NEUROGENESIS IN THE DENTATE GYRUS OF AGE-MATCHED CALCIUM ION CHANNEL MUTANT MICE, LEANER AND TOTTERING

A Thesis

by

SARAH ELLEN WILLS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2006

Major Subject: Veterinary Anatomy

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Approved by:

Chair of Committee,	Louise C. Abbott
Committee Members,	Newell McArthur
	Ursula Winzer-Serhan
Head of Department,	Evelyn Tiffany-Castiglioni

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ABSTRACT

Neurogenesis in the Dentate Gyrus of Age-Matched Calcium Ion Channel Mutant Mice, Leaner and Tottering. (August 2006)

Sarah Ellen Wills, B.S., Radford University

Chair of Advisory Committee: Dr. Louise C. Abbott

Homozygous leaner and tottering mice have a mutation in the α1A subunit, which cause a decrease in calcium ion current through these channels leading to altered calcium homeostasis. These channels are highly expressed in several areas of the brain including the hippocampus. Neurogenesis in adults happens only in two specific areas: the dentate gyrus of the hippocampus and the subventricular zone of the lateral ventricles. Since P/Q-type channels are highly expressed in the hippocampus, we expect altered calcium homeostasis might affect neural proliferation in the mutant mice. The aims of this thesis were: (1) to determine if there was a decrease in neural proliferation in the dentate gyrus of adult leaner and tottering mice when compared to age-matched wild-type mice and (2) to determine if the new cells are neurons or glial cells and if there is a difference in proportion when compared to wild-type mice.

We used bromodeoxyuridine (BrdU) to label the new cells. We examined proliferation rates in 100-150 day old mice and in 42-50 day old mice. We double-labeled some newly formed cells using two different fluorescent tags to determine if the cells were neurons or glial cells. These studies showed that cell proliferation is indeed decreased in leaner and tottering mice at three months of age when compared to the wild-type mice. However, when we looked at mice that were 42-50 days old, we found a

significant increase in cell proliferation in leaner and tottering mice compared to age-matched control mice. When we looked at the proportion of double-labeled cells (neurons versus glia), there was no difference among genotypes for either age group. For both age-groups, there were about 90% new neurons and 20% new glial cells for the P100-150 mice and 85% new neurons and 10% new glial cells for the P42-50 mice. Since we do not see a change in proportion of double-labeled cells at either age group, it is probable that differentiation patterns are not being affected. Based on these observations, we predict that the altered calcium homeostasis that is probably occurring in the mutant mice is affecting cell proliferation, but not differentiation.

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NOMENCLATURE

α1A	Alpha 1A subunit
ANOVA	Analysis of variance
BrdU	Bromodeoxyuridine
Ca^{++}	Calcium ion
CNS	Central nervous system
DAB	Diaminobenzadine
DCX	Doublecortin
DNA	Deoxyribonucleic acid
EtOH	Ethanol
GFAP	Glial fibrillary acidic protein
HCl	Hydrochloric acid
IP	Intraperitoneally
LRS	Lactated ringer's solution
NeuN	Neuronal nuclei protein
P	Postnatal day
PBS	Phosphate buffered solution
SSRI	Selective serotonin reuptake inhibitor
VGCC	Voltage-gated calcium ion channel

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

Page

LIST OF TABLES

Page

I. INTRODUCTION AND REVIEW OF THE LITERATURE

Neurogenesis

Until recently, adult neurogenesis was just a myth. Most scientists believed that all mammals including humans were born with all of the neurons that they were going to have, and once those were gone, they were not replaced. Adult neural stem cells were first isolated from the adult rodent central nervous system (CNS) by Reynolds and Weiss (1992). In 1998, Eriksson et al., by using bromodeoxyuridine (BrdU), found that there were a few specific regions of the brain in all mammals he examined, where neurogenesis did occur throughout life including adulthood. These specific regions in the brain that continue to produce neurons include the subgranular zone of the dentate gyrus in the hippocampus (See Figure 1) and the subventricular zone of the lateral ventricles (Gage et al., 1998; Rietze et al., 2000; Ming and Song, 2005). Neurogenesis outside these two areas of the CNS appears to be very limited or even non-existent in the normal adult mammalian CNS. However, pathological insult or traumatic injury to the CNS may initiate adult neurogenesis in regions that do not typically exhibit new neuron formation (Antel et al., 2000; Abrous et al., 2005; Ming and Song, 2005).

This thesis follows the journal Neuron.

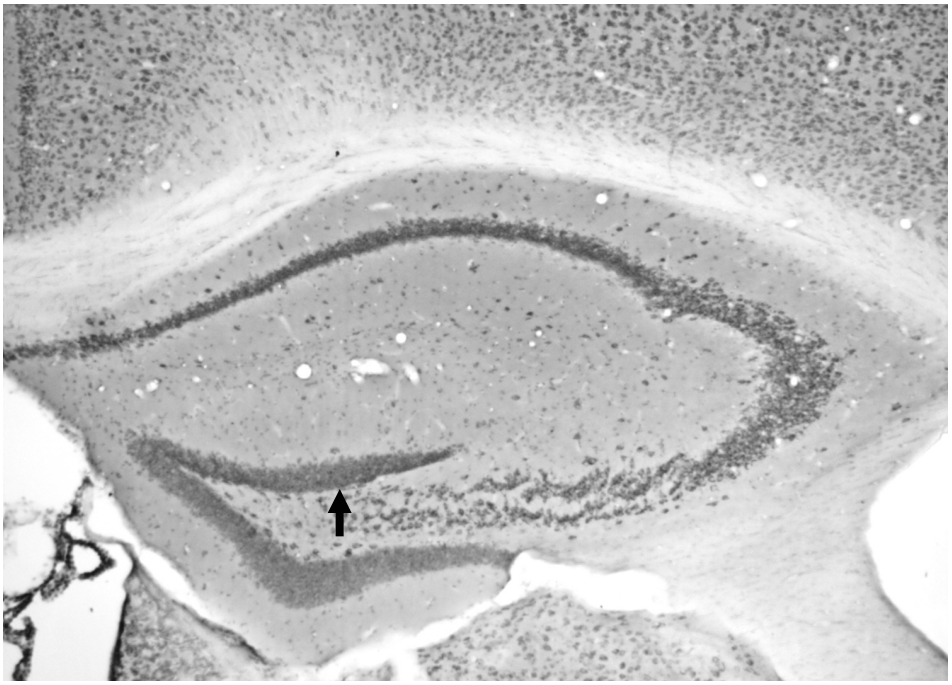

Figure 1. Histological coronal section of the right hippocampus including the subgranular zone of the dentate gyrus (Arrow indicates the subgranular zone).

One of the regions of significant adult neurogenesis, the dentate gyrus of the hippocampus, is of particular interest, due to the importance of the hippocampus in learning and memory as well as its contribution to the limbic system.

Hippocampus

The hippocampus is located in the temporal lobe and is part of the limbic system (Squire et al., 2004). The hippocampus begins in the caudal telencephalon and spans the cerebrum to about the rostral mesencephalon (most caudal part of the hippocampus). The middle and caudal parts of the pyriform lobe include the hippocampus. The hippocampus sends and receives information from the septal nuclei and the lateral mamillary nucleus (McArthur and Abbott, 2005). The hippocampus is connected ventrally and laterally with the olfactory cortex (Bear et al., 2001). Some other major sources of input to the hippocampus are the rhinal cortex, the parahippocampal cortex, and the fornix (Fell et al., 2006). There are two main sheets of neurons that are folded onto each other in the hippocampus. One of the sheets is referred to as the dentate gyrus. The other layer of neurons is called Ammon's horn and consists of four parts that are labeled CA1-4. Axons of dentate gyrus neurons synapse on neurons in the CA3 region of the hippocampus. These cells have axons that either leave the hippocampus via the fornix or synapse on the outer most region of the hippocampus, the CA1 region (Bear et al., 2001). The CA1 region of the hippocampus is important in long-term potentiation (LTP). LTP is a brief period of synaptic activity that causes a long-lasting increase in the strength of activated synapses (Thompson et al., 2005). This is important in the consolidation of recent events into long-term memories. It has been shown that treatments that block LTP also block the formation of long-term memories in the learning process (Morris et al., 2003).

The hippocampus is especially important in the formation of new memories and also plays a role in spatial memory formation and navigation(Shors et al., 2001; Wallenstein and Hasselmo, 1997). Lesions in the hippocampus have been found to impair not only memory formation, but the acquisition and consolidation of previous memories especially those associated with fear (Wallenstein and Vago, 2001). Rats with even partial damage to the CA1 region of the hippocampus show impaired spatial navigation and decreased learning and matching (Whishaw et al., 1994). The hippocampus has also been shown to play a large role in declarative memory (recalling everyday events and facts) and recognition memory formation and recollection (Eichenbaum, 2004; Cipolotti, et al., 2006; Zola et al., 2000).

It also has been noted that people with chronic depression ("unremitting depression") have decreased hippocampal size (Sheline et al., 2003; Videbech et al., 2004; Campbell et al., 2004). Smaller hippocampal volume is due to either one of two factors or a combination of both. Those two factors are: 1) decreased overall size of the cells or 2) a decreased number of cells, most probably due to a decline in adult neurogenesis.

In the adult hippocampus, constant reshaping occurs. There are new cells being formed and old ones are dying off (Phelps, 2006). However, if neurogenesis is decreased, but the amount of cell death remains the same, then the result will be a decrease in overall volume. Mice that were subjected to daily stress such as handling show a decrease in number of new neurons and depression-like behavioral symptoms, such as: a decrease in interaction or no interaction with other mice and/or a failure to

explore novel environments (Phelps, 2006; Santarelli et al., 2003). One would predict that depression could manifest as decreased locomotor activity in rodents. A common way to measure activity level in mice is using an open-field test (Crawley, 1999). This test measures a number of different variables such as: number of rearing movements and number of lines crossed in a given period of time. The mouse is placed in the chamber and the number of rearings and/or the number of lines crossed are counted in a given period of time.

Adult Neurogenesis

Most neurogenesis occurs before birth in humans. Once the closure of the neural tube has occurred, the rostral portion of the neural tube will differentiate and subdivide into three primary vesicles representing: forebrain (prosencephalon), midbrain (mesencephalon), and hindbrain (rhombencephalon). Two of the three primary vesicles are further subdivided into secondary vesicles. The forebrain is subdivided into the telencephalon and diencephalon. The midbrain continues to be the mesencephalon and the hindbrain subdivides into the metencephalon and the myelencephalon.

The Hox genes play an important role in establishing regional cell identity. The cerebral hemisphere arises from the telenecephalon region of the forebrain. Sonic Hegehog (SHH), produced in the ventral midline region, controls development of the basal ganglion and the medial and lateral ganglionic eminences. There are several major genes that code for proteins that act as different signaling factors involved with

development of the six major cortical layers (I-VI). Some of these proteins are: intergrin β1 in layer I, Reelin, VLDLR, ApoER2 in the cortical plate, and Cdk5/p35, Lis 1, and Dcx in the subplate and migrating zone. The first neurons of the cortex establish the preplate. Their axons along with ingrowing axons from the thalamus, establish the intermediate zone. The neurons of cortical layers II-VI establish the cortical plate, which splits into the marginal zone (future layer I) and the subplate. At the end of cortical development, the six cortical layers are seen overlying the white matter and the subplate nearly disappears. Neurons continue to be generated in the subventricular zone, which primarily migrate into the olfactory bulb. The neurons that are born in the ventricular zones follow pathways laid down by the radial glial cells. Later on neurons accumulate in the cortical plate and are ordered based on date of birth. Thus, older neurons are in the deep layers and newer neurons migrate through the deeper layers to be placed more superficially (See Figure 2, taken from Bear et al., 2001). This pathway continues to be used throughout life so that if you were to receive a transplant from someone younger, their cells would migrate through to be more superficial and vice versa for a transplant with older cells (Bear et al., 2001).

Neurons and glial cells are derived from either neuroblasts, glioblasts or from multipotent stem cells called neuroglioblasts (Udolph, et al., 1993, Bernardoni, et al., 1999; Roy et al., 2000; Namba et al., 2005). Neuroblasts from the midline and some neuroectodermal precursor cells exclusively produce neurons. Neuroblasts arise from the division of the neuroepithelial cells (See Figure 3, taken from Sadler, 2000). However, once the neuroblast is formed, they lose their ability to undergo further cell

division (Sadler, 2000). A few mesectodermal and lateral glioblasts give rise to only midline and lateral glial cells, respectively (Udolph, et al., 1993; Vincent et al., 1996). Other than those few cells, most of the neurons are generated from the multipotent progenitor stem cells, the neuroglioblasts. (Bernardoni, et al., 1999; Alvarez-Buylla et al., 1998).

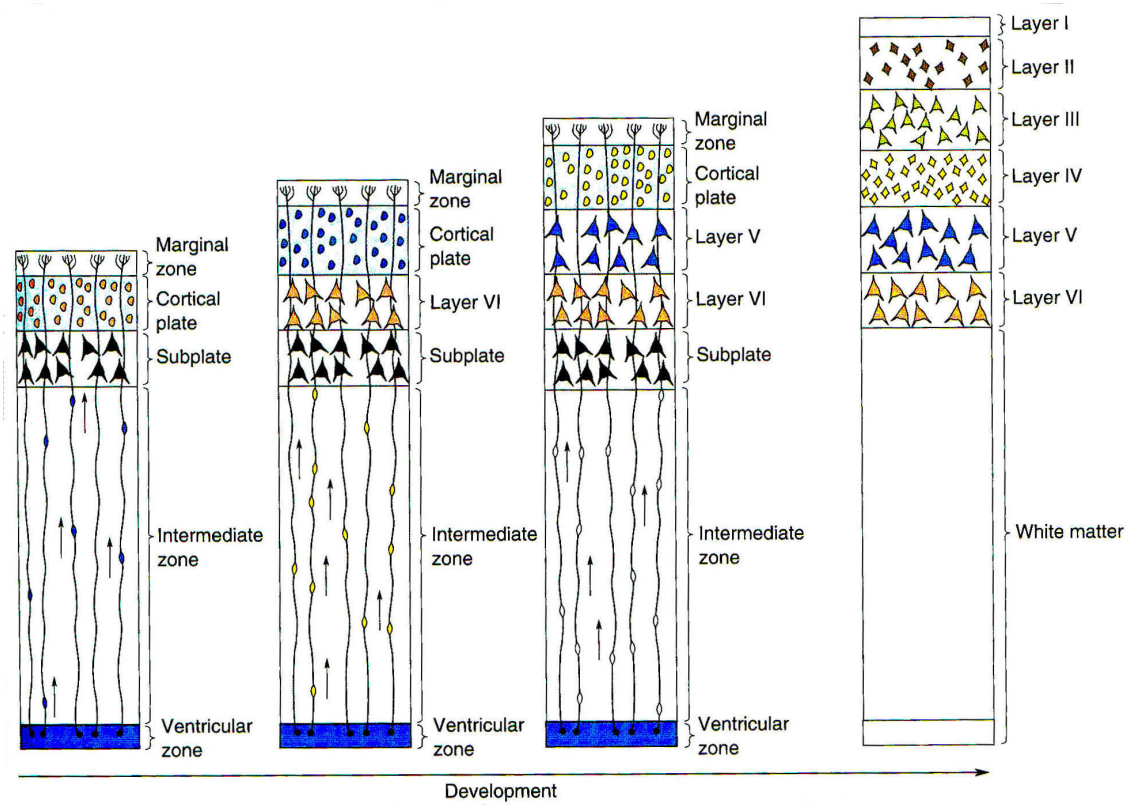

Figure 2. Schematic representation of cellular migration in the cortex (Bear et al., 2001).

