HIPPOCAMPAL NEURONAL MORPHOLOGY AND SPINE DENSITY IN A SEASONALLY REPRODUCING RODENT, RICHARDSON'S GROUND SQUIRREL (*UROCITELLUS RICHARDSONII)*

BENJAMIN EDWARD BRINKMAN
Bachelor of Science, University of Lethbridge, 2017

A thesis submitted
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

NEUROSCIENCE

Department of Neuroscience
University of Lethbridge
LETHBRIDGE, ALBERTA, CANADA

HIPPOCAMPAL NEURONAL MORPHOLOGY AND SPINE DENSITY IN A SEASONALLY REPRODUCING RODENT, RICHARDSON'S GROUND SQUIRREL (*UROCITELLUS RICHARDSONII)*

BENJAMIN EDWARD BRINKMAN

Date of Defence: December 9, 2019

Dr. A. Iwaniuk Supervisor	Associate Professor	Ph.D.
Dr. R. Sutherland Thesis Examination Committee Member	Professor	Ph.D.
Dr. D. Logue Thesis Examination Committee Member	Associate Professor	Ph.D.
Dr. M. Antle External Examiner University of Calgary Calgary, Alberta	Professor	Ph.D.
Dr. R. Gibb Chair, Thesis Examination Committee	Professor	Ph.D.

ABSTRACT

Both sex and reproductive status can alter the anatomy of brain regions within individuals. In mammals, these changes can be large in seasonally breeding species, but the extent to which fluctuations in brain region sizes are driven by neuron morphology has remained untested. I tested the hypothesis that sex-seasonal differences in hippocampus size are due, in part, to changes in neuronal morphology and spine density. Through analyzing Golgi-stained tissue from wild caught Richardson's ground squirrels (*Urocitellus richardsonii*), I found that season affects spine density in hippocampal neurons. In pyramidal cells, non-breeding squirrels had higher spine densities, but in granule cells non-breeding squirrels had lower spine densities. These seasonal effects on neuronal spine density likely reflect photoperiod, seasonal changes in stress, and activity levels in similar ways to studies of lab rodents. My results provide new insights into seasonal neuroplasticity in mammals and how it relates to their behaviour and environment.

ACKNOWLEDGEMENTS

I would like to begin with Dr. Iwaniuk, as he offered me this incredible experience and gave me the leadership, support, and encouragement to complete this project. I have grown as a person throughout my time in Andy's lab. I can now critically engage with science, I am more organized (somewhat), and I have learned the value of doing a task as soon as you say you will do it. Andy has given me unbelievable opportunities; including access to state-of-the-art equipment, and training in cutting edge research. I have been able to travel internationally to multiple conferences and have developed my personal scientific skills, which will assist me in all my future career goals. Andy taught me how to conduct effective fieldwork, and how to manage the huge amount of planning it takes, so that I was able to lead my own trips into the field. I have learned so much from him regarding science, however, I have also learned about many other facets of life including food, music, film etc. It was always a blast having discussions and I am so grateful for my time in the lab and for everything I have learned.

I would also like to say thank you to my committee members who challenged and encouraged me to think critically about my research, strengthing it through constructive feedback. In particular, I would like to thank Dr. Logue for his help with my statistical analysis. I want to apologize for what must have felt like teaching a tree stump when I struggled to learn R. His patience was (and still is) much appreciated!

I need to acknowledge Dr. Ngwenya for teaching me the steps to Golgi stain brain tissue, and for helping me to develop the standards of procedures that we follow for this methodology in the lab. She paved the way for me to take over and finish this research.

Another way I have been blessed is to have worked with amazing students during my degree. I need to acknowledge the contributions of Krista Fjordbotten and Olivia Stephen for the incredible assistance in both trapping and tracing hippocampal pyramidal cells. Their fantastic work helped me finish a task that at times felt insurmountable. Kelsey Raciot and Madison Hunter also provided invaluable help in trapping and processing animals in the field. Furthermore, they worked alongside me as I panicked to finish writing, so I am thankful for their support and sorry they had to see me in such a state. Lastly, I want to thank Felipe Cunha for his endless patience and support through my ups and downs during this project. I am beyond lucky to have had him as a lab mate and I will always appreciate his advice as I rambled about my problems and worries. I want to thank all the excellent students present during my time in the lab as they made it an amazing environment and a joy to be a part of.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE: GENERAL INTRODUCTION

The brain does not remain fixed throughout the lifespan of an individual. Specific brain areas can vary in size, usually in response to use, neglect, stress, or fluctuating levels of hormones (Czeh et al., 2001; Raz et al., 2005; Rosenzweig, Bennett, & Diamond, 1972; Woollett & Maguire, 2011). Individual neurons are also variable as they can change in both size and shape over time (Devoogd & Nottebohm, 1981; Hill & DeVoogd, 1991; Kolb et al., 2003). These changes in the brain are largely a result of two factors: behaviour and hormones. Hormones can cause significant changes in neuronal size and shape, numbers of neurons, and/or synaptic connectivity in specific brain regions (Nottebohm & Arnold, 1976). Behaviour has a direct impact as well. Overall activity levels along with changes in specific behaviours can influence the anatomical structure and/or size of brain regions and this is often manifested at the level of neuron size and shape (Kolb, et al., 2003; Perani & Abutalebi, 2005). Although many of these studies focus on lab animals, the most dramatic changes occur in wild species. Some wild species undergo large seasonal changes in brain region volumes, the most well-known example being male songbirds. As the breeding season approaches, longer photoperiods create a surge of testosterone that cause song nuclei (HVC, Area X, RA etc.) to dramatically increase in size, with some areas increasing in overall volume by more than 200% (Dawson et al., 2001; Nottebohm & Arnold, 1976). At the cellular level, the number of neurons in HVC increases with long days and testosterone (Nottebohm et al., 1987; Smith et al., 1997; Tramontin et al., 1998). In contrast, there is a decrease in neuronal density in RA due to neurons becoming larger, including the size of their dendritic trees (Devoogd & Nottebohm, 1981; Hill & DeVoogd, 1991). These volumetric and cellular changes in males are significant and far greater than what is reported in females of the same species (Hamilton et al., 1997). These sex and seasonal changes in the brain are directly

related to songbird behaviour as the males go from rarely singing, to singing thousands of songs a day in some species (Catchpole & Slater, 2003; Schlinger & Brenowitz, 2002).

Seasonal changes in brain anatomy are not limited to songbirds. Voles have been the focus of many studies on sex differences and seasonal changes in the brain for over 25 years (Sherry, 2006). Voles are small rodents closely related to the mouse (*Mus musculus*), but they have stouter bodies, shorter tails, and a more rounded head (O'Brien, 1994). One of the reasons that voles have been intensively studied is that their mating system varies across species; some are polygamous like the meadow vole (*Microtus pennsylvanicus*) whereas others are monogamous, like the pine (*M. pinetorum*) and prairie (*M. ochrogaster*) voles (Gaulin & Fitzgerald, 1986; 1989). The species differences in mating systems are associated with species and sex differences in spatial behaviour. For example, polygamous species have larger home ranges and perform better in spatial memory tests in the lab than monogamous species (Gaulin & Fitzgerald 1986, 1989). Within the polygamous meadow vole, there are also stark sex differences in spatial behaviours. Male meadow voles increase their territory size during the breeding season, whereas female territories stay the same size throughout the year (Gaulin & Fitzgerald, 1989). Males also outperform females in spatial memory performance and have significantly larger hippocampal volumes to mediate this behaviour (Jacobs et al., 1990). This sexual dimorphism in spatial ability appears during the breeding season when male ranges have expanded, so males have an advantage on spatial tests while breeding, but this disappears in the non-breeding season (Galea, Kavaliers, & Ossenkopp, 1996). Further, when female mate preference was tested, they preferred males with better spatial navigational skills (Spritzer, Meikle, & Solomon, 2005). Increasing spatial navigation allows males to better localize females in their environment and exploits a female preference for spatial ability in males (Spritzer et al.,

2005). Rates of neurogenesis however, do not seem to vary across sex, as males and females both show decreased levels of neurogenesis in the hippocampus during the breeding season (Spritzer et al., 2017). The theory is that intrasexual competition for females place information processing demands on the hippocampus to provide males with better spatial memory to reproduce successfully (Jacobs et al, 1990). Information processing is dependent on neuron survival, neuron numbers, neuron size and other aspects of anatomy; this is manifested as a sex difference in hippocampus size (Sherry, 2006). These sex and seasonal differences in the polygamous meadow voles are in stark contrast to the monogamous vole species. In pine and prairie voles, there are no sex differences in home range size as males and females occupy the same space and share parental care (Gaulin & Fitzgerald, 1986; 1989). There are no sex differences in spatial abilities in monogamous vole species (Gaulin & Fitzgerald, 1986, 1989), and no differences in hippocampal volume across season or sex (Jacobs et al, 1990). The conclusion of these vole studies is that mating system is playing a role in producing anatomical changes to brain that vary across sex and season (Sherry, 2006).

Although the vole studies are touted as an example of how species and sex differences in spatial behaviour are reflected in hippocampal anatomy, research in other small mammals is more variable. Eastern grey squirrels (*Sciurus carolinensis*) for example, are long-lived small mammals that scatter hoard food in the fall to survive the winter (Lavenex, Steele, & Jacobs, 2000a). Scatter hoarding food is associated with a larger hippocampus in other species (Jacobs & Spencer, 1994), but there were no significant seasonal differences in hippocampal anatomy and only a sex difference in the size of part of the hippocampus of grey squirrels (Lavenex et al, 2000). These results bring into question what selective pressures are responsible for seasonal

differences in some species and not others and the extent to which seasonal variation and sex differences interact to affect hippocampus anatomy across mammals.

Previous research on songbirds and voles has provided significant insights into neurogenesis, hormonal effects on brain anatomy, and the extent to which the brain changes anatomically under natural conditions. There is evidence that small mammals are experiencing seasonal changes in the brain, and, in at least one species, these changes interact with sex differences. However, we understand very little about seasonal neuroplasticity in mammals and several major questions have remained unanswered. First, how widespread are seasonal changes in brain anatomy in mammals and are they the same across species or are seasonal changes associated with specific behaviours and/or life histories? Second, what do these changes look like at the cellular level in the mammalian brain? As mentioned above, these kinds of changes are better understood in the avian brain as different song nuclei have different cellular mechanisms to mediate the change in size from the nonbreeding to breeding season. The seasonal change in HVC is largely due to neurogenesis while seasonal change in RA is largely due to increased neuron size (Devoogd & Nottebohm, 1981; Hill & DeVoogd, 1991; Nottebohm et al., 1987; Smith et al., 1997; Tramontin et al., 1998). Determining to what extent seasonal neuroplasticity in mammals is similar to or different from that of songbirds is important for understanding general principles of neuroplasticity in wild animals, a goal that cannot be readily achieved in the lab.

Richardson's ground squirrel *(Urocitellus richardsonii)* is a species that undergoes seasonal variation in the brain and exhibits seasonal changes in behaviour that differ from that of previously studied small mammals. Males are larger, cache food to survive the winter, and are asocial, usually becoming highly aggressive towards conspecifics around them throughout the

year (Michener, 1992). Males also occupy ranges 10-20x the size of females and spend most of their time fighting males and trying to mate with females during the breeding season (Michener & McLean, 1996). This contrasts with the more affiliative females that live in kin based social groups, do not cache food for winter and hibernate considerably longer than males (Michener & McLean, 1996). Females live in much smaller territories and rarely leave their home range (Michener & McLean, 1996). These behavioural sex differences are pronounced and are to some extent related to sex and seasonal differences in brain anatomy. Males, but not females, have larger hippocampal volumes during the nonbreeding season, possibly to mediate their larder food hoarding and memory of dense food locations (Burger et al., 2013). Males have increased rates of hippocampal neurogenesis during the breeding season, which could assist in finding females (Burger et al., 2014). This sexual dimorphism in behaviour is not only reflected in the hippocampus. Season, sex and the interaction between the two, also affect the sizes of several other brain regions, such as medial prefrontal cortex (mPFC), corpus callosum, anterior commissure, neocortex, and entorhinal cortex (Keeley et al., 2015).

Thus, there are seasonal and sex differences in the anatomy of Richardson's ground squirrel brains, however, what remains unknown is whether there are also sex and season differences in the shape and size of neurons in the hippocampus. Neuronal size can increase cell functionality and larger neuronal sizes can cause volumetric expansion as it does in the song bird song nucleus RA (Devoogd & Nottebohm, 1981). We would expect larger cells with higher spine densities in the sex, season, or sex by season interaction that requires a more functional hippocampus. Understanding the specific changes at the cellular level will give us insight into the mechanisms that mediate sex-season interaction effects on brain region sizes and determine the extent to which mammalian seasonal neuroplasticity is similar to or different from songbirds

(Devoogd & Nottebohm, 1981; Fernando Nottebohm et al., 1987; Smith et al., 1997; Tramontin et al., 1998).

In my thesis, I will address this knowledge gap by studying the sexually and seasonally dimorphic Richardson's ground squirrel. As outlined above, males and females differ in behaviour as they inhabit different sized ranges, males cache food and females do not, and they differ markedly in social behaviour (Michener, 1992, Michener & McLean, 1996). Based on previous studies on small mammals and songbirds (reviewed in Sherry, 2006), including recent studies on Richardson's ground squirrels (Burger et al., 2013, 2014) and shrews (Lazaro et al., 2019), I hypothesize that some of the sex and seasonal effects in the mammalian brain are due to changes in neuron morphology and spine density. Based on this hypothesis, I first predict that non-breeding males will have the largest and most complex hippocampal pyramidal neurons because they need to recall the locations of dense food when building their larders for hibernation. This will result in a sex difference in pyramidal neuron size and morphology during the non-breeding season. However, this sex difference will disappear during the breeding season when males are not caching food and are experiencing high stress levels, which can affect neuron morphology (Cameron & Schoenfeld, 2018).

Second, not only will neuron size and morphology vary between sexes and seasons, spine density will be higher in the non-breeding season males. Photoperiod length, enrichment, and dim light all affect spine density in the hippocampus as well as spatial learning and memory (Bedrosian et al. 2011; Pyter, Reader, & Nelson, 2005; Workman, Bowers, & Nelson, 2009). Again, if the hypothesis is true, I predict that the hippocampal pyramidal neurons of males in the non-breeding season will be the largest, the most dendritically complex, and have the highest

spine density. This non-breeding season sex difference will disappear during the breeding season when males are not caching food.

Third, I predict that males will have larger and more complex granule cells during the non-breeding season to support the increased spatial memory necessary for larder building. This sex difference will disappear during the breeding season when males have less selective pressure for spatial memory. Loss of these cells results in spatial memory deficits (Deng et al., 2009) and granule cells vary in morphology in response to photoperiod length and stress (Fonken et al., 2012). Lastly, I predict that as photoperiod increases, and stress decreases during the shift into the nonbreeding season, pyramidal and granule cells will be larger in both males and females (Fonken et al. 2012; Cameron and Schoenfeld, 2018) in the nonbreeding season as a seasonal effect. I predict that spine density will also follow this pattern (Pyter et al, 2005). These predictions are put to the test in the data chapter to follow.

CHAPTER TWO: QUANTIFYING HIPPOCAMPAL NEURONAL MORPHOLOGY AND SPINE DENSITY IN A SEASONALLY REPRODUCING RODENT, RICHARDSON'S GROUND SQUIRREL (*UROCITELLUS RICHARDSONII*)

Introduction

Seasonal effects on overall brain size, and in specific brain regions, have been well documented across vertebrate species. Seasonal breeding animals experience surges of steroid hormones at different times of the year, and this causes the size of the brain, or individual brain regions, to change (Bartkowska et al., 2008; Burger et al., 2013; Jacobs, 1996; Kabelik et al., 2006; Lázaro, Hertel, Sherwood, et al., 2018; Tramontin & Brenowitz, 2000; Yaskin, 1994). For example, in the song system of songbirds, some brain regions can increase in volume by up to 200% in breeding animals when compared with animals from the non-breeding season (Dawson et al., 2001; Tramontin & Brenowitz, 2000). Brain and brain region sizes can also change dramatically in seasonally breeding small mammals (Dark et al., 1990; Prendergast, Nelson, & Zucker, 2002; Weiler, 1992; Yaskin, 1994). For example, common shrews (*Sorex araneus*) experience massive changes in overall brain size throughout the year (Dehnel, 1949). This reduction in energy expensive brain tissue is hypothesized to be an adaptive alternative to hibernation or migration (Pucek, 1970). There is a decrease in overall brain mass by 10-26% as the animals move from summer to winter, and a subsequent regrowth of 9-16% in spring (Dehnel, 1949; Lázaro et al.,2018b; Yaskin, 1994)

Although the size of many brain regions can vary seasonally within species (Keeley et al., 2015; Lázaro, et al., 2018b), the majority of the research has focused on the hippocampus. This hippocampal formation is responsible for many functions but has been studied extensively in rodents for its role in spatial memory and navigation (Amaral et al. 2007). There are behaviours

in many species that require increased spatial memory at specific times of the year, making the hippocampus the focus of many studies on seasonal neuroplasticity (Burger et al., 2013; Jacobs et al., 1990; Sherry & Hoshooley, 2009). Many animals that experience seasonal changes in hippocampal volumes also have seasonal changes in home range and spatial abilities (Galea, Ossenkopp, & Kavaliers, 1994; Gaulin & FitzGerald, 1986; Workman et al., 2009). Studies have looked at the impact of season on general and specific features of hippocampal anatomy, such as changes in volume, dendritic branching, mossy fibre projection volume, size of CA1 pyramidal cells, spine density in CA1 and CA3 pyramidal cells, and neurogenesis in the dentate gyrus and how they vary between breeding and nonbreeding seasons (Burger et al., 2013; Burger et al., 2014; Galea & McEwen, 1999; Galea et al., 1999; Lavenex, Steele, & Jacobs, 2000b; Pyter, Reader, & Nelson, 2005; Workman et al., 2009; Workman et al., 2011).

