13 Regression analysis of log transformed values of cell proliferation and total granule cell number in the common mole-rat

3.5.6.2. Correlation and regression of total granule cell number and cell death in the common mole-rat

There was no significant correlation between total granule cell number and cell death in the common mole-rat (r=0.4, p-value=0.6) (Table 3.13 & Figure 3.14). This implies that as the total granule cells increase so the number of proliferative cells increases in the dentate gyrus of common mole-rat. The log transformed coefficient of determination, R^2 was -0.106. This suggested that, about 1 % of cell death obtained can be accounted for by the influence of granule cell numbers on cell death. However, it was not statistically significant (p=0.89). Regression log cell death= (0.2297x + 0.9374) log proliferative cells, (R^2 = 0.0121).

Table 3.13: Nonparametric correlation of total granule cell number against cell death in common mole-rat

			Total granule cell	**Cell proliferation**
Spearman's rho	Total granule cell	Correlation Coefficient	1.000	0.400
		Sig. (2-tailed)	0.0	0.600
	Cell proliferation	Correlation Coefficient	0.400	1.000
		Sig. (2-tailed)	0.600	0.0

Table 3.14: Log transformation for correlation of total granule cell numbers against cell death in the common mole-rat

Log total granule cell numbers	**Log cell death**
5.847988	2.454845
5.8154	2.176091
6.023308	2
6.066053	2.574031

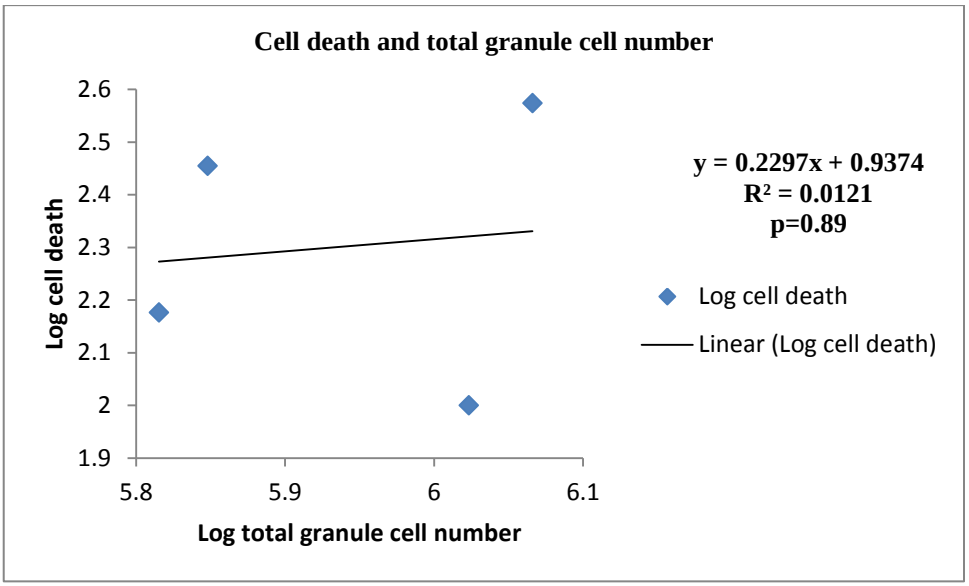


Figure 3.14 Regression analysis of log transformed values of cell death and total granule cell in the common mole-rat

3.5.6.3. Correlation and regression of cell death and cell proliferation in the common mole-rat

There was no significant correlation between cell death and cell proliferation in the dentate gyrus of the hippocampus ($p>0.05$) (Table 3.15; Figure 3.15). This implies that cell death is not proportional to cell proliferation in the dentate gyrus of common mole-rat. Regression analysis was performed on the log values of cell death and cell proliferation. The coefficient of variability (R^2) was 0.6563. This implies that about 65 % of proliferative cells obtained can be accounted for by the influence of pyknotic cells on cell proliferation. This was found to be statistically significant ($p=0.05$). Regression log cell death= (-2.4011x + 6.5972) log proliferative cells, ($R^2 = 0.6563$).

Table 3.15: Nonparametric correlation of cell proliferation against cell death in common mole-rat

			Cell death	Cell proliferation
Spearman's rho	Cell death	Correlation Coefficient	1.000	0.377
		Sig. (2-tailed)	0.0	0.461
	Cell proliferation	Correlation Coefficient	0.377	1.000
		Sig. (2-tailed)	0.461	0.0

Table 3.16: Log transformation for correlation of cell death against cell proliferation in the common mole-rat

Log cell death	Log cell proliferation
2.454845	1.60206
2.176091	1.812913
2	2.09691
2.574031	2.079181
	2.781755
	2.278754

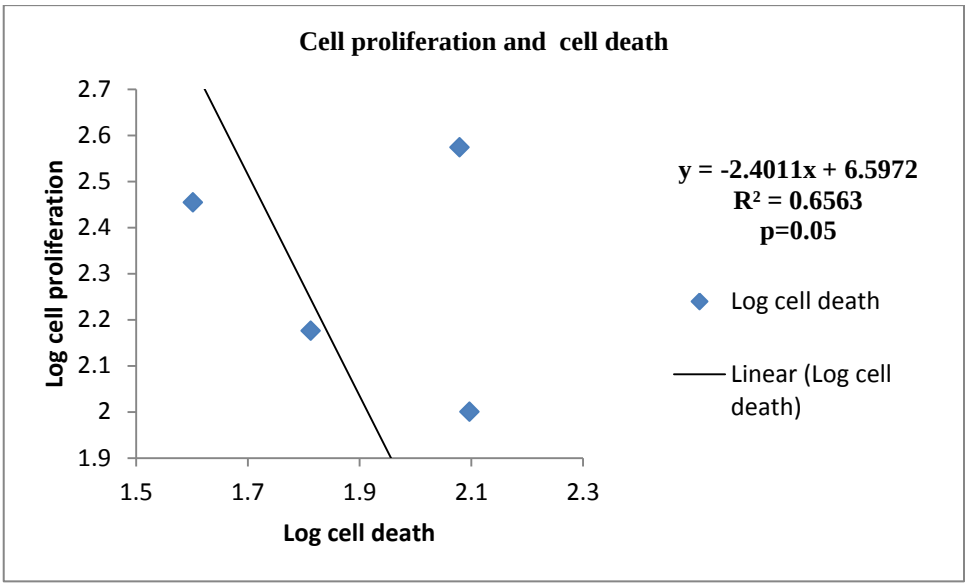


Figure 3.15 Correlation of cell proliferation and cell death in the common mole-rat upon log transformation

3.5.7. Comparison of means of variables in four-striped mouse and common mole-rat

There were significant differences between the mean body weight, mean brain weight, mean total granule cell number and the mean Ki-67 positive cells in the four-striped mouse and common mole-rat ($p<0.05$) (Table 3.17). The means of body weight and brain weight were significantly higher in common mole-rat than four-striped mouse ($p<0.05$). Conversely, the four-striped mouse had a greater mean in granule cell and Ki-67. Non-neuronal cells like glia, ependymal and epithelial cells could be a factor since they outnumber neurons in the central nervous system. There were no significant differences in DCX positive and pyknotic cells between four-striped mouse and common mole-rat ($p>0.05$) (Table 3.17).

Table 3.17: Summary of the comparison of means of the variables in four-striped mouse and common mole-rat

Variables	Specie	N	Mean	Std. Deviation	t-test	p-value
Body weight	FSM	6	80.53	3.38	-5.02	0.00
	CMR	7	127.93	22.76		
Brain weight	FSM	6	0.78	0.12	-10.94	0.00
	CMR	7	1.37	0.08		
Granule cell	FSM	6	1656366.67	252081.26	4.98	0.00
	CMR	7	511116.00	510569.88		
Proliferation	FSM	6	993.33	748.62	2.53	0.03
	CMR	6	190.83	209.51		
DCX	FSM	6	5843.17	3070.90	-0.83	0.43
	CMR	7	7830.86	5129.83		
Cell death	FSM	6	199.17	101.66	0.95	0.36
	CMR	7	227.5	150.58		

N= number of animals per group; **SD**= Standard deviation**; FSM** = Four-striped mouse; **CMR**= Common mole-rat

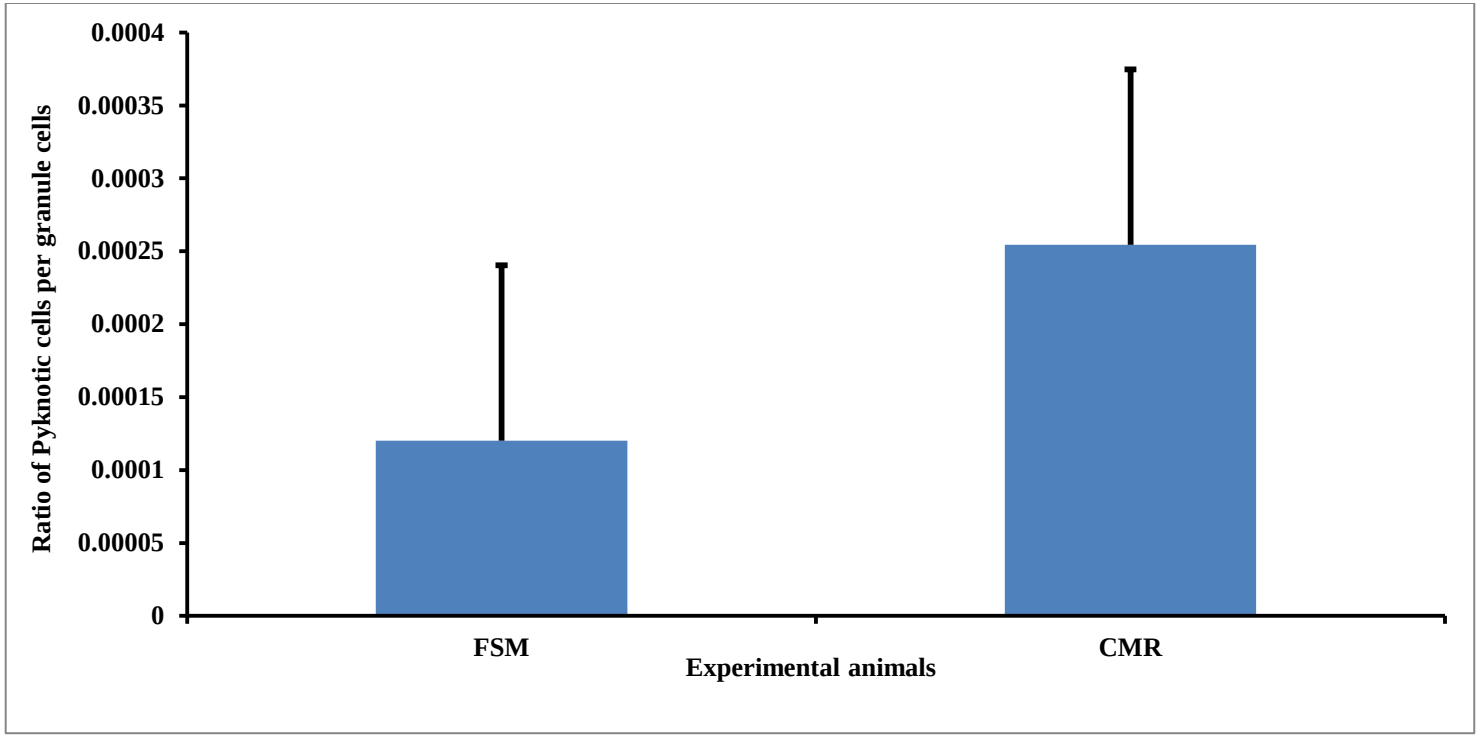
3.5.8. Ratio of pyknotic cell per granule cells in the DG of four-striped mouse and common mole-rat

The proportion of total granule cell number in relation to pyknotic cells in the four-striped mouse was 8316:1. While the proportion of granule cell number to pyknotic cell number was 2246:1 in common mole-rat (Table 3.18). Hence, there was a four-fold increase in the number of pyknotic cell observed in the common mole-rat as against four-striped mouse (Table 3.18; Figure 3.18).

