Spring 2017

Effects of predation and social interaction on spatial learning and brain cell proliferation in weakly electric fish, Apteronotus leptorhynchus

Elise A. Lasky
Trinity College, Hartford Connecticut, elise.lasky@trincoll.edu

TRINITY COLLEGE

EFFECTS OF PREDATION AND SOCIAL INTERACTION ON SPATIAL LEARNING AND BRAIN CELL PROLIFERATION IN WEAKLY ELECTRIC FISH, *APTERONOTUS LEPTORHYNCHUS*

BY

ELISE A. LASKY

A THESIS SUBMITTED TO
THE FACULTY OF THE DEPARTMENT OF BIOLOGY
IN CANDIDACY FOR THE BACCALAUREATE DEGREE
WITH HONORS IN BIOLOGY

DEPARTMENT OF BIOLOGY

HARTFORD, CONNECTICUT
5 MAY 2017

EFFECTS OF PREDATION AND SOCIAL INTERACTION ON SPATIAL LEARNING AND BRAIN CELL PROLIFERATION IN WEAKLY ELECTRIC FISH, *APTERONOTUS LEPTORHYNCHUS*

BY

ELISE A. LASKY

Honors Thesis Committee

Approved:

_____
Kent Dunlap, Advisor

_____
Daniel Blackburn

_____
Michael O'Donnell

Date: _____

Table of Contents

Abstract

Previous studies have determined that the stress of predation inhibits brain cell proliferation in two species of weakly electric fish, including, *Apteronotus leptorhynchus*. In this thesis, three experiments examined how predator stimuli and social interaction affect brain cell proliferation and spatial learning in *A. leptorhynchus*. The three questions that were explored were: 1) Is the decrease in brain cell proliferation seen after tail amputation in weakly electric fish due to the actual predation injury event or the subsequent regenerative process of their tail? 2) Does social interaction influence the effect of predator stimuli on brain cell proliferation? 3) Do predator stimuli in the form of chasing affect the spatial learning ability of the fish? In the first experiment the action of amputating the tail of the fish caused a drastic significant decrease in brain cell proliferation as compared to the fish allowed long-term recovery (17-18d) and the intact fish. This indicates that the actual predation injury event causes the decrease in cell proliferation, not the regenerative process of the tail. In the second experiment social interaction mitigated the negative effects of stress on brain cell proliferation. Finally in the third experiment the decrease in brain cell proliferation associated with chasing had no apparent effect on the spatial learning behavior of the fish.

Introduction

The Study of Neurogenesis

Until the 1960s it was believed that all neurons in the central nervous system of higher vertebrates were formed during embryonic development and that neurogenesis does not occur in adult vertebrates (Altman and Das, 1965). Through autoradiographic experiments in rats, Altman and Das (1965) demonstrated active neurogenesis within the dentate gyrus that proceeds at a low rate into adulthood. Kempermann and Gage (1999) defined neurogenesis as the cluster of events including proliferation of neuronal precursors or stem cells, survival of daughter cells and differentiation of the cells that results in the presence of new neurons. A variety of mammalian species display adult neurogenesis within the dentate gyrus (Opendak and Gould, 2015). The only mammals that have been studied that show little to no adult neurogenesis in the dentate gyrus are twelve tropical species of bats (Amrein et al., 2007; Schoenfeld and Gould, 2012).

Due to the involvement of the hippocampus with spatial memory and processing, it has been proposed that hippocampal neurogenesis may participate in the process of both spatial learning and encoding new spatial memories (LaDage, 2015). Such a proposal is based on observations such as an increase in the number of newborn neurons within the dentate gyrus in rats when doing hippocampus dependent learning (Gould et al., 1999). While the relationships between environmental stimuli, neurogenesis, and spatial learning have been examined in detail in mammals, relatively little is known about these processes in fish.

Neurogenesis can be defined as a two-part process: brain cell proliferation and neuronal differentiation. In this thesis I focus on the first part of the process, brain cell

proliferation, which increases with social interaction and decreases with stress (Dunlap, 2016). I seek to identify how predation affects brain areas involved in spatial cognition, spatial learning behavior, and social interaction in weakly electric fish.