These anatomical differences across seasons reflect both behaviour and hormone levels. Changes in hippocampal anatomy often occur in tangent with changes in behaviour. For example, many species that undergo seasonal changes in hippocampus volume score better in spatial tests (Galea et al., 1994; Gaulin & FitzGerald, 1986; Workman et al., 2009). Furthermore, sex hormones can impact the rates of neurogenesis in the dentate gyrus (Galea and McEwen, 1999) as well as the anatomy and physiology of pyramidal and granule cells (Gould et al., 1990; Woolley, Gould, & McEwen, 1990; Woolley & McEwen, 1993). In seasonally breeding species, sex and season can then interact to cause seasonal changes in hippocampus anatomy that reflect behaviour that also varies by both sex and season. This is best shown in polygynous rodents in which males expand their home range during the breeding season and cover greater distances daily to find mates (Jacobs et al., 1990; Jacobs, 1996b, 2011). Polygamous meadow voles (*Microtus pennsylvanicus*) are a great example because they experience different gonadal

hormone levels in the breeding season and the males develop large home ranges with a need to find females to mate with (Gaulin & Fitzgerald, 1986; 1989). The breeding season males have better spatial abilities and larger hippocampal and dentate gyrus volumes than nonbreeding males and all females (Gaulin and Fitzgerald, 1986, Galea et al. 1999 and Galea and McEwan, 1999). They also experience mate preference with females preferring males with better spatial skills, suggesting that scramble competition and mate choice both select for improved spatial ability in males (Spritzer, Meikle, & Solomon, 2005). Deer mice (*Peromyscus maniculatus*) also show sex differences in spatial ability with males performing better than females on spatial tasks, and young breeding males scoring highest in tests of spatial ability, experiencing the highest plasma testosterone levels, and the largest hippocampal volumes (Kavaliers et al., 1996; Perrot-Sinal, Kavaliers, & Ossenkopp, 1998). Thus, seasonally breeding, polygamous, small mammals undergo changes in hippocampal anatomy in relation to both sex and season that support a hippocampus-dependent behaviour: mate searching.

Although these studies on voles and deer mice provide an example of how sex and season can interact to affect hippocampus anatomy, comparable studies of other species are inconsistent. Eastern grey squirrels (*Sciurus carolinensis*), a long-lived rodent, have seasonal pressures on spatial ability as they seasonally cache food for hibernation, however, they experience no effect of season on volume or neuron number in any structure of the hippocampal complex (Lavenex, Steele, & Jacobs, 2000). In contrast, Richardson's ground squirrels (*Urocitellus richardsonii*) experience changes to hippocampal neuroanatomy across both sex and season in some, but not all, measurements of hippocampus anatomy. Nonbreeding males have significantly larger hippocampal volumes than breeding males or females of either season (Burger et al., 2013). Neurogenesis and dentate gyrus volume vary as well, with dentate gyrus volume and number of

DCX cells being larger in the breeding season when compared to the nonbreeding season, and brain volume and the number of DCX labeled cells being larger in males than females (Burger et al. 2014). Both grey squirrels and Richardson's ground squirrels are polygamous (Michener & McLean, 1996; Michener, 1995; Thompson, 1977), so these findings lead us to question what selective pressures and underlying mechanisms are responsible for neuroanatomical changes across seasons in some species but not others.

Despite the number of studies on the effects of sex and season on hippocampal neurogenesis and hippocampus size, neuronal morphology and spine density have been largely ignored in the context of seasonal neuroplasticity in mammals. The same hormones that are responsible for sex and seasonal effects on hippocampus volume can alter the spine density and morphology of hippocampal neurons (Gould et al., 1990; McEwen et al., 1991;Woolley et al., 1990). These hormone-induced changes to neuronal morphology could be responsible for some of the observed volumetric changes, as they are in the birdsong nucleus RA (Devoogd & Nottebohm, 1981; Hill & DeVoogd, 1991), or they could occur independently of hippocampal volume. Understanding how neurons change due to the effects of sex and season on the mammalian brain is necessary to identify consistent changes and whether they are region-specific, or general trends across the brain.

Here, I used Golgi staining to conduct the first test for the effects of sex and season on hippocampal neuron morphology in a wild mammal: Richardson's ground squirrel. Richardson's ground squirrels undergo seasonal changes in hippocampus anatomy (Burger et al. 2013, 2014) and exhibit marked sex and seasonal differences in behaviour. Males emerge from hibernation 2-4 weeks earlier than females to establish territories and prepare for the breeding season (Michener, 1983). During the breeding season, males have enlarged home ranges (10-20x)

relative to the nonbreeding males and females of both seasons to better compete for receptive females (Michener & McLean, 1996). They lose weight rapidly and the majority of males (50-79%) die during the breeding season. Females endure the costs of raising young alone, however, mating is more costly to males than the parental effort is for females (Michener & Locklear, 1990). In the nonbreeding season, both males and females spend most of their time feeding and creating a hibernaculum within their burrow to prepare for hibernation, which depending on age and sex, they can spend up to 8 months in this chamber (Michener, 1992). Only males build and maintain a larder of seeds in their burrow to eat during brief breaks from torpor to help account for the cost of thermogenesis (Michener, 1992). Thus, the sexes differ in behaviour in both breeding and non-breeding season in ways that could affect hippocampus anatomy.

Based on the ecology of Richardson's ground squirrels (Michener, 1992, 1998; Michener and Locklear 1990), and previous work on small mammals and songbirds (Sherry, 2006; Lazaro ref), I hypothesized that both neuronal morphology and spine density will differ according to sex and season. Based on this hypothesis, I made several predictions. First, pyramidal cells will be largest and most complex in nonbreeding males to account for the findings of Burger et al. (2013). This sex difference will decrease in the breeding season when males are not caching food and experiencing high levels of stress, as stress can impact the shape and size of neurons (reviewed in McEwen Harold & Milliken, 1999). Second, because granule cell morphology varies with photoperiod length and stress (Fonken et al. 2012), I predict that granule cells will be largest in the nonbreeding males and this difference will diminish in the breeding season when males experience high stress. Third, pyramidal and granule cell morphology, and spine densities, will increase from the breeding to nonbreeding season in all animals as photoperiod increases

(Pyter et al, 2005; Fonken et al. 2012) and the stress of the mating for males, and parental care for females, decreases (Michener and Locklear, 1990, Cameron and Schoenfeld, 2018).

Materials and Methods

Animals

All the procedures outlined below adhered to the Canadian Council for Animal Care guidelines and were approved by the University of Lethbridge Animal Welfare Committee (Protocol #1502). Permits for collections and research were issued by the Alberta Department of Sustainable Resource Development (permit #s: 55980, 55981, 53998, 53999, 18-226).

We captured 47 Richardson's ground squirrels (9 male, and 11 female breeding animals, 16 male and 11 female nonbreeding animals) at the University of Lethbridge and on private properties with the City of Lethbridge (49.683, -112.778). Ground squirrels were trapped from 2016-2018 during the breeding season (February/March) and the non-breeding season (June-August). These two times approximate the time of emergence from hibernation and the subsequent breeding season and the preparation for hibernation during the non-breeding season (Michener, 1998). All the squirrels were caught using single door wire traps (46 cm x 13 cm x 13 cm), baited with either peanut butter or dandelions (*Taraxacum officinale*) and placed near the burrow entrance after observing an animal retreat into it. The traps were always monitored by at least one observer with binoculars from 50m. Once an animal was trapped, the ground squirrel was removed from the trap as fast as possible and processed. If multiple animals were trapped in rapid succession, they remained in their trap and were placed inside an inverted plastic container to minimize stress. The squirrels were removed from the traps and placed into a cone-shaped

zippered bag after which sex was determined visually. If the squirrel was of the desired sex, then it was weighed to the nearest 5g with a 600g spring scale and given an intraperitoneal injection of sodium pentobarbital (100mg/kg). Once adequate anesthesia was achieved, the squirrels were transcardially perfused with 0.9% saline. The heads were removed, and the brains and eyes were extracted immediately. The eyes were placed into 4% PFA (in 0.1 M PBS, pH= 7.4) and the brains were placed into Golgi fixative (Gibb & Kolb, 1998) and transported back to the lab.

Histology

We followed a standard Golgi staining protocol (Gibb & Kolb 1998). The brains were placed into Golgi-Cox fixative for 14 days and then into 30% sucrose for a minimum of 5 days before processing. All brains were sectioned at a thickness of 200µm with a vibratome. The sections were mounted directly onto 3% gelatin coated slides and stored in a light-proof box, with damp paper towel to prevent drying, for 1-2 days. The brain sections were washed in distilled water, followed by ammonium hydroxide and then placed a 1:1 solution of Kodak Rapid Fix (Kodak, Rochester, NY) and distilled water. The slides were then dehydrated using a graded ethanol series, cleared with a 1:1:1 solution of chloroform, Hemo De (Electron Microscopy Sciences catalog No. 23410-04), and 100% ethanol followed by Hemo De. Finally, the slides were cover slipped with Permount (Fisher Scientific, Catalog No. SP15-500) and given at least one month to dry completely before being imaged .

Imaging and Neuron Tracing

To quantify neuron morphology, I first created virtual slides using an Olympus VS120 digital slide scanner with a 40x oil objective (UPlanFL N, 40x/1.30 oil, ∞/0.17/FN26.5) and

Olympus VS-ASW FL software (Figure 1). I constructed z-stacks of 147 images spaced 0.68µm apart throughout the section yielding 99.96µm of working distance. Only neurons that were fully impregnated, free from large truncations, centered within the z-stack and separated from dense labeling were selected. The virtual slides were then uploaded into Neurolucida 360 (MicroBrightField, Williston, VT, USA) to be traced (Figure 2). Four granule cell and four pyramidal neurons were traced in each animal. Pyramidal neurons were split between CA1 and CA3 (2 from each), and they were all sampled from ventral hippocampus as cells were more numerous and neurons that fit my criteria were available. This resulted in a total of 188 reconstructed neurons (CA1 = 94, CA3 = 94). From the traced neurons, I extracted the following measurements for the pyramidal neurons: (1) total convex hull volume, (2) total convex hull surface area, (3) total length, (4) basal dendrite length, (5) apical dendrite length, (6) total volume, (7) cell body volume, (8) basal volume, (9) apical volume, (10) total branch number, (11) basal branch number, and (12) apical branch number. Granule cell measurements were largely the same, apart from the basal dendrite measurements as rodent granule cells only have apical dendrites (Amaral, Scharfman, & Lavenex, 2007). As with pyramidal cells, 4 per animal were traced, resulting in a total of 188 granule cell reconstructions.

I created similar virtual slides to quantify spine density of the pyramidal and granule cells (Figure 3). Using an Olympus VS-120 Slide Scanner and Olympus VS-ASW FL software z-stacks of 149 images 0.59µm apart, for a working distance of 87.91µm, were created with a 100x oil objective (UPlanSApo, 100x/1.40 oil, ∞/0.17/FN26.5). Using Neurolucida 360 (MicroBrightField, Williston, VT, USA), distal dendritic spines were traced in each neuron at 100× (N.A. 1.40) in 4 apical and 4 basilar representative dendrite segments of at least 40 µm in length, and at least 50 µm distal to the cell body for basilar tracings and 100µm distal to the cell

body for apical tracings (Figure 4) (Megías et al., 2001). Spines were only traced if they were longer than the radius of the dendrite. Bifurcating spines were traced as if they were two separate spines as they both are separate points of connection. Proximal spines were traced at one 20µm segment on one side of the cell body and then two 20µm segments on both the basilar and apical dendrites within 50µm from the soma (Figure 4). Granule cell spine density was traced in the same way; however, selection of dendritic segments followed a slightly different methodology. The tracing of spines followed a similar methodology as (Yan, Wilson, & Haring, 1997) where the distance from the soma to the most distant dendrite was calculated and divided by three to create three sections of the dendrites. Within each section (proximal, middle, and distal) a clear 20µm section was traced and all three were combined to calculate total spine density of each granule cell (Figure 5).

One of the advantages of using virtual slides is that inter- and intra-observer error can be assessed readily through the retracing of neurons in an image file. For the hippocampal pyramidal neurons, all three tracers reconstructed the same 5 neurons and the tracings were each analyzed and compared to ensure consistency. The coefficient of variation was determined to calculate a percent value of the variation in each measure, and then all coefficients were averaged to determine the average variation between the tracers. The three hippocampal pyramidal tracers have a coefficient of variation of 11.68%. Intra-tracer comparisons were conducted by having the tracer reconstruct the same randomly selected cell or dendritic segment five times for both pyramidal cells and granule cells. For spine density, one 40µm distal dendritic segment and one 20µm proximal dendritic segment were traced 5 times in hippocampal cells, and one 20µm segment from a granule cell was traced 5 times. The coefficient of variation was calculated for every measure and averaged to determine the variation between the same tracings

from the same tracer. The coefficient for intra-tracer hippocampal morphology tracing was 4.74% averaged across all measures and tracers. the coefficient was 5.41% for granule cell morphology tracings, and for spine density it was an average of 3.4% averaged across all measures. Two of the three hippocampal pyramidal cell tracers were blind to both season and sex while I was blind only to sex as I could tell the season based on subtle differences in the staining. For all the CA1 and CA3 pyramidal cell spine measures, and the granule cell morphology and spine measures, I was blind only to sex.

Statistical analyses

After running the models I plotted the residuals for each measurement and determined if their distributions met the assumptions of normality. Although the majority did meet these assumptions, 12/41 measurements did not. These data were left untransformed because linear mixed models are robust when sample sizes are above 45 and to distributions that are slightly or moderately skewed (Arnau et al., 2013). All analyses were conducted in R v. 3.6.1 (R Core Team 2015) within the RStudio™ environment (RStudio 2019).

To determine the effects of sex, season, and the interaction of sex and season on the cellular morphology and spine density I used linear mixed models using the "lme4" (Bates et al., 2015) and lmerTest (Kuznetsova, Brockhoff, & Christensen, 2017) R packages. First, I used sex, season, and the interaction effect between the two, as the fixed effects and animal as a random effect to account for measuring multiple neurons from each brain. Next, I used the "dredge" function in the R package "MuMin" (Barton 2013) to compare every possible combination of fixed effects and then rank the best fitting models. All models were ranked using Akaike information criterion (AICc) scores, as it is preferred for analyses of smaller sample sizes

(Bedrick & Tsai, 1994). All models within 2 AICc units from the best fitting model were averaged. This produced a final model with weighted coefficients relevant to their rank. The "dredge" function produces two outputs for an averaged model, a "full average" which zeros out predictors in models where they are absent and then calculates the final weighted model, and a "conditional average" that ignores the predictors not present when models are averaged and simply takes the value from the component models where it is present (Barton, 2013). I utilized only the full average as it punishes effects that are not in every best fitting model and is more conservative overall (Barton, 2013). When the best fitting final model did not include a parameter, it is reported as "-" in the table (See tables 1,3,5, & 7). This method was applied to every morphological measurement, including spine densities, and the results from the final fitted model are reported. In all models $\alpha = 0.05$.

RESULTS

CA1 Pyramidal Cells

My morphological measurements of CA1 neurons were highly variable across individuals, with more inter-individual variation in the non-breeding season than the breeding season (Table 1). Examples of some of the reconstructed CA1 neurons are shown in Figure 6. The best fitting final model for every CA1 morphological measurement included all three fixed effects suggesting that they fit the data effectively. In these models, there were no significant sex differences in CA1 morphology and no instances of a significant sex-season interaction effect across all measurements (Table 2). The number of basal branches was 29.6% higher

in nonbreeding than breeding animals (Figure 1), but this was the only measurement that differed significantly according to season.

Spine density measurements of CA1 neurons tended be less variable within my four main groups, breeding males and females and non-breeding males and females (Table 3). Unlike the analyses of neuronal morphology, the best fit linear mixed models in my analyses of spine density did not include a sex-season interaction effect for any measurement and only one included sex (Table 4). Thus, for most measurements of spine density, only seasonal effects were tested. Distal spine density of both apical and basal dendrites was significantly higher in non-breeding animals. On average, spine density was 17.6% higher for basal dendrites and 18.2% higher for apical dendrites in the nonbreeding season than the breeding season (Figure 7). In contrast, proximal spine density of apical and basal dendrites did not differ significantly by season.