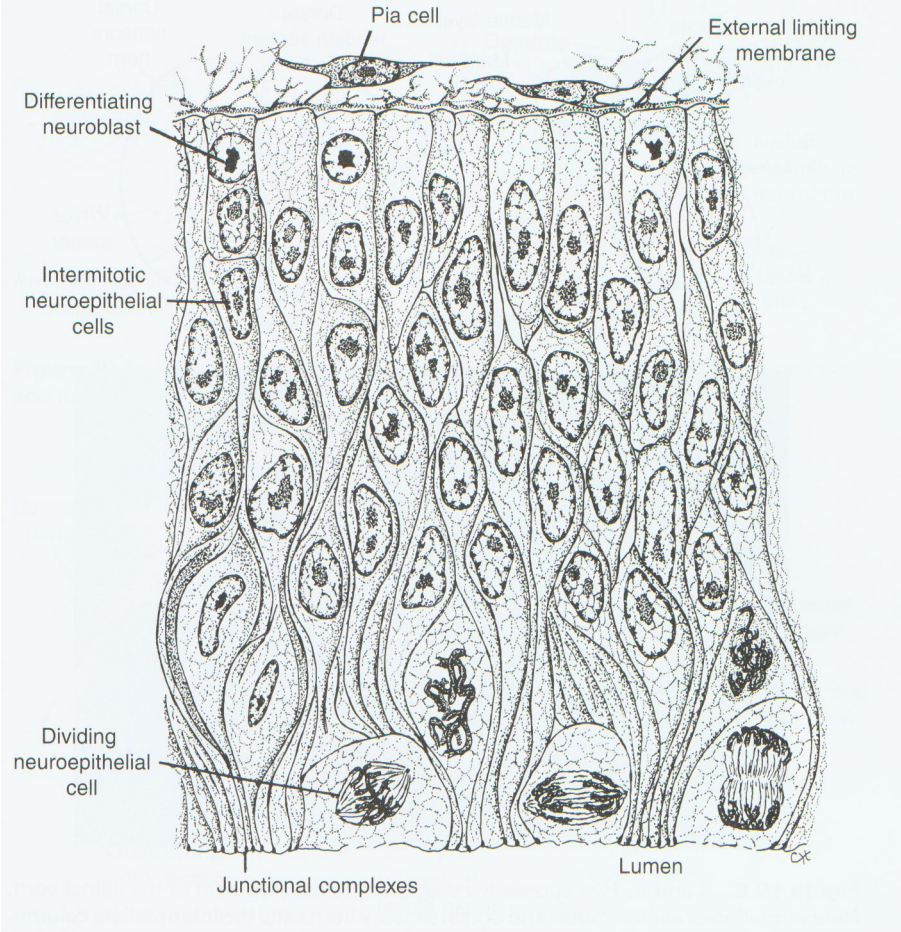

Figure 3. Schematic representation of neuronal differentiation (Sadler, 2000).

Although the majority of neurogenesis occurs prenatally, it has been shown that the development of the dentate gyrus happens over an extended period of time that varies among species (Guidi et al., 2005). In 2005, Guidi and his colleagues found that in guinea pigs postnatal neurogenesis peaks at postnatal day three (P3) and P5-6, and then again at P15-20. Starting at P30, a continuous drop in cell number occurs and continues into adulthood with the number of positive cells being eight times less.

Although the number of neurons being formed is decreased during adulthood, neurogenesis still occurs throughout life ((Cayre et al., 2002; Taupin, 2005).

A current hypothesis for the function of adult neurogenesis is that newly generated neurons in the adult CNS serve as mediators for structural plasticity (Ming and Song, 2005). Lack of new neurons being added to the dentate gyrus, for example, could lead to loss of normal hippocampal function leading to memory loss and/or the behavior changes associated with depression.

The Morris water maze is one of the most widespread and frequent tests used to monitor learning and memory (see Figure 4). The mouse has to find the platform each time hopefully by using a set of visual cues to learn and then remember where the platform was located. In a typical experimental protocol the first four days are training days to get the mouse to learn where the platform is located. By the fifth day, the control or normal mouse should have learned and be able to recall where the platform is using the same visual cues. Both rats and mice with hippocampal lesions or transgenic mice that express altered proteins that are associated with hippocampal neuron function do indeed show a decrease in learning and memory (Broadbent et al., 2006; Deacon and Rawlins, 2005; Sloan et al., 2006).

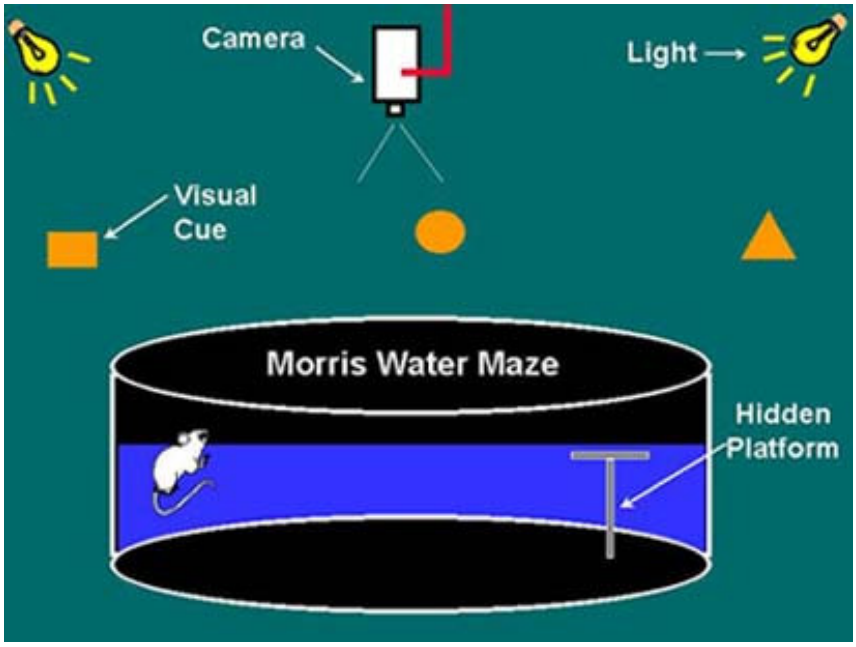

Figure 4. A schematic representation of the Morris water maze (www.mcg.edu/Core/Labs/sabc/Morriswatermaze.htm).

Leaner and Tottering Mice Phenotypic Expression

The focus of this investigation is on two mutations that have spontaneously occurred in mice. These mutant mice are called tottering mice and leaner mice. The tottering mutation and the leaner mutation both occur in the same gene that codes for a protein subunit of a calcium ion channel. Three distinct neurological disorders are exhibited by tottering and leaner mice, including: ataxia, absence epileptiform seizures, and paroxysmal dyskinesia (Lau et al., 1998; Lau et al., 2004). The range of severity of

all three disorders is dependant on the severity of the mutation, with leaner mice being the most affected and tottering mice being only mildly affected (See Table 1).

Table 1. Phenotypes of Cacna1a alleles

	Tottering	***Leaner***
Viability	Normal	Reduced
Age of onset of ataxia	P21-28	P8-10
Ataxia	+ (1)	+++ (1)
Paroxysmal dyskinesia	+ (2-4)	-
Absence seizures	+ (2-4)	+ (5)
Cerebellar cell death	- (7, 8)	+ (6)
Purkinje cell ectopic spines	+ (9)	+ (9)
Purkinje cell current density	60% of control (10)	40% of control (10, 11)

+++, Strong phenotype; ++, moderate phenotype; +, mild phenotype; -, no overt phenotype; ?, needs further study.

1, Meier and MacPike (1971); 2, Green and Sidman (1962); 3, Sidman et al. (1965); 4, Noebels and Sidman (1979); 5, Noebels (1984); 6, Levitt and Noebels (1981); 7, Nakane (1976); 8, Isaacs and Abbott (1995); 9, Rhyu et al. (1999a); 10, Rhyu et al. (1999b); 11, Wakamori et al. (1998); 22, Dove et al. (1998).

Adapted from Zwingman et al., 2001.

It has been shown that these two mutations affect neuronal intracellular calcium ion homeostasis (Dove et al., 2000). The severity of the phenotypic expression is most likely due to the difference in the specific mutation site with the leaner resulting in a truncation in the C-terminus of the protein and the tottering having a point mutation and thus an amino acid substitution. Because of these observations, one could speculate that survival of the neurons in either or both of these mutant mice could be compromised.

Leaner and Tottering Mutations

Leaner and tottering mice both carry a specific mutation in the gene coding for the α1A subunit (See Figure 5, taken from Catterall, 2000) of P/Q type voltage-activated calcium ion channels (Fletcher et al., 1996; Lau et al., 1998), which is the pore forming portion of P/Q-type calcium ion (Ca^{++}) channels. The P and Q voltage-gated calcium ion channel types constitute two of five high-threshold types of calcium ion channels (L, N, P, Q and R) that along with the low-threshold T-type channel form the major Ca^{++} entry pathways into neurons (Mori et al., 2000). The P/Q-type Ca^{++} channels are highly expressed in Purkinje cell somata and parallel fibers that emanate from granule cells in the cerebellum, but can also be found in other cell types throughout the CNS (Stea, et al., 1994, Westenbroek et al., 1995; Fletcher et al., 1996). According to Stea and his colleagues (1994) the α1A subunit was found to be expressed in the olfactory bulbs and throughout the cerebral cortex including the dentate gyrus region and the CA field of the hippocampus.

The tottering mutation is a point mutation where a cytosine base is exchanged for a thymine base. This leads to a non-conservative proline-to-leucine amino acid substitution (Fletcher et al., 1996). The leaner mutation occurs in the C-terminal region of the α1A gene and produces an out of frame splicing event (Mori et al., 2000), resulting in truncation of the C-terminus end of the protein. Both the leaner and tottering mutations cause a decrease in Ca^{++} currents in the neurons that express these channels. Calcium binding protein expression is also reduced in neurons in the leaner mouse

(Dove et al., 2000; Nahm et al., 2002). These observations show that there is an alteration in Ca^{++} homeostasis (Cicale et al., 2002), this can cause neurodegeneration When examining Morris water maze data obtained with tottering mice, we find that tottering mice do indeed show a decrease in learning and memory, which is probably due to decreased proliferation and increased cell death, that is occurring in the hippocampus. However, this test cannot be used to access learning and memory in the leaner mouse due to their severe ataxia, which prevents them from swimming.

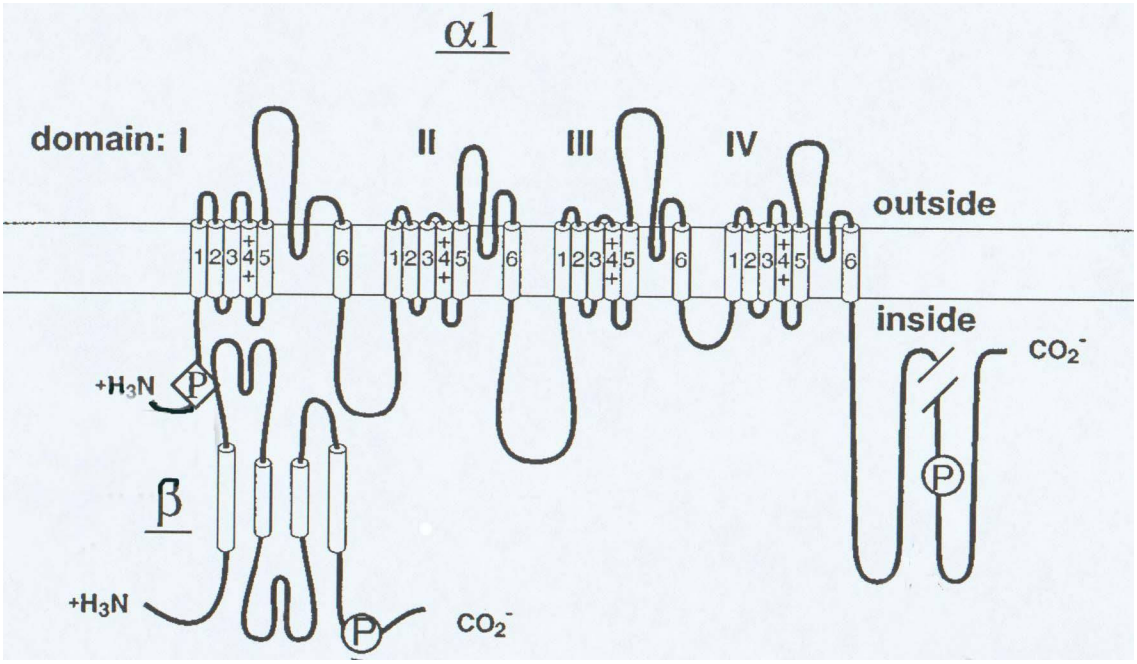

Figure 5. Schematic detailed representation of the structure of an α1 subunit of a voltage-gated calcium channel (adapted from Catterall, 2000).

Calcium Ion Channels

Calcium ions (Ca^{++}) have many functions in all cells. In neurons specifically, Ca^{++} regulate neurotransmitter release and excitability. Ca^{++} also plays a role in axon and dendrite plasticity and they also important for gene expression (Starr et al., 1991). Ca^{++} also plays a role in overall cell survival. When calcium ion homeostasis is altered cell death occurs. Under normal conditions calcium ion concentration is much higher outside of the cell than inside. Calcium ion channels allow controlled flow of Ca^{++} into and out of the cell as needed, thus maintaining homeostasis. This homeostasis is very highly regulated by calcium binding proteins and many other mechanisms. Basically, Ca^{++} enters the cell through calcium ion channels. These channels can be either voltage-gated or ligand-gated. There are two types of voltage-gated Ca^{++} channels (VGCC). The first type and most abundant are the high-voltage activated channels. These include the L, N, P/Q, and R-types. The other type is the low-voltage activated channel (T-type). Since we are interested in the P/Q-type channels, we will be focusing on the high-voltage activated type of channel.

Voltage-gated Ca^{++} channels are transmembrane proteins, thus they contain a pore forming unit (α1) which allows the Ca^{++} to pass through the neuronal membrane(Zhen et al., 2005). The ion channels are also composed of several other regulatory subunits including the α2, β, δ, and γ subunits (See Figure 6, from Randall et al., 1999). There are six known types of the α1-subunit. They are S, A, B, C, D, and E (Williams et al., 1992).

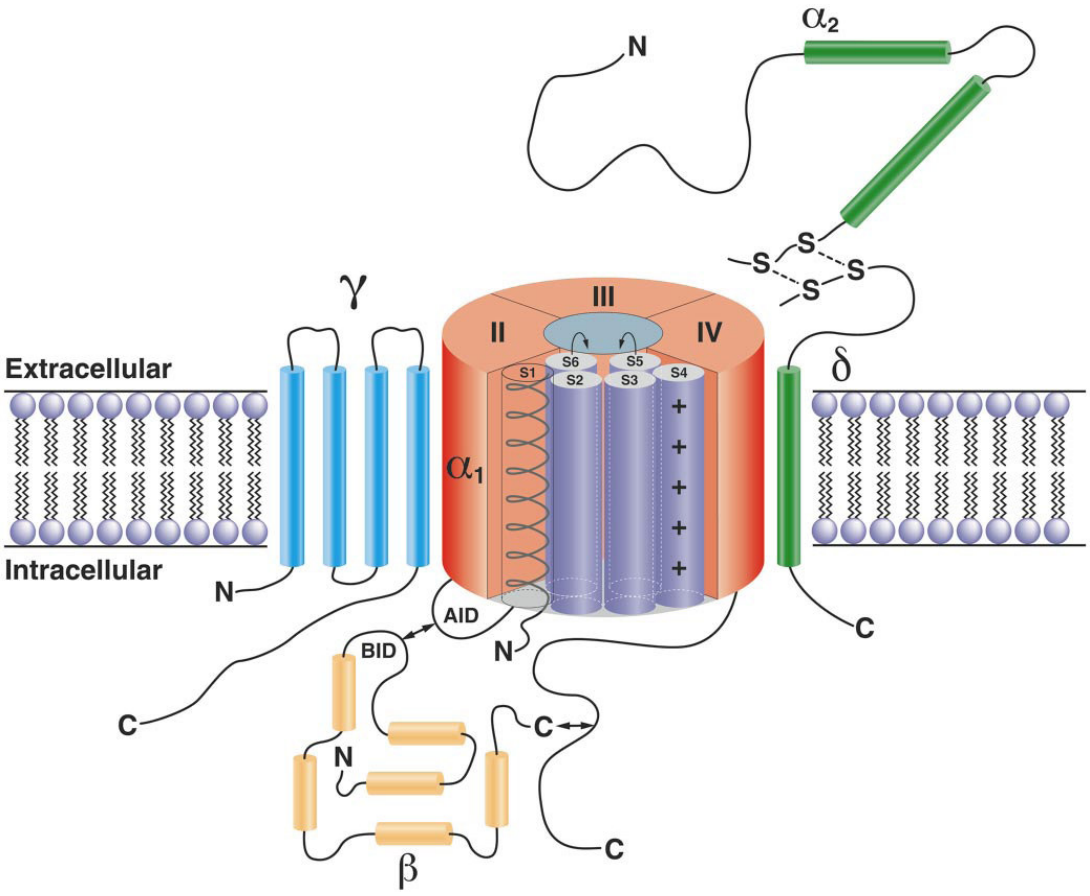

Figure 6. Schematic representation of a voltage-gated calcium ion channel (Randall et al., 1999).