Stress and the Brain

In rats, several thousand new hippocampal cells are produced each day, and this high rate of cell proliferation suggests biological relevance of hippocampal neurogenesis (Tanapat et al., 2001). Previous studies have shown that stress can suppress proliferation of progenitor cells that form new neurons. Stress inhibits adult neurogenesis by lowering the rate of brain cell proliferation (Tanapat et al., 2001; Schoenfeld and Gould, 2102), as demonstrated in a variety of mammalian species, including rats, mice, marmosets, and macaques (Opendak and Gould, 2015).

One stress that can inhibit brain cell proliferation is the threat of a predator. Exposure to predator odor (fox feces odor) but not other non-threatening odors decreased new cell formation in the rat dentate gyrus (Tanapat et al., 2001). Another study on rats showed that psychosocial stress decreased neurogenesis via the activation of the hypothalamic-pituitary-adrenal axis and glucocorticoid reception (Dranovsky and Hen, 2006). Many studies have focused on the effects of stress on cognition. Chronic stress, which can be predator related stress, is assumed to alter hippocampal structure and impairs spatial memory and learning in a maze that uses a food reward (Conrad, 2010). Brown and Braithwaite (2005) showed that fish (*Brachyrhaphis episcopi*) from a high predator environment demonstrate a decrease in cognitive abilities, solving spatial tasks at half the speed of fish from a low predation area (Brown and Braithwaite, 2005).

Although there is evidence that chronic stress impairs cognition, under certain conditions, chronically stressed rats have been shown to do as well or even better than the control rats (Conrad, 2010). Increased hippocampal neurogenesis as a result of coping with stress has also been demonstrated in squirrel monkeys (Lyons et al., 2009). Chronic stress decreases neurogenesis in squirrel monkeys (*Saimiri sciureus*), but if they are removed from the stressful scenario and allowed time to recover, their cognition improves (Lyons et al., 2009).

Predator Stress and the Brain

Predation has been identified as a strong selective force in evolution. Adaptations to this predator pressure include cryptic and aposematic coloration, chemical defenses, and protective armor (Lima and Dill, 1990). Throughout the life of an animal, the risk of being predated can change by the season, day, or even each minute (Lima and Dill, 1990). Change of risk means that behavior must vary and the brain must be plastic. This thesis seeks to identify the effects of long-term (~7 days) predator stress on brain cell proliferation and how this may affect the learning behavior of weakly electric fish.

In natural populations of the blunt-nosed electric fish, *Brachyhypopomus occidentalis*, predatory catfish (*Rhamdia quelen*) are their main predators because of the catfish's own electroreceptive abilities (Dunlap et al., 2016). Streams with large populations of *R. quelen* also have high incidence of tail injury in *B. occidentalis*. In the field, populations facing a high density of predators have lower rates of brain cell proliferation, but there was no way to determine whether the presence and activity of predators cause the decrease. The predatory stress that these catfish exert on electric fish can be experimentally recreated in the

lab through tail amputation and by tapping the tail of the electric fish using a plastic rod. In *B. occidentalis* that were captured from six different streams in the Republic of Panama, predation pressure correlated negatively with forebrain cell proliferation in natural populations of weakly electric fish (Dunlap et al., 2016). These effects were not only seen in the fish with their tail bitten off, but intact fish as well. These findings suggest that the simple action of detecting predators (non-injurious predation) can affect brain cell proliferation, not just fish that feel the direct effects of injury (injurious predation) (Dunlap et al., 2017). In the lab, we have experimental evidence in *Brachyhypopomus gauderio* that predator stimuli inhibit brain cell proliferation and elevate glucocorticoid secretions. However, this is not universal throughout all areas of the brain (Dunlap et al., 2016). The cell proliferation response to predation is specific to the forebrain (Dunlap et al., 2017). Part of this thesis examines whether experimental exposure to predator stimuli also affects *Apteronotus leptorhynchus*.