Season also had a significant effect on proximal spine density of CA1 neuron cell bodies, but in the opposite direction to that of the apical and basal dendrites. The sampled cells in breeding season animals had an average of 41.5% higher spine densities than nonbreeding animals (Figure 7).

CA3 Pyramidal Cells

The morphology of CA3 neurons was also variable across individuals (Figure 6) and this variation was greater in breeding than non-breeding animals (Table 5). The best fitting final model for every morphological measurement included all three fixed effects, but I detected no significant sex, season, or sex-season interaction effects for any of the CA3 measurements (Table 6).

As with CA1 neurons, spine density measurements of CA3 neurons were far less variable than the morphological measurements (Table 3 & 5). The best fit linear mixed models in my analyses of spine density did not include sex or sex-season interaction effects for any measurement (Table 6), so only seasonal effects were tested. Across my spine density measurements, proximal spine density in apical, basal, and cell body dendrites did not differ significantly by season, but distal basal and distal apical spine density was significantly higher in non-breeding animals. The distal basal dendrites were 21.8% and the distal apical dendrites were 17.7% more dense in the nonbreeding season than the breeding season (Figure 8).

Granule Cells

Granule cell morphology was far more consistent across seasons (Table 7) than the CA1 and CA3 neurons. Examples of some of the reconstructed granule cells are shown in Figure 9. As with my analyses of CA1 and CA3 neurons, all three fixed effects were included in the final model, but no significant effects of sex, season, or the interaction of the two were detected for any of my morphological measurements of granule cells (Table 3).

Spine density of granule cells were less variable within my four groups (Table 3), similar to that of CA1 and CA3 neurons. My final mixed model for granule cell spine density was best fit by including the effects of sex and season, but not the interaction effect (Table 4). There was a significant effect of season on granule cell spine density that followed the same pattern as CA1 cell body, where cells had more dense spines in the breeding season compared to the nonbreeding season. The breeding season animals had 20.6% higher spine density than their nonbreeding counterparts (Figure 10).

Discussion

Overall, I observed only one marginal seasonal difference in neuron morphology and many significant effects of season on spine density, but no significant differences between sexes or sex-season interactions. In CA1 pyramidal neurons, there was a marginal increase in the number of basal branches in nonbreeding than breeding animals. The distal spine density of both basal and apical dendrites of CA1 neurons was also higher in the nonbreeding than breeding season, but proximal cell body spines were the densest in the breeding season. The CA3 pyramidal cells were similar with no significant differences in neuronal morphology across seasons and both apical and basal distal spine densities highest in the nonbreeding season, but proximal spines did not differ between seasons. Last, the granule cells had significantly higher spine densities in the breeding season than the nonbreeding season.

Spine density is affected by stress, activity levels and other individual differences in behaviour (McEwen, Harold & Milliken, 1999; Michelsen et al., 2007; Mitra et al., 2005; Perez-Cruz et al. 2009) and these same variables likely contributed to the variability observed across individual ground squirrels. Although most animals (21 nonbreeding,18 breeding) were collected from the same location, the breeding animals were collected across two years. Inter-annual variation is thought to be responsible for inconsistent effects of season on hippocampal anatomy as stress response (Delehanty & Boonstra, 2012) and survivorship (Risch et al., 2007) vary from year to year. Major stressors like weather and disease can vary annually (Michener, 1998), and studies have reported changes in hippocampal volume one year, but not another in Richardson's ground squirrel (Burger et al. 2013; Burger et al., 2014) and other species (Hoshooley et al., 2007). Thus, a combination of individual and inter-annual variation may have contributed to the variability I observed within and across animals in neuronal morphology and spine densities and

affected my ability to detect sex or season effects. By using a linear mixed effects model, I was able to include individual animal as a random effect to account for at least some of the inter-individual variation within my sample. However, incorporating environmental and behavioural data across and within years, would allow me to determine to what extent these other sources of variation interact with population level sex and seasonal effects on hippocampal anatomy.

Another caveat is that I sampled entirely from ventral hippocampus. The ventral hippocampus has many afferent and efferent connections with limbic structures and fewer place cells than the dorsal hippocampus (Felix-Ortiz & Tye, 2014; Jung, Wiener, & McNaughton, 1994; Kheirbek & Hen, 2011). Ventral hippocampus place cells also have low spatial selectivity, resulting in lower firing resolution and less defined spatial maps (Jung, Wiener, & McNaughton, 1994; Royer et al., 2010). The ventral hippocampus plays some role in spatial cognition and memory (Jung, Wiener, & McNaughton, 1994; Royer et al., 2010), but not as strongly as the dorsal hippocampus. Thus, my ability to relate seasonal or sex differences in spatial behaviour to neuronal spine density is somewhat limited. Unfortunately, poor staining and a high percentage of truncated neurons, it was not possible to sample from sufficient neurons in the dorsal hippocampus across sexes, seasons or individuals. This limitation could be potentially overcome through sampling significantly more individuals and/or the use of alternative staining protocols (e.g., other Golgi-Cox rapid stains, intracellular fills) to ensure an adequate number of dorsal hippocampus neurons are sampled.

Neuronal Morphology

Sex hormones can cause changes to neuronal morphology, and these hormones can be variable between the sexes (Gould, Woolley, & McEwen, 1991; McEwen et al., 1991b). Seasonal surges in sex hormones should therefore result in both sex differences and sex-season

interaction effects on neuronal morphology in a seasonally breeding species like Richardson's ground squirrel. However, I observed no such effects and the only difference in neuronal morphology detected was a seasonal effect: CA1 basal branch number was higher in nonbreeding than in breeding season animals. This was contrary to my hypothesis that nonbreeding males would have larger and more complex neurons (Burger et al. 2013), but is consistent with the mixed results or lack of seasonal effects detected in previous studies of neuronal morphology in relation to hibernation, season and photoperiod in other small mammals.

Hibernation, and its impact on the brain, has been well-studied in ground squirrels, particularly in the hippocampus. The anatomy of hippocampal pyramidal neurons changes dramatically during hibernation: soma size decreases (Popov, Bocharova, & Bragin, 1992; von der Ohe, 2006); CA1 and CA3 cells have reduced spine density and dendritic branching (Popov & Bocharova, 1992; von der Ohe, 2006; Popov et al., 1992); there are fewer mossy fibre terminals (Arendt et al., 2003; Popov & Bocharova, 1992); and there is large scale synapse loss (Strijkstra et al., 2003; von der Ohe et al., 2007). Despite the magnitude and breadth of these anatomical changes, they can be reversed within a few hours of arousal from hibernation (Popov, Bocharova & Bragin, 1992; Popov & Bocharova, 1992; Strijkstra et al., 2003; Popov et al., 2007) and hippocampal functioning returns to normal (Weltzin et al., 2006). If this return to pre-hibernation neuronal morphology can occur rapidly, then perhaps there are only limited changes in neuronal morphology that happen throughout the active, above-ground period in which I sampled the animals.

Seasonal changes in brain region volumes are also not always reflected in neuronal morphology, as demonstrated by studies of the common shrew (*Sorex araneus*). The brain mass of common shrews decreases significantly (20% or more) from summer to winter, after which

there is a partial return to the original brain mass (15%) in the spring (Dehnel, 1949; Yaskin, 1994). This overall change in brain mass arises from a mosaic of changes in the size of individual brain regions, including the hippocampus (Lázaro et al., 2018). Some regions, such as the thalamus, hypothalamus and the hippocampus, decrease in volume in the winter and then regrow in the spring (Lázaro et al., 2018). However, other regions, such as the neocortex and striatum, do not regrow in the spring (Lázaro et al., 2018). An analysis of neuron morphology within some of these regions revealed that morphology varies with some of the changes in brain region volumes, but in an inconsistent fashion. Neurons underwent a decrease in at least some morphological measurements from the summer to winter, but in the subsequent regrowth there is either a further decrease in cell size and complexity (cautoputamen, anterior cingulate cortex) or no changes to neuron morphology (somatosensory cortex) (Lázaro et al., 2018). The morphology of cells in the hippocampus have yet to be examined in shrews, but these results at least indicate that not all neuronal populations change morphology with season in a predictable fashion. Thus, the lack of seasonal differences in the morphology of CA neurons and granule cells in the ground squirrels may demonstrate limited plasticity of neuronal morphology in some brain regions.

Experimentally manipulating photoperiod in the lab has also yielded minor or no changes to measures of neuronal morphology. In Siberian hamsters (*Phodopus sungorus*), short photoperiod groups had decreased CA1 soma area and proximal Scholl interactions than long photoperiod groups, but no effects were found on CA3 cells, and only spine density varied in granule cells (Workman et al., 2011). Disturbing regular photoperiods with a dim light condition in hamsters did not affect any measurements of dendritic complexity or soma size (Bedrosian et al., 2011). In male white footed mice (*Peromyscus leucopus*), short photoperiod groups did not show any changes to neuronal morphology in the hippocampus when compared with long

photoperiod animals (Pyter, Reader, & Nelson, 2005). Although short day lengths increased fear memory in white-footed mice, there were no significant changes to dendritic complexity or soma size (Walton et al., 2012). The lack of difference in neuronal morphology between breeding and nonbreeding seasons in the ground squirrels therefore parallels the minor or complete lack of effects of photoperiod manipulations on hippocampal neuron morphology.

CA cell body spine density

The number and shape of dendritic spines vary in relation to hormones, experience, stress and photoperiod (Gould et al., 1990; Kolb, Gibb, & Gorny, 2003; Martínez-Téllez et al., 2009; Pyter et al., 2005). Although in most studies (Bedrosian et al., 2011; Kolb et al., 2003; Pyter et al., 2005; Workman et al., 2009), the focus is often on distal spine densities, here I found significant seasonal differences in the spine density of CA1 cell bodies and in a direction contrary to the hypothesis: CA1 cell body spines were most dense in the breeding season compared to the nonbreeding season. Unlike distal spines, there has been minimal research on the plasticity of proximal spines and it is therefore unclear how or why this seasonal effect occurred. Dendritic spines are where most glutamatergic excitatory synapses onto the cell occur, while inhibitory synapses are more often targeting the axon and soma (Spruston, 2008). As distance is increased from the soma, the excitatory post synaptic potentials (EPSPs) decrease dramatically due to distance and impedance from dendrites with smaller diameters (Stuart & Spruston, 1998; Stuart et al., 1997). The most effective synapses for causing action potentials are close to, or on, the cell body (Golding et al., 2005; Spruston, 2008). I suggest that the change I found during the breeding season could be a way to ensure rapid firing of these cells during the breeding season. It may be necessary for meeting breeding season demands such as establishing territories, observing and remembering conspecifics, and locating food (Michener & McLean,

1996; Michener, 1992). Regardless of whether this is related to behaviour or not, why this seasonal effect was only observed in CA1 and not CA3 neurons is uncertain. CA3 ensembles are related to context representations and seem to play a critical role in maintaining stable representations, even with subtle changes to the environment (Danielson et al., 2016; Zemla & Basu, 2017). Ventral CA1 is thought to be associated with social and emotional memory due to connections with the amygdala, medial prefrontal cortex, and nucleus acumbens (Felix-Ortiz & Tye, 2014; Kheirbek & Hen, 2011). It could be this emotional and social associations of CA1 that demand more cell body spines for faster firing in the breeding season when females are social with kin and males must navigate other males and find sexually receptive females (Michener & McLean, 1996; Zemla & Basu, 2017). To fully understand this result, animals need to be sampled across years to determine the consistency of this effect and measuring *in vivo* firing rates of CA neurons of animals collected at different times of the year.

Granule Cells

As with the CA1 cell body spine density, granule cell spine density was also most dense in the breeding season. One potential explanation is that these results reflect an energetic trade-off experienced by ground squirrels in relation to neurogenesis (Amrein, 2015). Neurogenesis is energetically expensive (Vander et al., 2009) and situations that require increased resources, such as gestation, lactation, parental care, difficult environments, and winter, can impede adult hippocampal neurogenesis (Bartkowska et al., 2008; Cavegn et al., 2013; Galea & McEwen, 1999; Glasper, Kozorovitskiy, Pavlic, & Gould, 2011; Leuner et al., 2007; Pawluski & Galea, 2007). In Richardson's ground squirrels, the breeding season is energetically expensive with males competing for mates, females being responsible for reproduction (Michener & Locklear, 1990), while both sexes struggle to find food and evade predators. The combination of these

energetic costs with high cortisol (Delehanty & Boonstra, 2012; Hare et al., 2014; Ryan et al. 2012) and sex hormone levels (Delehanty & Boonstra, 2012), drives down neurogenesis rates in both male and female ground squirrels (Burger et al. 2014). The reduced daylight of the breeding season could further compound this phenomenon as short-day lengths can reduce neurogenesis in rodents (Huang, DeVries, & Bittman, 1998; Walton et al., 2014). The resource-intensive, short photoperiod, high stress breeding season not only impairs adult hippocampal neurogenesis, it likely encourages cell survival, allowing time for granule cells to potentiate and develop higher spine densities in the breeding season. Long term potentiation can significantly increase the spine density in rat granule cells (Trommald, Hulleberg, & Andersen, 1996), and a lower rate of cell death allows for a longer time for cells to potentiate and create connections. Nonbreeding animals experience lower resource demands and longer photoperiods which can increase neurogenesis (Galea & McEwen, 1999; Burger et al., 2014). This increased proliferation and cell death results in a population of granule cells with lower spine densities on average. A similar pattern occurs in Siberian hamsters where animals exposed to short photoperiods have higher granule cell spine density compared with those exposed to long photoperiods (Workman et al., 2011). Thus, although the direction of this seasonal effect on granule cell spine density is in the opposite direction to what I predicted if the hypothesis was true, it might reflect a more general phenomenon related to energetics, photoperiod and neurogenesis.

CA Distal Spine Density

The largest and most consistent effect in my study was a seasonal increase in CA1 and CA3 spine density from breeding to nonbreeding season. As with neuronal morphology (see above), levels of circulating sex hormones have significant effects on spine densities (Gould et al., 1990; Leranth, Hajszan, & MacLusky, 2004; Leranth, Petnehazy, & MacLusky, 2003) and

given the highly seasonal nature of reproduction in Richardson's ground squirrels (Michener & McLean, 1996), I expected sex and sex-season interaction effects on spine densities. However, no significant sex differences were detected. The overall effect of season, in the absence of sex effects, is therefore likely due to photoperiod, stress hormones and activity, all of which are shared between the sexes.

In a similar fashion to neuron morphology (see above), photoperiod is likely playing a large role in the seasonal effects on spine densities of CA1 neurons. In rodents, exposure to longer photoperiods increases distal spine densities and shorter photoperiods decreases distal spine densities in most studies (Bedrosian et al., 2011; Soler, et al., 2018; Walton et al., 2012; Workman et al., 2009). Although in a few studies, short photoperiods result in an increase in spine density in some neurons and decreases in others (Pyter et al., 2005), photoperiod seems to exert larger effects on spine densities in rodents than on neuron size or morphology. Photoperiod differed greatly between the breeding and nonbreeding seasons when my Richardson's ground squirrels were collected. During the breeding season, there was a minimum of 10.2 hours of daylight whereas the nonbreeding season had a maximum of 16.2 hours of daylight. Although this 6 hour difference is not as extreme as that implemented in many lab studies (Bedrosian et al., 2011; Pyter et al., 2005; Walton et al., 2012; Workman et al., 2011), it is the maximum difference in photoperiod that the ground squirrels can be exposed to after emerging from hibernation and would therefore be likely to have an effect on spine density.

Stress can also affect hippocampal distal spine density (Chen et al., 2010; Martínez-Téllez et al., 2009; Shors, Chua, & Falduto, 2001) and could be playing a role in the seasonal differences that I observed. Acute stress can have variable effects on spine density (Shors et al., 2001), but chronic stress causes spine loss in hippocampal cells (Leuner & Shors, 2013; Stewart

et al., 2005). During the breeding season, Richardson's ground squirrels experience high stress (Delehanty & Boonstra, 2012; Hare et al., 2014; Michener & Locklear, 1990) as a result of male-male competition for mates, female reproduction and both sexes recovering body condition after a long hibernation period (Michener & McLean, 1996; Michener, 1983; Michener & Locklear, 1990). Higher stress is thought to underlie lower neurogenesis rates in breeding males and females (Burger et al., 2014) and is also likely to contribute to lower spine densities in CA1 and CA3 in a similar fashion to that reported in chronically stressed laboratory rodents (Leuner & Shors, 2013; Stewart et al., 2005). This stress could be encouraging synapse shedding in the breeding season, but as it dissipates in the nonbreeding animals the processes may favor a different steady state with denser distal spine density.