3.5.9. Ratio of cell proliferation per granule cell in the DG of four-striped mouse and common mole-rat

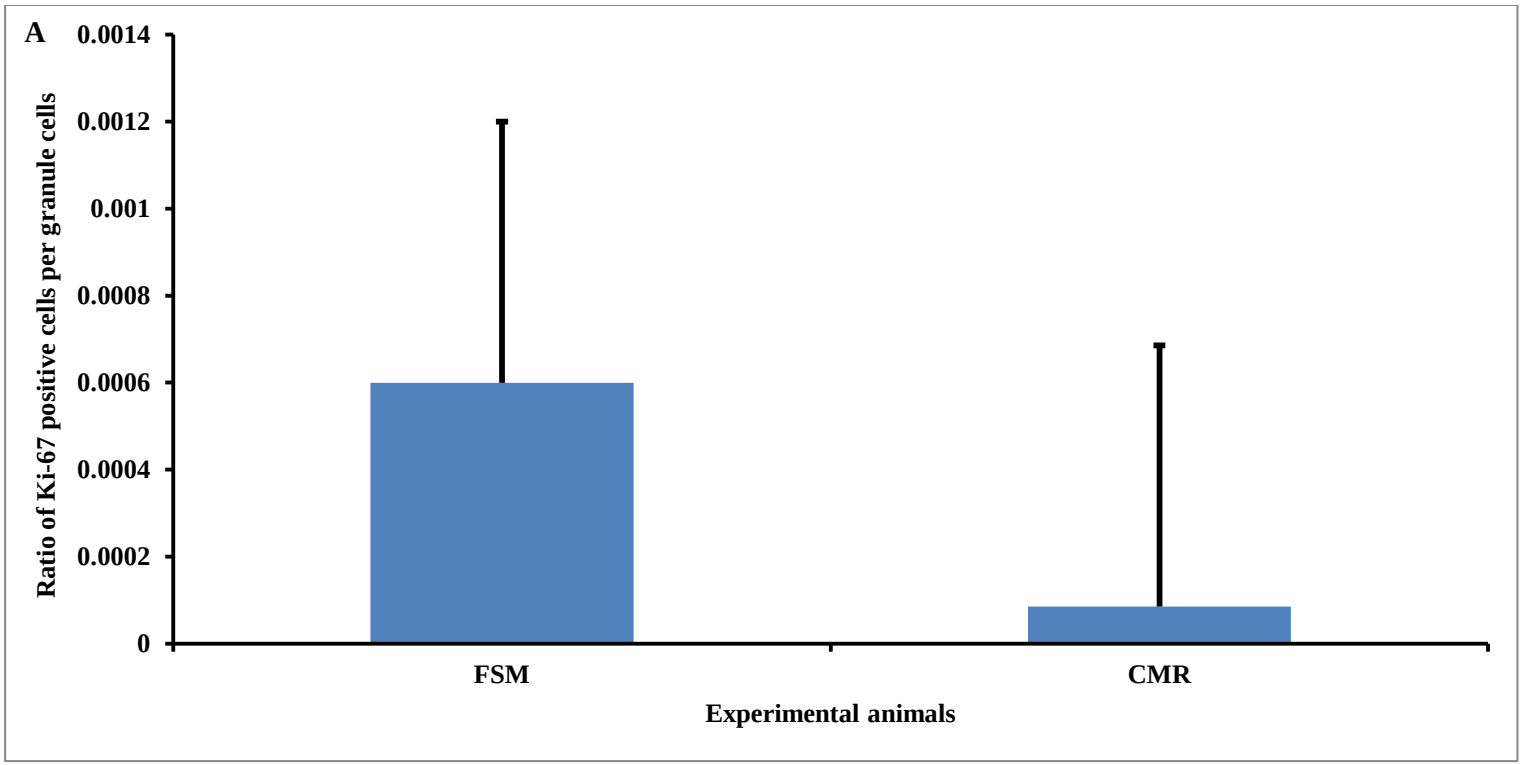
The ratio of the Ki-67 positive cells in relation to the total granule cell population was 1:1667 in the four-striped mouse (Table 3.18) whereas it was 1:2678 in the common mole-rat. This implies that the rate of cell proliferation is almost twice that of common mole-rat (Table 3.18). Therefore, there were more proliferative activities in the four-striped mouse than the common mole-rat as shown in the graph (Fig 3-18).

Figure 3.16 Cell death relative to granule cell number



Significantly more pyknotic cells were recorded in common mole-rat than four-striped mouse (p-value<0.05)

Figure 3.17 Proliferative cells relative to granule cell number



Significantly more proliferative cells were recorded in four-striped mouse than common mole-rat (p-value<0.05).

3.5.10. Ratio of pyknotic to proliferation rate in four-striped mouse and common mole-rat

The proliferative cell is five times more than pyknotic cell in four-striped mouse (5:1). While the ratio of pyknotic cell to proliferative cell is almost similar in common mole-rat (1:0.8) (Table 3.18).

3.5.11. Ratio of DCX positive to pyknotic cell number in the four-striped mouse and common mole-rat

A ratio of immature neuron to pyknotic cell was observed in common mole-rat (1:35) compared to four-striped mouse (1:29) (Table 3.18).

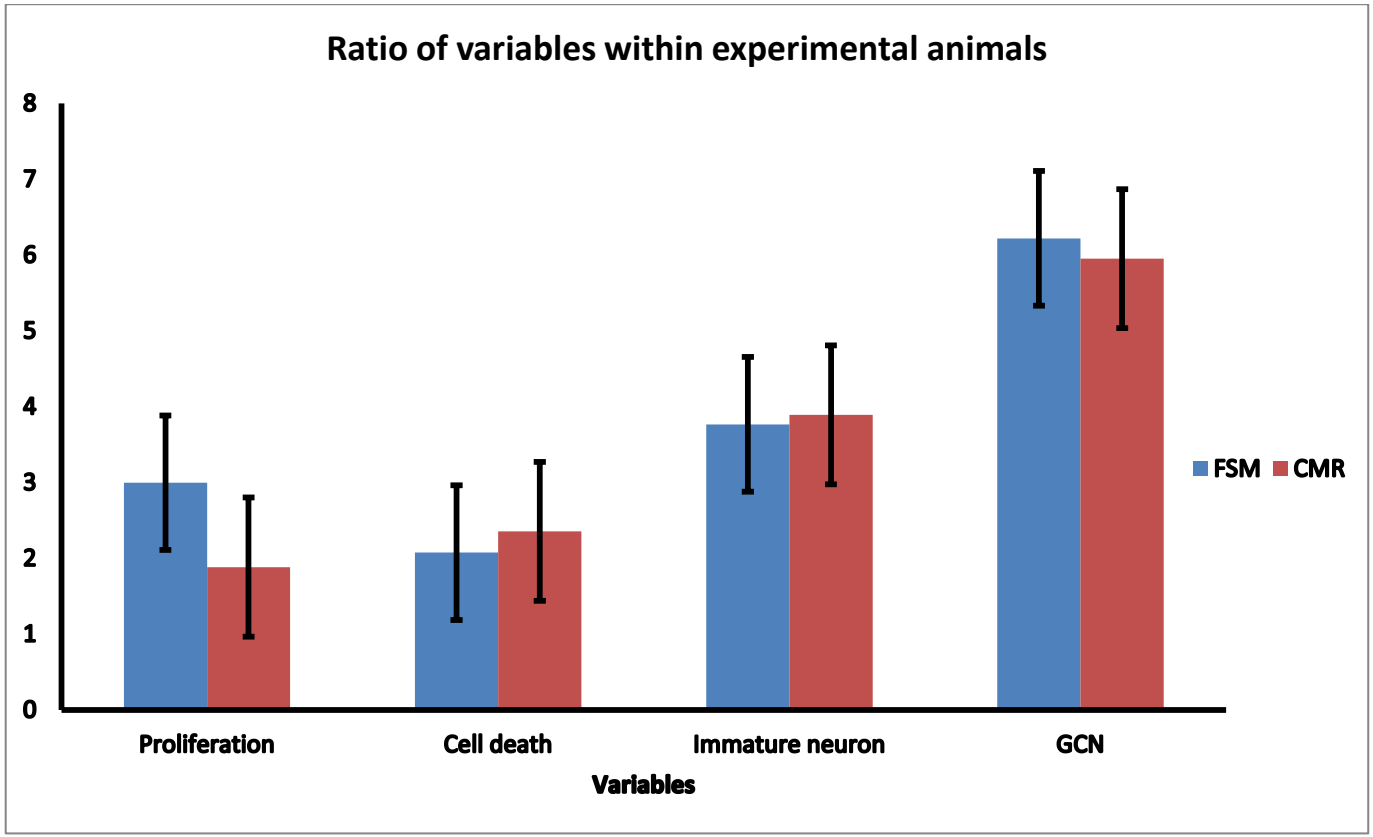
3.5.12. Ratio of body weight to brain weight in the four-striped mouse and common mole-rat

There was a similarity in the ratio of body weight to brain weight in both four-striped mouse (1:103) and common mole-rat (1:93) (Table 3.18).

Table 3.18: Ratio of the variables within the four-striped mouse and common mole-rat

Ratio of variables	FSM	CMR
Ratio of cell proliferation to total granule cell numbers	1:1667.49	1:2678.38
Ratio of pyknotic cells to total granule cell number	1:8316.35	1:2246.66
Ratio of pyknotic cells to cell proliferation	1:4.99	1:0.84
Ratio of DCX positive cells to pyknotic cells	1:29.36	1:34.49
Ratio of cell proliferation to pyknotic cell to total granule cell numbers	1:4.99:1667.49	1:0.84:2678.38
Body weight to brain weight	1:103.24	1:93.38

Figure 3.18 Comparison of ratio of cell proliferating, cell death, immature neuronal number and total granule cell number in the DG of four-striped mouse and common mole-rat



3.5.13. Comparison of results between four-striped mouse with reports from other mouse species

The total granule cell in the DG of the hippocampus in the four-striped mouse was similar to that of wood mice. However it was higher than the pine voles and laboratory mouse. The Gundersen coefficient of error (CE) was 0.05 in four-striped mouse which is considered to be excellent (Table 3.19).

Table 3.19: Comparison of results between four-striped mouse with reports from other mouse species

Species	**No. of sections analyzed**	**a (x,y step; µm)**	**Total granule cells ($x10^6$)**	**Gundersen Coefficient of Error (CE)**
***FSM	33	100 x 100	1.65	0.05
++Wood mice	31(28-33)	210 x 210	1.53	
++Bank voles	27 (25-30)	210 x 210	1.97	
++Pine voles	17.5 (16-19)	150 x 150	0.64	
++Laboratory mice	19.5 (18-20)	110 x 110	0.50	

++ Amrein et al., 2004
***** Current study

3.5.14. Comparison of results between common mole-rat with reports from other rat species

The total granule cells in the DG of the hippocampus in the common mole-rat were higher than the values reported in other rats (Table 3.18). The Gundersen coefficient of error (CE) was 0.06 in common mole-rat which is considered to be acceptable (Table 3.20).

Table 3.20: Comparison of results between common mole-rat with reports from other rat species

Species	**No. of sections analyzed**	**a (x,y step; µm)**	**Total granule cells ($x10^6$)**	**Gundersen Coefficient of Error (CE)**
*****CMR	36.8	100 x 100	0.90	0.06
++Highveld		140 x 140	0.62	0.10
++Cape mole-rat		250 x 250	0.47	0.12
++Naked mole-rat		110 x 110	0.39	0.13

++ Amrein et al., 2004
***** Current study

4. CHAPTER FOUR

4.0. DISCUSSION

4.1. Overview

The dentate gyrus (DG) of the hippocampus is responsible for memory, learning and cognitive functions in animals. The quantification and qualitative means of cell proliferation, cell death and total granule cells in the dentate gyrus could be used as a proxy to determine the extent of adult neurogenesis in mammals. Hence, this study was designed to provide qualitative and quantitative information on cell proliferation, cell death and total granule cell number in the DG of the hippocampus of the four-striped mouse *(Rhabdomys pumilio),* a captive bred animal originating from wild caught parents and the common mole-rat *(Cryptomys hottentotus),* wild caught animal. The findings were then compared with one another and also with findings previously reported inbred laboratory strains of rodents. Total granule cell number was used as a baseline to compare cell proliferation and cell death, the ratio of cell proliferation and cell death to total granule cell numbers. Also, correlation and regression analysis was carried out to determine the relationship and relative contribution of the variables to each other. A description of the evidence of adult neurogenesis in the four-striped mouse (Olaleye and Ihunwo, 2014) and common mole-rat (Olaleye, 2011) provided the background for the quantitative aspects of this study.