Serotonin

Serotonin is a hydrophilic molecule (Rapport et al., 1948) that does not readily pass through the lipophilic blood-brain barrier. Therefore, serotonin must be produced in the brain itself. Most of the serotonin containing cell bodies are produced in the raphe nuclei, however; not all of the cell bodies in the raphe nuclei are serotonergic (Dahlstrom and Fuxe, 1964; Frazer and Hensler 1999). Serotonergic connections from the raphe nuclei extend to many regions in the brain including the hippocampus (See Figure 7, taken from Frazer and Hensler, 1999) and the forebrain in general (Hatzidimitriou et al., 1999).

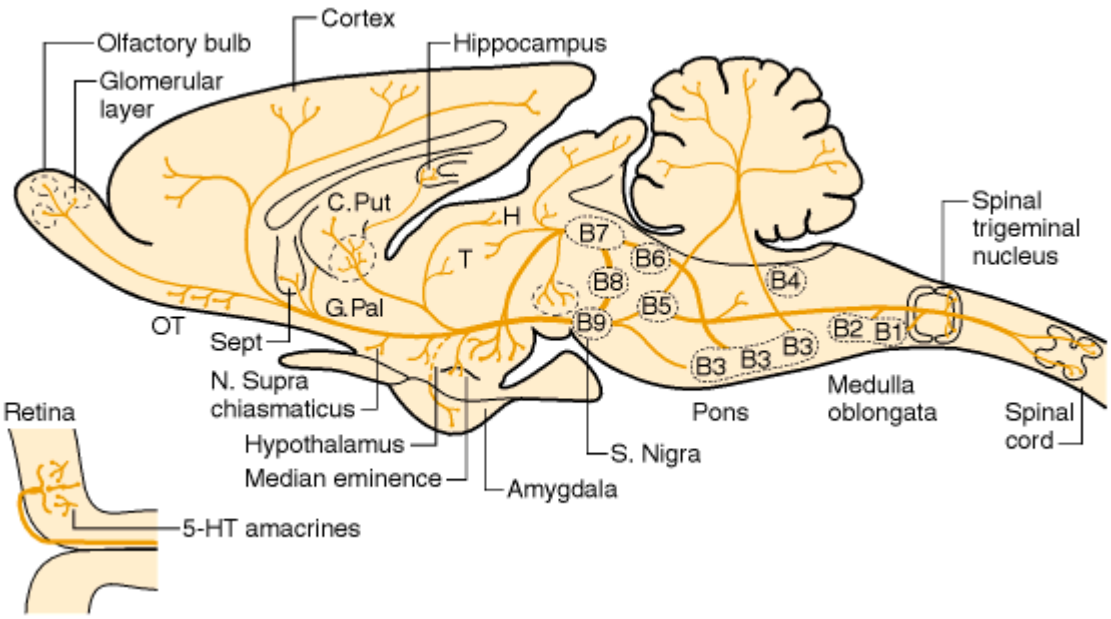

Figure 7. Schematic representation of the serotonergic connections found throughout the brain (Frazer and Hensler, 1999).

Serotonin is synthesized from tryptophan, a common amino acid found in many foods including: dietary protein, chocolate, oats, bananas, dried dates, milk, yogurt, cottage cheese, meat, fish, chicken, sesame, chickpeas, peanuts, and the well known turkey (Murry et al., 2003). Serotonin is stored in vesicles and is released with the serotonin binding proteins via a calcium dependant process. Calcium ion influx either with or without membrane depolarization has been reported to stimulated fusion of the vesicle to the plasma membrane (Frazer and Hensler, 1999). Serotonin is implicated to have a role in regulating many behaviors including rage, aggression, sleep disorder, and of course depression (Ansorge et al., 2004; Hassanain et al., 2003; Holtzheimer and Nemeroff, 2006; Malatynska et al., 2005). Depression has been linked to lower hippocampal volume as well presumable due to the lack of serotonin (Sheline et al., 2003; Videbech et al., 2004; Campbell et al., 2005; Saylam et al., 2006). Serotonin can be reduced either by lack of production, increased re-uptake, or lack of tranmission.

Rationale for This Study

We know from previous studies that altered Calcium ion homeostasis via impaired P/Q-type voltage-gated Ca^{++} channels causes neurodegeneration via cell death (Herrupetal., 1982, Lau et al., 2004). We also know that P/Q-type Ca^{++} channels are expressed throughout the rest of the CNS particularly in the dentate gyrus of the hippocampus (Stea et al., 1994). It is feasible that progenitor neurons that have altered

Ca^{++} homeostasis may show a decrease in proliferation. Finally, we know that altered calcium ion homeostasis will affect synaptic vesicle transport and therefore, neurotransmitter release (Ayata et al., 1999). In addition, since P/Q-type channels are expressed mainly pre-synaptically, we can hypothesize that a decrease in release of serotonin might add to decreased proliferation. We already know that people who are depressed have a decreased amount of serotonin in the CNS and also show decreased hippocampal size (Sheline et al., 2003; Videbech et al., 2004; Campbell et al., 2005). We also know that when given a selective serotonin reuptake inhibitor (SSRI) and a few other antidepressant medications, that neurogenesis increases in the dentate gyrus region of the hippocampus (Clark, et al., 2006; Encinas, et al., 2006; Saylam, et al., 2006; Grote, et al., 2005; Malberg and Schechter, 2005; Chen et al., 2000).

We have observed a decrease in forebrain weight in adult leaner and tottering mice as well (Abbott, unpublished data), therefore the general hypothesis for this study is that the decreased forebrain weight may be due, at least in part, to a decline in adult neurogenesis. We tested this hypothesis in three specific ways:

1. We determined that there are a decreased number of new cells in the dentate gyrus of the hippocampus of the leaner and tottering mouse as compared to age-matched wild type (control) mice over time.

2. We determined what percentage of the new cells that form in the dentate gyrus are neurons or the supportive glial cells.

3. We monitored the activity level of both leaner and tottering adult mice and age-matched wild-type mice before and after a two-week period of handling. We wanted to determine if there was a difference in response due to handling.

This study is important to help determine if there is a difference in adult neurogenesis due to altered calcium ion homeostasis because there is a correlation between decreased neuronal proliferation and depression. We could then use these mice as a model for depression with hope of developing more effective treatments for depression and several other neurodegenerative diseases.

II. MATERIALS AND METHODS

Animals

We used C57BL/6J:+/+ control (wild type), and mutant genotype, C57Bl/6J: *tg/tg* (homozygous tottering) and C57Bl/6J:tg^{la}/tg^{la} (homozygous leaner) mice. This particular mouse lineage was originally obtained from The Jackson Laboratory in Bar Harbor, ME and then bred and housed in the Laboratory Animal Research and Resource (LARR) facility on the Texas A&M University campus. All mice were kept on a constant 12 hour light/dark cycle with constant humidity (45-50%) at a constant temperature of 23-24°C. All mice were provided with commercial rodent chow and deionized water *ad libitum*. Due to the severity of the ataxia exhibited by homozygous leaner mice beginning at postnatal day 10-12 and the leaner dams' decreased lactation; on the day of birth, leaner pups were fostered to lactating Swiss White Webster dams. If leaner pups are not fostered, they usually die from starvation and/or dehydration by day 18. Both adults and pups were given moistened rodent chow as a supplement in a Petri dish for easy access. The moistened rodent chow was changed daily. Homozygous leaner mice were also housed in cages that allow the water bottles to be closer to the bedding since they do not rear very well and cannot hold that position long enough to intake an adequate amount of water. All mice were weaned between 30-40 days of age. Once the mice were weaned from their mothers, they were housed with littermates of the same gender. Male and female mice of all genotypes at ages postnatal day (P) 21, P42-50 and P100-150 and were used for this study.

Drugs

Sterile lactated Ringer's solution (LRS) was given intraperitoneally (IP) every day for 14 days at a dose of 0.01 ml/gm of body weight respectively. LRS is given mainly as extra supportive care for the leaner mice since they often times become dehydrated, however; it is given at the same dose to all mice to avoid a confounding variable. All animals received bromodeoxyuridine (BrdU) (Sigma, St. Louis, Missouri, USA) made in sterile LRS on days 13 and 14. BrdU is a pyrimidine analogue of thymidine that is selectively incorporated into DNA during the S-phase of the cell cycle. BrdU is a common marker for newly dividing cells (Ledergerber et al., e-publish ahead of print 2006, Mandairon et al., e-publish ahead of print 2006, Shimazu et al., 2006). BrdU was administered IP every eight hours on those two days at a dose of 100mg/kg of body weight. On the final day, day 15, one final BrdU injection was given IP one hour before perfusion.

Animals were anesthetized with a ketamine/xylazine (Vetco, St.Joseph, MO, USA) solution also given IP. Ketamine is a dissociative anesthetic and analgesic. Xylazine is another common anesthetic and is commonly used in conjunction with ketamine. Xylazine is a sedative and muscle relaxant and when combined with ketamine, muscle relaxation and visceral analgesia are improved, and emergence from anesthesia is smoother.

Histological Procedures

Perfusion

Once the mice were in a deep surgical plane of anesthesia, they were perfused intracardially with Tyrode's saline (pH 7.4) at room temperature, followed with 300-350 ml of cold 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.2-7.4). The bodies were wrapped in foil and placed in the cold room (4-12°C) overnight. The following day, the brains were removed from the skull and placed in a vial of 4% phosphate buffered paraformaldehyde at 4°C for 24 hours. The fixative was replaced with a 20% sucrose solution made in autoclaved phosphate-buffered saline (PBS) and left at 4°C until the brains sink to the bottom of the vial. The sucrose is important because it acts as a cryoprotectant. After the brains sink in the sucrose, we assume the sucrose has completely infiltrated the brain, and they can be frozen. The brains were rapidly frozen using powdered dry ice and stored at -70°C until sectioned.

Once the brains were frozen, they were sectioned coronally at a thickness of 18 µm, using a SLEE cryostat (SLEE Mainz, Germany). Serial sections were taken beginning at the rostral hippocampus. Four sections were placed on each gelatin coated glass microscope slide in consecutive order and organized into groups of 5 slides per set. Sections were collected until the caudal most portion of the hippocampus is reached (approximately 25 slides total were used per brain). The slides were stored in slide boxes wrapped in foil at -70°C until we were ready to stain them.

Immunohistochemistry

All sections were stained using standard immunohistochemistry with DAB labeling. Two slides for each mouse (male and female) were sorted in a cryo-chamber at –35°C and then thawed for 15 minutes. Slides were stained in two sets of 18 animals to avoid having too many slides at once. After the slides were dry, they were placed in a slide rack and submerged in 2N hydrochloric acid (HCl) at 37°C for 1 hour. The HCl step is necessary for anti-BrdU staining to break down the histone proteins to allow access to the BrdU incorporated in the DNA. Following the HCl step, slides were dipped 5-6 times in (0.01M) sodium borate in water to neutralize the HCl. Then the slides were put into phosphate-buffered saline (PBS). Washes with PBS were done three times for five minutes each. Next, the slides went into 0.3% Triton X-100 for 1 hour. Triton X-100 is a detergent that is used to permeabilize cellular membranes to allow the antibody better access to the intracellular components. Following the triton step, slides are again washed in PBS this time 2 times for 15 minutes each. Once all of the washes are complete, slides are placed back to back in plastic slide mailers called "antibody jackets" filled with 5% normal horse serum in autoclaved PBS for 1 hour. Then the slides are removed and place in clean antibody jackets that are filled with the primary antibody made in autoclaved PBS and 2% normal horse serum (Equitech, Kerrville, TX, USA). Our primary antibody is anti-BrdU (Sigma) made at a dilution of 1:5000. Slides were then incubated in the primary antibody in the refrigerator overnight for at least 12 hours. On day two, the slides were removed and rinsed with PBS three times for 10 minutes each. They were then placed into antibody jackets with the secondary antibody

which in our case was biotinylated horse anti-mouse (BHAM) (Vector Laboratories, Burlingame, CA, USA) made in autoclaved PBS at 1:400 for two hours at room temperature. The slides were then rinsed again three times 10 minutes each and then placed in 1:5000 streptavidin with horseradish peroxidase (HRP) (KLP, Gaithersburg, MD, USA) in autoclaved PBS at room temperature for two hours. The slides were once again rinsed, this time only two times in PBS for 10 minutes each. Next the slides were put into 0.05 M Tris HCl for 10 minutes. They were then placed in antibody jackets with diaminobenzidine (DAB) (Fisher Scientific, Fairlawn, NJ, USA) made in 0.05 M Tris with 30% H_2O_2, 5% nickel ammonium sulfate to increase the staining intensity. The DAB reaction usually occurred within five minutes of submersion. Slides were then removed from the DAB solution after five minutes and placed in 0.05 M Tris HCl for five minutes then transferred to PBS for another five minutes. Slides were then ready for dehydration and counterstaining.

Slides were dehydrated using ethanol (EtOH). Slides spent five minutes in each of the following alcohols in consecutive order, 70% and 80%. The slides were then dipped four times in 10% eosin solution made in 95% EtOH. The slides were then placed for five minutes each in 95% EtOH and two times in 100% EtOH to remove excess stain and further dehydration. The slides then went into xylene two times for five minutes each and were then coverslipped using permount mounting media (Fisher) and number 1 rectangular glass coverslips (Clay Adams, Parsippany, NJ, USA). Once the slides dried overnight, they were coded using a random number generator by someone in the lab not associated with the counting. The BrdU positive cells, which are new cells,

were counted along the subgranular layer of the dentate gyrus using a Nikon E400 research microscope for the light microscopy. The number of BrdU positive labeled nuclei per each dentate gyrus was recorded into Microsoft excel and analyzed using SPSS.

Controls

We also took samples of intestines from male and female mice and testes from male mice to use as positive controls for our immunohistochemistry. Testes and intestines were obtained, sectioned, and stained following the same protocol as the hippocampal sections. BrdU incorporates itself into the S-phase of DNA replication of all cells, not just neurons. Therefore, as expected we were able to find positively labeled BrdU cells in the body where there is a rapid turnover in cell proliferation. Two places that we know have a high constant proliferation rate are the testes and the small intestine. A negative control for immunohistochemistry that we used was to leave out the primary antibody to BrdU. When we leave out the primary antibody for BrdU then we expect that we will not see any positive cells labeled for BrdU.

Immunofluorescence

The standard immunohistochemistry procedure tells us how many new cells were present in the dentate gyrus of the hippocampus. However, it does not tell us if those new cells are neurons of if they are glial cells. In order to establish which type of cell was formed, we needed to double label the cells with anti-BrdU and either anti-NeuN (a

neuron specific protein) (Chemicon International, Temecula, CA, USA) or anti-GFAP (glial fibrillary acidic protein, a glial cell specific protein) (Sigma) with a fluorescent tag for each and then we used a fluorescence microscope (Zeiss Axioplan Microscope, Axioplan 2) to determine which cells were double labeled. NeuN labels the nuclear cytoplasm in neuronal cells (Mullen et al., 1992; Wolf, 1996) whereas GFAP labels the astrocyte cytoplasm.

The procedure was very similar to the standard anti-BrdU immunohistochemistry that we used. In fact, the first day was exactly the same as the standard first day except we used normal goat serum (Equitech) to replace the normal horse serum. On the second day, slides were once again rinsed in PBS three times for 10 minutes each. All steps after that must be done in the dark to maintain the integrity of the fluorescent tag. They were then placed in the first secondary which was the first fluorescent labeled tag (Alexa 568) (Invitrogen, Eugene, OR, USA) made in 1% bovine serum albumin (BSA) (Sigma) in PBS at a dilution of 1:1000. Slides were left for six hours at room temperature. The slides were then rinsed again three times for 10 minutes each time and then placed in 5% normal donkey serum in autoclaved PBS for one hour. Following that, slides were removed and placed in the second primary antibody (either anti-NeuN or anti-GFAP) at a dilution of 1:1000 in the refrigerator overnight. On day three, slides were removed and rinsed (same procedure) and then placed in second secondary fluorescent tag (Alexa 488) (Invitrogen) for six hours at room temperature at a dilution of 1:1000. The slides were washed again and then coverslipped using either prolong antifade or slow fade mounting media (both from Invitrogen). The slides were examined using a Zeiss

axioplan fluorescence microscope and images were captured using the Carl Zeiss axiovision 4.0 software. Each picture was coded and then opened individually. BrdU positive nuclei were counted along the sub-granular layer of the dentate gyrus. Once all of the BrdU positive nuclei were counted and recorded, the second picture was overlaid to show the double labeling. All double labeled cells were counted and recorded. All data were entered into an excel spread sheet.