The correlation between increased predation (both direct injury and perceived threat) and decreased brain cell proliferation raises the question of whether the decreased brain cell proliferation is a cost or an adaptation (Dunlap et al., 2016). Two possible adaptive benefits of this decrease in brain cell proliferation are that it may cause 1) an increase in anxiety behaviors, such as rapid retreat and the inclination to stay hidden longer when exposed to a predator, and 2) a decrease in exploratory behaviors, behaviors which would create higher possible exposure to predators (Dunlap et al., 2016). Research by Geoffrey Keane in the Dunlap lab at Trinity College sought to answer this question by monitoring the ability of *Apteronotus leptorhynchus* to seek shelter by swimming through a hole in a plastic divider, both before and after the simulated stress of predation, through chasing. Fish exposed to

simulated predation stimuli retreated faster than control fish, providing evidence that the decrease in brain cell proliferation may be an adaptive response to predation pressure.

Predation does not always result in death. When the predation results in tail loss (or in the laboratory, experimental tail amputation), there is a question of whether the decrease in brain cell proliferation can be attributed to the stress of tail loss or if it is due to a redistribution of energy to cell proliferation in the tail at the expense of brain cell proliferation. These fish are highly regenerative, and changes in the brain following somatic injury may be due to elevated proliferative rates of cells in the tissue that must be regenerated, indicating that brain changes may be due to regeneration rather than the act of injury (Dunlap, 2016). One aim of my research is to clarify this by examining brain cell proliferation at different time points after amputation, allowing us to determine whether it is the act of injury or the subsequent regeneration that causes the observed decrease in brain cell proliferation.

Social Interaction and Brain Cell Proliferation

Many animals exhibit social behaviors, therefore studying the way in which social interaction changes the brain is important. It is already known that social isolation can adversely influence neurogenesis (Holmes, 2016). The reduction in neurogenesis is associated with increased glucocorticoid secretion as well as anxiety behaviors (Holmes, 2016). Mitra and Sapolsky (2008) examined environmental enrichment during stress and how it changes the ability of rats to discriminate contextual cues in comparison with isolated rats. They found that when chronic stress and enrichment are combined, the effects of enrichment supersede the effects of stress. The effects of a social environment have also been studied in

primates. After putting a squirrel monkey in a socially stressful situation, isolation, placing them in a new social setting with a novel partner allowed for stress coping. This resulted in an increase in hippocampal neurogenesis (Lyons et al., 2009). This experiment showed that social stress followed by a more ideal social situation may also be beneficial, rather than just keeping these animals completely out of stressful social situations.

Brain cell proliferation is greater in *B. gauderio* living freely than in captive fish and even when compared to fish living in semi natural lab conditions (Dunlap et al., 2016). In another species of electric fish, *A. leptorhynchus,* social interaction increased cell addition in the midbrain, within regions that are associated with electrocommunication (Dunlap et al., 2016). Pairing electric fish increased cell addition, specifically in the periventricular zone that lies adjacent to the pre-pacemaker nucleus. This social enhancement of cell addition coincides with an increase in chirping behavior, a type of electrocommunication. Dunlap et al. (2013) showed that after seven days of being paired the rate of cell addition increased, but at one, four, and 14 days of pairing there was no difference in brain cell proliferation.

Brain Cell Proliferation in Weakly Electric Fish

Weakly electric fish are a good study subject for investigating the link between social environment and neurogenesis (Dunlap et al., 2013). Within the electric fish brain specific regions control communication signals, and the activity of these brain regions is connected closely to the behavioral output of the fish (Dunlap et al., 2013). A region that is easily studied is the pre-pacemaker nucleus, which controls certain electrocommunication signals and is only two synapses removed from the cells that generate the communication signal (Dunlap et al., 2013). Neurogenesis can be monitored in this brain region, allowing researchers to quantify this relationship between social interaction and brain cell proliferation

(Dunlap et al., 2013). The homologues of forebrain regions to structures in the brains of other vertebrates, as described below, also make findings in electric fish brains applicable to other vertebrate taxa.

Forebrain Cell Proliferation in Electric Fish

The forebrain is particularly relevant because it contains the regions that most likely coordinate behavioral response to predators in teleost fish (Dunlap et al., 2016). Research has focused on brain cell proliferation in three sections of the forebrain, the dorsolateral telencephalon, dorsomedial telencephalon, and ventral telencephalon, because of their homology to mammalian brain structures (Figure 1).