In the previous studies, the four-striped mouse (*Rhabdomys pumilio*), common mole-rat (*Cryptomys hottentotus*) (Olaleye 2011) and laboratory strains of rodents (Nacher *et al.*, 2001; Kee *et al.*, 2002; McDonald and Wojtowicz, 2005; Plumpe *et al.*, 2006; Yang *et al.*, 2015), a combination of Ki-67 and DCX immunohistochemistry was used to provide evidence of adult neurogenesis in the active sites (dentate gyrus of the hippocampus and

lateral wall of the subventricular zone) and in some potential sites (striatum, substantia nigra, cortex and olfactortory bulb) in the four-striped mouse and common mole-rat (Olaleye, 2011).

The present study, estimated the number of proliferating cells (Ki-67 and DCX positive cells), cell death (pyknotic cells) and total granule cell population using techniques which were discussed in the materials and methods section. Correlation and regression analysis test was carried out to determine the relationship between cell proliferation and cell death, cell death and total granule cell numbers and cell proliferation and total granule cell numbers in four-striped mouse (*Rhabdomys pumilio*) and common mole-rat (*Cryptomys hottentotus*).

4.2. Estimates of cell proliferation in the dentate gyrus of the hippocampus in the four-striped mouse

Proliferating cells are newly formed neurons in the dentate gyrus of adult brain and they are responsible for memory and cognitive functions (Ngwenya *et al.*, 2015). The dentate gyrus of the hippocampus is one of the only two regions where significant cell proliferation has been explicitly shown to occur even in adulthood (Hauser *et al.*, 2009; Ihunwo and Schliebs, 2010; Olaleye and Ihunwo, 2014; Bergmann *et al.*, 2015). The more the proliferating cells compared to other cells the better the cognitive function. Therefore, animals that require a high adaptive behaviour for survival are more likely to show adult neurogenesis than animals that live in an enriched environment (Rambau *et al.*, 2003). However, findings from previous reports (Nacher *et al.*, 2001; Schauwecker, 2006; Kim *et al.*, 2009; Hauser *et al.*, 2009; Olaleye and Ihunwo, 2014) using different markers for cell proliferation including Ki-67 to assessed the dentate gyrus of the four-striped mouse and other mouse models were not consistent. The reasons for the variation have been the subject of studies by many scholars (Hauser *et al.*, 2009; Lieberwirth and Wang, 2012).

The value of proliferative cells in the adult four-striped mouse in the present study also showed variations compared to previous studies. The number was found to be higher than the values reported in laboratory mouse (Amrein *et al.,* 2004b), but lower than the value reported by Hauser *et al.,* (2009) in the wild caught long tailed wood mouse. One possible explanation for this variation is the effect of social and environmental factors on the animals (Lieberwirth and Wang, 2012). Social interactions among conspecifics such as adult-adult and adult –offspring interactions are an integral part of mammalian society and it affect the psychological, physiological and behavioural functions. Studies on animal models have demonstrated the effect of social interactions on the brain especially the neuronal activation, morphology, neurotransmitter system activity as well as regulation of bio-behavioural functions (Lieberwirth and Wang, 2012). For example in prairie voles, mating induced pair bonds are associated with neuroplastic changes in several neurotransmitter systems which in turn play a role in social behaviours such as selective aggression against novel conspecifics and enhanced parental care towards offspring (Fowler *et al.,* 2002). Another study showed that in captivity, males from both mesic and xeric populations display parental care and show more social interactions than the wild ones. However in the wild, they may display solitary lifestyle with their females rearing their offspring on their own thus facilitating adult neurogenesis (Lieberwirth and Wang, 2012). Study by Hauser *et al.,* (2009) did not show more cell proliferation in the wild caught long tail wood mice compared to house mice even when such mice were exposed to voluntary running and environmental changes. They concluded that environmental factors does not influence cell proliferation but that there are other regulatory mechanisms involved in cell proliferation in wild mouse specie compared to laboratory mouse. Regulatory factors such as glutamate receptor activation (Cameron *et al.,* 1995; Gould *et al.,* 1997; Cameron *et al.,* 1998; Bernabeu and Sharp, 2000), dietary restriction (Lee *et al.,*

2000; Lee *et al.*, 2002a; 2002b), growth factors (Palmer *et al.*, 1995; Scharfman *et al.*, 2005), stress (Brunson *et al.*, 2005; Nichols *et al.*, 2005) and neuronal injury (Parent, 2003; Cooper-Kuhn *et al.*, 2004) and cellular degeneration were reported to cause cell proliferation and granular degeneration concurrently in the dentate gyrus of Yellow-necked wood mouse, bank voles, European pine vole and laboratory mouse (Amrein *et al.*, 2004a).

Genetic influence on the animals could also be a source of the variation in the values of proliferative cell in the mouse. The adult four-striped mouse used in this study was captive reared. The captive reared are offsprings of wild caught parents which have been shown to display adult cell proliferation (Chetty *et al.*, 2009). Hence, there may be a genetic tendency towards more cell proliferation. Whereas, in the laboratory strains, the enriched environment may not require a complex adaptive behaviour needed for survival (Kempermann *et al.*, 1997). This may explain why there was higher number of cell proliferation in the captive reared four-striped mouse used in the present study.

4.3. Estimates of pyknotic cell number in the dentate gyrus of the hippocampus in the four-striped mouse

Adult neurogenesis has been positively related to performance in hippocampal-dependent learning tasks. This could be verified by the numbers of pyknotic cell which represents the number of cell death in the brain. By either eradicating excess number of new neurons or already established neurons in the brain, the cell death population is believed to re-invent the entire lifespan of neurons in mammalian animal species (Jabès *et al.*, 2010). The rate of cell death in the four-striped mouse was found to be lower than other non-laboratory mouse (Amrein *et al.*, 2004a & 2004b) and other species like wild wood mice and bank voles (Amrein *et al.*, 2004a). The reason for this could be due to environmental exposure.

The four-striped mice used in this study were captive reared and they spent a greater part of their life in social interactions with their species.

Down regulation in adult neurogenesis has been reported in some wild caught rodents (Amrein *et al.*, 2004a; 2011) and in an Alzheimer's disease animal model, the Tg2576 (Ihunwo and Schliebs, 2010). This implies that there is an age related increase in the number of pyknotic cell compared to cell proliferation in the four-striped mouse. Conversely, this study showed low number of pyknotic cells in the dentate gyrus compared to proliferative cell. The low number of pyknotic cells in four-striped mouse is in consonant with lower cell death seen in animals from an enriched environment (Kempermann *et al.*, 1997) and laboratory bred (Ihunwo and Schliebs, 2010) compared to higher number of cell death seen in the wild caught long-tailed wood mouse (Hauser *et al.*, 2009). By implication, a non-diseased environment could be good for less cell death.

4.4. Estimates of total granule cell numbers in the dentate gyrus of the four-striped mouse

The estimation of the total granule cell number in this study constituted the baseline for comparing cell proliferation and cell death in the four-striped mouse. Researches are still ongoing in the maturation, integration and functional relevance of granules cells in the dentate gyrus. In the yellow-necked wood and old-bank voles mice, granule cell numbers were significantly higher than that of laboratory mouse (Amrein *et al.*, 2004) and in an Alzheimer's diseases Tg2576 mouse (Ihunwo and Schliebs, 2010). The current study showed that the number of granule cells was higher than those reported by Amrein *et al.*, (2004) and, Ihunwo and Schliebs, (2010) with the exception of bank voles. The possible reason for the high number of granule cell could be attributed to the ages of the four-striped mice used which were all adults. The reliability and repeatability of the granule cell

is proven by the coefficient of error (CE). Slomianka and West (2005) reported that the CE of an estimation procedure expresses the validity and reliability of the result obtained. It could provide information about the precision of stereological estimates. The Gundersen–Jensen coefficient of error obtained in this study is within the acceptable limit of 0.05. This shows that the result obtained for this study is valid.

4.5. Categorization of DCX- positive neurons in the dentate gyrus of hippocampus in the four-striped mouse

Doublecortin is an associated phosphoprotein that regulates neuronal migration during development while in adult brain. DCX is associated with neurite and axon elongation and synaptogenesis (Ribak *et al.*, 2004; Deuel *et al.*, 2006; Kempermann, 2006; Plumpe *et al.*, 2006). DCX is classified as a marker for identifying new neurons in the adult hippocampus (D'Alessio *et al.*, 2010; Rosenzweig and Wojtowicz, 2011) and its expression has been reported only in cells that are responsible for adult neurogenesis (Plumpe *et al.*, 2006). There was an abundance of DCX- positive cells in the DG of the hippocampus of four-striped mouse. Even though, it is believed that DCX is associated with the inception of neuronal differentiation and migration in the adult DG (Kempermann, 2006; Plumpe *et al.*, 2006; D'Alessio *et al.*, 2010).

The morphological categorization of DCX-positive cells (Plumpe *et al.*, 2006) in the DG was achieved by observing the structure of the cells as recorded in previous work done (Ribak *et al.*, 2004; Plumpe *et al.*, 2006; D'Alessio *et al.*, 2010; Olaleye, 2011). The typical immature neurons observed in the DG of the four-striped mouse was not different from what was observed in the literature (Plumpe *et al.*, 2006; D'Alessio *et al.*, 2010). The DCX- positive cells identified in the four-striped mouse showed the soma of the immature neuron located in the subgranular layer of the DG of the hippocampus and their processes

projecting into the molecular layer where synaptogenesis is expected to occur as reported in previous studies (Ribak *et al.*, 2004; Plumpe *et al.*, 2006; D'Alessio *et al.*, 2010; Olaleye, 2011). At higher magnification some of the cell bodies (soma) were located in the granular layer as well as the subgranular layer (Figure 3-3), with their processes reaching as far as the molecular layer of the hippocampus. It is quite interesting also to see in the four-striped mouse that some of the processes were directed towards the hilar region of the hippocampus. The DCX positive cells observed in four-striped mouse could be classified as postmitotic stage and had either a one thick dendrite or a delicate branched dendritic tree with few major branches close to the soma or within the granule cell layer. Majority of the DCX positive cells were located in the suprapyramidal region of the DG in the four-striped mouse.

4.6. Comparison of cell proliferation, cell death and total granule cell numbers in the four-striped mouse

The dentate gyrus of the hippocampus is important for learning tasks and could be used to directly identify underlying memory deficit in mammals. Therefore, it is important to investigate the relationship of cell proliferation, cell death and total granule cell numbers in the dentate gyrus of the hippocampus with a view to understand the degree of adult neurogenesis in any particular specie. If the rate of cell proliferation is more than cell death, then there is a strong tendency towards adult neurogenesis which leads to a better memory and learning performance. Study by Amrein and colleagues (2004a) showed no differences in the ratio of cell proliferation and cell death. This is contrary to the findings of this study which showed proliferating cells to be 5 times more than pyknotic cells. However, Kozorovitskiy and Gould, (2004) identified some factors such as position of the animal within a group hierarchy to affect cell death but not cell proliferation. The reason for this difference is not clear. However, environmental factor could have played a role in

the higher number of proliferative cell compared to cell death observed in this study. Kempermann *et al.,* (1997) and van Praag, *et al.,* (1999) identified environmental complexities and physical exercise as factors that could increase cell proliferation.