A third factor that could contribute to the seasonal difference in spine density is activity level. Exercise is correlated with increased distal spine density in the medial prefrontal cortex, entorhinal cortex, dentate gyrus, and CA1 region (Eadie, Redila, & Christie, 2005; Eddy & Green, 2017; Stranahan, Khalil, & Gould, 2007; Stranahan et al., 2009). The breeding season of Richardson's ground squirrel coincides with their emergence from hibernation, during which they have no or limited movements. So emergence is associated with a rapid increase in activity and an expanse of home range size (Michener & McLean, 1996). As the year progresses, home range size decreases in both sexes (Michener & McLean, 1996), but ground squirrels are regularly active during daylight hours and the progressive increase in daylength means that activity can increase for several months. This activity also increases as ground squirrels find sufficient food to fatten up (both sexes) and store food (males only) for hibernation. The ongoing activity of ground squirrels throughout the breeding and nonbreeding seasons could be sufficient

to also stimulate the formation of dendritic spines, ultimately resulting in spine density being higher in the nonbreeding season than the breeding season.

It is important to note that these three potential causes of changes in spine density, stress, photoperiod and activity level, are not mutually exclusive of one another and might all be driving an increase in spine density in CA neurons in the nonbreeding season. Further, these three factors acting in concert could explain why I observed large and uniform seasonal effects on distal spine density whereas laboratory studies that manipulate only one of these factors yield less uniform results (Pyter et al., 2005).

Conclusion

Contrary to my hypothesis, Richardson's ground squirrels do not exhibit any sex-season interactions on neuronal morphology or spine density in the hippocampus. Instead, they primarily undergo seasonal changes in spine density that vary in direction depending on the location of the spines and neuron type. The variability observed across ground squirrels and lack of sex-season interaction effects highlights several aspects of hippocampus anatomy. First, hippocampal neuronal morphology is highly variable within and among individuals. Second, the sex by season patterns observed in other rodents are not applicable to all species. Their landmark-sparse habitat, and the spacing of animals close together in spatial groups, could result in less demand on the hippocampus in the breeding season than the other studied rodent species. Lastly, seasonal plasticity in spine density is high, and it differs across cell types and locations. Future studies using wild animals and different brain regions are required to understand why seasonal neuroplasticity takes place in some species and not others. Wild animals are sampled from the environment in which they have been evolutionarily selected for, so wide-scale and diverse research may reveal fundamental aspects of seasonal plasticity in the mammalian brain.

Table 1. The average (± standard deviation) measurements of CA1 neurons of males and females in breeding and non-breeding seasons.

Measurement	Breeding Males (n =9)	Breeding Females (n =11)	Non-breeding males (n =16)	Non-breeding females (n = 11)
Total Convex Hull Volume (µm³)	10305166.67±5958246.895	8127211±3255081	11491136.25±6272639.88	11009994.55±4934250.98
Total Convex Hull Surface (µm^2)	390930.3±158164.4	322921.5±96007.52	432755.91±195202.35	421999.82±149443.8
Total Length (µm)	4899.18±952.824	4640.33±1001.49	5780.45±1916.24	5446.39±1898.31
Basal Length (µm)	2089.23±616.703	1984.60±460.93	2633.18±1047.89	2532.76±913.02
Apical Length (µm)	2809.95±822.19	2655.72±830.51	3147.27±1222.11	2913.63±1704.24
Total Volume (µm³)	11482.72±6961.9	11224.02±4836.29	14749.28±7882.67	13304.23±4642.01
Cell Body Volume (µm³)	6044.15±3732.57	6462.17±3120.49	8142.55±3988.41	8090.63±3748.17
Basal Volume (µm³)	1835.28±1589.79	1566.34±1175.31	2423.32±1874.40	1646.99±848.24
Apical Volume (µm³)	3603.3±2433.87	3195.51±1815.71	4237.63±3335.62	3566.61±2037.95
Total Branch Number	74.56±10.41	74.82±14.6	88.66±19.58	82.50±23.41
Basal Branch Number	32.28±9.11	32.77±6.35	43.16±13.76	41.18±14.55
Apical Branch Number	42.28±11.72	42.05±14.81	45.5±14.66	41.32±19.9

Table 2- Results of a linear mixed model analysis of sex, season, and sex by season interaction and their effects on 12 measures of CA1 pyramidal neuron morphology: Linear mixed model results are reported with the fixed effects estimate, the standard error, and the P-values where (*) denotes a significant effect (p-values <0.05).

Measurement	**Sex (Male)**			**Season (Nonbreeding)**			**Sex*Season**			**Intercept**	
	Estimate	**SE**	**P-value**	**Estimate**	**SE**	**P-value**	**Estimate**	**SE**	**P-value**	**Estimate**	**SE**
Total Convex Hull Volume (µm³)	2177955	2027840	0.289	2581542	1923778	0.187	1395573	2689755	0.607	8127211	1360316
Total Convex Hull Surface (µm²)	68009	58438	0.251	99078	55439	0.081	-57253	77512	0.464	322921	68009
Total Length (µm)	258.86	637.27	0.687	806.06	604.57	0.189	75.2	845.29	0.93	4640.33	258.86
Basal Length (µm)	104.625	312.22	0.739	548.152	296.201	0.071	-4.203	414.138	0.992	1984.603	209.446
Apical Length (µm)	154.23	478.53	0.749	257.91	453.98	0.573	79.41	634.73	0.901	2655.72	154.23
Total Volume (µm³)	258.7	2269.1	0.91	2080.2	2152.7	0.339	1240.6	3009.8	0.682	11224	1522.2
Cell Body Volume (µm³)	-418	1333.5	0.755	1628.5	1265	0.205	469.9	1768.7	0.792	6462.2	894.5
Basal Volume (µm³)	268.94	561.77	0.635	80.66	532.94	0.880	507.38	745.14	0.5	1566.34	376.84
Apical Volume (µm³)	407.8	982.1	0.68	371.1	931.7	0.692	263.2	1302.7	0.841	3195.5	407.8
Total Branch Number	-0.263	6.682	0.969	7.682	6.339	0.232	6.419	8.863	0.473	74.818	4.483
Basal Branch Number	-0.495	4.031	0.903	8.409	3.825	**0.033 ***	2.469	5.347	0.647	32.773	2.704
Apical Branch Number	0.232	5.759	0.968	-0.727	5.463	0.895	3.950	7.639	0.608	42.046	3.863

Table 3. The average (± standard deviation) measurements of hippocampal cell spine density of males and females in breeding and non-breeding seasons.

Measurement	**Breeding Males (n =9)**	**Breeding Females (n =11)**	**Non-breeding males (n =16)**	**Non-breeding females (n = 11)**
CA1 Basal Distal Spines	1.50±0.27	1.67±0.37	1.90±0.38	1.80±0.37
CA1 Apical Distal Spines	1.51±0.19	1.54±0.26	1.84±0.38	1.76±0.22
CA1 Basal Proximal Spines	0.97±0.26	1.01±0.32	1.06±0.32	0.95±0.30
CA1 Apical Proximal Spines	0.74±0.33	0.75±0.30	0.76±0.41	0.79±0.34
CA1 Proximal Cell Body Spines	0.61±0.29	0.60±0.19	0.42±0.10	0.43±0.15
CA3 Basal Distal Spines	1.47±0.32	1.59±0.35	1.87±0.44	1.86±0.37
CA3 Apical Distal Spines	1.58±0.36	1.41±0.36	1.77±0.36	1.74±0.32
CA3 Basal Proximal Spines	0.75±0.19	0.72±0.24	0.71±0.22	0.73±0.27
CA3 Apical Proximal Spines	0.79±0.23	0.82±0.20	0.71±0.21	0.71±0.20
CA3 Proximal Cell Body Spines	0.51±0.15	0.51±0.13	0.46±0.15	0.46±0.12
Granule Cell Spine Density	1.19±0.31	1.36±0.27	1.00±0.29	1.14±0.33

Table 4- Results of linear mixed model analysis of sex, season, and sex by season interaction and their effects on 11 measurements of cellular spine density. Linear mixed model results are reported with the fixed effects estimate, the standard error, and the P-values where (*) denotes a significant effect (p-values <0.05). Fixed effects that were not included in the best fitting final model are denoted with a (-).

Measurement	**Sex (Male)**			**Season (Nonbreeding)**			**Sex*Season**			**Intercept**	
	Estimate	**SE**	**P-value**	**Estimate**	**SE**	**P-value**	**Estimate**	**SE**	**P-value**	**Estimate**	**SE**
CA1 Basal Distal Spine Density (1/µm)	-	-	-	0.278	0.093	**0.005***	-	-	-	1.583	0.071
CA1 Apical Distal Spine Density (1/µm)	-	-	-	0.278	0.073	**>0.001***	-	-	-	1.526	0.055
CA1 Basal Proximal Spine Density (1/µm)	0.044	0.070	0.529	-	-	-	-	-	-	0.979	0.051
CA1 Apical Proximal Spine Density (1/µm)	-	-	-	0.028	0.096	0.769	-	-	-	0.744	0.073
CA1 Proximal Cell Body Spine Density (1/µm)	-	-	-	-0.177	0.048	**0.001***	-	-	-	0.601	0.036
CA3 Basal Distal Spine Density (1/µm)	-	-	-	0.334	0.104	**0.002***	-	-	-	1.53	0.079
CA3 Apical Distal Spine Density (1/µm)	-	-	-	0.265	0.088	**0.004***	-	-	-	1.495	0.067
CA3 Basal Proximal Spine Density (1/µm)	-	-	-	-0.016	0.052	0.768	-	-	-	0.731	0.040
CA3 Apical Proximal Spine Density (1/µm)	-	-	-	-0.095	0.054	0.082	-	-	-	0.808	0.041
CA3 Proximal Cell Body Spine Density (1/µm)	-	-	-	-0.048	0.031	0.125	-	-	-	0.511	0.023
Granule Cell Spine Density (1/µm)	-0.079	0.088	0.371	-0.210	0.063	**0.001***	-	-	-	1.314	0.065

Table 5. The average (± standard deviation) measurements of CA3 neurons of males and females in breeding and non-breeding seasons.

Measurement	Breeding Males (n =9)	Breeding Females (n =11)	Non-breeding males (n =16)	Non-breeding females (n = 11)
Total Convex Hull Volume (µm³)	17241726.11±10757705	15840739.09±5985666	15941845.63±6816279	14079090.91±6202622
Total Convex Hull Surface (µm²)	566464.22±217697.4	553930.05±176349.3	572059.81±190800.5	537034.64±164754.6
Total Length (µm)	7053.44±1918.55	7094.28±2234.39	7011.53±2172.29	6887.82±1691.59
Basal Length (µm)	3731.66±1073.72	3436.99±1518.74	3207.02±1280.7	3154.34±862.92
Apical Length (µm)	3321.78±1323.1	3657.29±1219.74	3804.50±1763.5	3733.48±1093.46
Total Volume (µm³)	18616.14±7977.34	17399.65±6089.82	18590.61±4839.67	14751.25±4968.07
Cell Body Volume (µm³)	9225.08±3893.54	8826.36±3581.25	10503.87±3754.72	8103.69±3762.02
Basal Volume (µm³)	3833.14±1896.26	3523.85±2174.39	3197.98±1495.25	2527.44±942.94
Apical Volume (µm³)	5557.92±3125.5	5049.44±2360.66	4888.76±2367.68	4120.13±1845.93
Total Branch Number	90.39±24.08	86.09±21.61	86.91±21.59	91.59±19.48
Basal Branch Number	51.61±16.25	43.27±12.26	41.44±12.58	44.59±11.16
Apical Branch Number	38.78±14.81	42.82±14.29	45.47±22.67	47.00±13.33

Table 6- Results of a linear mixed model analysis of sex, season, and sex by season interaction and their effects on 12 measures of CA3 pyramidal neuron morphology. Linear mixed model results are reported with the fixed effects estimate, the standard error, and the p-values where (*) denotes a significant effect (p-values <0.05).

Measurement	**Sex (Male)**			**Season (Nonbreeding)**			**Sex*Season**			**Intercept**	
	Estimate	**SE**	**P-value**	**Estimate**	**SE**	**P-value**	**Estimate**	**SE**	**P-value**	**Estimate**	**SE**
Total Convex Hull Volume (µm³)	1400987	2751716	0.613	-1761648	2610507	0.503	461768	3649916	0.9	15840739	1400987
Total Convex Hull Surface (µm²)	12534	70641	0.86	-16895	67016	0.802	22491	93699	0.811	553930	12534
Total Length (µm)	-40.84	753.17	0.957	-206.46	714.52	0.774	164.55	999.02	0.87	7094.28	-40.84
Basal Length (µm)	294.7	425.6	0.492	-282.6	403.8	0.488	-242	564.5	0.67	3437	294.7
Apical Length (µm)	-335.51	508.71	0.513	76.19	482.6	0.875	406.53	406.53	0.55	3657.29	341.25
Total Volume (µm³)	1216	2295	0.599	-2648	2177	0.23	2623	3044	0.394	17400	1539
Cell Body Volume (µm³)	398.7	1432.8	0.782	-722.7	1359.3	0.598	2001.5	1900.5	0.298	8826.4	961.2
Basal Volume (µm³)	309.3	662.2	0.643	-996.4	628.2	0.12	361.3	878.4	0.683	3523.8	444.2
Apical Volume (µm³)	508.5	987.6	0.609	-929.3	937	0.327	260.1	1310	0.844	5049.4	662.5
Total Branch Number	4.298	8.005	0.594	5.5	7.594	0.473	-8.983	10.618	0.402	86.091	5.37
Basal Branch Number	8.338	4.839	0.092	1.318	4.591	0.775	-11.492	6.418	0.08	43.273	3.246
Apical Branch Number	-4.04	5.798	0.49	4.182	5.501	0.451	2.509	7.691	0.746	42.818	3.89

Table 7. The average (± standard deviation) measurements of granule cells of males and females in breeding and non-breeding seasons.

Measurement	Breeding Males (n =9)	Breeding Females (n =11)	Non-breeding males (n =16)	Non-breeding females (n = 11)
Total Convex Hull Volume (µm³)	1445379±718009	1417369.86±979090.9	1849959.58±787675.7	1623384.86±923986
Total Convex Hull Surface (µm²)	84437.84±31452.24	80780.13±33676.61	100544.60±31081.05	93640.77±35251.24
Apical Length (µm)	1802.22±705.81	1696.74±734.77	1862.78±564.04	1949.14±925.14
Total Volume (µm³)	6973.92±4331.71	7405.08±3955.58	6748.91±3213.87	8218.39±5509.32
Cell Body Volume (µm³)	5088.13±3608.96	5637.54±3705.65	5485.63±3232.25	6926.25±5189.91
Apical Volume (µm³)	1885.79±1325.88	1767.54±716.87	1263.28±970.86	1292.13±832.31
Apical Branch Number	32.03±14.9	29.14±12.93	29.52±10.5	30.50±16.16

Table 8- Results of linear mixed model analysis of sex, season, and sex by season interaction and their effects on 7 measures of granule cell morphology. Linear mixed model results are reported with the fixed effects estimate, the standard error, and the p-values where (*) denotes a significant effect (p-values <0.05).