Controls

For our controls for immunofluorescence, we again used the testes and intestine slides to make sure that our BrdU was still labeling the newly divided cells. We also ran a set of slides for each genotype with each single antibody with either the alexa-fluor 568 or 488 to make sure our antibodies and our tags were working correctly. The same staining protocol was used except we just used the single-label and all images were captured and saved in the same manner.

Hippocampal Length Measurements

Six–week-old mouse brains were removed and frozen using the same protocol as previously described. The brains were mounted and sectioned at a thickness of 25 micrometers using a SLEE cryostat. Sections were counted beginning at the most rostral hippocampus until the caudal most part. Sections were counted from male and female

mice of all genotypes. Data were entered into an excel sheet and analyzed using SPSS software.

Behavior

Data collection occurred on the two days that immediately precede initiation of the LRS injections and the two days immediately before the BrdU injections begin. Two days before initiation of LRS injections, the mice were weighed and run individually in the manual activity monitoring chamber. The manual activity monitoring chamber consists of a wooden box with gridlines on the bottom (See Figure 8).

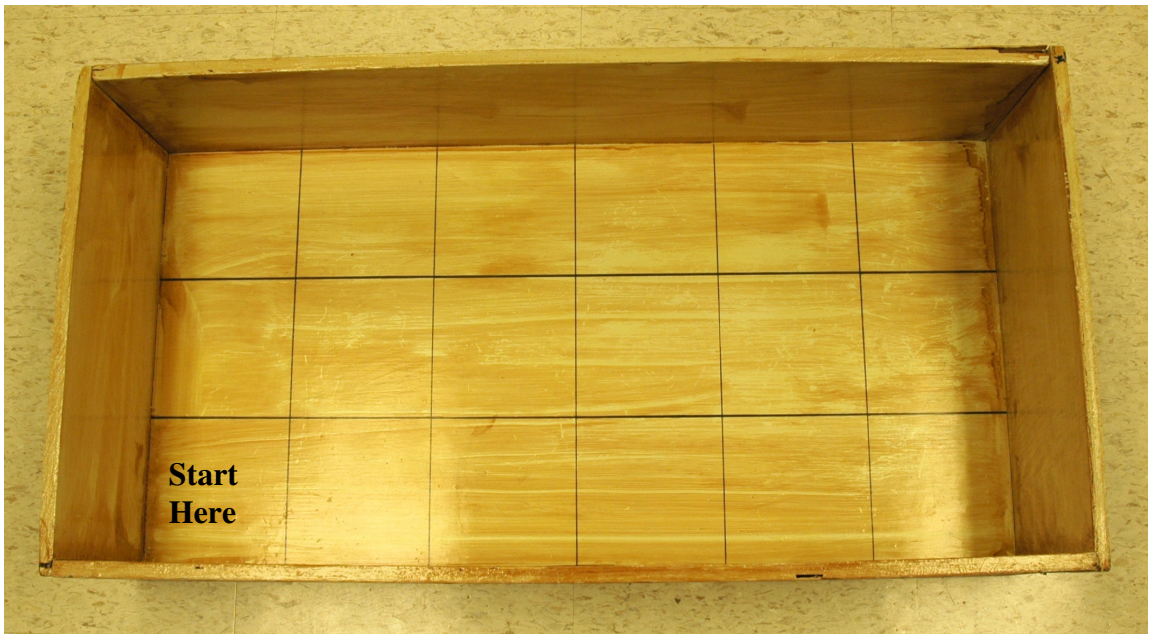

Figure 8. Manual activity monitoring chamber (Picture courtesy of Kerry Thuett).

Each mouse was tested for five minutes. The mouse was placed in the upper left-hand corner of the box and released. Each researcher had to maintain absolute silence. The number of lines crossed by each mouse was counted. Crossing a line was determined by both front feet crossing over a line. Each rearing event was also counted. A rearing event was classified each time both front feet came completely off the ground. The next rearing event will not be counted until both front feet touch the ground and then come off the ground again. These data were recorded manually and transferred to an excel spreadsheet. The mice were run for two consecutive days before any injections began. The injections were then given as previously described. Once all of the data were collected, it was transferred into an excel spreadsheet and analyzed using SPSS

software. Data were analyzed by doing a repeated measures test, followed by a Tukey's post hoc test for individual group differences.

Statistical Analysis

Cell Counts

Cell counts were done the same way for the three-month-old animals and the six-week-old mice. Cells were counted blindly and entered into an excel spreadsheet. The data were copied into SPSS 12.0 for Windows and a two-way ANOVA was run to test for gender difference and gender/genotype interactions. There was no difference or interaction for either age group so, the data were then combined and a one-way ANOVA for genotypic differences with a Tukey's post hoc test was run. The p-value was set at 0.05.

Double Fluorescence Cell Counts

All cells were counted the same way and entered into an excel spreadsheet. Numbers were then transferred into SPSS and analyzed using a simple one-way ANOVA to test for genotypic differences with a tukey's post hoc test. A p-value of 0.05 was used to determine statistical significance. Also, in excel, we obtained a percent of cells that were double labeled by taking the number of double labeled cells and dividing them by total number of positive cells and then multiplying by 100. We did this for each mouse and each genotype overall.

Hippocampal Length measurements

Sections were counted as previously described and then entered into SPSS and analyzed using a two-way ANOVA to test for gender and genotypic differences. There was a significant difference between genders, so two one-way ANOVA's were run looking for genotypic differences for each gender. P-value was set at 0.05.

Behavior

Behavior was only collected and analyzed for the three-month-old mice. Number of rearing events and number of lines crossed before and after handling were observed and recorded into an excel spreadsheet. The data for each activity were entered into SPSS and analyzed using a repeated measures test, first for gender, genotype, and before and after differences. For the number of rearing movements, there was no difference between genders. Therefore, the males and females were combined and a pairwise repeated measures test was run with out gender as a variable to look for before and after and genotypic differences. The p-value was set at 0.05 to establish significance.

Number of lines crossed was also analyzed using a repeated measures test to evaluate gender, genotypic, and before/after interactions. We found there were no differences between genders and no interaction, so we combined the genders and ran a pairwise repeated measures test. All p-values were set at 0.05.

III. RESULTS

Behavior

Mice that are subject to stress tend to exhibit behaviors that are classified as depression such as, lack of interaction and a decreased willingness to explore environments (Pham et al., 2003; Rosenbrock et al., 2005; Sloan et al., 2006; Warner-Schmidt, 2006). Daily handling could qualify as stressful to the mice and we could therefore expect their activity level to be decreased after consecutive days of handling. To test this possibility we assessed the activity exhibited by the three-month-old mice in an open field test. Specifically, we manually counted the number of rearing events and number of lines crossed in an open field chamber. We did not find a difference between genders for either activity test ($p=0.317$ for rearing and $p=0.914$ for number of lines crossed), so data from male and female mice were combined for both data sets. This allowed us to increase our sample size, which is important to decrease variability.

When the results were combined and analyzed for differences in number of rearings before and after handling, we found no statistical significant difference ($p=0.206$) (See Figure 9). These data suggest that there was not a handling effect. However, when we analyzed the number of lines crossed, we did find a small but significant difference in before and after results ($p=0.053$). We also observed a trend towards a genotypic before and after interaction ($p=0.067$), which we did not observe with the rearing activity (See Figure 10).

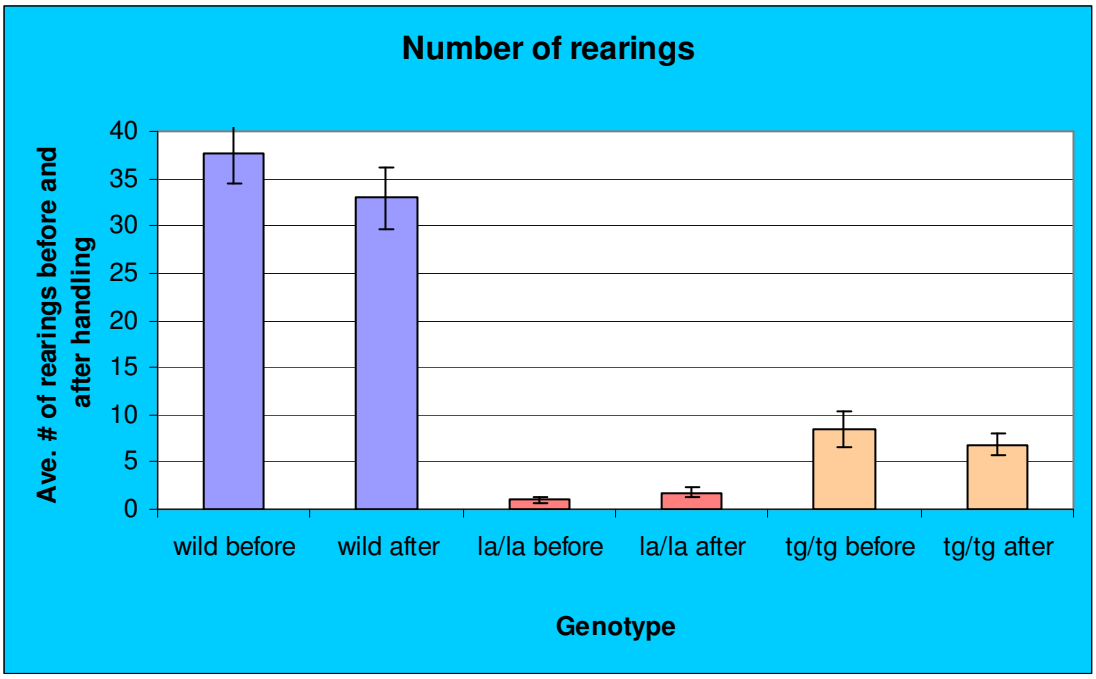

Figure 9. Rearing activity before and after handling. Handling occurred once a day for 14 days. (N=12 for wild-type and leaner mice and N=13 for tottering mice).

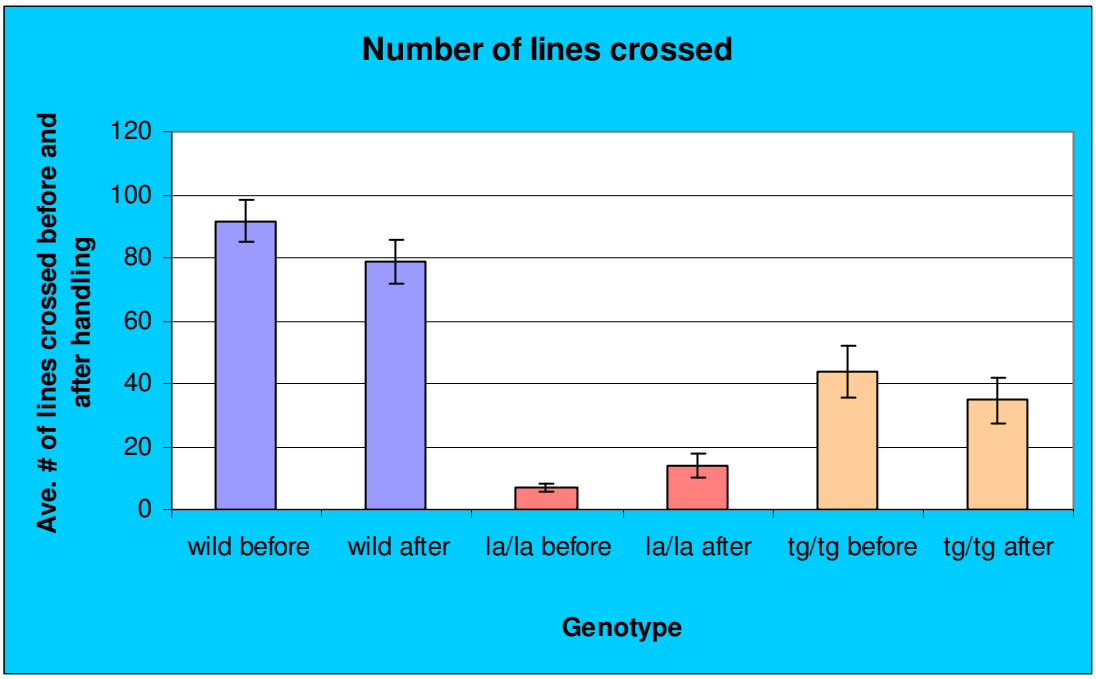

Figure 10. Number of lines crossed before and after handling. Handling occurred once a day for 14 days. (N=12 for wild-type and leaner mice and N=13 for tottering mice).

Adult Neurogenesis at P100-150

To examine whether proliferation in the subgranular zone of the hippocampal dentate gyrus was decreased in leaner and tottering mice compared to the wild-type mice, we tested six female leaner, five female tottering, and five female wild-type mice and 17 male mice (six in each genotype except tottering, which only had five mice) for

BrdU labeling. Injections, perfusion, immunostaining, and cell counts were done as previously described. Cell counts were analyzed using a two-way ANOVA with a Tukey's post hoc test to determine if there was a gender difference (See Table 2). There was no gender difference nor was a gender/genotype reaction observed, so we combined the data from males and females and ran a one-way ANOVA to determine if there was a genotypic difference in numbers of newly formed cells.

Table 2. Two-way ANOVA tests of between-subjects effects

Source	Mean Square	F	Sig.
gender	34.198	.714	.406
gender * genotype	42.554	.888	.423

The one-way ANOVA did reveal a statistically significant difference between wild-type and leaner mice with the leaner mice exhibiting fewer numbers of BrdU positive cells ($p=0.006$). There was also a statistically significant difference in the mean number of BrdU positive cells between tottering mice and wild-type mice ($p=0.002$). However, there was no difference between the leaner and tottering mice in number of BrdU positive cells, which we expected (See Figure 11).

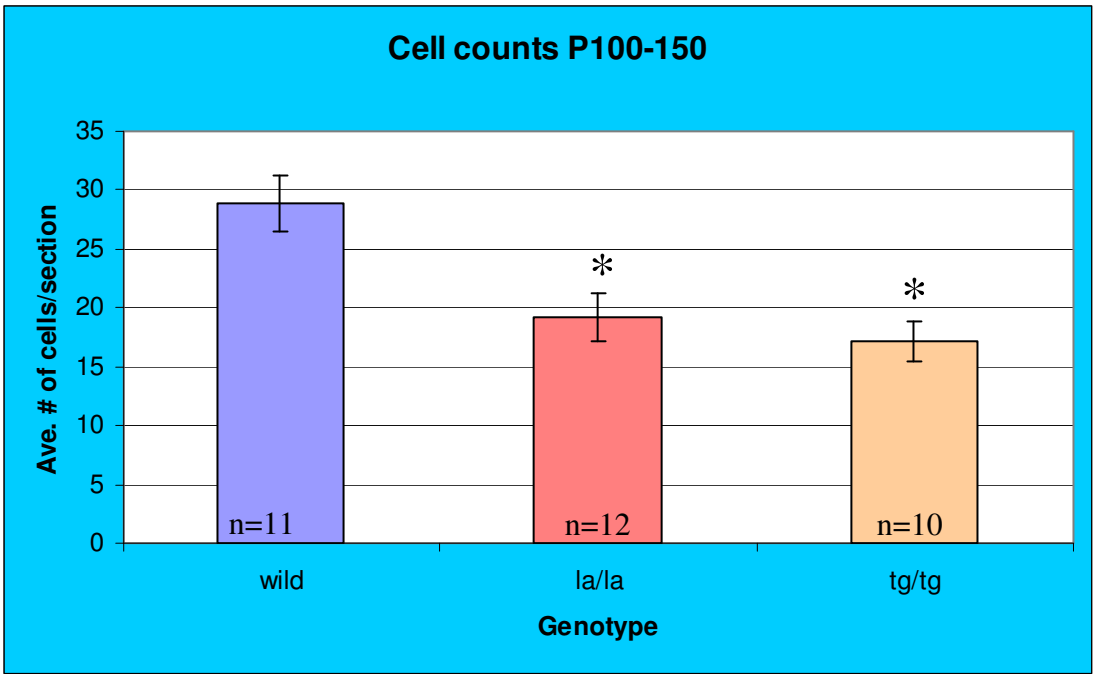

Figure 11. Three month old mice combined cell counts for average number of BrdU positive cells per section. Asterisks indicate significance.

The graph seen here in Figure 11 shows that there is a statistically significant difference in the average number of new cells forming in the dentate gyrus of the hippocampus in both mutant mouse genotypes as compared to age-matched wild type mice. For representative images of the BrdU positive cells see Figure 12.