It is generally known that granule cell number is significantly higher in the DG than both cell proliferation and cell death (Amrein *et al.,* 2004; Ihunwo and Schliebs, 2010). In this study, there were a higher number of granule cells to proliferative cells compared to previous study (Amrein *et al.,* 2004a). Similarly there was a higher granule cell to pyknotic cell in four-striped mouse compared to previous study (Amrein *et al.,* 2004). Again the reason for the differences could be due to environmental factors such as physical exercise, mating pair and rearing of offspring as seen in previous studies (Amrein *et al.,* 2004a & 2004b; Epp *et al.,* 2009; Hauser *et al.,* 2009 and Lieberwirth and Wang, 2012).

4.7. Correlation of cell proliferation, cell death and total granule cell numbers in the four-striped mouse

There was no significant correlation between cell death and cell proliferation in the dentate gyrus of the hippocampus. This implies that cell proliferation was not directly proportional to cell death in the dentate gyrus. However, the relative contribution of cell death to cell proliferation was obtained after log transformation of the values was done although they were not statistically significant. The correlation coefficient obtained suggested that, 25 % of newly formed cells could be accounted for by the indirect influence of the number of cell death recorded. Similarly, Amrein *et al.,* (2004a) found no correlation between cell proliferation and cell death except in pine voles. No reason could easily be adduced for the difference, but genetic factor could play a role. Presently there are few studies that correlated cell proliferation with cell death (Amrein *et al.,* 2014; Ngwenya *et al.,* 2015).

Further studies needs to be done to determine the relative influence of cell death on cell proliferation.

Also, no significant correlation was found between cell death, cell proliferation and total granule cell number in the dentate gyrus of the hippocampus. This also shows that the total granule cell number is not directly proportional to cell death and cell proliferation in the dentate gyrus. However, a log transformed correlation coefficient obtained suggested that 21 % of cell death and 28 % of cell proliferation could be accounted for by the indirect influence of total granule cell numbers. Again these were not statistically significant. The search of literature did not yield any evidence on the relationship of total granule cell to cell death and cell proliferation in mouse specie. A study done on monkeys showed a significant correlation between dentate gyrus cell proliferation and cell death. This implies that as the cell proliferation increases, cell death decreases.

4.6. Estimates of cell proliferation in the dentate gyrus of the hippocampus in the common mole-rat

The common mole-rat *(Cryptomys hottentotus)* is the most common species of mole-rat in Southern Africa and it has been little researched. Most researches were done on the naked mole-rat *(Heterocsphalus glaber)* because it shows extended habitat adaptations like hairlessness and ectothermy. Estimate of cell proliferation in the common mole-rat was higher than the naked mole-rat but lower compared to the estimates reported in cape mole-rat and Highveld mole-rat (Amrein *et al.,* 2014). One would have expected similar cell proliferation numbers in both naked mole-rat and common mole-rat because of their similar complex tunnel system which provides safe environment. However, it may be that the hairlessness and ectothermy in naked mole-rat makes it more suitable to adapt to its immediate environment and hence less neurogenesis is required. For survival on the other

hand, common mole-rats must display higher behavioural flexibility to adapt to similar environmental conditions. It is well known that experimental adjustments of the social context influence adult hippocampal neurogenesis in several mammals (Gheusi *et al.*, 2009; Lieberwirth and Wang, 2012), but there are limited studies that compared solitary with social species. Fowler *et al.*, (2002) found a higher density of proliferating cells in solitary meadow voles compared to the social prairie voles. In contrast, Barker *et al.*, (2005) found more proliferating cells in social eastern gray squirrels than in yellow-pine chipmunks. Likewise, Snyder *et al.*, (2009) found higher proliferation in laboratory rats compared to laboratory mice. Under natural settings rats would live in mixed-gender groups, while mice consolidate in groups of one territorial male with several females. Another reason for the differences in the adult cell proliferation within species may be due to genetic factors or differences in body weight.

4.7. Estimates of pyknotic cell number in the dentate gyrus of the hippocampus in the common mole-rat

There is paucity of data and report on the rate of cell death in the common mole-rat. The value of cell death was found to be higher than cell proliferation in the common mole-rat in the present study. This shows that less proliferative cells get into the maturity or post mitotic stage of cell development in common mole-rat. Amrein *et al.*, (2014) observed similar trend when they reported that the ratio of the high cell death to cell proliferation could be due to environmental factors. The environment of common mole-rats under study which was strictly solitary could have been responsible for the high rate of cell death observed.

4.8. Estimates of total granule cell number in the dentate gyrus of common mole-rat

Most of the mole-rat species show a small granule cell layer in the dentate gyrus compared to other rodents. The compact hilar polymorphic cell layer merges with CA3 pyramidal layer in naked mole-rats, whereas the hilar region in common mole-rat is similar to what was reported in cape and social highveld mole-rats by Amrein *et al.,* (2014).

There were a high number of granule cell populations seen in the dentate gyrus of the hippocampus of the common mole-rat used for this study. This finding is corroborated by the report of Bayer *et al.,* (1982) who reported an upto 43% increase in the number of granule cells in adult laboratory rats. Furthermore, the estimated granule cell numbers in the dentate gyrus of common mole-rat was higher than the values reported by Amrein *et al.,* (2014) for the naked, cape and social highveld mole-rats. The bigger brain weight of the common mole-rat used for this study could be a reason for the high values of granule cells observed.

Some authors reported that newly formed granule cells are added to the granular cell population which receive inputs from already established ones making them stable overtime (Ngwenya *et al.,* 2015). They assumed that the stability of the total granule cell numbers in the brain of adult rhesus monkeys is balanced out by cell proliferation and cell death which could be a reasonable argument to support the finding in the common mole-rat. The study of total granule cell numbers through the life span reveals that a fraction of cell proliferation and cell death cause a stable increase in number of neurons in the dentate gyrus (Vivar and Van Praag, 2013; Ngwenya *et al.,* 2015). One of the limitations of the present study is that the study did not look at the count of granule cell by age of the rat. Hence this study could not prove the assertion of Ngwenya and colleagues, (2015) that granule cell decreases with age.

4.9. Categorization of DCX- positive neurons in the dentate gyrus of hippocampus in the common mole-rat

Similar to reports of previous studies (Ribak *et al.*, 2004; Plumpe *et al.*, 2006; D'Alessio *et al.*, 2010; Olaleye, 2011), DCX positive cells identified in the common mole-rat showed the soma of the immature neuron located in the subgranular layer of the DG of the hippocampus with their processes projecting into the molecular layer where synaptogenesis was expected to occur. The immature neurons in the DG of adult mammalian brain are presumed to display the phenotype of differentiated granule cells (Rao and Shetty, 2004). The cell bodies were located in the granule cell layer as well as the subgranular layer with their processes reaching as far as the molecular layer of the hippocampus. Rao and Shetty, (2004) also found similar morphological architecture in laboratory rats. Furthermore, the DCX positive cells in the DG of the hippocampus of common mole-rat showed more matured appearance classified by Plumpe *et al.*, (2006) as a postmitotic stage of neuron development. The DCX positive cells observed in the common mole-rat had either a one thick dendrite or a delicate branched dendritic tree with few major branches close to the soma or within the granule cell layer. The majority of the DCX positive cells were located in the suprapyramidal region of the DG in the common mole-rat, an indication that many of the immature neurones tend towards maturity and integration. The value of DCX positive cell in the common mole-rat was similar to the adult Sprague-Dawley rat (Epp *et al.*, 2009). Epp *et al.*, (2009) found similarity in dentate gyrus cell proliferation and young neuron survival in free-living adult and captive-bred rats even though the animals live in extremely different environments. A possible explanation for this is that wild,captive, and laboratory rats are exposed to a balance of opposing regulatory factors such as, glutamate receptor activation (Cameron *et al.*, 1995; Gould *et al.*, 1997; Cameron *et al.*, 1998; Bernabeu and Sharp, 2000), dietary restriction (Lee *et al.*, 2000; Lee *et al.*, 2002a; 2002b),

growth factors (Palmer *et al.,* 1995; Scharfman *et al.,* 2005), stress (Brunson *et al.,* 2005; Nichols *et al.,* 2005) and neuronal injury (Parent, 2003; Cooper-Kuhn *et al.,* 2004), resulting in no net difference between the two populations of cell proliferation and cell death.

In free-living rats, for example, high activity levels and enrichment may enhance neurogenesis, but only relative to a concurrent stress-induced suppression of neurogenesis (Brunson *et al.,* 2005; Nichols *et al.,* 2005). Similarly, in captive-bred rats, the effects of lower levels of both activity and stress could counteract each other, resulting in a stable basal rate of neurogenesis that closely resembles that of their wild counterparts (Epp *et al.,* 2009).

Controversies exist on the reason for variation in cell proliferation in free living rats and laboratory rats. One would have thought that the more stressful environment to which free-living adult rats are constantly exposed to, would lead to less cell proliferation and/or cell survival in comparison to laboratory rats. On the other hand one might also presume that enhanced exercise and enrichment experienced by free-living rats could lead to increase in cell proliferation and cell survival in adulthood. Hence, the present result of high rate of cell proliferation in common mole-rats could be ascribed to the memorizing of the complex burrowing as described by Bennet and Faulkes, (2000). However, studies are needed to put these controversies to rest.

Although the findings of this study was similar to that of laboratory rat, it was higher than the values reported for Highveld, Cape and Naked mole-rats (Amrein *et al.,* 2014) which are free-living rats like common mole-rats. The reason for the disparities in the DCX positive cell values across the wild mole rats species may not be readily explained but could be due to genetic rather than environmental factors.

It could also be inferred from the present study, that there was an increase in the survival of immature neuron in common mole-rat. A search of literature did not yield any report on similar study of immature neurons in rat species. This is a novel finding and more studies should be done to confirm this findings.

4.10. Correlation coefficient of cell death, cell proliferation and total granule cell number in the common mole-rat

The estimation of the total granule cell number in this work constituted the baseline for comparing cell proliferation and cell death in the common mole-rat. In the common mole-rat, cell proliferation was not proportional to cell death in the dentate gyrus. This is similar to the findings of by Amrein and colleagues, (2014) on naked mole-rat and Highveld common mole-rat with the exception of pine voles (Amrein *et al.,* 2004). The reason may be due to environmental and genetic influences on the animals.

However, the relative contribution of cell death to cell proliferation was obtained after log transformation of the values was done. The correlation coefficient obtained suggested that, 65 % of newly formed cells could be accounted for by the number of cell death. Presently there are few studies (Amrein *et al.,* 2004a) that correlated cell proliferation with cell death in common mole-rat. Further studies needs to be done to determine the relative influence of cell death to cell proliferation.

No significant correlation was found between cell death, cell proliferation and total granule cell number in the dentate gyrus of the hippocampus of the common mole-rat. This also shows that the total granule cell number is not directly proportional to cell death and cell proliferation in the dentate gyrus. However, a log transformed correlation coefficient obtained suggested that 1 % of cell death and 62 % of cell proliferation could be accounted for by the indirect influence of total granule cell numbers. Other authors have reported that

several regulatory mechanisms such as glutamate receptor activation (Cameron *et al.,* 1995; Gould *et al.,* 1997; Cameron *et al.,* 1998; Bernabeu and Sharp, 2000), dietary restriction (Lee *et al.,* 2000; Lee *et al.,* 2002a; 2002b), growth factors (Palmer *et al.,* 1995; Scharfman *et al.,* 2005), stress (Brunson *et al.,* 2005; Nichols *et al.,* 2005) and neuronal injury (Parent, 2003; Cooper-Kuhn *et al.,* 2004) are involved in cell proliferation and cell death in the dentate gyrus. The reason for the findings is not clear due to the design of this study which did not identify the factors responsible for the results obtained.

The reliability and repeatability of the granule cell is proven by the coefficient of error (CE). Slomianka and West (2005) reported that the CE of an estimation procedure expresses the validity and reliability of the result obtained. It thus provided information about the precision of the results from the stereological estimates. The Gundersen coefficient of error obtained in this study is within the acceptable limit of 0.07.