Measurement	Sex (Male)			Season (Nonbreeding)			Sex*Season			Intercept	
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE
Total Convex Hull Volume (µm³)	28009	233352	0.905	206015	221377	0.357	198566	309521	0.525	1417370	156537
Total Convex Hull Surface (µm²)	3658	9702	0.708	12956	12861	0.921	3246	12869	0.802	80780	6509
Apical Length (µm)	105.5	193.2	0.588	252.4	183.3	0.176	-191.8	256.2	0.458	1696.7	129.6
Total Volume (µm³)	-431.2	1425.8	0.764	804.1	1352.6	0.555	-1032.2	1891.2	0.588	7405.1	956.4
Cell Body Volume (µm³)	-549.4	1346.8	0.685	1288.7	1277.6	0.319	-891.2	1786.3	0.62	5637.5	903.4
Apical Volume (µm³)	118.2	284.4	0.68	-484.6	269.8	0.08	-140.9	377.2	0.711	1767.5	190.8
Apical Branch Number	2.891	3.477	0.41	1.364	3.298	0.681	-3.876	4.612	0.405	29.136	2.332

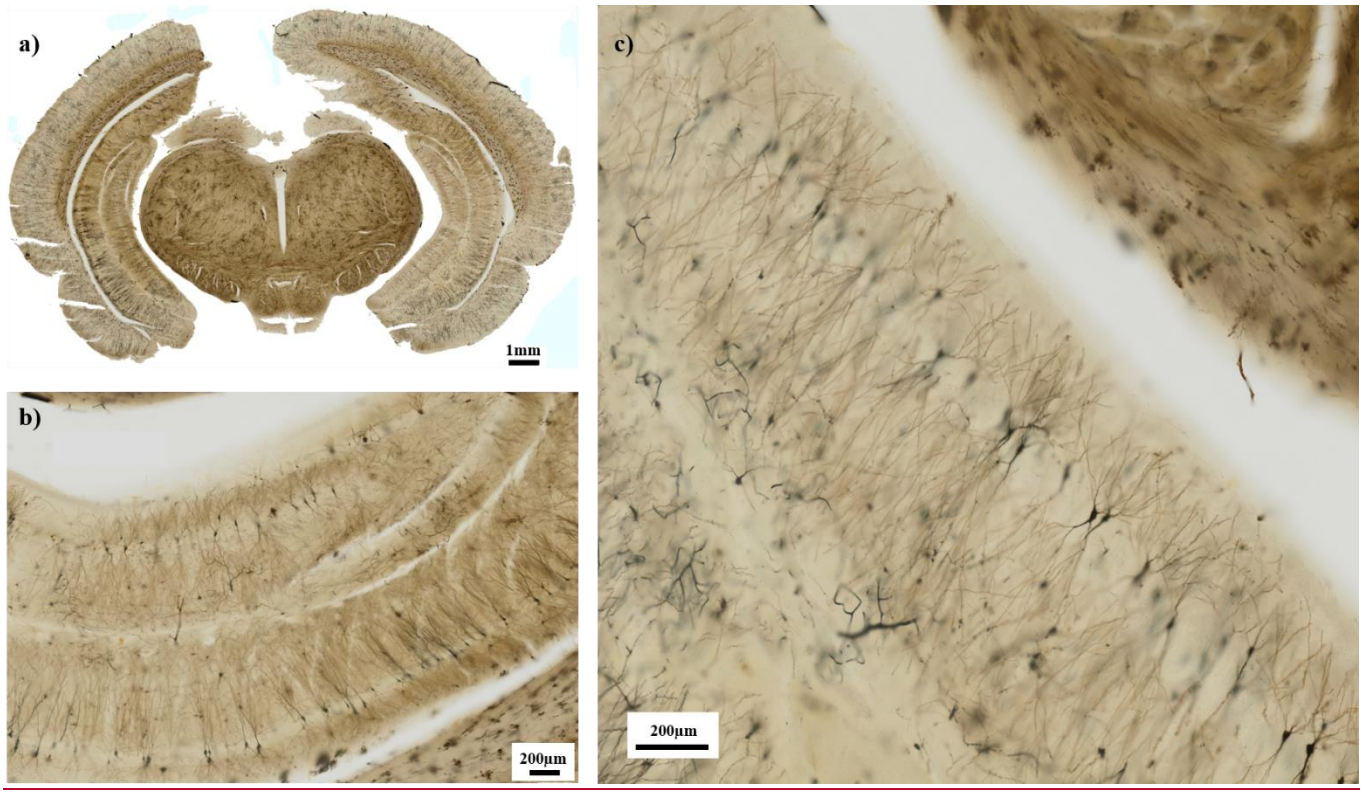

Figure 1- Examples of Golgi stained tissue. **a)** Golgi stained section of a Richardson's ground squirrel brain. **b)** A Golgi stained section detailing the hippocampus. **c)** Golgi Stained CA3 region of the hippocampus in a Richardson's ground squirrel.

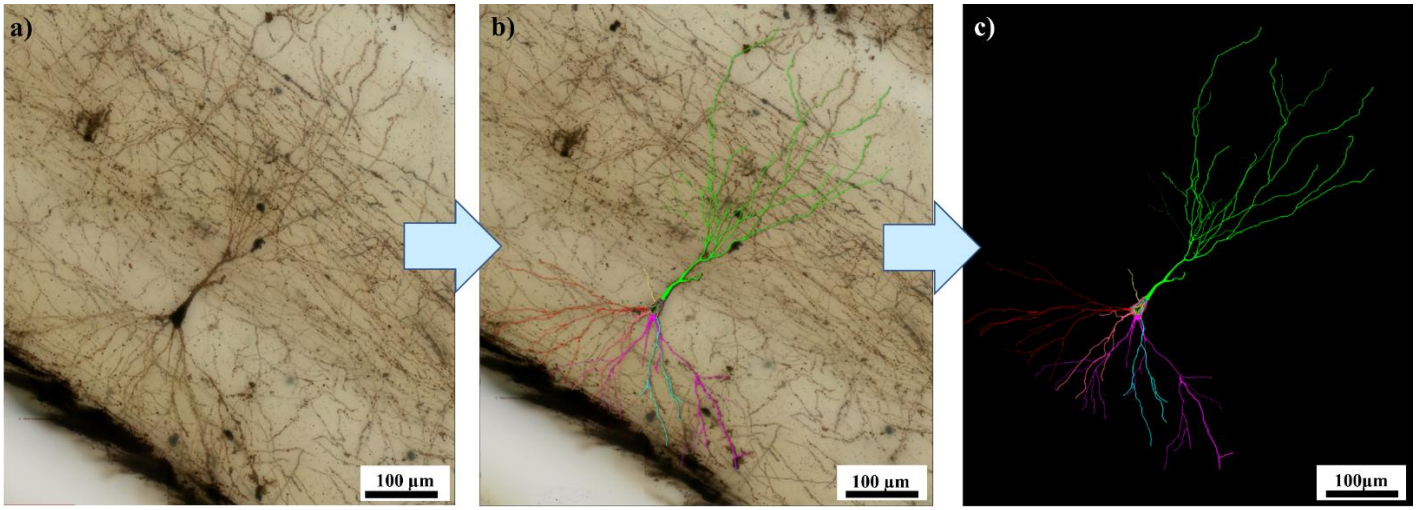

Figure 2. **a)** A clear, adequately stained neuron free from truncations. **b)** A tracing on top of the selected neuron with Neurolucida 360 software. **c)** The final reconstruction ready to be analyzed with Neurolucida Explorer.

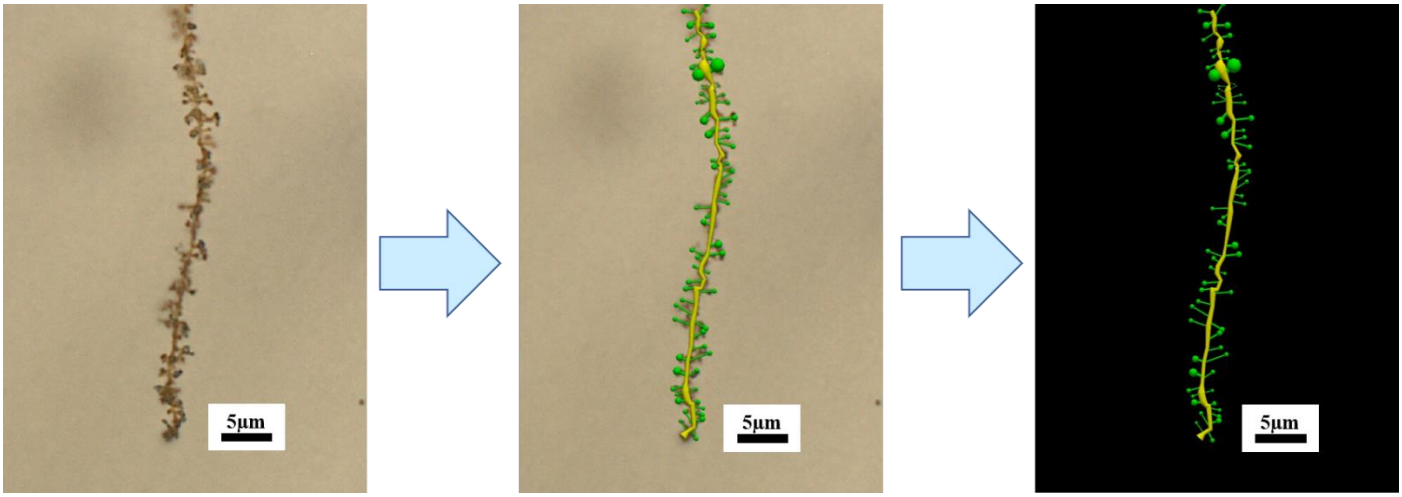

Figure 3. **a)** A clear distal dendritic segment not obscured by background staining. **b)** Tracing with spines placed on the segment. **c)** The reconstruction ready to be analyzed for spines per µm.

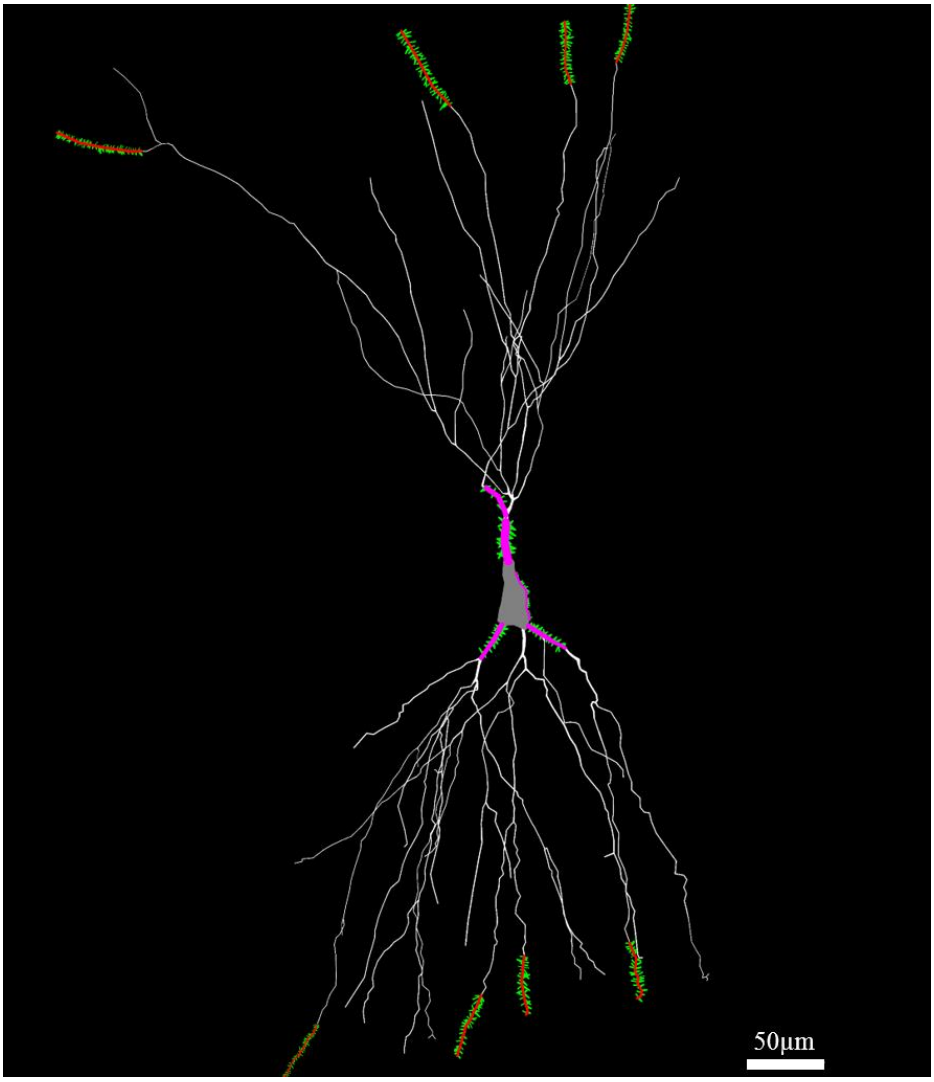

Figure 4- Example cell highlighting the sampling protocol for hippocampal pyramidal cell spine density measurements. Pink sections are sample sites for proximal spines and red sections are sites for distal spine tracing.

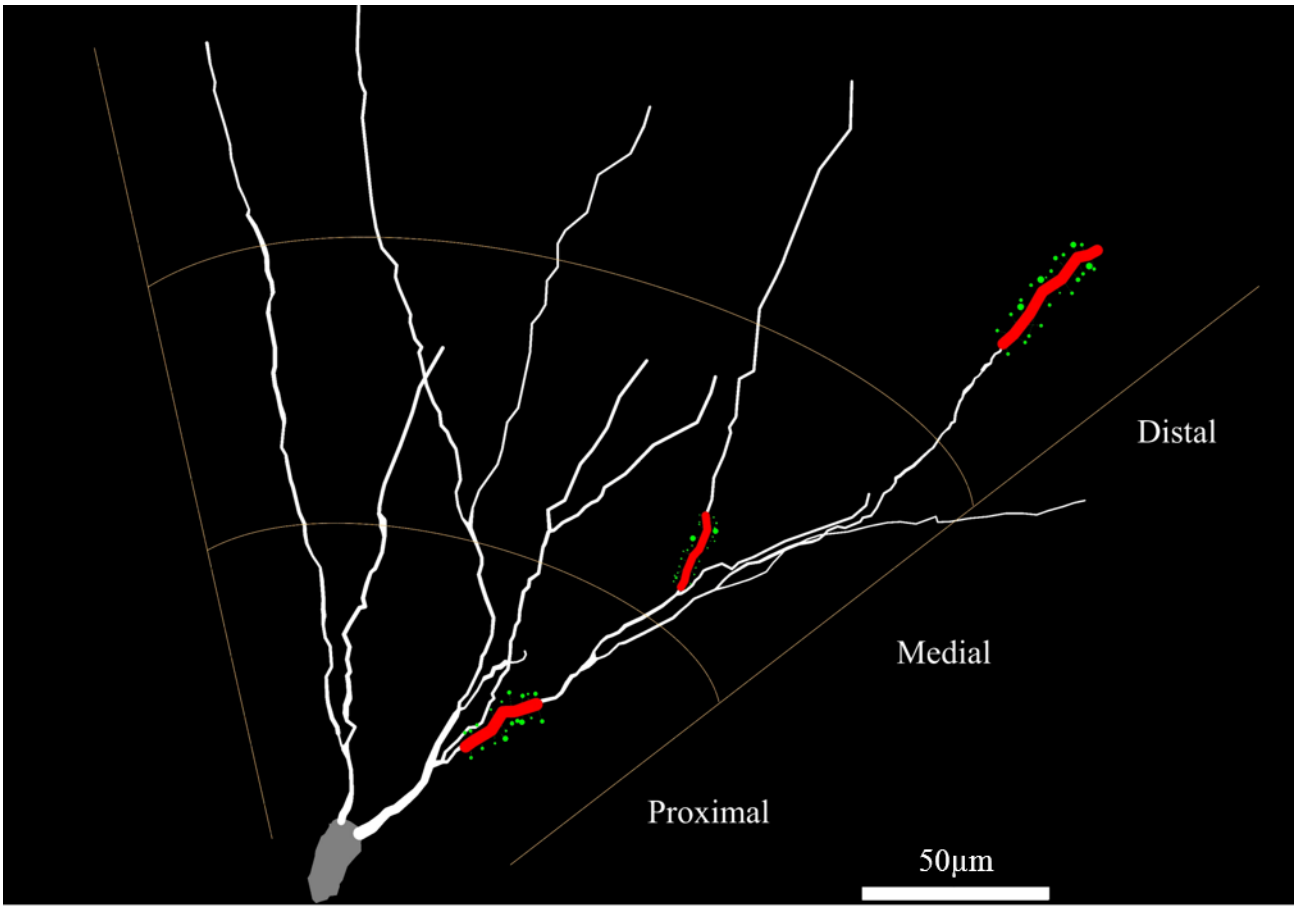

Figure 5- Example granule cell detailing the sampling protocol for granule cell spine density. One clear 20µm dendritic segment in the proximal, medial, and distal portions of the dendrites was identified and traced and then combined to get a total spine density measure per cell.