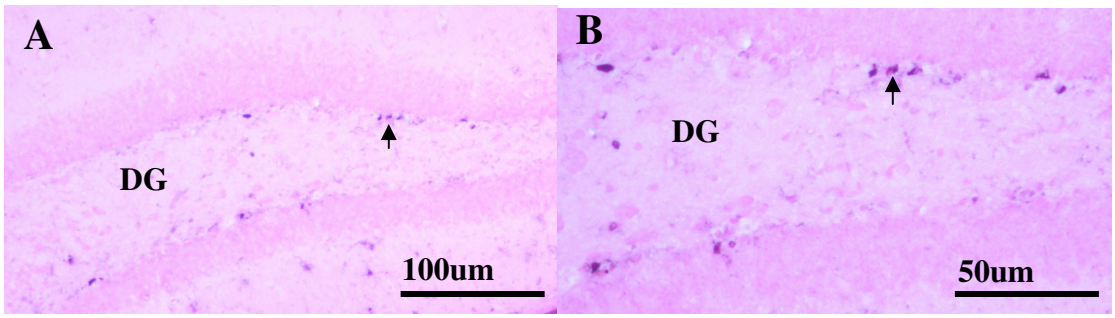

Figure 12. Representative images of BrdU positive-labeled cells in the dentate gyrus region of the hippocampus of P100-150 wild-type mice. Arrows indicate BrdU positive cells. DG-dentate gyrus. A-10x. B-20x.

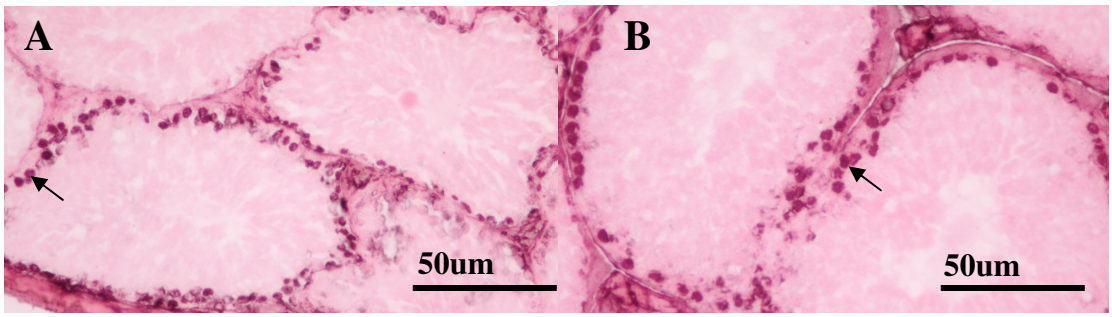

Figure 13. Representative images of BrdU positive-labeled cells in the testes. Arrows indicate BrdU positive cells. DG-dentate gyrus. A-P100-150 wild-type mouse at 20x. B-P42-50 leaner mouse at 20x.

Adult Neurogenesis at P42-50

Based on data published for the guinea pig, postnatal neurogenesis peaks at postnatal day three (P3) and P5-6, and then again at P15-20 and then starting at P30, a continuous drop in cell number occurs and continues into adulthood (Guidi, 2005). Also, in C57BL/6 mice, it has been shown that postnatal neurogenesis peaks at day 35 and then begins to decline starting at day 36 (Hayes et al., 2005).

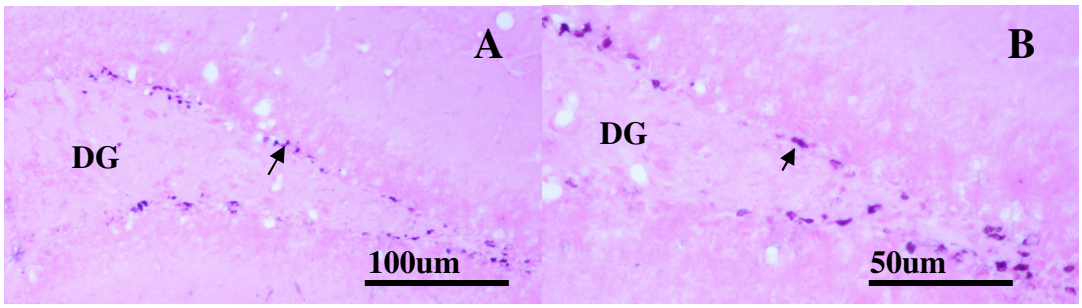

Figure 14. Representative images of BrdU positive-labeled cells in the dentate gyrus region of the hippocampus of P42-50 tottering mice. Arrows indicate BrdU positive cells. DG-dentate gyrus. A-10x. B-20x.

We also know that in the leaner mouse, ataxia associated with dysfunctional calcium ion channels that result in reduced calcium ion currents begins at around P10-12. Because of these observations and the fact that there is significant decreased proliferation in the adult mutant mice at P100-150, we decided to examine the rate of proliferation in the subgranular zone of the hippocampal dentate gyrus at P42-50. For representative images of the BrdU positive cells see Figure 14.

We ran a two way ANOVA on data collected at P42-50 and found once again that there was no significant difference between the male and female mice of any genotype ($p=0.660$), and could therefore combine their data. When we looked at genotype differences, we found that the tottering and leaner mice do have a significantly different number of new cells that are formed when compared to age-matched wild type mice. However, both tottering and leaner mice at P42-50 had a significantly higher number of cells ($p=0.0001$ for both) when compared to the wild type mice (See Figure 15). The numbers of proliferating cells observed in tottering and leaner mice were not statistically different from each other.

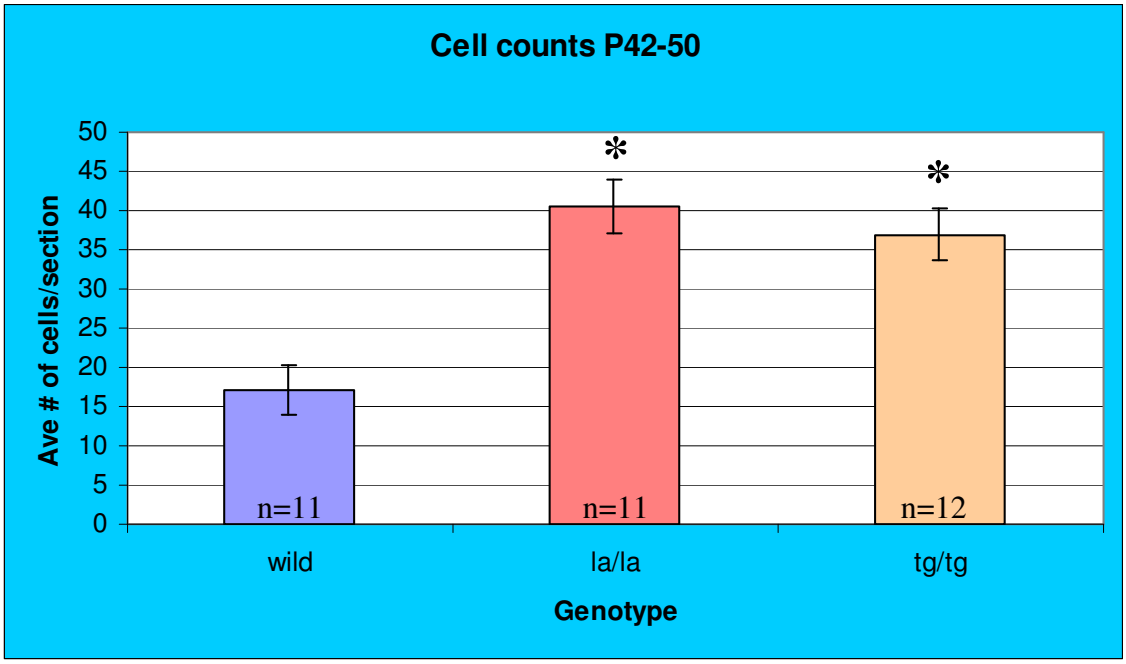

Figure 15. Six week old mice cell counts for average number of BrdU positive cells per section. Asterisks indicate a significant difference with the mutant mice having more cells than the wild-types.

Neurogenesis at P21

Members of the Abbott lab examined the rate of proliferation in leaner and wild type mice at P21 in order to determine whether the increased rate of proliferation was present in leaner mice at an even younger age when compared to age-matched wild type mice. These are data that were collected by Dana Tomlinson and counted by Dr. Abbott. Only P21 leaner and wild type mice were used in this experiment. We observed that the number of new cells that were labeled with BrdU in leaner mice at P21 was much lower when compared to age-matched P21 wild type mice (see Figure 16).

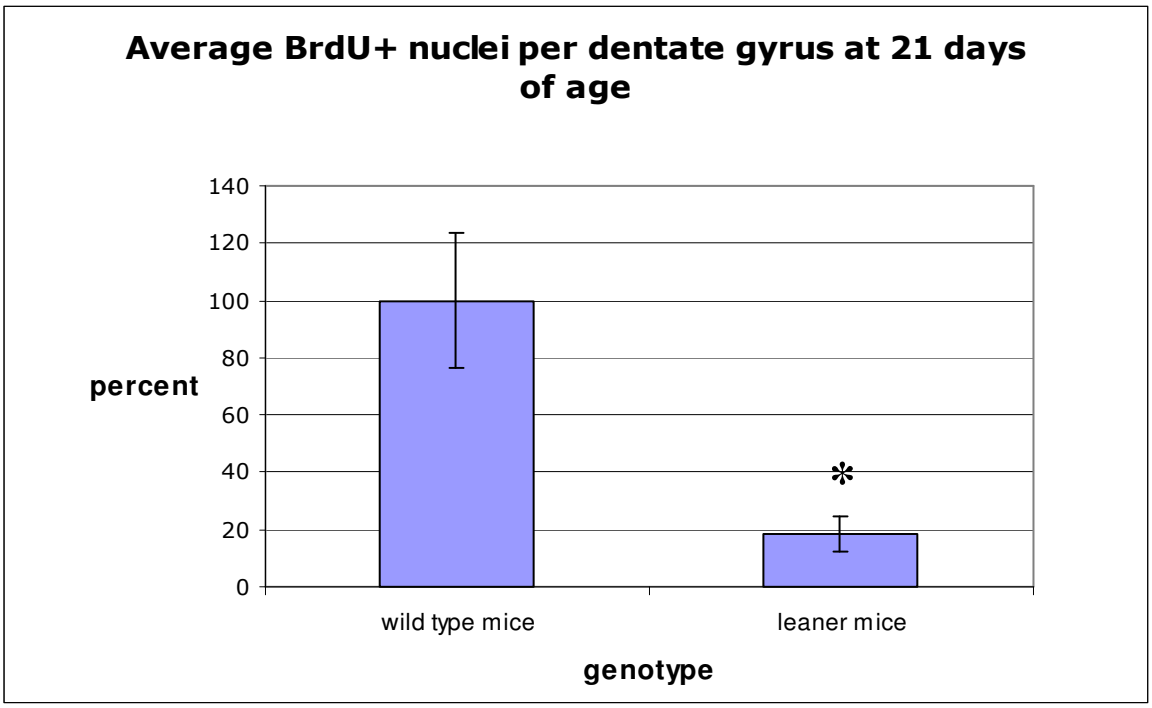

Figure 16. Twenty-one day old mice cell counts for average number of BrdU positive cells. These data are presented as percent difference from wild type mice since the data were collected several years before the current study was initiated. It is not possible to compare actual numbers of cells between these data and data from the current set of experiments. Asterisks indicate that leaner mice have a significantly lower number of BrdU positive cells.

Hippocampus Measurement

To determine if there was a difference in hippocampal size, which could be expected due to the increase in proliferation in the 42-50 day old mice, we examined the length of the hippocampus. It is possible that a difference in hippocampal size might be reflected in a difference in overall length of the hippocampus. To assess hippocampal length we counted the number of sections required to cut through the entire length of the hippocampus in six week old animals. We ran a two way ANOVA comparing genotype and gender and found that there was a difference in males and females ($p=0.014$), but no genotype/gender interaction ($p=0.167$), so we separated out males and females and analyzed them separately.

When we looked at female hippocampus length, we did not find any differences between the different genotypes ($p=0.887$). When we looked at hippocampal length in the male mice there also was no statistically significant difference. However, there was a trend for the male leaner mice to have a longer hippocampus than the age matched wild type mice ($p=0.080$) (see figure 17). We would need to increase our sample size to determine whether or not this trend reflects a significant difference.

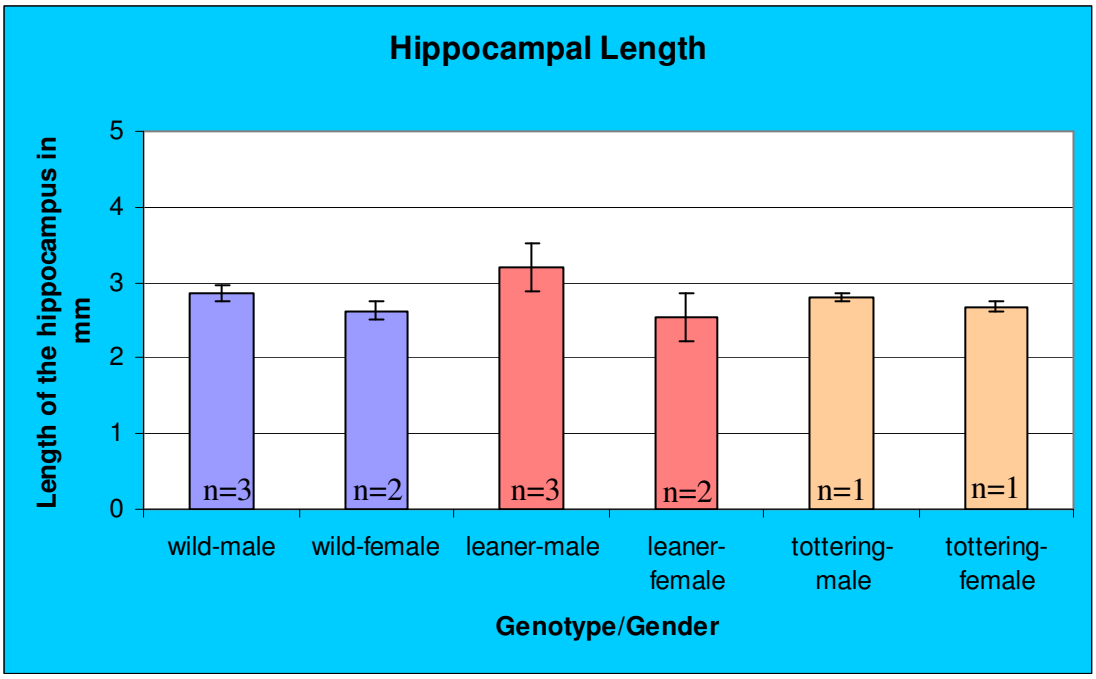

Figure 17. Hippocampal length in six week old mice.

Fluorescence Double-Labeling P100-150

Once the number of BrdU positive cells were counted, we determined the percentage of those cells that were neurons and what percent were glial supportive cells. We determined whether there were genotypic differences in the percentage of neurons vs. glia. To make sure that our tags were working properly, we used single-label immunohistochemistry on sections for each of the three antibodies (BrdU, NeuN, and GFAP).

When we double-labeled with anti-BrdU and anti-NeuN for new neurons we observed that wild-type, leaner, and tottering mice have approxmately 90% of the BrdU positive cells double-labeled with NeuN, meaning that about 90% of the new cells that were formed were actually neurons. When we double-labeled with anti-BrdU and anti-GFAP for new glial cells we observed that wild-type, leaner, and tottering mice have approxmately 16% of the BrdU positive cells double-labeled with GFAP, meaning that about 16% of the new cells that were formed were actually glial cells (See figure 18). For representative images of immunofluorescence see Figures 19, 20, and 21.