4.11. Comparison of cell proliferation in four-striped mouse and common mole-rat

The dentate gyrus has been assessed by different markers for cell proliferation including Ki-67 (Nacher *et al.,* 2001; Schauwecker, 2006; Kim *et al.,* 2009; Hauser *et al.,* 2009; Olaleye and Ihunwo, 2014). Despite other neurogenic sites (Bernier *et al.,* 2002; Zhao *et al.,* 2003; Takemura, 2005; Luzzati *et al.,* 2006, 2007), the dentate gyrus of the hippocampus remains one of the most active neurogenic sites in the adult mammalian brain (Hauser *et al.,* 2009; Ihunwo and Schliebs, 2010; Olaleye and Ihunwo, 2014; Bergmann *et al.,* 2015).

The occurrence of new cells is four times more than that of pyknotic cell in the four-striped mouse whereas, the ratio of the two cells in common mole-rate was equal. The difference in the ratio of pyknotic cell to cell proliferation in the two animals may be due to environmental factors and genetic influence.

The four-striped mouse has a flexible social organization and mating system which is controlled mainly by resource availability and population density. This might have accounted for the increase in the number of cell proliferation. One would have expected an increase in the ratio of cell proliferation to pyknotic cell due to the complex burrowing system and other environmental and adaptive behaviour in common mole-rats. The reason for the equal ratio may be that they use other sensory in memorizing their complex burrowing system rather than the hippocampus. For example, DCX positive cells were observed in the cortex region of common mole-rat (Olaleye, 2011) which may indicate that dentate gyrus may not be involved in memory and cognition needed in the subterranean lifestyle. The decline in the Ki-67 positive cells in common mole-rat could also be related to a decrease in proliferation of granule cell precursor (Kuhn *et al.*, 1996). Regulatory factors and cellular degeneration could cause cell proliferation and granular degeneration concurrently in the dentate gyrus of common mole-rats (Cameron and Gould, 1996; Amrein *et al.*, 2004b). Chronic and acute socio-sexual interaction has shown to facilitate cell proliferation in mammalian brain (Lieberwirth and Wang, 2012). Howbeit, the social influence such as an enriched environment and lack of exposure to opposite sex (Fowler *et al.*, 2002) in the two experimental animals could have played a vital role in the outcome of cell proliferation in relation to sustained active and challenging environments (Sherman *et al.*, 1999; Lacey *et al.*, 2000; Schradin and Pillay, 2004 & 2005).

4.12. Comparison of pyknotic cell number in the dentate gyrus of the hippocampus in four-striped mouse and common mole-rat

Pyknotic cell numbers represents the number of cell death in the brain. The rate of cell death in common mole-rat was higher than four-striped mouse. The rate of cell death has been reported in a number of mammalian species (Amrein *et al.*, 2011; Spalding *et al.*, 2013). The rate of cell death in the four-striped mouse and common mole-rat does not

differ from other non-laboratory rodents reported (Amrein *et al.*, 2004a & 2004b) but differ in comparison to species like wood mice and bank voles where cell death population is considerable very low. This could have been as a result of environmental exposure since the common mole-rat is a solitary living rodent which tends to spend a greater part of its life in burrows. In the common mole-rat, the granule cell layer of the DG is relatively small in comparison to that of the four-striped mouse; hence this might also affect other areas including the visual areas of the brain. Brain weight could have contributed to the findings of this study. However, when the rate of cell death in the four-striped mouse was compared to common mole-rat by their brain size the pyknotic cell number was found to be two-fold higher in the common mole-rat than the four-striped mouse despite the larger brain weight of the common mole-rat. This reveals that the relative proportion of cell death to the brain weight between four-striped mouse and common mole-rat are inversely related.

New cells are being added to the hippocampus everyday (Spalding *et al.*, 2013) and most of these cell undergo cell degeneration which are represented by the presence of pyknotic cells (Zupanc, 1999; Biebl *et al.*, 2000; Jabès *et al.*, 2010). The low number of pyknotic cells is an indication of lower cell death in animals from an enriched environment (Kempermann *et al.*, 1997) compared to common mole-rat which is directly from the wild (Amrein *et al.*, 2004a & 2004b, 2007; Amrein *et al.*, 2011).

4.13. Comparison of total granule cell in the dentate gyrus of four-striped mouse and common mole-rat

The estimate of granule cells in the dentate gyrus of four-striped mouse was higher than common mole-rat despite the fact that the brain size in the common mole-rat was larger than that of the four-striped mouse. The reason for this may be due to the larger granule cells seen in the common mole-rats compared to the small cells seen in the four-striped

mouse. Newly formed granule cells are believed to be added to the granular cell population and they receive inputs from already established ones (Vivar and Van Praag, 2013). In the four-striped mouse, cell proliferation has more influence on the total granule cell number by stabilising the granule cell population in the dentate gyrus (Ngwenya *et al.,* 2015). Whereas in the common mole-rat, pyknotic cell number has a direct influence on the total granule cell number due to the values recorded against the values of cell proliferation.

4.14. Categorization of DCX- positive neurons in the dentate gyrus of hippocampus in the four-striped mouse and common mole-rat

The morphological categorization of DCX-positive cells in the DG was achieved by observing the structure of the cells as recorded in previous works (Ribak *et al.,* 2004; Plumpe *et al.*, 2006; D'Alessio *et al.,* 2010; Olaleye, 2011). The typical immature neurons observed in the DG of the two experimental animals, four-striped mouse and common mole-rat, were not different from what was observed in the literature (Plumpe *et al.,* 2006; D'Alessio *et al.,* 2010). The DCX- positive cells identified in the four-striped mouse and common mole-rat showed the soma of the immature neuron located in the subgranular layer of the DG of the hippocampus and their processes projecting into the molecular layer where integration is expected to occur. Some of the cell bodies (soma) were located in the granular layer as well as the subgranular layer (Figure 3-3). Their processes reach as far as the molecular layer of the hippocampus. It is quite interesting also to see in the four-striped mouse that some of the processes were directed towards the hilar region of the hippocampus (Figure 3.2b). In the DCX positive cells for category E (Plumpe *et al.,* 2006), they had a comparatively sparse branching in the molecular layer. There is a continuum that exists between the different categories. Majority of the DCX positive cells were located in the suprapyramidal region of the DG in the four-striped mouse and common mole-rat.

4.15. Correlation coefficient of cell proliferation and cell death in the four-striped mouse and common mole-rat

It is no longer in doubt that adult cell proliferation exists in the DG of the four-striped mouse (Olaleye and Ihunwo, 2014) and common mole-rat (Olaleye, 2011) which corroborates previous report on adult cell proliferation in rodents (Eriksson *et al.*, 1998; Nacher *et al.*, 2001; Amrein *et al.*, 2004a & 2004b; Schauwecker, 2006; Kim *et al.*, 2009). The newly formed cells are believed to receive inputs from established cells upon incorporation mostly for memory function (Amrein *et al.*, 2007; Vivar and Van Praag, 2013). Regulatory factors such as seizures and environmental manipulation (van Praag *et al.*, 2000), administration of drugs such as antidepressants (Malberg *et al.*, 2000; Santarelli *et al.*, 2003), exercise such as wheel-running (van Praag *et al.*, 1999; Creer *et al.*, 2010) and environmental enrichment (Kempermann *et al.*, 2002; Brown *et al.*, 2003; Ehninger and Kempermann, 2003) are believed to increase adult cell proliferation. However, stress (Gould *et al.*, 1997; Cameron *et al.*, 1998) and social isolation (Lu *et al.*, 2003; Lievajová *et al.*, 2011) have been shown to affect cell proliferation. The regulatory factors that promotes proliferation in one animal could lead to their degeneration in another (Cameron and Gould, 1996; Amrein *et al.*, 2004a). Also, cell death regulation could have been more tissue- or cell-type specific (Amrein *et al.*, 2004b). Some cells are believed to unusually switch from normal cell cycle pathway into apoptotic pathway (Heintz, 1993; Thomaidou *et al.*, 1997; Timsit *et al.*, 1999; Liu and Greene, 2001; Janumyan *et al.*, 2003) which maybe due to programmed cell death (Timsit *et al.*, 1999).

Opposite relationship was found in the four-striped mouse and common mole-rat in the relationship of cell proliferation to cell death. In the four-striped mouse, cell proliferation was not proportional to cell death in the dentate gyrus even though a negative but

insignificant correlation was observed similar to a previous report (Amrein *et al.*, 2004a). This meant that as cell proliferation is increasing cell death decreases in the four-striped mouse. Only about 24.7 % of the population of newly generated cells can be accounted for by indirect influence of pyknotic cell population in the dentate gyrus of four-striped mouse. Similarly, in the common mole-rat, there was no significant correlation between cell death and cell proliferation in the dentate gyrus of the hippocampus however, a positive correlation coefficient was observed. This meant that as new cell are being generated this is directly proportional to the pyknotic population in the dentate gyrus of common mole-rat.

The populations of pyknotic to granule cell numbers in the two experimental animals is similar to previous reports (Amrein *et al.*, 2004a & 2004b; Ihunwo and Schliebs, 2010). The estimate population of pyknotic cells observed in the four-striped mouse and common mole-rat could have been a small amount in relation to the total granule cell population as reported in (Kerr *et al.*, 1972; Amrein *et al.*, 2004a). The result revealed no significant correlation between cell death and total granule cell number in the dentate gyrus of the hippocampus in the four-striped mouse. However, based on the result of negative correlation observed, it could be inferred that as the population of pyknotic cell decreases proportionally, the number of granule cell increases and vice versa. In addition, the total granule cell number is not proportional to cell death in the dentate gyrus in four-striped mouse which points to the fact that 24.7 % of dead cells can be explained by the indirect influence of the total number of granule cells. In contrast to four-striped mouse, there was a positive and significant correlation between total granule cell number and cell death in the common mole-rat. Based on the outcome, the granule cell population is directly affected by the pyknotic cell number despite the brain size in comparison to the four-striped mouse brain because as granule cell increases so is the pyknotic cell population.

5. CHAPTER FIVE

5.0. Conclusion

The study has provided a quantification data on estimation of cell proliferation, cell death and total granule cell number as a baseline in the four-striped mouse *(Rhabdomys pumilio)* and common mole-rat *(Cryptomys hottentotus).*

Ki-67 positive cells appeared in clusters along the whole region of the DG of the four-striped mouse and common mole-rat and were used to estimate the cell proliferation number. A larger portion of these proliferating cells were relatively located in the supra-pyramidal, than the infra-pyramidal region of the dentate gyrus, a few were located in the crest region in the dentate gyrus of hippocampus.

The DCX positive cells indicated cells in the proliferating, mitotic and postmitotic phases. Majority of the DCX positive cells observed in the four-striped mouse and common mole-rat were in their post mitotic phase. The DCX positive cells were mostly located in the suprapyramidal region of the DG and a relative amount in the crest and infra-pyramidal regions.

Using Giemsa staining two classes of cells were identified and quantified. First, the total granule cells in the granule cell layer and secondly, pyknotic cells which represent cell death in the dentate gyrus of the hippocampus in the four-striped mouse and common mole-rat.

Using the estimated total granule cell number as a baseline data, the influence of cell proliferation and cell death was analysed. The combinations of cell death and cell

proliferation do have a direct impact on the entire lifespan of the four-striped mouse and the common mole-rat.

There was no correlation between cell proliferation and cell death, cell proliferation and total granule cell, and cell death and total granule cell number in the four-striped mouse and common mole-rat reveals that there was no correlation. However, the regression analysis revealed a statistical significant in cell proliferation and cell death in the common mole-rat.

The ratio of cell proliferation was five times higher than pyknotic cell in four-striped mouse while it was almost equal in common mole-rat. The total granule cell was higher in common mole-rat than four-striped mouse. Also, the number of DCX positive cells was higher in the common mole-rat than the four-striped mouse.

Therefore, this study demonstrated adult neurogenesis in both animals. However, more adult neurogenesis was found in the four-striped mouse than the common mole-rat. This could be due to combination of social, environmental and genetic influence.