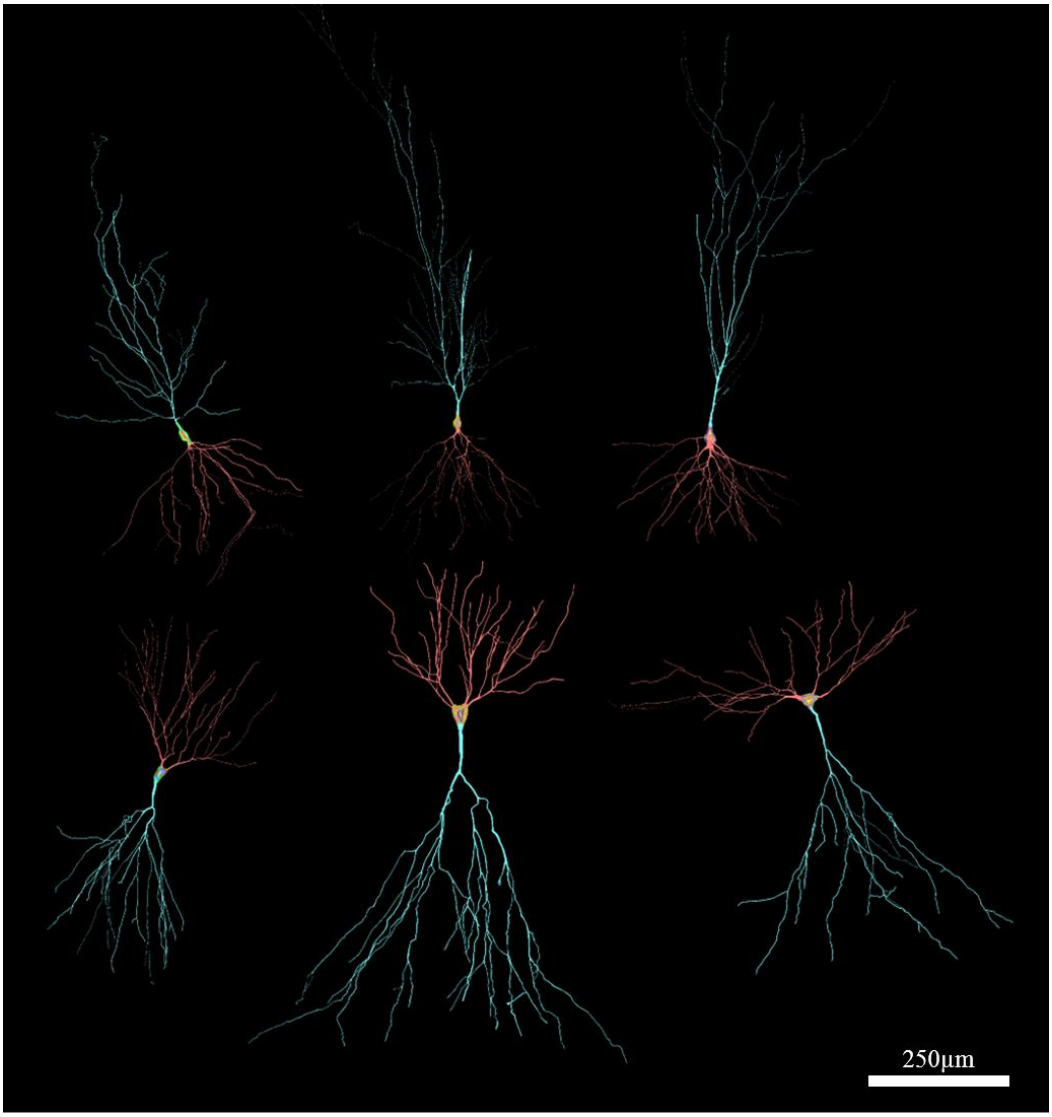

Figure 6- Examples of reconstructed hippocampal pyramidal cells. CA1 cells are across the top and CA3 cells are across the bottom. Reconstructions were done in Neurolucida 360.

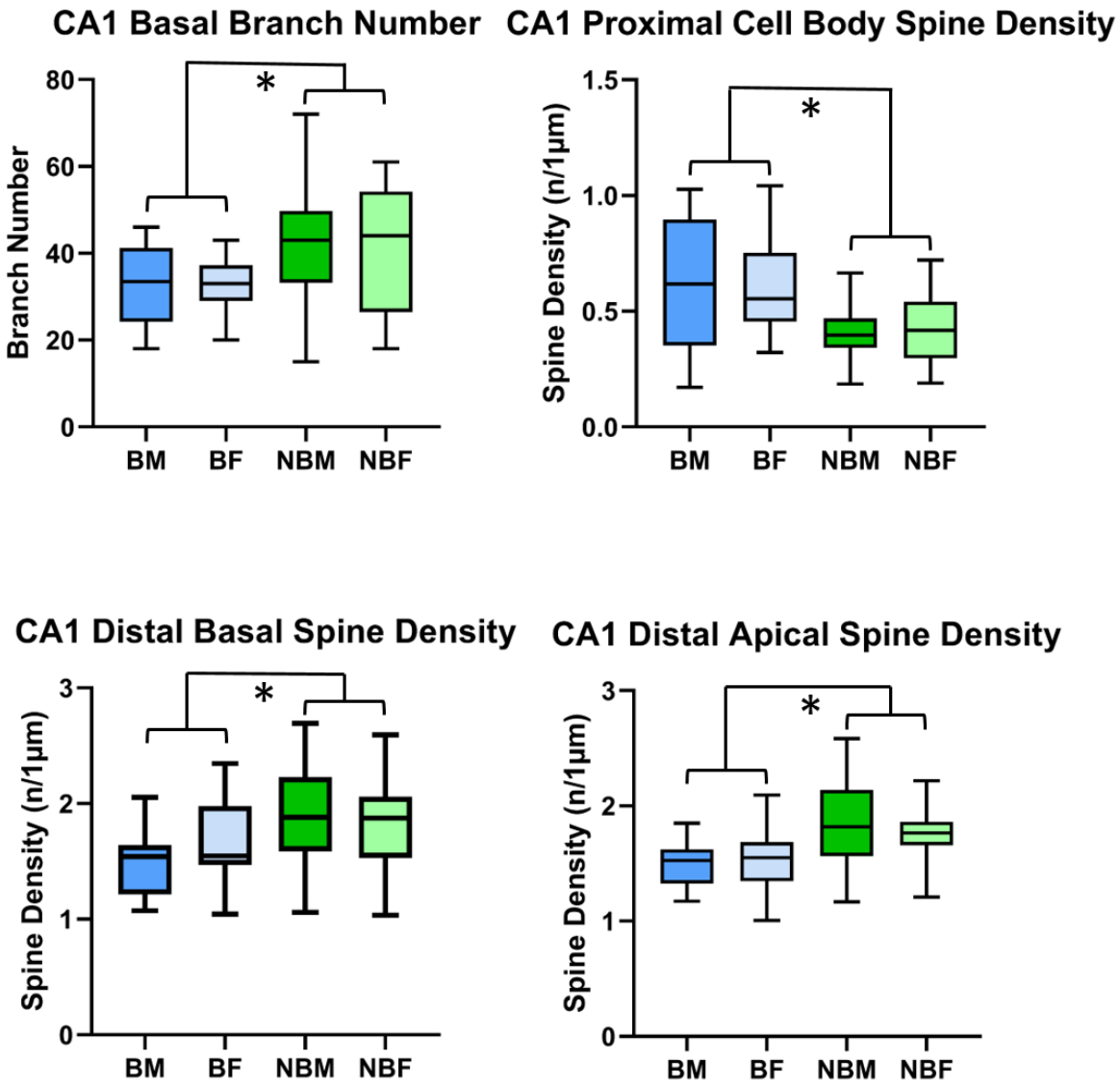

Figure 7- Box and whisker plots of the significant effects within CA1 morphology and spine density. Blue reflects animals sampled from the breeding season and green reflects animals sampled from the nonbreeding season. The darker shades represent males and the lighter shades represent females. The box displays the interquartile range of the data and the whiskers capture the minimum and maximum data points. * denotes a significant difference between the seasons. This shows an effect of season on the CA1 cells where the majority of significant measures, branch number, distal basal and apical spine densities, all increase in the nonbreeding animals. Proximal cell body spine density shows the opposite effect where the cell body spines are most dense in the breeding season.

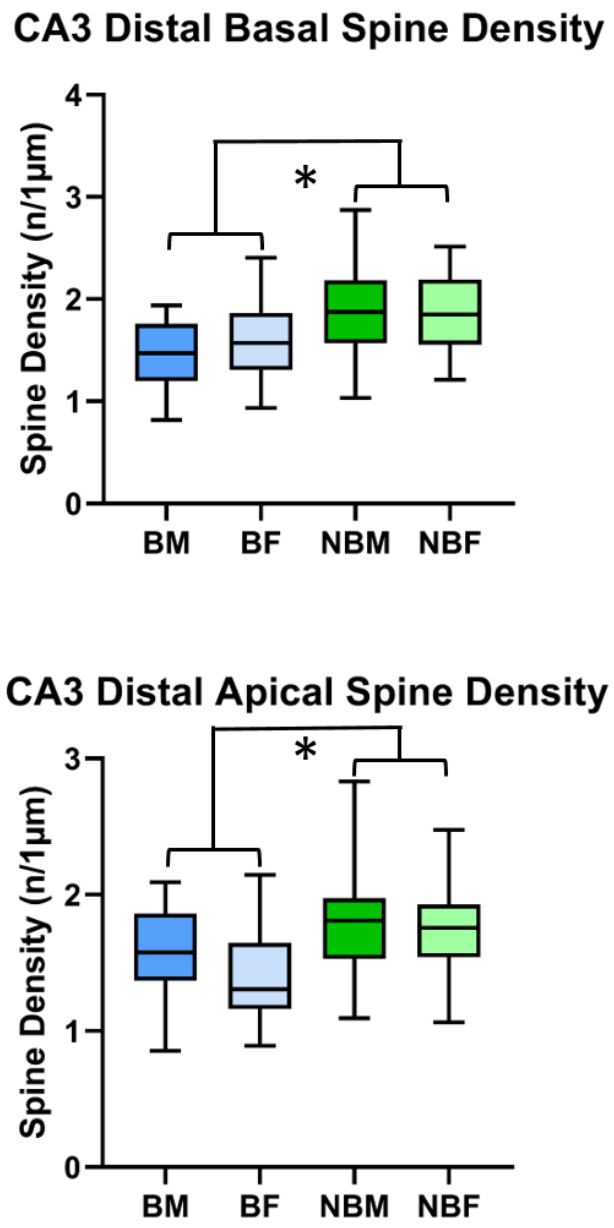

Figure 8- Box and whisker plots of the significant effects within CA3 spine density. Blue reflects animals sampled from the breeding season and green reflects animals sampled from the nonbreeding season. The darker shades represent males and the lighter shades represent females. The box displays the interquartile range of the data and the whiskers capture the minimum and maximum data points. * denotes a significant difference between the seasons. This shows an effect of season on the CA3 cells where spine density increases in both basal and apical dendrites in non breeding animals.

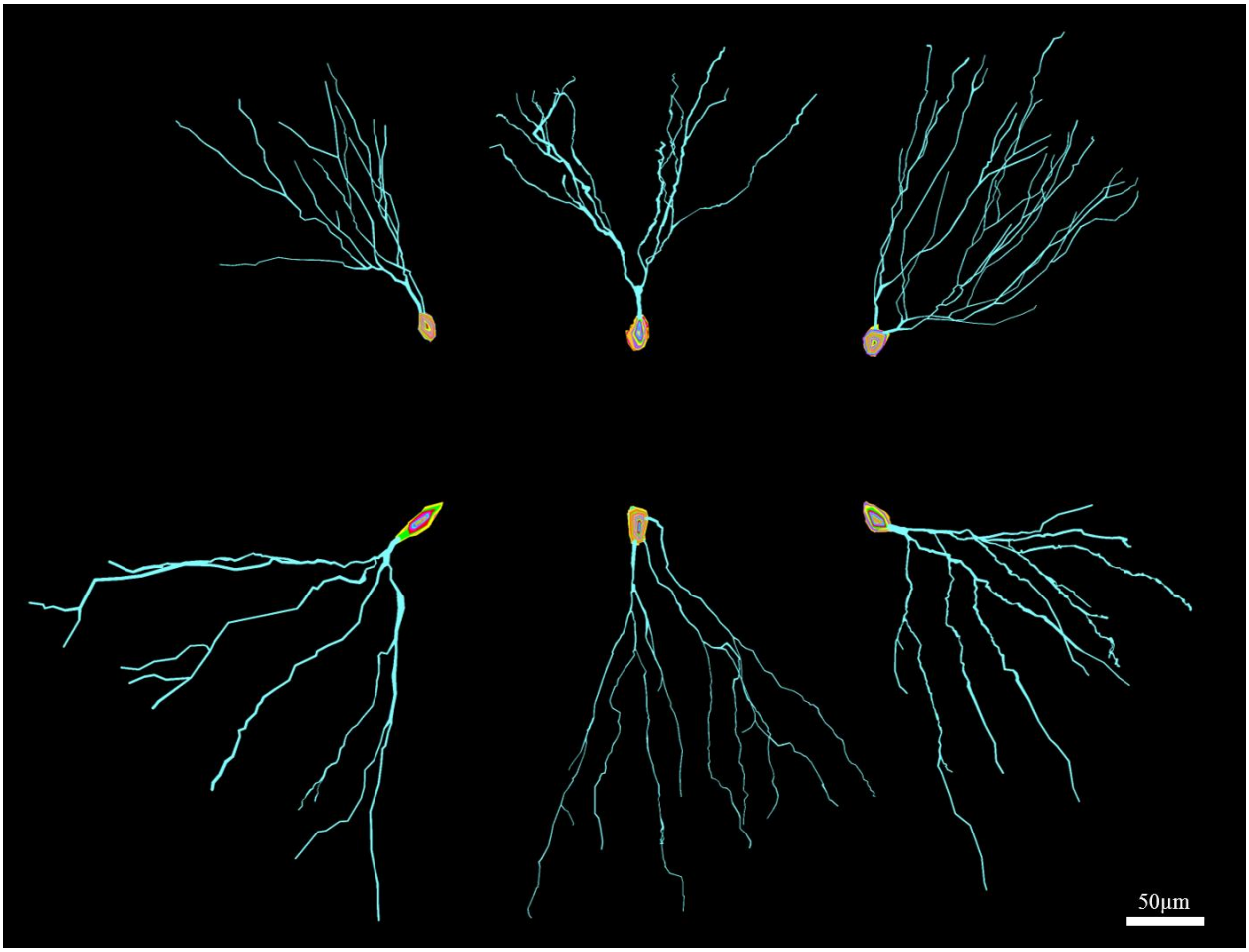

Figure 9- Examples of 6 reconstructed granule cells in the dentate gyrus.

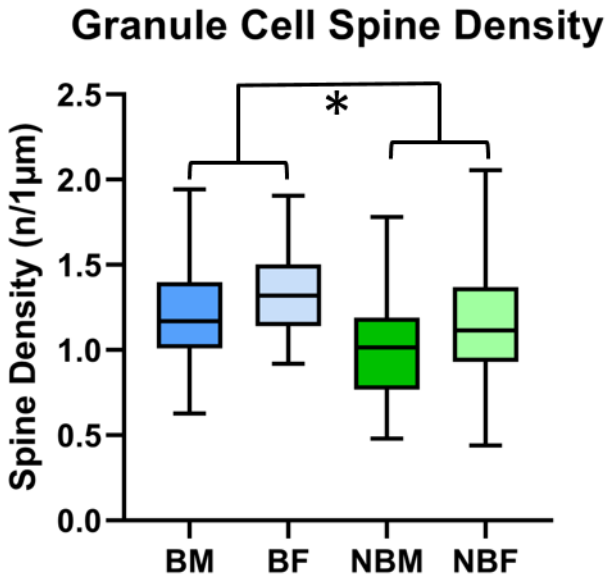

Figure 10- Box and whisker plots of the significant effects within dentate granule cell spine density. Blue reflects animals sampled from the breeding season and green reflects animals sampled from the nonbreeding season. The darker shades represent males and the lighter shades represent females. The box displays the interquartile range of the data and the whiskers capture the minimum and maximum data points. * denotes a significant difference between the seasons. This shows an effect of season on the granule cells where spine density is highest in the breeding season when compared to the nonbreeding season.

CHAPTER THREE: GENERAL DISCUSSION

Sex and seasonal differences in brain anatomy of songbirds provided some of the first strong evidence of neuroplasticity and the effects of sex hormones on the brain (Devoogd & Nottebohm, 1981; Nottebohm & Arnold, 1976; Nottebohm et al., 1987; Smith et al., 1997; Tramontin et al., 1998). Within the songbird vocal control system, individual brain regions undergo seasonal differences in males through different mechanisms. In HVC, the volumetric increase in the breeding season is coupled with an increase in neuron numbers (Nottebohm et al., 1987; Smith et al., 1997; Tramontin et al., 1998) However, in the robust nucleus of the arcopallium (RA) the neuronal density decreases when there is a breeding season volumetric expansion due to neurons increasing in size and dendritic trees becoming larger (Devoogd & Nottebohm, 1981). Thus, there are two different mechanisms by which individual brain regions can vary seasonally: increases in neuron numbers or increases in neuron size.

In some seasonally breeding small mammals, overall brain size and individual brain regions can also undergo seasonal and sex-season changes in size. The brains of some shrew species experience the largest seasonal variation in individual brain mass and architecture in any known mammal (Dehnel, 1949). As they move from summer to winter, they experience a decrease in overall brain mass by 20% or more, and a subsequent regrowth of 15% as they transition from winter to spring (Dehnel, 1949; Yaskin, 1994; Lázaro et al., 2018). This is the most extreme case of seasonal neuroplasticity in mammals, but it remains unclear to what extent these seasonal changes in brain region sizes occur as a result of increases in neuron numbers, neuron sizes or both.

In the hippocampus of other small, seasonally breeding mammals, it also remains unclear whether seasonal differences arise from changes in neuron sizes or numbers. Males of

polygamous voles and deer mice undergo an increase in hippocampus size during the breeding season (Jacobs et al., 1990), which is thought to support mate searching behaviour (Gaulin & Fitzgerald, 1989; Jacobs et al., 1990), but the extent to which this seasonal increase arises from the addition of granule cells to the dentate gyrus, expansion of cell bodies and/or dendritic trees or both mechanisms remains unclear.