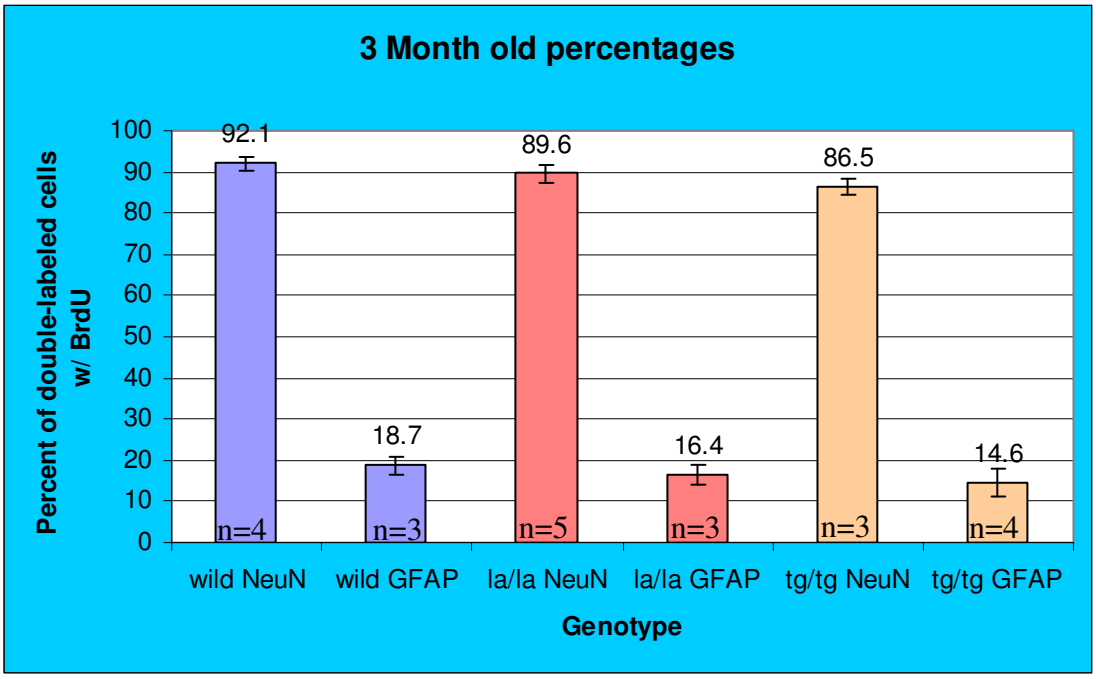

Figure 18. Percentage of double-labeled BrdU positive cells for three month old animals.

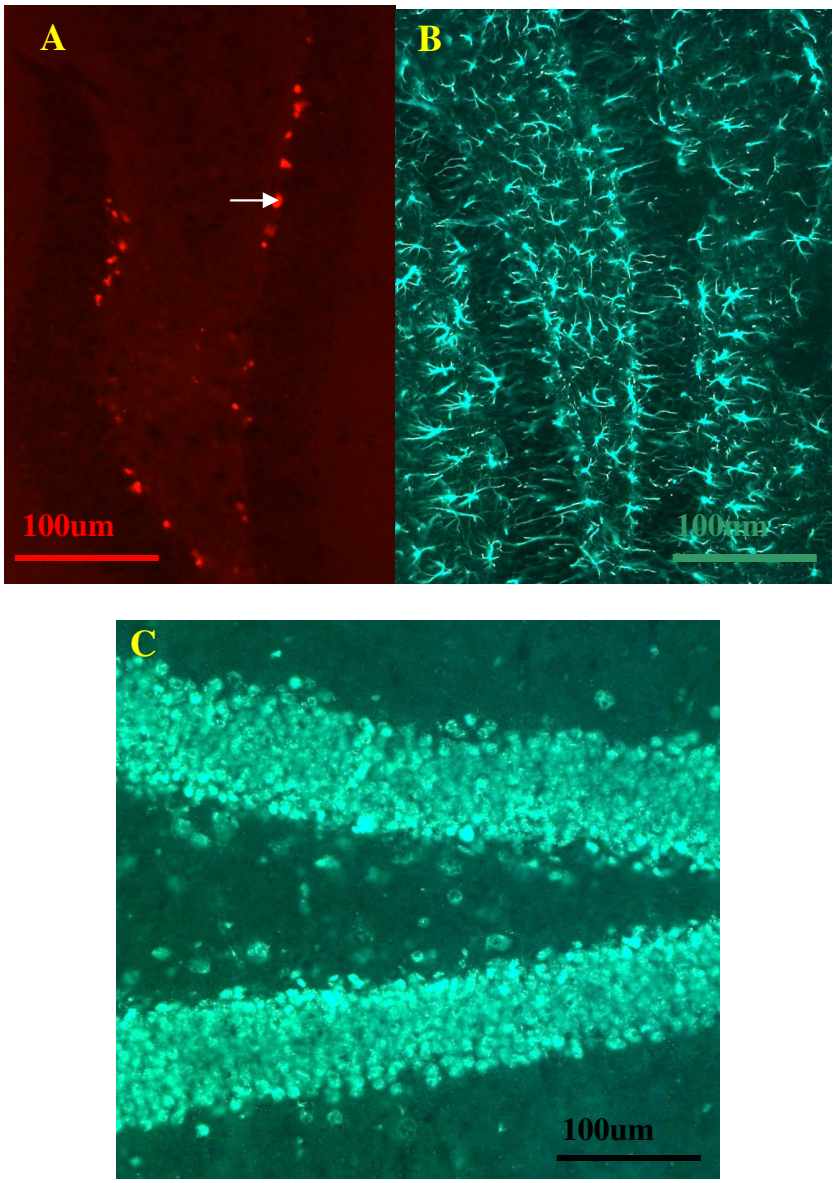

Figure 19. Representative images of single-labeled cells in the dentate gyrus region of the hippocampus. A- BrdU only labeled cells, leaner at 16x. B- GFAP only labeled cells, leaner at 16x. C- NeuN only labeled cells, tottering at 16x. White arrows indicate single labeled cells with BrdU.

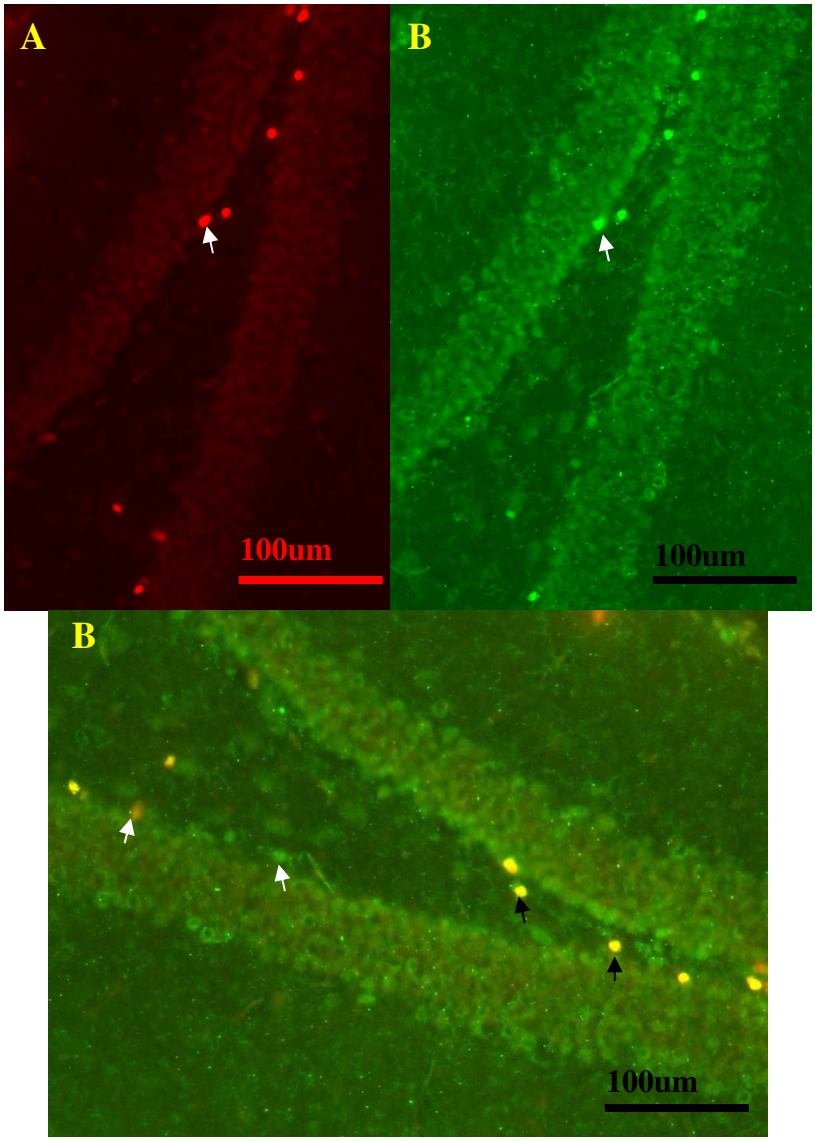

Figure 20. Representative images of double immunofluorescence (NeuN/ BrdU) in the dentate gyrus. A-Single-labeled BrdU positive cells. B-Single-labeled NeuN positive cells. C-Double-labeled cells with NeuN and BrdU. White arrows indicate single labeled cells. Black arrow indicates double-labeled cells.

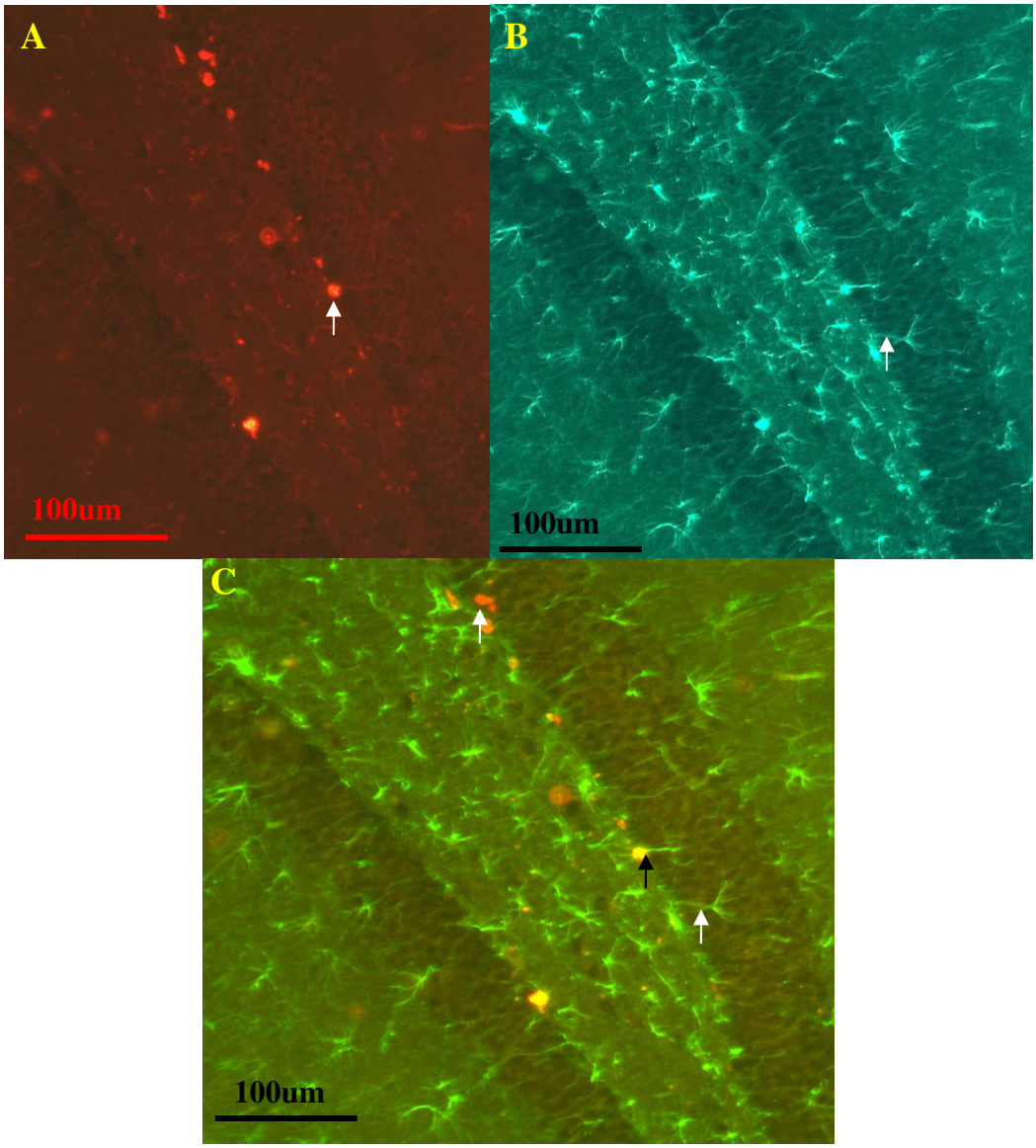

Figure 21. Representative images of double immunofluorescence (GFAP/BrdU) in the dentate gyrus. A-Single-labeled BrdU positive cells. B-Single-labeled GFAP positive cells. C-Double-labeled cells with GFAP and BrdU. White arrows indicate single labeled cells. Black arrow indicates a double-labeled cell.

Fluorescence Double-Labeling P42-50

We wanted to determine the percentages of double labeled cells for the six week old animals as well so we followed the same procedure for these mice as was used for the three month old mice. We found that overall for all three genotypes; the percent of double-labeled BrdU/NeuN cells was about 85% for the leaner and tottering mice and approximately 80% double-labeled for the wild-type mice (See Table 3 and Figure 22). The genotypes were not significantly different from each other (p=0.370) overall.

Table 3. Percentage of BrdU/NeuN double-labeled cells in six week old mice

Genotype	Average # of BrdU positive cells/section in dentate gyrus	Average # of double labeled cells/section in dentate gyrus	Percentage
Leaner	24.8	21.208	85.4% +/- 2
Wild-type	21.6	17.4	80.5% +/- 1.8
Tottering	27.8	24.1	86.7% +/- 1.9

We also looked at BrdU positive cells double-labeled with GFAP to determine the percentage of new cells that were glial cells. We found that BrdU/GFAP double-labeled cells composed approximately 10% of the BrdU positive cell population in the leaner and wild-type mice and 11% in tottering mice (See Table 4 and Figure 22). The genotypes were not statistically significantly different from each other (p=0.813) overall.

Table 4. Percentage of BrdU/GFAP double-labeled cells-six week old mice

Genotype	Average # of BrdU positive Cells/section in dentate gyrus	Average # of double labeled cells/section in dentate gyrus	Percentage
Leaner	18.7	1	11.5 % +/- 2.4
Wild-type	18.8	1.8	9.5 % +/- 2.2
Tottering	21	2.4	11.9 % +/- 3.5

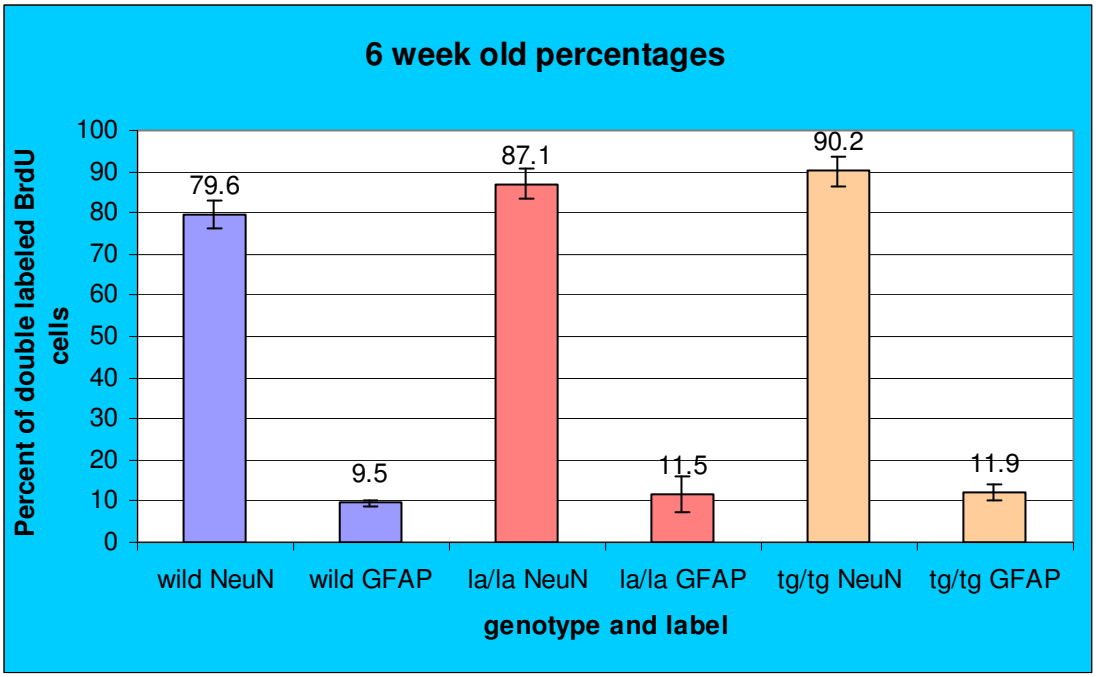

Figure 22. Percentage of double-labeled BrdU positive cells in six week old animals. N=3 for all genotypes and groups.