One section of the forebrain that may participate in spatial learning and orientation is the dorsolateral telencephalon. This region is thought to be homologous to the mammalian hippocampus, a part of the brain in mammals that is also influenced by predator stimuli (Dunlap et al., 2016). Much research on adult neurogenesis in mammals has focused on the hippocampus because of it role in learning and memory, as well as other important functions (Opendak and Gould, 2015). The hippocampus and specifically the dentate gyrus, also demonstrates a large degree of structural plasticity in adulthood compared to other brain regions, as has been shown in a variety of mammalian species, including humans (Opendak

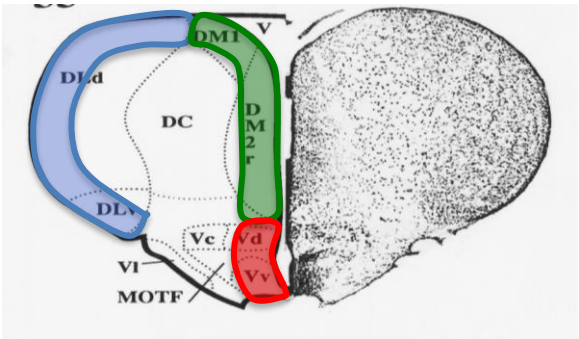


Figure 1. Transverse section of the forebrain of a weakly electric fish *Apteronotus leptorhynchus* (Dunlap et al., 2016) Blue designates the dorsolateral telencephalon, homologous to the hippocampus. Red designates the ventral telencephalon, homologous to the basal ganglia. Green designates the dorsomedial telencephalon, homologous to the amygdala.

and Gould, 2015). The dorsomedial telencephalon is postulated to be the homologue of the mammalian amygdala because of its involvement in conditioned avoidance, and the ventral telencephalon is considered to be homologous to the basal ganglia because of its involvement in selecting motor actions and evaluating their outcome (Dunlap et al., 2016).

Learning and Brain Cell Proliferation

Past studies in the Dunlap lab have observed weakly electric fish exhibiting shelter-seeking behavior. In the field, shelter limits their interactions with predators. In the lab, the fish spend most of their time within a PVC tube. Studies on optimal foraging in ungulates have shown that individuals modify their behavior in the presence of predators by using a time minimizing approach when searching for food in order to limit exposure to predators (Kie, 1999). In this thesis, shelter-seeking behavior, referred to as tube-seeking behavior, is used as a measure for spatial learning tasks.

Studies on rodents have investigated ways in which changes in neurogenesis affect spatial learning abilities. Vaneema et al. (2007) proposed that continuous brain cell proliferation allows the hippocampus to adapt more readily to challenges, such as novelty. When learning is stressful or challenging for a rodent, it can cause a decrease in brain cell proliferation (Schoenfeld and Gould, 2012). Opendak and Gould (2015) report that stress-induced decreases in brain cell proliferation in rats are associated with impaired performance on cognitive tasks that require the hippocampus, such as spatial navigation tasks and learning and object memory tasks. Conversely, running increases brain cell proliferation and neurogenesis in mice and has a positive influence on spatial navigation (Van Praag et al., 1999).

Experimental Question

In this study, I examine the effects of predator stimuli, including tail amputation, on brain cell proliferation in *A. leptorhynchus*, as well as the combined effects of predatory stress and social interaction on brain cell proliferation. I also examine whether predatory stress affects learning. This thesis seeks to determine: 1) whether predatory tail amputation causes a decrease in brain cell proliferation because of the actual action of tail amputation, or if it is due to the subsequent regeneration of the tail; 2) if social interaction influences the brain proliferative response to predator stimuli; and 3) if the predator stress of chasing affects the spatial learning abilities of *A. leptorhynchus.*

Materials and Methods

Overview

This thesis explores three questions through three separate experiments using weakly electric fish, *Apteronotus leptorhynchus*. In experiment 1, I investigate how tail amputation affects brain cell proliferation at two time points of recovery. In experiment 2, I investigate the interactive effects of social and predator stimuli on brain cell proliferation. Finally, in experiment 3, I investigate the effect of predator stimuli on spatial learning. In the first two experiments, I quantified the density of new brain cells using an immunohistochemistry protocol to label proliferating cell nuclear antigen (PCNA), a marker of proliferating cells.