Previous studies of Richardson's ground squirrels found that hippocampus was larger in nonbreeding males (Burger et al., 2013) and dentate gyrus volume, granule cell number, and overall brain size varied by season while brain volume and DCX cells varied by sex (Burger et al., 2014). In a similar fashion to other mammalian species studied to date, data on neuron sizes and morphology was lacking in Richardson's ground squirrel, leaving the question of whether neuron sizes are affected by sex and season unanswered. In my thesis, I not only addressed this knowledge gap, I also provided the first test of the effects of sex and season on hippocampal neuron morphology in a wild mammal species. Even comprehensive studies of seasonal neuroplasticity of the hippocampus in other mammals (Lavenex et al., 2000a; Lázaro et al., 2018) failed to examine neuron morphology or spine density in any hippocampal neurons. Although I predicted sex-season interaction effects on the morphology and spine density of granule and pyramidal cells, my analyses yielded only seasonal effects, the majority of which were on spine density. These results nevertheless indicate two important aspects of seasonal neuroplasticity in the mammalian hippocampus. First, pyramidal neuron morphology is highly variable within and across individuals. The cells varied dramatically in every measure, suggesting that the size and dendritic complexity of the cells can be diverse and dramatic. The difference makes sense in the dentate gyrus, a region that experiences adult hippocampal neurogenesis and can have young and old cells, however, why hippocampal pyramidal cells vary

so dramatically is unclear. Second, spine density appears to vary more consistently with respect to season. This suggests that the connectivity of the cells can change most readily to seasonal pressures in the mammalian brain.

Studying wild species is critical to understanding neuroplasticity in a natural context. The results from wild animals produce data that is more variable than that of lab animals because their individual life experiences vary. However, the study of wild species yields data that is more ecologically valid and possibly more akin to humans, where life experience and genetic background can also result in high variability in structural analyses of the brain (Amos, 1998; Kemper, Pasquier, & Drazen, 1978; Kotrschal, Deacon, Magurran, & Kolm, 2017; Krech, Rosenzweig, & Bennett, 1966). Captivity can affect brain anatomy, and if it is for long enough the changes become irreversible (O'Regan & Kitchener, 2005). Further, lab animals have been selected over time to produce breeds/strains that differ from the ancestral wild-type in brain anatomy (Kruska, 1988). Although there are clear logistic and economic benefits to experimentation with lab animals, comparable studies of wild animals are important for understanding fundamental aspects of brain anatomy and function. Using natural populations allows us to address questions, such as the sex and seasonal impacts on cellular morphology, at the level of individuals, species, and taxa. This allows for results to be expanded on and for wholistic conclusions to be drawn. Research using wild animals should be considered and encouraged, as it is performed on individuals that have lived their lives in the context of what they were selected for (Amrein, 2015).

Virtual Microscopy

A major innovation in my thesis was the use of virtual slides to complete neuron reconstructions and morphological analyses. Virtual microscopy is the method of digitizing

traditional glass slide specimens into image files (Lundin, 2004). A digital representation of a slide at a resolution of a high magnification objective is referred to as a virtual slide (Lundin, 2004). In contrast to live tracing of neurons with analytical software or *camera lucida*, the use of virtual slides allows for the image files to be stored and reviewed. Databases of virtual slides can be created, and the exact same samples can be viewed and annotated by other researchers in ways that traditional neuron tracing and live single user tracing software do not allow. This adheres to the recent demands of higher transparency in scientific research to encourage discourse and reproducibility (Baker & Penny, 2016). Virtual slides are created and stored at the same quality that they were analyzed, preventing degradation of the sample and stain or accidental destruction. Being able to store and pull up the exact same image files allows for comparisons to be made across observers, as I demonstrated in my thesis. The variation within individual tracers can also be calculated to ensure they are reliably making the same decisions while tracing. This is important as potential error from inconsistent tracing is usually not reported in studies of Golgi stain cellular reconstructions (cite some examples here)(Devoogd & Nottebohm, 1981; Kolb et al., 2003; Pyter et al., 2005; Workman et al., 2011). This is a cause for concern as we cannot verify that the tracings have been produced consistently or accurately. Lastly, depth of field and focus ability are not lost (Lundin, 2004) by creating Z-stacks, or series of images taken a set distance apart throughout an objectives depth of field. This creates virtual slides of very high resolution that can be focused through and encompass stained neurons to their full dendritic extent.

Although slide scanner technology offers many advantages to ‘traditional’ methods, it does have issues of its own. First, producing Z-stacks at high magnification is time consuming even for images of highly specific regions of relatively small size (1200µm by 1200µm). For

example, 40x Z-stacks of a hippocampal subregion can take 0.75-2 hours to create and 100x Z-stacks can take up most of a day (5-8 hours). Within this same length of time, an observer could trace 2.5-4 neurons or quantify spine density of 24-36 dendritic segments with live counting. Second, auto-feeding and automated focusing options can prove to be problematic when scanning Golgi stained sections. Section thickness, the nature of Golgi staining (i.e., irregular, 'messy') and need to acquire z-stacks throughout the section prevent the use of automated or semi-automated functions of slide scanners that could reduce the time spent scanning. Third, the resulting size of image files can be extremely large. A single Z-stack image file of a hippocampal subregion under a 40x objective with step sizes of 0.68 microns can be as high as 9 GB. Although the costs of memory have decreased significantly over time, making these files easy to store, the file size can exceed the computational capacity of many software packages used for image quantification. Despite these issues, virtual microscopy is slowly becoming an integral part of anatomical research and I would implore labs to consider adopting this methodology. The openness and transparency it provides will assist with the current reproducibility crisis in science (Baker & Penny, 2016), the storage can allow image files to be reused indefinitely without any loss to tissue quality, and easy comparisons between and within tracers will increase reliability in methodologies that require cellular tracing.

Future Directions

Despite the lack of support for many of my predictions, the line of research represented by my thesis can and should be continued in two distinct, but equally important, ways. First, initiating a field study of a ground squirrel population in which individuals are marked and observed over time. Subsequent sampling of brains from this population would allow the study of more subtle effects on brain anatomy in relation to individual life histories. I could adequately

consider stress, development, and specific age and how these different variables do or do not impact aspects of cellular morphology. At the same time, this observed population, if maintained over years, could be used to test for interannual variation and determine what annual factors cause seasonal effects in some years, but not others. The second line of research involves finding similar rodents and model species that exhibit different behaviours across sex and season. Richardson's ground squirrels are one of many ground dwelling squirrels worldwide, each of which have distinct seasonal and sex differences in behaviour (Anthony, 1953; Ferron, 1985; Michener & McLean, 1996; Murie, 1995; Owings, Borchert, & Virginia, 1977; Watton & Keenleyside, 1974; Weltzin et al., 2006). Through conducting similar comparisons of neuronal morphology in the hippocampus, and other brain regions, it might be possible to develop a more coherent theory of how and why some mammals exhibit seasonal neuroplasticity in brain anatomy and others do not. Doing so would aid significantly in our general understanding of mammalian brain plasticity and how it relates to natural behaviour in the environment.

REFERENCES

Dehnel, A. (1949). Studies on the genus Sorex L. *Ann Univ Mariae Curie-Skodowska, C 4*.

Amaral, D. G., Scharfman, H. E., & Lavenex, P. (2007). The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). *Progress in brain research*, 163, 3-790.

Amos, W. (1998). Factors affecting levels of genetic diversity in natural populations. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *353*(1366), 177–186.

Anthony, A. (1953). Seasonal reproductive cycle in the normal and experimentally treated male prairie dog, Cynomys ludovicianus. *Journal of Morphology*, *93*(2), 331–369.

Arendt, T., Stieler, J., Strijkstra, A. M., Hut, R. A., Rüdiger, J., Van der Zee, E. A., … Härtig, W. (2003). Reversible paired helical filament-like phosphorylation of tau is an adaptive process associated with neuronal plasticity in hibernating animals. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *23*(18), 6972–6981.

Arnau, J., Bendayan, R., Blanca, M. J., & Bono, R. (2013). The effect of skewness and kurtosis on the robustness of linear mixed models. *Behavior Research Methods*, *45*(3), 873–879.

Baker, M., & Penny, D. (2016). Is there a reproducibility crisis? *Nature*, *533*, 452–454.

Bartkowska, K., Djavadian, R. L., Taylor, J. R. E., & Turlejski, K. (2008). Generation recruitment and death of brain cells throughout the life cycle of Sorex shrews (Lipotyphla). *European Journal of Neuroscience*, *27*(7), 1710–1721.

Bates, D., Kliegl, R., Vasishth, S., & Baayen, H. (2015). Parsimonious mixed models. arXiv preprint arXiv:1506.04967.

Barton, K., & Barton, M. K. (2013). Package 'MuMIn'. *Version, 1*, 18.

Bedrosian, T. A., Fonken, L. K., Walton, J. C., Haim, A., & Nelson, R. J. (2011). Dim light at night provokes depression-like behaviors and reduces CA1 dendritic spine density in female hamsters. *Psychoneuroendocrinology*, *36*(7), 1062–1069.

Burger, D. K., Gulbrandsen, T., Saucier, D. M., & Iwaniuk, A. N. (2014). The effects of season and sex on dentate gyrus size and neurogenesis in a wild rodent, Richardson's ground squirrel (Urocitellus richardsonii). *Neuroscience*, *272*, 240–251.

Burger, D. K., Saucier, J. M., Iwaniuk, A. N., & Saucier, D. M. (2013). Seasonal and sex differences in the hippocampus of a wild rodent. *Behavioural Brain Research*, *236*(1), 131–138.

Cameron, H. A., & Schoenfeld, T. J. (2018). Behavioral and structural adaptations to stress. *Frontiers in Neuroendocrinology*, *49*, 106–113.

Cavegn, N., van Dijk, R. M., Menges, D., Brettschneider, H., Phalanndwa, M., Chimimba, C. T., ... & Amrein, I. (2013). Habitat-specific shaping of proliferation and neuronal differentiation in adult hippocampal neurogenesis of wild rodents. *Frontiers in*

neuroscience, 7, 59.

Chen, Y., Rex, C. S., Rice, C. J., Dubé, C. M., Gall, C. M., Lynch, G., & Baram, T. Z. (2010). Correlated memory defects and hippocampal dendritic spine loss after acute stress involve corticotropin-releasing hormone signaling. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(29), 13123–13128.

Czeh, B., Michaelis, T., Watanabe, T., Frahm, J., de Biurrun, G., van Kampen, M., … Fuchs, E. (2001). Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. *Proceedings of the National Academy of Sciences*, *98*(22), 12796–12801.

Danielson, N. B. B., Kaifosh, P., Zaremba, J. D. D., Lovett-Barron, M., Tsai, J., Denny, C. A. A., … Kheirbek, M. A. A. (2016). Distinct contribution of adult-born hippocampal granule cells to context encoding. *Neuron*, *90*(1), 101–112.

Dark, J., Spears, N., Whaling, C. S., Wade, G. N., Meyer, J. S., & Zucker, I. (1990). Long day lengths promote brain growth in meadow voles. *Developmental Brain Research*, *53*(2), 264–269.

Dawson, A., King, V. M., Bentley, G. E., & Ball, G. F. (2001). Photoperiodic control of seasonality in birds. *Journal of Biological Rhythms*, *16*(4), 365–380.

Delehanty, B., & Boonstra, R. (2012). The benefits of baseline glucocorticoid measurements: Maximal cortisol production under baseline conditions revealed in male Richardon's ground squirrels (Urocitellus richardsonii). *General and Comparative Endocrinology*, *178*(3), 470–476. 9

Deng, W., Saxe, M. D., Gallina, I. S., & Gage, F. H. (2009). Adult-born hippocampal dentate granule cells undergoing maturation modulate learning and memory in the brain. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *29*(43), 13532–13542.

Devoogd, T. J., & Nottebohm, F. (1981). Sex differences in dendritic morphology of a song control nucleus in the canary: A quantitative Golgi study. *Journal of Comparative Neurology*, *196*(2), 309–316.

Eadie, B. D., Redila, V. A., & Christie, B. R. (2005). Voluntary exercise alters the cytoarchitecture of the adult dentate gyrus by increasing cellular proliferation, dendritic complexity, and spine density. *Journal of Comparative Neurology*, *486*(1), 39–47.

Eddy, M. C., & Green, J. T. (2017). Running wheel exercise reduces renewal of extinguished instrumental behavior and alters medial prefrontal cortex neurons in adolescent, but not adult, rats. *Behavioral Neuroscience*, *131*(6), 460–469.

Felix-Ortiz, A. C., & Tye, K. M. (2014). Amygdala inputs to the ventral hippocampus bidirectionally modulate social behavior. *Journal of Neuroscience*, *34*(2), 586–595.

Ferron, J. (1985). Social behaviour of the golden-mantled ground-squirrel (Spermophilus lateralis). *Canadian Journal of Zoology*, *63*(11), 2529–2533.

Fonken, L. K., Kitsmiller, E., Smale, L., & Nelson, R. J. (2012). Dim nighttime Light Impairs

Cognition and Provokes Depressive-Like Responses in a Diurnal Rodent. *Journal of Biological Rhythms*, *27*(4), 319–327.

Galea, L. A. M., Kavaliers, M., & Ossenkopp, K. P. (1996). Sexually dimorphic spatial learning in meadow voles Microtus pennsylvanicus and deer mice Peromyscus maniculatus. *The Journal of Experimental Biology*, *199*(Pt 1), 195–200.

Galea, L.A.M., Ossenkopp, K.-P., & Kavaliers, M. (1994). Developmental changes in spatial learning in the Morris water-maze in young meadow voles, Microtus pennsylvanicus. *Behavioural Brain Research*, *60*(1), 43–50.

Galea, L. A. M., & McEwen, B. (1999). Sex and seasonal changes in the rate of cell proliferation in the dentate gyrus of adult wild meadow voles. *Neuroscience*, *89*(3), 955–964.

Galea, L. A. M, Perrot-Sinal, T. S., Kavaliers, M., & Ossenkopp, K.-P. (1999). Relations of hippocampal volume and dentate gyrus width to gonadal hormone levels in male and female meadow voles. *Brain Research*, *821*(2), 383–391.

Gaulin, S. J. C., & Fitzgerald, R. W. (1989). Sexual selection for spatial-learning ability. *Animal Behaviour*, *37*(PART 2), 322–331.

Gaulin, S. J. C., & FitzGerald, R. W. (1986). Sex Differences in Spatial Ability: An Evolutionary Hypothesis and Test. *The American Naturalist*, *127*(1), 74.

Gibb, R., & Kolb, B. (1998). A method for vibratome sectioning of Golgi-Cox stained whole rat brain. *Journal of Neuroscience Methods*, *79*(1), 1–4.

Glasper, E. R., Kozorovitskiy, Y., Pavlic, A., & Gould, E. (2011). Paternal experience suppresses adult neurogenesis without altering hippocampal function in Peromyscus californicus. *The Journal of Comparative Neurology*, *519*(11), 2271–2281.

Golding, N. L., Mickus, T. J., Katz, Y., Kath, W. L., & Spruston, N. (2005). Factors mediating powerful voltage attenuation along CA1 pyramidal neuron dendrites. *The Journal of Physiology*, *568*(1), 69–82.

Gould, E., Westlind-Danielsson, A., Frankfurt, M., & McEwen, B. S. (1990). Sex differences and thyroid hormone sensitivity of hippocampal pyramidal cells. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *10*(3), 996–1003.

Gould, E., Woolley, C. S., Frankfurt, M., & McEwen, B. S. (1990). Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *10*(4), 1286–1291.

Gould, E., Woolley, C. S., & McEwen, B. S. (1991). The hippocampal formation: Morphological changes induced by thyroid, gonadal and adrenal hormones. *Psychoneuroendocrinology*, *16*, 67–84.

Hamilton, K. S., King, A. P., Sengelaub, D. R., & West, M. J. (1997). A Brain of Her Own: A Neural Correlate of Song Assessment in a Female Songbird. *Neurobiology of Learning and Memory*, *68*(3), 325–332.

Hare, J. F., Ryan, C. P., Enright, C., Gardiner, L. E., Skyner, L. J., Berkvens, C. N., & Anderson,

W. G. (2014). Validation of a radioimmunoassay-based fecal corticosteroid assay for Richardson's ground squirrels Urocitellus richard-sonii and behavioural correlates of stress. *Current Zoology*, *60*(5), 591–601.

Hill, K. M., & DeVoogd, T. J. (1991). Altered daylength affects dendritic structure in a song-related brain region in red-winged blackbirds. *Behavioral and Neural Biology*, *56*(3), 240–250.

Hoshooley, J. S., Phillmore, L. S., Sherry, D. F., & MacDougall-Shackleton, S. A. (2007). Annual cycle of the black-capped chickadee: seasonality of food-storing and the hippocampus. *Brain, Behavior and Evolution*, *69*(3), 161–168.

Huang, L., DeVries, G. J., & Bittman, E. L. (1998). Photoperiod regulates neuronal bromodeoxyuridine labeling in the brain of a seasonally breeding mammal. *Journal of Neurobiology*, *36*(3), 410–420.