IV. DISCUSSION

Behavior

We examined the activity levels of three to four month old mice before and after a two week period of time in which the mice were handled daily. We hypothesized that handling was stressful to the mice and that one of the responses to stress could be a change in activity levels, which might be indicative of depression. We carried out two different activity measurements, number of rearing movements and numbers of lines crossed in an open field that served as a measure of general locomotor activity. No differences were observed in the number of rearing movements in the mice. However, when we analyzed the number of lines crossed in the open field, we observed a small but significant difference in the number of lines crossed when before handling results and after handling results for each mouse were compared. This effect was not specifically related to the genotype of the mice but we did observe a trend towards a genotypic interaction when the results from before handling were compared to the results obtained after two weeks of handling. It is interesting to note that for the leaner mice the observed tendency was to increase their level of activity after two weeks of handling while both the wild type mice and the tottering mice tended to decrease their activity levels after two weeks of handling. Additional testing is required to understand these observations. However, we are tempted to speculate that the leaner mice are not responding in the same manner as the other two genotypes due to the increased severity of the effects of the mutation on calcium ion channel function. Further testing is

necessary to determine if the trend towards increased activity in the leaner mice is due to perhaps increased anxiety or potentially decreased depressive tendencies. For example, monitoring in an automated activity chamber would allow determination of time spent in the center of the open field versus time spent in the periphery, which can indicate level of anxiety for the mice.

One also needs to take into consideration the fact that both leaner and tottering mice experience frequent absence seizures (a range of 20-40 brief 1-3 second seizures per hour have been recorded (Fletcher et al., 1996) and they have different degrees of ataxia. In addition, the three month old tottering mice exhibit paroxysmal dyskinesia when confronted with stressful situations such as being handled or being given injections, but the paraxoysmal dyskinesia is reduced in adult leaner mice relative to the number of events seen in tottering mice (Rhyu, et al., 1999). The observed differences in activity levels could be due to the epileptiform seizures, ataxia or the paroxysmal dyskinesia that these mutant mice experience. Therefore, we can not conclude that there is a major handling effect on the activity levels of leaner and tottering mice when just examining the number of lines crossed as an indication of activity level of these three-month-old mice.

Adult Neurogenesis P100-150

After completing the behavioral analysis on the three to four month old leaner and tottering mice as compared to age-matched wild type mice, we examined the proliferation of cells in the subgranular zone of the hippocampal dentate gyrus in these same mice, using BrdU to label the DNA found in the nuclei of newly formed cells. We hypothesized that there would be decreased proliferation in the mutant mice as compared to age-matched wild type mice. Both leaner and tottering mice at three to four months of age were observed to have a significant decrease in number of new cells being born in the dentate gyrus of the hippocampus as compared to age and gender matched wild type mice. We speculate that the smaller numbers of BrdU-labeled cells found in the adult mutant mice was in some way related to the decrease in calcium ion currents that occurs in leaner and tottering mice. It has already been shown that a significant amount of cell death happens in the cerebellum of the leaner mouse due to the mutation in the α1A subunit of P/Q-type calcium ion channels (Isaacs and Abbott, 1995; Frank et al., 2003). We therefore expected that the decrease in calcium current and accompanying alterations in intracellular calcium homeostasis could also be causing progenitor cell degeneration or a decrease in cell cycling and therefore fewer cells are being produced or surviving in the dentate gyrus of the hippocampus. We know that P/Q-type calcium ion channels are expressed mainly pre-synaptically. It has been shown that a decrease in serotonin levels result in a decrease in cell proliferation, and when subjects were given selective serotonin reuptake inhibitors (SSRI's), neurogenesis in the dentate gyrus of the

hippocampus increased (Clark, et al., 2006; Encinas, et al., 2006; Saylam, et al., 2006; Grote, et al., 2005; Malberg and Schechter, 2005; Chen et al., 2000). Therefore, it is possible that altered calcium ion homeostasis in these mutant animals is causing altered synaptic vesicle transmission and neurotransmitter release. Therefore, it is tempting to speculate that serotonin release could be affected in tottering and leaner mice that would cause lower levels of serotonin in the dentate gyrus of the hippocampus and subsequently result in decreased neurogenesis. We need to examine the brains of tottering and leaner mice for serotonin levels and/or give these mice some sort of SSRI's to see if this is what is really occurs and if we can increase proliferation in the dentate gyrus of the adult leaner and tottering mouse hippocampus. However, it is important to note that serotonin is not the only neurotransmitter released from the neurons. The hippocampus has many other inputs from other parts of the brain such as the substantia nigra which releases a large amount of the neurotransmitter dopamine. Therefore, it is probably safe to assume that other neurotransmitter release is being affected as well.

Adult Neurogenesis P42-50

In addition to examining cell proliferation in hippocampi of three to four month old mice we extended our observations to include younger mice of the age range P42-50. In contrast to the observed decrease in proliferation rate seen in both leaner and tottering mice at P100-150, we observed an increase in the formation of new cells in both mutants at this younger age. A normal decrease in cell proliferation in the dentate gyrus of the hippocampus occurs sometime around day 36 in mice (Hayes et al., 2005). The altered

calcium homeostasis that we see in neurons that express αA1 channels has the possibility of affecting many aspects of cellular function, proliferation and survival, including growth factors. Therefore, we hypothesize that the decrease in proliferation that is observed at P100-150 is delayed for some reason in the younger mutant mice. However, this increased proliferation rate is not maintained in the mutant mice eventually the proliferation rate drops below the normal proliferation rates seen in the adult wild-type mice. We need to look more extensively into additional age groups of these mutant mice to see when exactly the plateau period for cell proliferation occurs and when the drop in proliferation begins in the mutant mice when compared to age-matched wild-type mice.

Neurogenesis P21

In the P21 leaner and wild type mice that were studied, we observed that the leaner mice also had substantially fewer numbers of newly formed cells when compared to the age-matched wild type mice. When examining mitochondrial membrane potential for cerebellar granule cells from leaner and wild type mice at different postnatal ages (unpublished data from Bhupinder Bawa, 2006), we observed a similar trend to the observed decreased proliferation in the dentate gyrus of the hippocampus. Mitochondrial membrane potential was used as an indicator of mitochondrial function in cerebellar granule cells such that the decreased mitochondrial membrane potential is thought to indicate decreased mitochondrial function, which may be a potential cause of neuronal apoptosis. The mitochondrial membrane potential was low in leaner cerebellar

granule cells at an early age (P20), then increased around P30 to level or mitochondrial membrane potential that was comparable to that observed in age-matched wild type cerebellar granule cells. The potential dropped again at P40 in leaner cerebellar granule cells relative to age-matched wild type cerebellar granule cells and stayed decreased into adulthood. It would seem that some type of compensatory mechanism may be occurring in the leaner mouse cerebellar granule cells at P30 such that the leaner cerebellar granule cell mitochondria were able to achieve a normal membrane potential. However, this compensation could not be maintained and the membrane potential in mitochondria of p40 granule cells of leaner and tottering mice decreased subsequently to P30.

Based on these mitochondrial membrane potential data we postulate that both leaner and tottering mice have some sort of compensatory mechanism that in the short term may allow for an increase in proliferation rates in the hippocampus, but cannot be maintained over the life of the animals. Therefore, leaner and tottering mice can only maintain the increased rate of cell proliferation in the hippocampus for a short period of time before decreasing to a level that is significantly lower that the rate observed in age-matched wild type mice.

Hippocampal Length

The increased proliferation rate that was observed in the P42-50 tottering and leaner mice raised the question of whether the hippocampus might be, at least temporarily larger in tottering and leaner mice at P42-50 when compared to age-matched wild type mice. As a first estimate of size we examined the length of the hippocampus

in all three genotypes at P42-50. When we examined the length of the hippocampus in female mice, we did not find any differences between the different genotypes. When we examined hippocampal length in male mice there also was no statistically significant difference between the different genotypes. However, there was a trend for male leaner mice to have a longer hippocampus than the age matched wild type mice (refer to Figure 17). We will need to increase our sample size to determine whether or not this trend reflects a significant difference. While length could be a reasonable indicator of a change in size of the hippocampus, it is not the best indicator of size. We will need to examine the volume (length x area) and/or neuronal density in the hippocampus to get a better idea of the overall size of the hippocampus in order to determine whether the hippocampus is indeed increased in size in leaner mice at P42-50.

Double Fluorescence

We examined double-labeling of BrdU-positive cells in the hippocampi of leaner, tottering and wild type mice at both P42-50 and P100-150. We double labeled hippocampal sections for BrdU and NeuN, which is a nuclear marker for neurons and other sections were double-labeled for BrdU and GFAP, which is a cytoplasmic marker for glial cells, specifically, astrocytes. From the literature, we expect to see about 80% of new cells double-labeled with NeuN and about 20% double-labeled with GFAP. In both age groups and in all three genotypes, we observed percentages of double labeled cells that were similar to these published percentages. While there was no significant difference in percentage of double-labeled GFAP/BrdU positive cells (new astrocytes)

among genotypes, we observed that in both age groups, but especially the P-42-50 mice, that both tottering and leaner mice seemed to have more astrocytes than the age-matched wild-type mice. This is supported by previous observations and data collected in the laboratory (unpublished data, LC Abbott). The fact that we did not find a difference in proportion among genotypes and age groups for the double-labeled cells indicates that even though there is a difference in overall number of new cells that are being produced at both ages examined in this study, the differentiation patterns are probably not being affected. However, we gave the final BrdU injections one hour before the mice were perfused, and this is not sufficient time for the BrdU-labeled cells to differentiate into neurons or glia. Therefore in future studies we plan to look at mice that survived for two weeks after their final BrdU injection and look at the proportion of double-labeled cells after differentation should essentially be complete. This should provide a better determination as to the number of neurons being generated as opposed to the number of glial cells. We could also use an early differentiation pattern marker such as doublecortin, which labels immature neurons to detect differentiation patterns (Koketsu, et al., 2006).

V. CONCLUSIONS

In conclusion, we see a decrease of cell proliferation in the dentate gryus of the hippocampus in the mutant mice compared to the wild-type mice at 100-150 days old. This decrease in neurogenesis could lead to lower hippocampal function eventually leading to learning and memory loss. However, when we look at mice that are 42-50 days old, there is an increase in cell proliferation in the same area in the mutants compared to the wild-type mice. This tells us that there is a delay in the normal decrease in the proliferation rate based on previous studies. However, the mutant mice can not maintain this level of increased proliferation and eventually fall below the wild-type mice (See Figure 23). Based on our data, we need to look at other age groups to determine when exactly the plateau period and the drop off in proliferation is actually occurring. Also, it would be interesting to look at much older mice to see if we continue to see a decrease in the amount of proliferation in the mutants compared to the wild-type mice. There is correlation between decreased proliferation and depression; therefore these adult mice could prove to be a good model for depression.

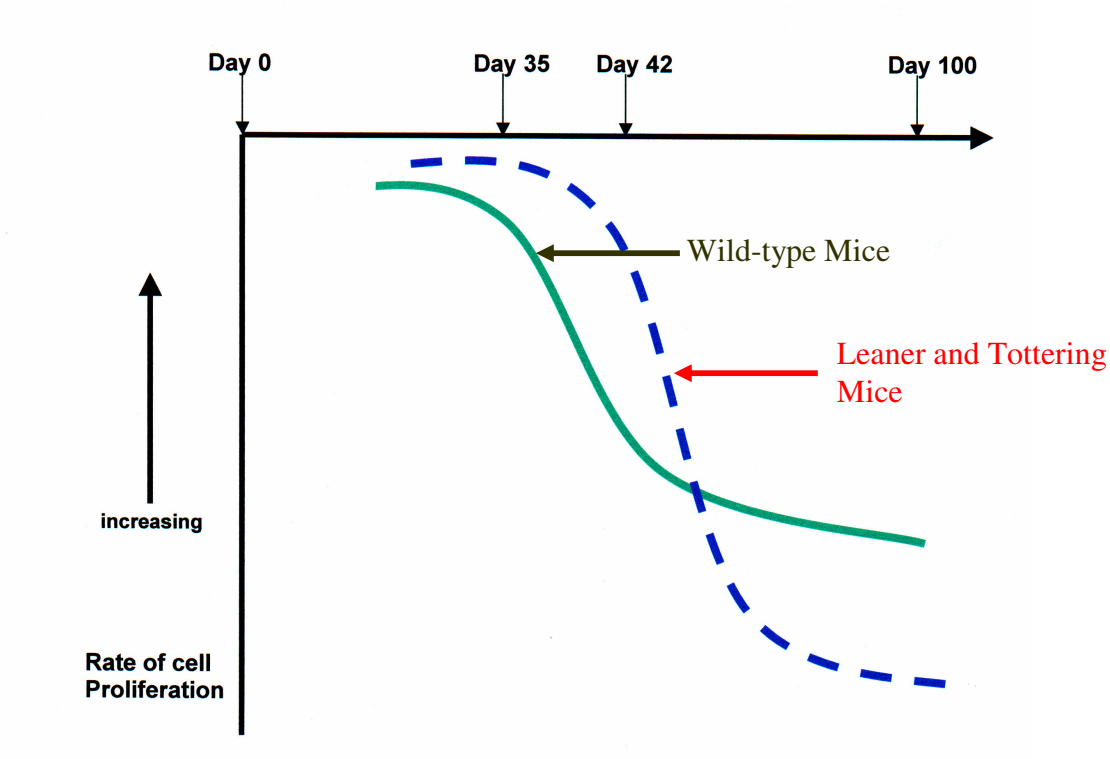

Figure 23. Schematic representation of the proliferation rates in wild-type and mutant mice.

REFERENCES

Afzalpurkar, Abhijit (2003). Morris water maze. Medical College of Georgia. Online. www.mcg.edu/Core/Labs/sabc/Morriswatermaze.htm.

Abrous, D. N., Koehl, M., and Le Moal, M. (2005). Adult neurogenesis: from precursors to network and physiology. Physiol Rev *85*, 523-569.

Alvarez-Buylla, A., Temple, S. (1998). Stem cells in the developing and adult nervous system. Neural Stem Cells *36*, 105-110.

Ansorge, M. S., Zhou, M., Lira, A., Hen, R., and Gingrich, J. A. (2004). Early-life blockade of the 5-HT transporter alters emotional behavior in adult mice. Science *306*, 879-881.

Antel, J., Nalbantoglu, J., Olivier, A. (2000). Neuronal progenitors-learning from the hippocampus. Nature Medicine *6*, 249-250.

Ayata, C., Shimizu-Sasamata, M., Lo, E. H., Noebels, J. L., and Moskowitz, M. A. (1999). Impaired neurotransmitter release and elevated threshold for cortical spreading depression in mice with mutations in the [alpha]1A subunit of P/Q type calcium channels. Neuroscience *95*, 639-645.

Bear, M., Connors, B., Paradiso, M. (2001). Neuroscience: exploring the brain, 2nd ed (Baltimore, MD, Lippincott, Williams, and Wilkins).

Bernardoni, R., Kammerer, M., Vonesch, J.-L., and Giangrande, A. (1999). gliogenesis depends on glide/gcm through asymmetric division of neuroglioblasts. Developmental Biology *216*, 265-275.

Broadbent, N. J., Squire, L. R., and Clark, R. E. (2006). Reversible hippocampal lesions disrupt water maze performance during both recent and remote memory tests. Learn Mem *13*, 187-191.

Campbell, S., Marriott, M., Nahmias, C., and MacQueen, G. M. (2004). Lower hippocampal volume in patients suffering from depression: a meta-analysis. American Journal of Psychiatry *161*, 598-607.

Catterall, W. A. (2000). Structure and regulation of voltage-gated Ca2+ channels. Annual Review of Cell and Developmental Biology *16*, 521-555.

Cayre, M., Malaterre, J., Scotto-Lomassese, S., Strambi, C., and Strambi, A. (2002). The common properties of neurogenesis in the adult brain: from invertebrates to vertebrates. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology *132*, 1-15.

Chen, G., Rajkowska, G., Du, F., Seraji-Bozorgzad, N., and Manji, H. K. (2000). Enhancement of hippocampal neurogenesis by lithium. Journal of Neurochemistry *75*, 1729-1734.

Cicale, M., Ambesi-Impiombato, A., Cimini, V., Fiore, G., Muscettola, G., Abbott, L. C., and de Bartolomeis, A. (2002). Decreased gene expression of calretinin and ryanodine receptor type 1 in tottering mice. Brain Research Bulletin *59*, 53-58.