5.1. Further studies

Analysis on correlation studies of cell proliferation and cell death need to be carried out in neurogenic regions (SVZ) and non-neurogenic regions with neurogenic potential:

- By conducting a quantification study of cell proliferation and cell death in the subventricular zone of lateral ventricle (SVZ). The SVZ is very important due to the fact that majority of the newly formed cells migrate to the olfactory bulb (OB) via the rostral migratory stream.
- Electron microscopic study of newly formed adult neural cells needs to be studied.

- Isolation of adult neural stem cells for the dentate gyrus for *invitro* study on cell proliferation and cell death need to be investigated.
- An evaluation of the functionality (synapse activity) of newborn neurons needs to be investigated.

REFERENCES

1. Abdipranoto, A., Wu, S., Stayte, S. and Vissel, B. (2008) 'The Role of Neurogenesis in Neurodegenerative Diseases and its Implications for Therapeutic Development', *CNS & Neurological Disorders - Drug Targets*, 7(2), pp. 187–210.

2. Ajao, M. S., Olaleye, O. and Ihunwo, A. O. (2010) 'Melatonin Potentiates Cells Proliferation in the Dentate Gyrus Following Ischemic Brain Injury in Adult Rats', *Journal of Animal and Veterinary Advances*, 9(11)1633–1638.

3. Akers, K. G., Martinez-Canabal, A., Restivo, L., Yiu, A. P., Cristofaro, A. D., Hsiang, H.-L. (Liz), Wheeler, A. L., Guskjolen, A., Niibori, Y., Shoji, H., Ohira, K., Richards, B. A., Miyakawa, T., Josselyn, S. A. and Frankland, P. W. (2014) 'Hippocampal Neurogenesis Regulates Forgetting During Adulthood and Infancy', *Science*, 344(6184)598–602.

4. Altman, J. and Das, G. D. (1967) 'Postnatal neurogenesis in the guinea-pig', *Nature*, 214(5093)1098–1101.

5. Alvarez-Buylla, A. and Garcia-Verdugo, J. M. (2002) 'Neurogenesis in adult subventricular zone', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 22(3)629–634.

6. Amrein, I., Dechmann, D. K., Winter, Y. and Lipp, H. P. (2007) 'Absent or low rate of adult neurogenesis in the hippocampus of bats (Chiroptera)', *PloS one*, 2(5)455.

7. Amrein, I., Isler, K. and Lipp, H.-P. (2011) 'Comparing adult hippocampal neurogenesis in mammalian species and orders: influence of chronological age and life history stage', *European Journal of Neuroscience*, 34(6)978–987.

8. Amrein, I., Slomianka, L. and Lipp, H. P. (2004a) 'Granule cell number, cell death and cell proliferation in the dentate gyrus of wild-living rodents', *The European journal of neuroscience*, 20(12)3342–3350.

9. Amrein, I., Slomianka, L., Poletaeva, I. I., Bologova, N. V. and Lipp, H. P. (2004b) 'Marked species and age-dependent differences in cell proliferation and neurogenesis in the hippocampus of wild-living rodents', *Hippocampus*, 14(8)1000–1010.

10. Arsenijevic, Y., Villemure, J. G., Brunet, J. F., Bloch, J. J., Deglon, N., Kostic, C., Zurn, A. and Aebischer, P. (2001) 'Isolation of multipotent neural precursors residing in the cortex of the adult human brain', *Experimental neurology*, 170(1), pp. 48–62.

11. Barker, J. M., Wojtowicz, J. M. and Boonstra, R. (2005) 'Where's my dinner? Adult neurogenesis in free-living food-storing rodents', *Genes, Brain and Behavior*, 4(2), pp. 89–98.

12. Bayer, S. A., Yackel, J. W. and Puri, P. S. (1982) 'Neurons in the rat dentate gyrus granular layer substantially increase during juvenile and adult life', *Science*, 216(4548)890–892.

13. Bayer, S. A., Yackel, J. W. and Puri, P. S. (1982) 'Neurons in the rat dentate gyrus granular layer substantially increase during juvenile and adult life', *Science*, 216(4548), pp. 890–892.

14. Bennett, N. C. and Faulkes, C. G. (2000) *African Mole-Rats: Ecology and Eusociality*. Cambridge University Press.

15. Bergmann, O., Spalding, K. L. and Frisén, J. (2015) 'Adult Neurogenesis in Humans', *Cold Spring Harbor Perspectives in Biology*, 7(7)018994.

16. Bernabeu, R. and Sharp, F. R. (2000) 'NMDA and AMPA/kainate glutamate receptors modulate dentate neurogenesis and CA3 synapsin-I in normal and ischemic hippocampus', *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*, 20(12)1669–1680.

17. Bernier, P. J., Bedard, A., Vinet, J., Levesque, M. and Parent, A. (2002) 'Newly generated neurons in the amygdala and adjoining cortex of adult primates', *Proceedings of the National Academy of Sciences of the United States of America*, 99(17)11464–11469.

18. Biebl, M., Cooper, C. M., Winkler, J. and Kuhn, H. G. (2000a) 'Analysis of neurogenesis and programmed cell death reveals a self-renewing capacity in the adult rat brain', *Neuroscience Letters*, 291(1)17–20.

19. Bordiuk, O. L., Smith, K., Morin, P. J. and Semënov, M. V. (2014) 'Cell Proliferation and Neurogenesis in Adult Mouse Brain', *PLoS ONE*, 9(11)111453.

20. Brooks, P. M. (2009) 'Aspects of the reproduction, growth and development of the four-striped field mouse, Rhabdomys pumilio (Sparrman, 1784)', *Mammalia*, 46(1)53–64.

21. Brown, J., Cooper-Kuhn, C. M., Kempermann, G., Van Praag, H., Winkler, J., Gage, F. H. and Kuhn, H. G. (2003) 'Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis', *European Journal of Neuroscience*, 17(10)2042–2046.

22. Bruening, S. and Bruening, S. (2001) *Cryptomys hottentotus (African mole rat)*, *Animal Diversity Web*. Available at: http://animaldiversity.org/accounts/Cryptomys_hottentotus/ (Accessed: 8 January 2015).

23. Cameron, H. A. and Gould, E. (1996) 'Distinct populations of cells in the adult dentate gyrus undergo mitosis or apoptosis in response to adrenalectomy', *The Journal of Comparative Neurology*, 369(1)56–63.

24. Cameron, H. A. and Mckay, R. D. G. (2001) 'Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus', *The Journal of Comparative Neurology*, 435(4), pp. 406–417.

25. Cameron, H. A., McEwen, B. S. and Gould, E. (1995) 'Regulation of adult neurogenesis by excitatory input and NMDA receptor activation in the dentate gyrus', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 15(6)4687–4692.

26. Cameron, H. A., Tanapat, P. and Gould, E. (1998) 'Adrenal steroids and N-methyl-D-aspartate receptor activation regulate neurogenesis in the dentate gyrus of adult rats through a common pathway', *Neuroscience*, 82(2)349–354.

27. Chetty, T., Ajao, M.S., Manger, P.R. and Ihunwo, A.O., 2009. Behavioural Patterns and Corticosterone Levels Induced by Chronic Psychosocial Stress in the Four-Striped Mice (Rhabdomys pumilio). *Journal of Animal and Veterinary Advances*, *8*(3), pp.459-463.

28. Cooper-Kuhn, C. M., Winkler, J. and Kuhn, H. G. (2004) 'Decreased neurogenesis after cholinergic forebrain lesion in the adult rat', *Journal of neuroscience research*, 77(2)155–165.

29. Creer, D. J., Romberg, C., Saksida, L. M., Praag, H. van and Bussey, T. J. (2010) 'Running enhances spatial pattern separation in mice', *Proceedings of the National Academy of Sciences*, 107(5), pp. 2367–2372.

30. D'Alessio, L., Konopka, H., López, E. M., Seoane, E., Consalvo, D., Oddo, S., Kochen, S. and López-Costa, J. J. (2010) 'Doublecortin (DCX) immunoreactivity in hippocampus of chronic refractory temporal lobe epilepsy patients with hippocampal sclerosis', *Seizure*, 19(9)567–572.

31. Deuel, T. A. S., Liu, J. S., Corbo, J. C., Yoo, S.-Y., Rorke-Adams, L. B. and Walsh, C. A. (2006) 'Genetic interactions between doublecortin and doublecortin-like kinase in neuronal migration and axon outgrowth', *Neuron*, 49(1), pp. 41–53.

32. Ehninger, D. and Kempermann, G. (2003) 'Regional Effects of Wheel Running and Environmental Enrichment on Cell Genesis and Microglia Proliferation in the Adult Murine Neocortex', *Cerebral Cortex*, 13(8)845–851.

33. Epp, J. R., Barker, J. M. and Galea, L. A. (2009) 'Running wild: neurogenesis in the hippocampus across the lifespan in wild and laboratory-bred Norway rats', *Hippocampus*, 19(10), pp. 1040–1049.

34. Eriksson, P. S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A. and Gage, F. H. (1998) 'Neurogenesis in the adult human hippocampus', *Nature medicine*, 4(11)1313–1317.

35. Four-striped grass mouse' (2014) *Wikipedia, the free encyclopedia*. Available at: http://en.wikipedia.org/w/index.php?title=Four-striped_grass_mouse&oldid=608615334 (Accessed: 7 January 2015).

36. Fowler, C. D., Liu, Y., Ouimet, C. and Wang, Z. (2002) 'The effects of social environment on adult neurogenesis in the female prairie vole', *Journal of Neurobiology*, 51(2)115–128.

37. Frielingsdorf, H., Schwarz, K., Brundin, P. and Mohapel, P. (2004) 'No evidence for new dopaminergic neurons in the adult mammalian substantia nigra', *Proceedings of the National Academy of Sciences of the United States of America*, 101(27), pp. 10177–10182.

38. Gerdes, J. (1990) 'Ki-67 and other proliferation markers useful for immunohistological diagnostic and prognostic evaluations in human malignancies', *Seminars in Cancer Biology*, 1(3)199–206.

39. Gheusi, G., Ortega-Perez, I., Murray, K. and Lledo, P.-M. (2009) 'A niche for adult neurogenesis in social behavior', *Behavioural Brain Research*. (Pheromonal communication in higher vertebrates and its implication for reproductive function), 200(2), pp. 315–322.

40. Gil-Perotin, S. G., Alvarez-Buylla, A. and Garcia-Verdugo, J. M. (2009) *Identification and Characterization of Neural Progenitor Cells in the Adult Mammalian Brain*. Springer Science & Business Media.

41. Gleeson, J. G., Lin, P. T., Flanagan, L. A. and Walsh, C. A. (1999) 'Doublecortin is a microtubule-associated protein and is expressed widely by migrating neurons', *Neuron*, 23(2)257–271.

42. Gould, E., Beylin, A., Tanapat, P., Reeves, A. and Shors, T. J. (1999) 'Learning enhances adult neurogenesis in the hippocampal formation', *Nature Neuroscience*, 2(3)260–265.

43. Gould, E., McEwen, B. S., Tanapat, P., Galea, L. A. and Fuchs, E. (1997) 'Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial

stress and NMDA receptor activation', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 17(7)2492–2498.

44. Gould, E., Reeves, A. J., Graziano, M. S. A. and Gross, C. G. (1999) 'Neurogenesis in the Neocortex of Adult Primates', *Science*, 286(5439)548–552.

45. Gould, E., Tanapat, P., McEwen, B. S., Flugge, G. and Fuchs, E. (1998) 'Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress', *Proceedings of the National Academy of Sciences of the United States of America*, 95(6)3168–3171.

46. Gritti, A., Bonfanti, L., Doetsch, F., Caille, I., Alvarez-Buylla, A., Lim, D. A., Galli, R., Verdugo, J. M., Herrera, D. G. and Vescovi, A. L. (2002) 'Multipotent neural stem cells reside into the rostral extension and olfactory bulb of adult rodents', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 22(2)437–445.

47. Grote, H. E. and Hannan, A. J. (2007) 'Regulators of Adult Neurogenesis in the Healthy and Diseased Brain', *Clinical and Experimental Pharmacology and Physiology*, 34(5-6), pp. 533–545.