All fish were obtained commercially, housed in 28°C ± 1°C water and isolated in 38-L aquaria that are part of a 1230-L circulating aquatics facility. The fish were fed brine shrimp and blackworms and were acclimated in these conditions for at least 7 d before undergoing predation stimuli and/or social pairing.

Experiment 1: Experimental Tail Amputation and Brain Cell Proliferation

In the field, the majority of predation related injuries in *Brachyhypopomus occidentalis* are in the tail region (Dunlap et al., 2016). On average, tail injury in the wild results in the loss of about 20% of the body length of the fish *B. occidentalis* (Tran, 2014). Our experimental amputation was designed to mimic this natural injury. All fish, including the control fish, were anesthetized (0.5% 2-phenoxyphenol in aquarium water) and body length was measured (mean ± SEM: 12.3 cm ± 1.8 cm, range: 10.5-14.5 cm). The tails of the experimental fish were cut with a scalpel, removing the caudal 20% of their body. Control fish were handled similarly, but their tails were left intact. All fish were returned to their tanks for recovery. The short-term recovery group was sacrificed 1 d (28-29 h) post

amputation (N=6). The long-term recovery group was sacrificed 18 d post tail amputation (N=5) and the control group was left intact and sacrificed with the long-term recovery fish. Removal and fixation of the brain was performed as described below. The brains were labeled for PCNA using the immunohistochemistry protocol described below.

Experiment 2: Social Environment and Predator Induced Changes in Cell Proliferation

To expose fish to social stimuli, fish were paired in a 38-L aquarium and placed on either side of a mesh divider. A mesh divider was used to prevent the fish from hurting each other while still allowing electric signals and other social signals to travel between fish. Before being placed into treatment groups, all 24 fish were anaesthetized (0.5% 2-phenoxyphenol in aquarium water) and weighed (mean body mass ± SEM: 2.73 ± 0.77 g, range: 1.86 – 3.94 g). The fish were then put into three different experimental groups: 1) paired fish with neither fish chased (N=6), 2) paired fish with one fish chased (N=6), and 3) isolated fish that were chased (N=6). Chasing began within 24 h after the fish were paired. Predator stress was simulated by utilizing the observation that, as stated above, the majority of predation-related injuries in *B. occidentalis* in the wild are in the tail region (Dunlap et al., 2016). Therefore, a physical stimulus was applied to the tail of the fish using a Plexiglas rod. Each predation stimulus event consisted of tapping the tail four times in one minute. There were three predation stimulus events each day, performed 1.5-3.0 h apart. The chase treatment duration was 7 d. After treatment, the brains were collected, fixed, and analyzed using the procedures described below.

Experiment 3:Simulated Predation and Spatial Learning

Predation was simulated as described above. Spatial learning tasks were performed to determine whether the stress of predator stimuli changes the ability of the fish to navigate a

spatial maze test after a change has been made. A clear Plexiglas divider with a 3 cm diameter hole divided the 38-L aquaria. The learning task consisted of four trials. In the first three trials, the shelter tube of the fish was removed from the side of the tank with the fish to the other side of the divider. In the fourth trial, the divider was rotated so that the hole in the divider was on the opposite side of the tank from where it started, requiring the fish to locate the hole in a different place. Success in a learning trial was defined as the fish making it halfway through the hole in the divider on its way to find the tube; we referred to this success as the latency to find the hole. The learning trials were performed within 1.5-3.0 h of each other. These learning trials were performed on the first and last day of the 9 d experimental period. On days two through eight, the experimental group (N= 10) underwent simulated predation, as described above and the control group (N=10) was left alone. Brain collection and fixation, as well as immunohistochemistry and analysis were performed for all of the fish.