Cipolotti, L., Bird, C., Good, T., Macmanus, D., Rudge, P., and Shallice, T. (2006). Recollection and familiarity in dense hippocampal amnesia: A case study. Neuropsychologia *44*, 489-506.

Clark, S., Schwalbe, J., Stasko, M. R., Yarowsky, P. J., and Costa, A. C. S. (2006). Fluoxetine rescues deficient neurogenesis in hippocampus of the Ts65Dn mouse model for Down syndrome. Experimental Neurology, In Press.

Crawley, J. N. (1999). Behavioral phenotyping of transgenic and knockout mice: experimental design and evaluation of general health, sensory functions, motor abilities, and specific behavioral tests. Brain Research *835*, 18-26.

Dahlstrom, A., Fuxe, K. (1964). Localization of monoamines in the lower brainstem. Experimenta *20*, 398-399.

Deacon, R. M. J., and Rawlins, J. N. P. (2005). Hippocampal lesions, species-typical behaviours and anxiety in mice. Behavioural Brain Research *156*, 241-249.

Dove, L. S., Abbott, L. C., and Griffith, W. H. (1998). Whole-cell and single-channel analysis of P-Type calcium currents in cerebellar purkinje cells of leaner mutant mice. Journal of Neuroscience *18*, 7687-7699.

Dove, L. S., Nahm, S.-S., Murchison, D., Abbott, L. C., and Griffith, W. H. (2000). Altered calcium homeostasis in cerebellar purkinje cells of leaner mutant mice. J Neurophysiol *84*, 513-524.

Eichenbaum, H. (2004). Hippocampus: cognitive processes and neural representations that underlie declarative memory. Neuron *44*, 109-120.

Encinas, J. M., Vaahtokari, A., and Enikolopov, G. (2006). Fluoxetine targets early progenitor cells in the adult brain. Proc.Natl.Acad.Sci.USA *103*, 8233-8238.

Eriksson, P. S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A.-M., Nordborg, C., Peterson, D. A., and Gage, F. H. (1998). Neurogenesis in the adult human hippocampus. Nat Med *4*, 1313-1317.

Fell, J., Fernandez, G., Lutz, M. T., Kockelmann, E., Burr, W., Schaller, C., Elger, C. E., and Helmstaedter, C. (2006). Rhinal-hippocampal connectivity determines memory formation during sleep. Brain *129*, 108-114.

Fletcher, C. F., Lutz, C. M., O'Sullivan, T. N., Shaughnessy, J. J. D., Hawkes, R., Frankel, W. N., Copeland, N. G., and Jenkins, N. A. (1996). Absence epilepsy in tottering mutant mice is associated with calcium channel defects. Cell *87*, 607-617.

Frank, T. C., Nunley, M. C., Sons, H. D., Ramon, R., and Abbott, L. C. (2003). Fluoro-jade identification of cerebellar granule cell and purkinje cell death in the [alpha]1A calcium ion channel mutant mouse, leaner. Neuroscience *118*, 667-680.

Frazer, A., Hensler, J. (1999). Serotonin, 264-292. Basic neurochemistry: molecular, cellular, and medical aspects. (Philadelphia, PA, Lippincott, Williams, and Wilkins).

Gage, F., Kempermann, G., Palmer, T., Peterson, D., Ray, J. (1998). Multipotent progenitor cells in the adult dentate gyrus. Journal of Neurobiology *36*, 249-266.

Grote, H. E., Bull, N. D., Howard, M. L., van Dellen, A., Blakemore, C., Bartlett, P. F., and Hannan, A. J. (2005). Cognitive disorders and neurogenesis deficits in Huntington's disease mice are rescued by fluoxetine. European Journal of Neuroscience *22*, 2081-2088.

Guidi, S., Ciani, E., Severi, S., Contestabile, A., Bartesaghi, R. (2005). Postnatal neurogenesis in the dentate gyrus of the guinea pig. Hippocampus *15*, 285-301.

Hassanain, M., Bhatt, S., and Siegel, A. (2003). Differential modulation of feline defensive rage behavior in the medial hypothalamus by 5-HT1A and 5-HT2 receptors. Brain Research *981*, 201-209.

Hatzidimitriou, G., McCann, U. D., and Ricaurte, G. A. (1999). Altered serotonin innervation patterns in the forebrain of monkeys treated with (±)3,4-methylenedioxymethamphetamine seven years previously: factors influencing abnormal recovery. J Neurosci *19*, 5096-5107.

Hayes, N. L., and Nowakowski, R. S. (2005). The genetics of adult neurogenesis: identification of two independently regulated phases of aging in the stem/progenitor cell population in the dentae gyrus of C57Bl/6J and BALB/cbyj mice. In 2005 Abstract Viewer/Internary Planner (Washington, DC, Society for Neuroscience), Program No. 141.111.

Herrup, K., and Wilczynski, S. L. (1982). Cerebellar cell degeneration in the leaner mutant mouse. Neuroscience *7*, 2185-2196.

Holtzheimer, and Nemeroff (2006). Advances in the treatment of depression. NeuroRX *3*, 42-56.

Isaacs, K. R., and Abbott, L. C. (1995). Cerebellar volume decreases in the tottering mouse are specific to the molecular layer. Brain Research Bulletin *36*, 309-314.

Koketsu D., Furuichi Y, Maeda M, Matsuoka N, Miyamoto Y, and T., H. (2006). Increased number of new neurons in the olfactory bulb and hippocampus of adult non-human primates after focal ischemia. Experimental Neurology. In press.

Lau, F. C., Frank, T.C., Nahm, S., Stoica, G., Abbott, L.C. (2004). Postnatal apoptosis in cerebellar granule cells of homozygous leaner (tg^{la}/tg^{la}) mice. Neurotoxicity Research *6*, 267-280.

Lau, F. C., Abbott, L. C., Rhyu, I. J., Kim, D. S., and Chin, H. (1998). Expression of calcium channel [alpha]1A mRNA and protein in the leaner mouse (tgla/tgla) cerebellum. Molecular Brain Research *59*, 93-99.

Ledergerber, D., Fritschy, J.-M., and Kralic, J. E. Impairment of dentate gyrus neuronal progenitor cell differentiation in a mouse model of temporal lobe epilepsy. Experimental Neurology, In Press.

Malatynska, E., Rapp, R., Harrawood, D., and Tunnicliff, G. (2005). Submissive behavior in mice as a test for antidepressant drug activity. Pharmacology Biochemistry and Behavior *82*, 306-313.

Malberg, J., Schechter, L. (2005). Increasing hippocampal neurogenesis: a novel mechanism for antidepressant drugs. Current Pharmaceutical Design *11*, 145-155.

Mandairon, N., Sacquet, J., Jourdan, F., and Didier, A. (2006). Long-term fate and distribution of newborn cells in the adult mouse olfactory bulb: influences of olfactory deprivation. Neuroscience, In Press.

McArthur, N., and Abbott, L. C. (2005). Neuroanatomy: Notes and Guide VAPH 603 (College Station, Texas, Texas A&M University).

Ming, G.-l., and Song, H. (2005). Adult neurogenesis in the mammalian central nervous system. Annual Review of Neuroscience *28*, 223-250.

Mori, Y., Wakamori, M., Oda, S.-i., Fletcher, C. F., Sekiguchi, N., Mori, E., Copeland, N. G., Jenkins, N. A., Matsushita, K., Matsuyama, Z., and Imoto, K. (2000). Reduced voltage sensitivity of activation of P/Q-Type Ca2+ channels is associated with the ataxic mouse mutation rolling nagoya (tgrol). J Neurosci *20*, 5654-5662.

Morris, R. G. (2003). Long-term potentiation and memory. Philosophical Transactions of the Royal Society of London: Series B *358*, 643-647.

Mullen, R. J., Buck, C. R., and Smith, A. M. (1992). NeuN, a neuronal specific nuclear protein in vertebrates. Development *116*, 2

Murrary, R., Granner, D., Mayes, P., Rodwell, V. (2003). Lange: harper's illustrated biochemistry, 26th ed, (New York: McGraw-Hill).

Nahm, S., Thomlinson, D., and Abbott, L. C. (2002). Decreased calretinin expression in cerebellar granule cells in the leaner mouse. Journal of Neurobiology *51*, 313-322.

Nahm, S., Jung, K., Krause-Enger, M., Griffith, W., Abbott, L.C. (2005). Differential expression of T-type calcium channels in P/Q-type calcium channel mutant mice with ataxia and absence epilepsy. Journal of Neurobiology *62*, 352-360.

Namba, T., Mochizuki, H., Onodera, M., Mizuno, Y., Namiki, H., and Seki, T. (2005). The fate of neural progenitor cells expressing astrocytic and radial glial markers in the postnatal rat dentate gyrus. European Journal of Neuroscience *22*, 1928-1941.

Pham, K., Nacher, J., Hof, P. R., and McEwen, B. S. (2003). Repeated restraint stress suppresses neurogenesis and induces biphasic PSA-NCAM expression in the adult rat dentate gyrus. European Journal of Neuroscience *17*, 879-886.

Phelps, J. (2006). Stress mechanisms, from molecules to brains (Online) www.psycheducation.org.

Randall, A., and Benham, C. D. (1999). Recent advances in the molecular understanding of voltage-gated Ca2+ channels.[erratum appears in Mol Cell Neurosci 2000 Jan;15(1):112]. Molecular & Cellular Neurosciences *14*, 255-272.

Rapport, M. M., Green, A. A., and Page, I. H. (1948). Serum vasoconstrictor, (Serotonin). IV. Isolation and characterization. J Biol Chem *176*, 1243-1251.

Reynolds, B., and Weiss, S. (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science *255*, 1707-1710.

Rhyu, I. J., Abbott, L. C., Walker, D. B., and Sotelo, C. (1999). An ultrastructural study of granule cell/Purkinje cell synapses in tottering (tg/tg), leaner (tgla/tgla) and compound heterozygous tottering/leaner (tg/tgla) mice. Neuroscience *90*, 717-728.

Rietze, R., Poulin, P., Weiss, S. (2000). Mitotically active cells that generate neurons and astrocytes are present in multiple regions of the adult mouse hippocampus. The Journal of Comparative Neurology *424*, 397-408.

Rosenbrock, H., Koros, E., Bloching, A., Podhorna, J., Borsini, F. (2005). Effect of chronic intermittent restraint stress on hippocampal expression of marker proteins for synaptic plasticity and progenitor cell proliferation in rats. Brain Research *1040*, 55-63.

Roy, N., Wang, S., Jiang, L., Kang, J., Benraiss, A., Harrison-Restelli, C., Fraser, R. A. R., Couldwell, W. T., Kawaguchi, A., Okano, H. (2000). In vitro neurogenesis by progenitor cells isolated from the adult human hippocampus. Nat Med *6*, 271-277.

Sadler, T. (2000). Langman's medical embryology, Eighth ed. (Philadelphia, PA, Lippincott, Williams, and Wilkins).

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O.*, et al.* (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science *301*, 805-809.

Saylam, C., Ucerler, H., Kitis, O., Ozand, E., and Gonul, A. (2006). Reduced hippocampal volume in drug-free depressed patients. Surgical and Radiologic Anatomy *28*, 82-87.

Sheline, Y. I., Gado, M. H., and Kraemer, H. C. (2003). Untreated depression and hippocampal volume loss. American Journal of Psychiatry *160*, 1516-1518.

Shimazu, K., Zhao, M., Sakata, K., Akbarian, S., Bates, B., Jaenisch, R., and Lu, B. (2006). NT-3 facilitates hippocampal plasticity and learning and memory by regulating neurogenesis. Learn Mem *13 (3)*, 307-315.

Shors, T. J., Miesegaes, G., Beylin, A., Zhao, M., Rydel, T., and Gould, E. (2001). Neurogenesis in the adult is involved in the formation of trace memories. Nature *410*, 372-376.

Sloan, H. L., Good, M., and Dunnett, S. B. (2006). Double dissociation between hippocampal and prefrontal lesions on an operant delayed matching task and a water maze reference memory task. Behavioural Brain Research *171*, 116-126.

Squire, L. R., Stark, C. E. L., and Clark, R. E. (2004). The medial temporal lobe. Annual Review of Neuroscience *27*, 279-306.

Starr, T. V. B., Prystay, W., and Snutch, T. P. (1991). Primary structure of a calcium channel that is highly expressed in the rat cerebellum. Proc.Natl.Acad.Sci.USA *88*, 5621-5625.

Stea, A., Tomlinson, W. J., Soong, T. W., Bourinet, E., Dubel, S. J., Vincent, S. R., and Snutch, T. P. (1994). Localization and functional properties of a rat brain 1A calcium channel reflect similarities to neuronal Q- and P-Type channels. Proc.Natl.Acad.Sci.USA *91*, 10576-10580.

Taupin, P. (2005). Adult neurogenesis in mammalian central nervous system system: functionality and potential clinical interest. Medical Science Monitor *11*, 247-252.

Thompson, S., Mattison, H., Nestor, M. (2005). Morphological correlates of long-term potentiation and depression. Cell Science *2,* 58-78.

Udolph, G., Prokop, A., Bossing, T., and Technau, G. M. (1993). A common precursor for glia and neurons in the embryonic CNS of Drosophila gives rise to segment-specific lineage variants. Development *118*, 765-775.

Videbech, P., and Ravnkilde, B. (2004). Hippocampal volume and depression: a meta-analysis of MRI studies. American Journal of Psychiatry *161*, 1957-1966.

Vincent, S., Vonesch, J. L., and Giangrande, A. (1996). Glide directs glial fate commitment and cell fate switch between neurones and glia. Development *122*, 131-139.

Wallenstein, G. V., and Hasselmo, M. E. (1997). Functional transitions between epileptiform-like activity and associative memory in hippocampal region CA3. Brain Research Bulletin *43*, 485-493.

Wallenstein, G. V., and Vago, D. R. (2001). Intrahippocampal scopolamine impairs both acquisition and consolidation of contextual fear conditioning. Neurobiology of Learning and Memory *75*, 245-252.

Warner-Schmidt, J., Duman, R. (2006). Hippocampal neurogenesis: Opposing effects of stress and antidepressant treatment. Hippocampus *16*, 239-249.

Westenbroek, R., Sakurai, T., Elliott, E., Hell, J., Starr, T., Snutch, T., and Catterall, W. (1995). Immunochemical identification and subcellular distribution of the alpha 1A subunits of brain calcium channels. J Neurosci *15*, 6403-6418.

Whishaw, I. Q., Rod, M. R., and Auer, R. N. (1994). Behavioral deficits revealed by multiple tests in rats with ischemic damage limited to half of the CA1 sector of the hippocampus. Brain Research Bulletin *34*, 283-289.

Wolf, H., Buslei, R., Schmidt-Kastner, R., Schmidt-Kastner, P., Pietsch, T., Wiestler, O., Blumcke, I. (1996). NeuN: A useful neuronal marker for diagnostic histopathology. The Journal of Histochemistry and Cytochemistry *44*, 1167-1171.

Zhen, X., Xie, C., Fitzmaurice, A., Schoonover, C. E., Orenstein, E. T., and Yang, J. (2005). Functional architecture of the inner pore of a voltage-gated Ca2+ channel. J Gen Physiol *126*, 193-204.

Zola, S. M., Squire, L. R., Teng, E., Stefanacci, L., Buffalo, E. A., and Clark, R. E. (2000). Impaired recognition memory in monkeys after damage limited to the hippocampal region. J Neurosci *20*, 451-463.

Zwingman, T. A., Neumann, P. E., Noebels, J. L., and Herrup, K. (2001). Rocker is a new variant of the voltage-dependent calcium channel gene Cacna1a. J Neurosci *21*, 1169-1178.

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M.S. Veterinary Anatomy, Texas A&M University, College Station, Texas, August 2006

EMPLOYMENT:

2004-2006: Teaching Assistant, Biomedical Anatomy, Texas A&M University, College Station, Texas.

2003-2004: Veterinary Assistant, Read Mountain Animal Hospital, Roanoke, Virginia

2003-2004: Veterinary Assistant, Botetourt Veterinary Hospital, Botetourt, Virginia

2002: Avian Intern, The Tracy Aviary, Salt Lake City, Utah

2001-2002: Reserves Assistant, McConnell Library, Radford University, Radford, Virginia

2000-2002: Cashier, Burger King Corporation, Roanoke, Virginia

1995-2004: Assistant Manager, Sonic Drive In, Roanoke, Virginia

AFFILIATIONS:

Beta Beta Beta, Biology Honor Society
Pi Gamma Mu, Social Science Honor Society
Sigma Kappa Sorority
Graduate Student Association