48. Gueneau, G., Privat, A., Drouet, J. and Court, L. (1982) 'Subgranular zone of the dentate gyrus of young rabbits as a secondary matrix. A high-resolution autoradiographic study', *Developmental neuroscience*, 5(4), pp. 345–358.

49. Gundersen, H. J. and Osterby, R. (1981) 'Optimizing sampling efficiency of stereological studies in biology: or "do more less well!"', *Journal of Microscopy*, 121(Pt 1), pp. 65–73.

50. Gundersen, H. J., Jensen, E. B., Kieu, K. and Nielsen, J. (1999) 'The efficiency of systematic sampling in stereology--reconsidered', *Journal of microscopy*, 193(Pt 3), pp. 199–211.

51. Hauser, T., Klaus, F., Lipp, H. P. and Amrein, I. (2009) 'No effect of running and laboratory housing on adult hippocampal neurogenesis in wild caught long-tailed wood mouse', *BMC neuroscience*, 10(Journal Article)43–2202–10–43.

52. Heintz, N. (1993) 'Cell death and the cell cycle: a relationship between transformation and neurodegeneration?', *Trends in Biochemical Sciences*, 18(5)157–159.

53. Hou, L. and Hong, T. (2008) 'Stem cells and neurodegenerative diseases', *Science in China Series C: Life Sciences*, 51(4), pp. 287–294.

54. Ihunwo, A. O. and Pillay, S. (2007) 'Neurogenic Sites in the Adult Mammalian Central Nervous System'. (Research Journal of Biological Sciences), 2(2)170–177.

55. Ihunwo, A. O. and Schliebs, R. (2010) ‘Cell proliferation and total granule cell number in dentate gyrus of transgenic Tg2576 mouse’, *Acta Neurobiologiae Experimentalis*, 70(4)362–369.

56. Iñiguez, C., Gayoso, M. J. and Carreres, J. (1985) ‘A versatile and simple method for staining nervous tissue using Giemsa dye’, *Journal of Neuroscience Methods*, 13(1), pp. 77–86.

57. Jabès, A., Lavenex, P. B., Amaral, D. G. and Lavenex, P. (2010) ‘Quantitative analysis of postnatal neurogenesis and neuron number in the macaque monkey dentate gyrus’, *European Journal of Neuroscience*, 31(2)273–285.

58. Janumyan, Y. M., Sansam, C. G., Chattopadhyay, A., Cheng, N., Soucie, E. L., Penn, L. Z., Andrews, D., Knudson, C. M. and Yang, E. (2003) ‘Bcl-xL/Bcl-2 coordinately regulates apoptosis, cell cycle arrest and cell cycle entry’, *The EMBO Journal*, 22(20)5459–5470.

59. Jun, H., Mohammed Qasim Hussaini, S., Rigby, M. J. and Jang, M. H. (2012) ‘Functional role of adult hippocampal neurogenesis as a therapeutic strategy for mental disorders’, *Neural plasticity*, 2012(Journal Article), p. 854285.

60. Kaplan, M. S. and Bell, D. H. (1984) ‘Mitotic neuroblasts in the 9-day-old and 11-month-old rodent hippocampus’, *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 4(6)1429–1441.

61. Kaplan, M. S. and Hinds, J. W. (1977) ‘Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs’, *Science (New York, N.Y.)*, 197(4308)1092–1094.

62. Kee, N., Sivalingam, S., Boonstra, R. and Wojtowicz, J. M. (2002) ‘The utility of Ki-67 and BrdU as proliferative markers of adult neurogenesis’, *Journal of neuroscience methods*, 115(1), pp. 97–105.

63. Kempermann, G. (2006) *Adult Neurogenesis: Stem Cells and Neuronal Development in the Adult Brain*. Oxford University Press.

64. Kempermann, G. and Gage, F. H. (2002) ‘Genetic influence on phenotypic differentiation in adult hippocampal neurogenesis’, *Brain research.Developmental brain research*, 134(1-2)1–12.

65. Kempermann, G., Kuhn, H. G. and Gage, F. H. (1997) ‘Genetic influence on neurogenesis in the dentate gyrus of adult mice’, *Proceedings of the National Academy of Sciences of the United States of America*, 94(19)10409–10414.

66. Kerr, J. F. R., Wyllie, A. H. and Currie, A. R. (1972) ‘Apoptosis: A Basic Biological Phenomenon with Wideranging Implications in Tissue Kinetics’, *British Journal of Cancer*, 26(4)239–257.

67. Kim, J.-S., Jung, J., Lee, H.-J., Kim, J. C., Wang, H., Kim, S.-H., Shin, T. and Moon, C. (2009) ‘Differences in immunoreactivities of Ki-67 and doublecortin in the adult hippocampus in three strains of mice’, *Acta Histochemica*, 111(2)150–156.

68. Kornack, D. R. and Rakic, P. (1999) ‘Continuation of neurogenesis in the hippocampus of the adult macaque monkey’, *Proceedings of the National Academy of Sciences of the United States of America*, 96(10)5768–5773.

69. Kozorovitskiy, Y. and Gould, E. (2004a) ‘Dominance Hierarchy Influences Adult Neurogenesis in the Dentate Gyrus’, *The Journal of Neuroscience*, 24(30), pp. 6755–6759.

70. Kuhn, H. G., Dickinson-Anson, H. and Gage, F. H. (1996) ‘Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation’, *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 16(6)2027–2033.

71. Lacey, E. A., Patton, J. L. and Cameron, G. N. (2000) *Life Underground: The Biology of Subterranean Rodents*. University of Chicago Press.

72. Lagace, D. C., Donovan, M. H., DeCarolis, N. A., Farnbauch, L. A., Malhotra, S., Berton, O., Nestler, E. J., Krishnan, V. and Eisch, A. J. (2010) ‘Adult hippocampal neurogenesis is functionally important for stress-induced social avoidance’, *Proceedings of the National Academy of Sciences of the United States of America*, 107(9), pp. 4436–4441.

73. Lagace, D. C., Noonan, M. A. and Eisch, A. J. (2007) ‘Hippocampal neurogenesis: a matter of survival’, *The American Journal of Psychiatry*, 164(2)205.

74. Latchney, S. E. and Eisch, A. J. (2012) ‘Therapeutic application of neural stem cells and adult neurogenesis for neurodegenerative disorders: regeneration and beyond’, *European journal of neurodegenerative disease*, 1(3), pp. 335–351.

75. Lee, J., Duan, W. and Mattson, M. P. (2002a) ‘Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice’, *Journal of neurochemistry*, 82(6)1367–1375.

76. Lee, J., Duan, W., Long, J. M., Ingram, D. K. and Mattson, M. P. (2000) ‘Dietary restriction increases the number of newly generated neural cells, and induces BDNF expression, in the dentate gyrus of rats’, *Journal of molecular neuroscience : MN*, 15(2)99–108.

77. Lee, J., Seroogy, K. B. and Mattson, M. P. (2002b) 'Dietary restriction enhances neurotrophin expression and neurogenesis in the hippocampus of adult mice', *Journal of neurochemistry*, 80(3)539–547.

78. Lennington, J. B., Yang, Z. and Conover, J. C. (2003) 'Neural stem cells and the regulation of adult neurogenesis', *Reproductive biology and endocrinology : RB&E*, 1(Journal Article)99.

79. Lichtenwalner, R. J., Forbes, M. E., Bennett, S. A., Lynch, C. D., Sonntag, W. E. and Riddle, D. R. (2001) 'Intracerebroventricular infusion of insulin-like growth factor-I ameliorates the age-related decline in hippocampal neurogenesis', *Neuroscience*, 107(4)603–613.

80. Lieberwirth, C. and Wang, Z. (2012) 'The Social Environment and Neurogenesis in the Adult Mammalian Brain', *Frontiers in Human Neuroscience*, 6.

81. Lievajová, K., Blaško, J., Martončíková, M., Cigánková, V. and Račeková, E. (2011) 'Delayed maturation and altered proliferation within the rat rostral migratory stream following maternal deprivation', *European Journal of Histochemistry : EJH*, 55(4).

82. Liu, D. X. and Greene, L. A. (2001) 'Neuronal apoptosis at the G1/S cell cycle checkpoint', *Cell and Tissue Research*, 305(2)217–228.

83. Lu, L., Bao, G., Chen, H., Xia, P., Fan, X., Zhang, J., Pei, G. and Ma, L. (2003) 'Modification of hippocampal neurogenesis and neuroplasticity by social environments', *Experimental neurology*, 183(2)600–609.

84. Luzzati, F., De Marchis, S., Fasolo, A. and Peretto, P. (2006) 'Neurogenesis in the caudate nucleus of the adult rabbit', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 26(2)609–621.

85. Luzzati, F., De Marchis, S., Fasolo, A. and Peretto, P. (2007) 'Adult neurogenesis and local neuronal progenitors in the striatum', *Neuro-degenerative diseases*, 4(4)322–327.

86. Maini, P. K. (2003) 'How the mouse got its stripes', *Proceedings of the National Academy of Sciences of the United States of America*, 100(17)9656–9657.

87. Malberg, J. E., Eisch, A. J., Nestler, E. J. and Duman, R. S. (2000) 'Chronic Antidepressant Treatment Increases Neurogenesis in Adult Rat Hippocampus', *The Journal of Neuroscience*, 20(24), pp. 9104–9110.

88. McDonald, H. Y. and Wojtowicz, J. M. (2005) 'Dynamics of neurogenesis in the dentate gyrus of adult rats', *Neuroscience letters*, 385(1), pp. 70–75.

89. Merrill, D. A., Karim, R., Darraq, M., Chiba, A. A. and Tuszynski, M. H. (2003) 'Hippocampal cell genesis does not correlate with spatial learning ability in aged rats', *The Journal of Comparative Neurology*, 459(2)201–207.

90. Ming, G. and Song, H. (2011) 'Adult Neurogenesis in the Mammalian Brain: Significant Answers and Significant Questions', *Neuron*, 70(4)687–702.

91. Moyse, E., Bauer, S., Charrier, C., Coronas, V., Krantic, S. and Jean, A. (2006) 'Neurogenesis and neural stem cells in the dorsal vagal complex of adult rat brain: new vistas about autonomic regulations--a review', *Autonomic Neuroscience : Basic & Clinical*, 126-127(Journal Article) 50–58.

92. Mummery, C., Wilmut, S. I., van de Stolpe, A. and Roelen, B. A. J. (2011) 'Chapter 3 - What Are Stem Cells?', in *Stem Cells*. San Diego: Academic Press, pp. 45–57.

93. Nacher, J., Crespo, C. and McEwen, B. S. (2001) 'Doublecortin expression in the adult rat telencephalon', *European Journal of Neuroscience*, 14(4), pp. 629–644.

94. Ngwenya, L. B., Heyworth, N. C., Shwe, Y., Moore, T. L. and Rosene, D. L. (2015) 'Age-related changes in dentate gyrus cell numbers, neurogenesis, and associations with cognitive impairments in the rhesus monkey', *Frontiers in Systems Neuroscience*, p. 102.

95. Nichols, N. R., Agolley, D., Zieba, M. and Bye, N. (2005) 'Glucocorticoid regulation of glial responses during hippocampal neurodegeneration and regeneration', *Brain research.Brain research reviews*, 48(2)287–301.

96. Okano, H. (2002) 'Stem cell biology of the central nervous system', *Journal of neuroscience research*, 69(6), pp. 698–707.

97. Olaleye, O. and Ihunwo, A. (2014) 'Adult neurogenesis in the four-striped mice (Rhabdomys pumilio)', *Neural Regeneration Research*, 9(21), p. 1907.

98. Olaleye, O. O. (2011) *Adult neurogenesis in the four-striped mouse (Rhabdomys pumilio) and common mole-rat (Cryptomys hottentotus)*. Thesis. Available at: http://wiredspace.wits.ac.za/handle/10539/10072 (Accessed: 27 January 2015).