Brain Collection and Fixation

Fish were anesthetized (0.75% 2-phenoxyphenol) and the brains were dissected and placed immediately in paraformaldehyde (4% in PBS). For fixation, the brain was kept in paraformaldehyde for 80 min at 4°C. The brains were then washed in PBS (3 x 20 min), and then transferred to a sucrose solution (25%) for cryoprotection and kept at 4°C overnight. The following day the brains were frozen in cold (-80°C) isopentane. The brains were sectioned (30 μm) using a freezing microtome, and the sections were mounted on slides and stored at 4 °C until immunolabeling.

Immunohistochemistry and Analysis

As stated above, anti-PCNA was used for immunolabeling. Slides were placed in 2N HCl at 37 °C for 30 min and then washed in borate buffer solution (0.1M, pH=8.5) (2 x 10min). The slides were moved into PBS for 1 h and then placed into a humidity chamber. Blocking solution (5% Normal Donkey Serum and 0.3% Triton X in PBS) was then applied for 1 h. The primary antibody (FL-261, Santa Cruz Biotechnology, 1:50 dilution in blocking solution) was applied overnight at room temperature in the dark. The following day, the slides were washed with PBS (3 x 20min) and the secondary antibody (Donkey anti-Rabbit, Jackson Immunoresearch, 1:300 in PBS) was applied for 2 h in the dark. The slides were washed with PBS (3 x 20min), and cover-slipped. Brain tissue was examined using a Nikon E600 epifluorescence scope at 200 X, and the density of PCNA-labeled cells was quantified in portions of the forebrain and the midbrain using the brain atlas of the electric fish, *Apteronotus leptorhynchus* (Maler et al., 1991). Within the forebrain the abundance of proliferating cells was quantified by counting unilaterally the PCNA+ cells in three forebrain regions (Dl, Dm, V) in sections corresponding to section 30-36 in the brain atlas of the electric fish, *Apteronotus leptorhynchus*. In the midbrain, the density of PCNA+ cells mm^{-3} was measured in the periventricular zone by counting within a 100 μm band in sections 17-19 in the brain atlas and dividing the count by the area of each region and the section thickness (30 μm).

Statistical Analysis

Experiment 1: The effect of predator stimuli on brain cell proliferation was determined using two-way repeated measure ANOVA with treatment (amputated vs. intact) as the independent variable, brain region (dorsolateral telencephalon, dorsomedial

telencephalon, ventral telencephalon) as the repeated measure and density of PCNA+ cells as the dependent variable using Prism 7.0 software. The overall proliferating cell density across the telencephalon was calculated and then the analysis was repeated using the telencephalon and diencephalon as brain regions.

Experiment 2: Data were analyzed using the same procedure as described in experiment 1. In this case the independent variable was the treatment brain region as the repeated measure, while the density of PCNA+ cells was the dependent variable.

Experiment 3: Repeated measure two-way ANOVA was also used. The predator stimuli treatment (chase vs. no chase) was the independent variable, time was the repeated measure and time to find and complete the learning task was the dependent variable.

Results

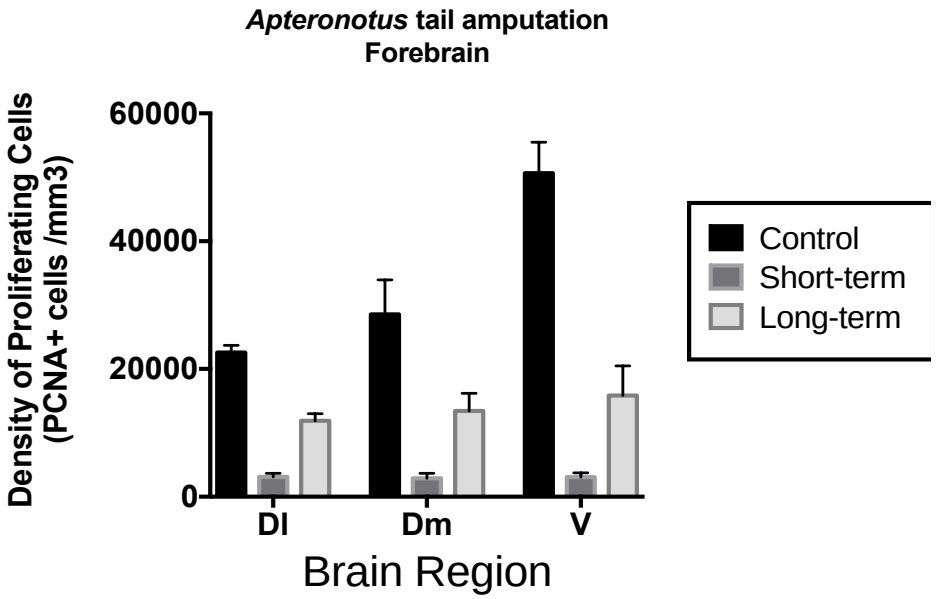
Experiment 1: Tail Amputation and Brain Cell Proliferation