Irmgard A. (2015). Adult hippocampal neurogenesis in natural populations of mammals. *Cold Spring Harb Perspect Biol*, 7:a021295.

Jacobs, L. F., Gaulin, S. J., Sherry, D. F., & Hoffman, G. E. (1990). Evolution of spatial cognition: sex-specific patterns of spatial behavior predict hippocampal size. *Proceedings of the National Academy of Sciences*, *87*(16), 6349–6352.

Jacobs, L. F. (1996). Sexual selection and the brain. *Trends in Ecology & Evolution*, *11*(2), 82–86.

Jacobs, L. F., & Spencer, W. D. (1994). Natural space-use patterns and hippocampal size in kangaroo rats. *Brain, Behavior and Evolution*, *44*(3), 125–132.

Jung, M. W., Wiener, S. I., & McNaughton, B. L. (1994). Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat. *Journal of Neuroscience*, *14*(12), 7347-7356.

Kabelik, D., Weiss, S. L., & Moore, M. C. (2006). Steroid hormone mediation of limbic brain plasticity and aggression in free-living tree lizards, Urosaurus ornatus. *Hormones and Behavior*, *49*(5), 587–597.

Keeley, R. J., Burger, D. K., Saucier, D. M., & Iwaniuk, A. N. (2015). The size of non-hippocampal brain regions varies by season and sex in Richardson's ground squirrel. *Neuroscience*, *289*, 194–206.

Kemper, T. L., Pasquier, D. A., & Drazen, S. (1978). Effect of a low protein diet on the anatomical development of subcortical formations. *Brain Research Bulletin*, *3*(5), 443–450.

Kheirbek, M. A., & Hen, R. (2011). Dorsal vs ventral hippocampal neurogenesis: Implications for cognition and mood. *Neuropsychopharmacology*, *36*, 373–374.

Kolb, B., Gorny, G., Söderpalm, A. H. V, & Robinson, T. E. (2003). Environmental complexity has different effects on the structure of neurons in the prefrontal cortex versus the parietal cortex or nucleus accumbens. *Synapse (New York, N.Y.)*, *48*(3), 149–153.

Kotrschal, A., Deacon, A. E., Magurran, A. E., & Kolm, N. (2017). Predation pressure shapes

brain anatomy in the wild. *Evolutionary Ecology*, *31*(5), 619–633.

Krech, D., Rosenzweig, M. R., & Bennett, E. L. (1966). Environmental impoverishment, social isolation and changes in brain chemistry and anatomy. *Physiology and Behavior*, *1*(2), 99–104.

Kruska, D. (1988). Mammalian domestication and its effect on brain structure and behavior. In *Intelligence and evolutionary biology* (pp. 211-250). Springer, Berlin, Heidelberg.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: tests in linear mixed effects models. *Journal of Statistical Software*, *82*(13).

Lavenex, P., Steele, M. A., & Jacobs, L. F. (2000a). Sex differences, but no seasonal variations in the hippocampus of food-caching squirrels: a stereological study. *The Journal of Comparative Neurology 425*, 152-166

Lavenex, P., Steele, M. A., & Jacobs, L. F. (2000b). The seasonal pattern of cell proliferation and neuron number in the dentate gyrus of wild adult eastern grey squirrels. *European Journal of Neuroscience*, *12*(2), 643–648.

Lázaro, J., Hertel, M., Sherwood, C. C., Muturi, M., & Dechmann, D. K. N. (2018). Profound seasonal changes in brain size and architecture in the common shrew. *Brain Structure and Function*, *223*(6), 2823–2840.

Leranth, C., Hajszan, T., & MacLusky, N. J. (2004). Androgens increase spine synapse density in the CA1 hippocampal subfield of ovariectomized female rats. *Journal of Neuroscience*, *24*(2), 495–499.

Leranth, C., Petnehazy, O., & MacLusky, N. J. (2003). Gonadal hormones affect spine synaptic density in the CA1 hippocampal subfield of male rats. *Journal of Neuroscience*, *23*(5), 1588–1592.

Leuner, B, & Shors, T. J. (2013). Stress, anxiety, and dendritic spines: what are the connections? *Neuroscience*, *251*, 108–119.

Leuner, B., Mirescu, C., Noiman, L., & Gould, E. (2007). Maternal experience inhibits the production of immature neurons in the hippocampus during the postpartum period through elevations in adrenal steroids. *Hippocampus*, *17*(6), 434–442.

Lundin, M. (2004). Virtual microscopy. *Journal of Clinical Pathology*, *57*(12), 1250–1251.

Martínez-Téllez, R. I., Hernández-Torres, E., Gamboa, C., & Flores, G. (2009). Prenatal stress alters spine density and dendritic length of nucleus accumbens and hippocampus neurons in rat offspring. *Synapse*, *63*(9), 794–804.

McEwen, B. S., Coirini, H., Westlind-Danielsson, A., Frankfurt, M., Gould, E., Schumacher, M., & Woolley, C. (1991). Steroid hormones as mediators of neural plasticity. *The Journal of Steroid Biochemistry and Molecular Biology*, *39*(2), 223–232.

McEwen, B. S. (1999). Stress and hippocampal plasticity. *Annual review of neuroscience*, *22*(1), 105-122.

Megías, M., Emri, Z., Freund, T. ., & Gulyás, A. . (2001). Total number and distribution of

inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. *Neuroscience*, *102*(3), 527–540.

Michelsen, K. A., van den Hove, D. LA, Schmitz, C., Segers, O., Prickaerts, J., & Steinbusch, H. W. (2007). Prenatal stress and subsequent exposure to chronic mild stress influence dendritic spine density and morphology in the rat medial prefrontal cortex. *BMC Neuroscience*, *8*(1), 107.

Michener, G. R., & McLean, I. G. (1996). Reproductive behaviour and operational sex ratio in Richardson's ground squirrels. *Animal Behaviour*, *52*(1985), 743–758.

Michener, G. R. (1983). Spring emergence schedules and vernal behavior of Richardson's ground squirrels: why do males emerge from hibernation before females? *Behavioral Ecology and Sociobiology*, *14*(1), 29–38.

Michener, G. R., & Locklear, L. (1990). Differential Costs of Reproductive Effort for Male and Female Richardson's Ground Squirrels. *Ecology*, *71*(3), 855–868.

Michener, G. R. (1992). Sexual differences in over-winter torpor patterns of Richardson's ground squirrels in natural hibernacula. *Oecologia*, *89*(3), 397-406..

Michener, G. R. (1998). Sexual differences in reproductive effort of Richardson's ground squirrels. *Journal of Mammalogy*, *79*(1), 1-19.

Mitra, R., Jadhav, S., McEwen, B. S., Vyas, A., & Chattarji, S. (2005). Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(26), 9371–9376.

Murie, J. O. (1995). Mating behavior of Columbian ground squirrels. I. Multiple mating by females and multiple paternity. *Canadian Journal of Zoology*, *73*(10), 1819–1826.

Nottebohm, F, & Arnold, A. (1976). Sexual dimorphism in vocal control areas of the songbird brain. *Science*, *194*(4261), 211–213.

Nottebohm, F., Nottebohm, M. E., Crane, L. A., & Wingfield, J. C. (1987). Seasonal changes in gonadal hormone levels of adult male canaries and their relation to song. *Behavioral and Neural Biology*, *47*(2), 197–211.

O'Brien, J. (1994). Voles. *The Handbook: Prevention and Control of Wildlife Damage*.

O'Regan, H. J., & Kitchener, A. C. (2005). The effects of captivity on the morphology of captive, domesticated and feral mammals. *Mammal Review*, *35*(3–4), 215–230.

Owings, D. H., Borchert, M., & Virginia, R. (1977). The behaviour of California ground squirrels. *Animal Behaviour*, *25*(PART 1), 221–230.

Pawluski, J. L., & Galea, L. A. M. (2007). Reproductive experience alters hippocampal neurogenesis during the postpartum period in the dam. *Neuroscience*, *149*(1), 53–67.

Perani, D., & Abutalebi, J. (2005). The neural basis of first and second language processing. *Current Opinion in Neurobiology*, *15*(2), 202–206.

Perez-Cruz, C., Simon, M., Czéh, B., Flügge, G., & Fuchs, E. (2009). Hemispheric differences in

basilar dendrites and spines of pyramidal neurons in the rat prelimbic cortex: activity- and stress-induced changes. *European Journal of Neuroscience*, *29*(4), 738–747.

Popov, V. I., & Bocharova, L. S. (1992). Hibernation-induced structural changes in synaptic contacts between mossy fibres and hippocampal pyramidal neurons. *Neuroscience*, *48*(1), 53–62.

Popov, V. I., Bocharova, L. S., & Bragin, A. G. (1992). Repeated changes of dendritic morphology in the hippocampus of ground squirrels in the course of hibernation. *Neuroscience*, *48*(1), 45–51.

Prendergast, B. J., Nelson, R. J., & Zucker, I. (2002). Mammalian seasonal rhythms: behavior and neuroendocrine substrates. *Hormones, Brain and Behavior*, *4*, 93–156.

Pyter, L. M., Reader, B. F., & Nelson, R. J. (2005). Short photoperiods impair spatial learning and alter hippocampal dendritic morphology in adult male white-footed mice (Peromyscus leucopus). *Journal of Neuroscience*, *25*(18), 4521–4526.

Raz, N., Lindenberger, U., Rodrigue, K. M., Kennedy, K. M., Head, D., Williamson, A., … Acker, J. D. (2005). Regional brain changes in aging healthy adults: General trends, individual differences and modifiers. *Cerebral Cortex*, *15*(11), 1676–1689.

Rosenzweig, M. R., Bennett, E. L., & Diamond, M. C. (1972). Brain changes in response to experience. *Scientific American*, *226*(2), 22–29.

Ryan, C. P., Anderson, W. G., Gardiner, L. E., & Hare, J. F. (2012). Stress-induced sex ratios in ground squirrels: Support for a mechanistic hypothesis. *Behavioral Ecology*, *23*(1), 160–167.

Schlinger, B. A., & Brenowitz, E. A. (2002). Neural and Hormonal Control of Birdsong. *Hormones, Brain and Behavior*, 799–839.

Sherry, D. F. (2006). Neuroecology. *Annual Review of Psychology*, *57*(1), 167–197.

Sherry, D. F., & Hoshooley, J. S. (2009). The seasonal hippocampus of food-storing birds. *Behavioural Processes*, *80*, 334–338.

Shors, T. J., Chua, C., & Falduto, J. (2001). Sex differences and opposite effects of stress on dendritic spine density in the male versus female hippocampus. *The Journal of Neuroscience*, *21*(16), 6292–6297.

Smith, G. T., Brenowitz, E. A., Beecher, M. D., & Wingfield, J. C. (1997). Seasonal Changes in Testosterone, Neural Attributes of Song Control Nuclei, and Song Structure in Wild Songbirds. *Journal of Neuroscience*, *17*(15), 6001–6010.

Soler, J. E., Robison, A. J., Núñez, A. A., & Yan, L. (2018). Light modulates hippocampal function and spatial learning in a diurnal rodent species: A study using male nile grass rat (*Arvicanthis niloticus*). *Hippocampus*, *28*(3), 189–200.

Spritzer, M. D., Meikle, D. B., & Solomon, N. G. (2005a). Female choice based on male spatial ability and aggressiveness among meadow voles. *Animal Behaviour*, *69*(5), 1121–1130.

Spritzer, M. D., Panning, A. W., Engelman, S. M., Prince, W. T., Casler, A. E., Georgakas, J. E.,

… Wagner, B. A. (2017). Seasonal and sex differences in cell proliferation, neurogenesis, and cell death within the dentate gyrus of adult wild-caught meadow voles. *Neuroscience*, *360*, 155–165.

Spruston, N. (2008). Pyramidal neurons: dendritic structure and synaptic integration. *Nature Reviews Neuroscience*, *9*(3), 206–221.

Stewart, M. G., Davies, H. A., Sandi, C., Kraev, I. V, Rogachevsky, V. V, Peddie, C. J., Popov, V. I. (2005). Stress suppresses and learning induces plasticity in CA3 of rat hippocampus: a three-dimensional ultrastructural study of thorny excrescences and their postsynaptic densities. *Neuroscience*, *131*(1), 43–54.

Stranahan, A. M., Khalil, D., & Gould, E. (2007). Running induces widespread structural alterations in the hippocampus and entorhinal cortex. *Hippocampus*, *17*(11), 1017–1022.

Stranahan, A. M., Lee, K., Martin, B., Maudsley, S., Golden, E., Cutler, R. G., & Mattson, M. P. (2009). Voluntary exercise and caloric restriction enhance hippocampal dendritic spine density and BDNF levels in diabetic mice. *Hippocampus*, *19*(10), 951–961.

Strijkstra, A. M., Hut, R. A., de Wilde, M. C., Stieler, J., & Van der Zee, E. A. (2003). Hippocampal synaptophysin immunoreactivity is reduced during natural hypothermia in ground squirrels. *Neuroscience Letters*, *344*(1), 29–32.

Stuart, G., & Spruston, N. (1998). Determinants of voltage attenuation in neocortical Pyramidal Neuron Dendrites. *Journal of Neuroscience*, *18*(10), 3501–3510.

Stuart, G., Spruston, N., Sakmann, B., & Häusser, M. (1997). Action potential initiation and backpropagation in neurons of the mammalian CNS. *Trends in Neurosciences*, *20*(3), 125–131.

Thompson, D. C. (1977). Reproductive behavior of the grey squirrel. *Canadian Journal of Zoology*, *55*(7), 1176–1184.

Tramontin, A. D., & Brenowitz, E. A. (2000). Seasonal plasticity in the adult brain. *Trends in Neurosciences*, *23*(6), 251–258.

Tramontin, A. D., Smith, G. T., Breuner, C. W., & Brenowitz, E. A. (1998). Seasonal plasticity and sexual dimorphism in the avian song control system: Stereological measurement of neuron density and number. *Journal of Comparative Neurology*, *396*(2), 186–192.

Trommald, M., Hulleberg, G., & Andersen, P. (1996). Long-term potentiation is associated with new excitatory spine synapses on rat dentate granule cells. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *3*(2–3), 218–228.

Vander Heiden, M. G., Cantley, L. C., & Thompson, C. B. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science (New York, N.Y.)*, *324*(5930), 1029–1033.

von der Ohe, C. G., Garner, C. C., Darian-Smith, C., & Heller, H. C. (2007). Synaptic protein dynamics in hibernation. *The Journal of Neuroscience*, *27*(1), 84–92.

Walton, J. C., Aubrecht, T. G., Weil, Z. M., Leuner, B., & Nelson, R. J. (2014). Photoperiodic

regulation of hippocampal neurogenesis in adult male white-footed mice (Peromyscus leucopus). *European Journal of Neuroscience*, *40*(4), 2674–2679.

Walton, J. C., Haim, A., Spieldenner, J. M., & Nelson, R. J. (2012). Photoperiod alters fear responses and basolateral amygdala neuronal spine density in white-footed mice (Peromyscus leucopus). *Behavioural Brain Research*, *233*(2), 345–350.

Watton, D. G., & Keenleyside, M. H. A. (1974). Social behaviour of the arctic ground s quirrel, Spermophil us und ula Tus. *Behaviour*, *50*(1–2), 77–99.

Weiler, E. (1992). Seasonal changes in adult mammalian brain weight. *Naturwissenschaften*, *79*(10), 474–476.

Weltzin, M. M., Zhao, H. W., Drew, K. L., & Bucci, D. J. (2006). Arousal from hibernation alters contextual learning and memory. *Behavioural Brain Research*, *167*(1), 128–133.

Woollett, K., & Maguire, E. A. (2011). Acquiring "the knowledge" of London's layout drives structural brain changes. *Current Biology*, *21*(24), 2109–2114.

Woolley, C. S., Gould, E., Frankfurt, M., & McEwen, B. S. (1990). Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *10*(12), 4035–4039.

Woolley, C. S., Gould, E., & McEwen, B. S. (1990). Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. *Brain research*, *531*(1-2), 225-231.

Woolley, C. S., & McEwen, B. S. (1993). Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. *Journal of Comparative Neurology*, *336*(2), 293-306..

Workman, J. L., Bowers, S. L., & Nelson, R. J. (2009). Enrichment and photoperiod interact to affect spatial learning and hippocampal dendritic morphology in white-footed mice (Peromyscus leucopus). *European Journal of Neuroscience*, *29*(1), 161–170.

Workman, J. L., Manny, N., Walton, J. C., & Nelson, R. J. (2011). Short day lengths alter stress and depressive-like responses, and hippocampal morphology in Siberian hamsters. *Hormones and Behavior*, *60*(5), 520–528.

Yan, W., Wilson, C. C., & Haring, J. H. (1997). Effects of neonatal serotonin depletion on the development of rat dentate granule cells. *Developmental Brain Research*, *98*(2), 177–184.

Zemla, R., & Basu, J. (2017, April 1). Hippocampal function in rodents. *Current Opinion in Neurobiology*, *43*, 187–197.