99. Pagano, S. F., Impagnatiello, F., Girelli, M., Cova, L., Grioni, E., Onofri, M., Cavallaro, M., Etteri, S., Vitello, F., Giombini, S., Solero, C. L. and Parati, E. A. (2000) 'Isolation and characterization of neural stem cells from the adult human olfactory bulb', *Stem cells (Dayton, Ohio)*, 18(4), pp. 295–300.

100. Palmer, T. D., Ray, J. and Gage, F. H. (1995) 'FGF-2-responsive neuronal progenitors reside in proliferative and quiescent regions of the adult rodent brain', *Molecular and cellular neurosciences*, 6(5)474–486.

101. Parent, J. M. (2003) ‘Injury-induced neurogenesis in the adult mammalian brain’, *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry*, 9(4)261–272.

102. Patzke, N., Spocter, M. A., Karlsson, K. Æ., Bertelsen, M. F., Haagensen, M., Chawana, R., Streicher, S., Kaswera, C., Gilissen, E., Alagaili, A. N., Mohammed, O. B., Reep, R. L., Bennett, N. C., Siegel, J. M., Ihunwo, A. O. and Manger, P. R. (2015) ‘In contrast to many other mammals, cetaceans have relatively small hippocampi that appear to lack adult neurogenesis’, *Brain Structure and Function*, 220(1), pp. 361–383.

103. Pawluski, J. L. and Galea, L. A. (2006) ‘Hippocampal morphology is differentially affected by reproductive experience in the mother’, *Journal of neurobiology*, 66(1), pp. 71–81.

104. Pillay, N. (2000) ‘Reproductive isolation in three populations of the striped mouse rhabdomys pumilio (rodentia, muridae): Interpopulation breeding studies’, *Mammalia*, 64(4)461–470.

105. Plümpe, T., Ehninger, D., Steiner, B., Klempin, F., Jessberger, S., Brandt, M., Römer, B., Rodriguez, G. R., Kronenberg, G. and Kempermann, G. (2006) ‘Variability of doublecortin-associated dendrite maturation in adult hippocampal neurogenesis is independent of the regulation of precursor cell proliferation’, *BMC Neuroscience*, 7(1)7.

106. Rakic, P. (2002) ‘Neurogenesis in adult primate neocortex: an evaluation of the evidence’, *Nature reviews.Neuroscience*, 3(1)65–71.

107. Rakic, P. and Nowakowski, R. S. (1981) ‘The time of origin of neurons in the hippocampal region of the rhesus monkey’, *The Journal of Comparative Neurology*, 196(1)99–128.

108. Rambau, R. V., Robinson, T. J. and Stanyon, R. (2003) ‘Molecular genetics of Rhabdomys pumilio subspecies boundaries: mtDNA phylogeography and karyotypic analysis by fluorescence in situ hybridization’, *Molecular Phylogenetics and Evolution*, 28(3)564–575.

109. Rao, M. S. and Shetty, A. K. (2004) ‘Efficacy of doublecortin as a marker to analyse the absolute number anddendritic growth of newly generated neurons in the adult dentate gyrus’, *European Journal of Neuroscience*, 19(2), pp. 234–246.

110. Rapp, P. R. and Gallagher, M. (1996) ‘Preserved neuron number in the hippocampus of aged rats with spatial learning deficits’, *Proceedings of the National Academy of Sciences*, 93(18)9926–9930.

111. Rasmussen, T., Schliemann, T., Sørensen, J. C., Zimmer, J. and West, M. J. (1996) 'Memory impaired aged rats: no loss of principal hippocampal and subicular neurons', *Neurobiology of Aging*, 17(1)143–147.

112. Reynolds, B. A. and Weiss, S. (1992) 'Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system', *Science (New York, N.Y.)*, 255(5052), pp. 1707–1710.

113. Ribak, C. E., Korn, M. J., Shan, Z. and Obenaus, A. (2004) 'Dendritic growth cones and recurrent basal dendrites are typical features of newly generated dentate granule cells in the adult hippocampus', *Brain Research*. (Brain Research Volume 1000), 1000(1–2)195–199.

114. Rochefort, C., Gheusi, G., Vincent, J.-D. and Lledo, P.-M. (2002) 'Enriched Odor Exposure Increases the Number of Newborn Neurons in the Adult Olfactory Bulb and Improves Odor Memory', *The Journal of Neuroscience*, 22(7)2679–2689.

115. Rosenzweig, S. and Wojtowicz, J. M. (2011) 'Analyzing Dendritic Growth in a Population of Immature Neurons in the Adult Dentate Gyrus Using Laminar Quantification of Disjointed Dendrites', *Frontiers in Neuroscience*, 5.

116. Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C. and Hen, R. (2003) 'Requirement of Hippocampal Neurogenesis for the Behavioral Effects of Antidepressants', *Science*, 301(5634), pp. 805–809.

117. Scharfman, H., Goodman, J., Macleod, A., Phani, S., Antonelli, C. and Croll, S. (2005) 'Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats', *Experimental neurology*, 192(2)348–356.

118. Scharfman, H., Goodman, J., Macleod, A., Phani, S., Antonelli, C. and Croll, S. (2005) 'Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats', *Experimental neurology*, 192(2), pp. 348–356.

119. Schauwecker, P. E. (2006) 'Genetic influence on neurogenesis in the dentate gyrus of two strains of adult mice', *Brain research*, 1120(1)83–92.

120. Schradin, C. and Pillay, N. (2003) 'Paternal care in the social and diurnal striped mouse (Rhabdomys pumilio): laboratory and field evidence', *Journal of Comparative Psychology (Washington, D.C.: 1983)*, 117(3)317–324.

121. Schradin, C. and Pillay, N. (2004) 'The striped mouse (Rhabdomys pumilio) from the succulent karoo, South Africa: a territorial group-living solitary forager with communal

breeding and helpers at the nest', *Journal of comparative psychology (Washington, D.C.: 1983)*, 118(1)37–47.

122. Schradin, C. and Pillay, N. (2005) 'The influence of the father on offspring development in the striped mouse', *Behavioral Ecology*, 16(2)450–455.

123. Seaberg, R. M. and van der Kooy, D. (2002) 'Adult rodent neurogenic regions: the ventricular subependyma contains neural stem cells, but the dentate gyrus contains restricted progenitors', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 22(5), pp. 1784–1793.

124. Sherman, P. W., Braude, S. and Jarvis, J. U. M. (1999) 'Litter Sizes and Mammary Numbers of Naked Mole-Rats: Breaking the One-Half Rule', *Journal of Mammalogy*, 80(3)720–733.

125. Sims, K. B., Hoffman, D. L., Said, S. I. and Zimmerman, E. A. (1980) 'Vasoactive intestinal polypeptide (VIP) in mouse and rat brain: An immunocytochemical study', *Brain Research*, 186(1)165–183.

126. Slomianka, L. and West, M. J. (2005) 'Estimators of the precision of stereological estimates: An example based on the CA1 pyramidal cell layer of rats', *Neuroscience*. (Quantitative Neuroanatomy: from molecules to systemsA special issue in honor of the late Professor Theodor W. Blackstad), 136(3), pp. 757–767.

127. Snyder, J. S., Choe, J. S., Clifford, M. A., Jeurling, S. I., Hurley, P., Brown, A., Kamhi, J. F. and Cameron, H. A. (2009) 'Adult-born hippocampal neurons are more numerous, faster-maturing and more involved in behavior in rats than in mice', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 29(46), pp. 14484–14495.

128. Spalding, K. L., Bergmann, O., Alkass, K., Bernard, S., Salehpour, M., Huttner, H. B., Boström, E., Westerlund, I., Vial, C., Buchholz, B. A., Possnert, G., Mash, D. C., Druid, H. and Frisén, J. (2013) 'Dynamics of Hippocampal Neurogenesis in Adult Humans', *Cell*, 153(6)1219–1227.

129. Takemura, N. U. (2005) 'Evidence for neurogenesis within the white matter beneath the temporal neocortex of the adult rat brain', *Neuroscience*, 134(1)121–132.

130. Taupin, P. (2006) 'The therapeutic potential of adult neural stem cells', *Current opinion in molecular therapeutics*, 8(3)225–231.

131. Temple, S. and Alvarez-Buylla, A. (1999) 'Stem cells in the adult mammalian central nervous system', *Current opinion in neurobiology*, 9(1)135–141.

132. Thomaidou, D., Mione, M. C., Cavanagh, J. F. and Parnavelas, J. G. (1997) ‘Apoptosis and its relation to the cell cycle in the developing cerebral cortex’, *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 17(3)1075–1085.

133. Timsit, S., Rivera, S., Ouaghi, P., Guischard, F., Tremblay, É., Ben-Ari, Y. and Khrestchatisky, M. (1999) ‘Increased cyclin D1 in vulnerable neurons in the hippocampus after ischaemia and epilepsy: a modulator of in vivo programmed cell death?’, *European Journal of Neuroscience*, 11(1)263–278.

134. van Praag, H., Kempermann, G. and Gage, F. H. (1999) ‘Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus’, *Nature Neuroscience*, 2(3), pp. 266–270.

135. van Praag, H., Kempermann, G. and Gage, F. H. (2000) ‘Neural consequences of enviromental enrichment’, *Nature Reviews Neuroscience*, 1(3), pp. 191–198.

136. Vivar, C. and Van Praag, H. (2013) ‘Functional circuits of new neurons in the dentate gyrus’, *Frontiers in Neural Circuits*, 7:15.

137. Vollmayr, B., Simonis, C., Weber, S., Gass, P. and Henn, F. (2003) ‘Reduced cell proliferation in the dentate gyrus is not correlated with the development of learned helplessness’, *Biological psychiatry*, 54(10), pp. 1035–1040.

138. Watts, C., McConkey, H., Anderson, L. and Caldwell, M. (2005) ‘Anatomical perspectives on adult neural stem cells’, *Journal of anatomy*, 207(3)197–208.

139. Weiss, S., Dunne, C., Hewson, J., Wohl, C., Wheatley, M., Peterson, A. C. and Reynolds, B. A. (1996) ‘Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis’, *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 16(23), pp. 7599–7609.

140. West, M. J., Slomianka, L. and Gundersen, H. J. (1991) ‘Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator’, *The Anatomical Record*, 231(4), pp. 482–497.

141. Willan, K. and Meester, J. (1989) ‘Life-history styles of southern African Mastomys natalensis, Otomys irroratus and Rhabdomys pumilio (Mammalia, Rodentia)’, in Bruton, M. N. (ed.) *Alternative Life-History Styles of Animals*. Springer Netherlands (Perspectives in vertebrate science, 6)421–439. Available at: http://link.springer.com/chapter/10.1007/978-94-009-2605-9_21 (Accessed: 7 January 2015).

142. Wyss, J. M. and Sripanidkulchai, B. (1985) ‘The development of Ammon’s horn and the fascia dentata in the cat: a [3H]thymidine analysis’, *Brain Research*, 350(1-2), pp. 185–198.

143. Xu, Y., Tamamaki, N., Noda, T., Kimura, K., Itokazu, Y., Matsumoto, N., Dezawa, M. and Ide, C. (2005) ‘Neurogenesis in the ependymal layer of the adult rat 3rd ventricle’, *Experimental neurology*, 192(2)251–264.

144. Yang, Y., Zhang, M., Kang, X., Jiang, C., Zhang, H., Wang, P. and Li, J. (2015) ‘Impaired adult hippocampal neurogenesis and cognitive ability in a mouse model of intrastriatal hemorrhage’, *Neuroscience Letters*.

145. Zhao, M., Momma, S., Delfani, K., Carlen, M., Cassidy, R. M., Johansson, C. B., Brismar, H., Shupliakov, O., Frisen, J. and Janson, A. M. (2003) ‘Evidence for neurogenesis in the adult mammalian substantia nigra’, *Proceedings of the National Academy of Sciences of the United States of America*, 100(13)7925–7930.

146. Zupanc, G. K. (1999) ‘Neurogenesis, cell death and regeneration in the adult gymnotiform brain’, *Journal of Experimental Biology*, 202(10)1435–1446.