In *Apteronotus leptorhynchus*, tail amputation followed by long-term recovery (18 d) significantly decreased the density of proliferating cells in the telencephalon by about one-half (Table 1 and Figure 2, $F = 4.7$, $P < 0.001$). This decrease in proliferation did not differ between the three regions of the telencephalon (Figure 2, $F = 1.5$, $P > 0.05$). The telencephalon responded significantly as a whole, but the diencephalon did not respond to tail amputation, showing that the effect is regionally specific (Table 1). In fish with tail amputation and short-term recovery (1 d), brain cell proliferation was drastically lower than in control or intact fish (Table 1). This effect occurred across all regions examined in the telencephalon and the diencephalon. Fish with long-term recovery (18 d) had cell proliferation rates lower than those of intact fish, but higher than those of fish with short-term recovery (1 d).

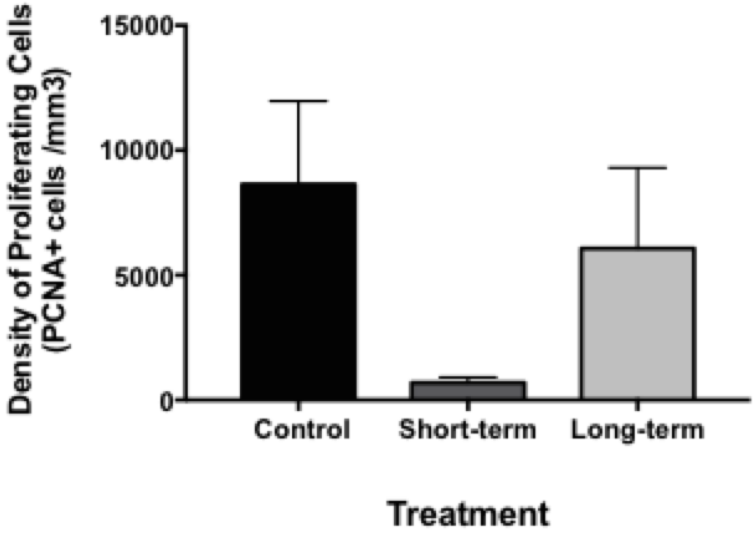
Table 1. Effect of tail amputation on brain cell proliferation in *Apteronotus leptorhynchus* The density of proliferating cell (PCNA+ cells/mm^3) was quantified in four regions of the brain, three within the telencephalon and one within the diencephalon in all treatment groups. The mean density ± SEM of proliferating cells for each treatment group is reported. Short-term is defined as 1 d recovery period post tail amputation, while long-term is defined as 18 d recovery period post amputation.

	Density of Proliferating Cells (PCNA+ cells/mm^3)			
	Telencephalon			Diencephalon
Treatment group (N)	Dorsolateral	Dorsomedial	Ventral	PVZ
Amputated-Short Term (6)	1548 ±270	1444 ± 385	1532 ± 334	684 ±213
Amputated-Long term (5)	5941 ±558	6709 ±1384	7919 ±2315	6073 ±1145
Intact (6)	11278 ±575	142740 ±2695	25325±2430	8636±1365

A



B



Figures 2 A and B. Density of proliferating cells within the three sections of the forebrain (A) and the midbrain (B) for the three treatment groups A) Density of proliferating cells within three sections of the forebrain, the dorsolateral telencephalon (Dl), the dorsomedial telencephalon (Dm), and the ventral telencephalon (V) for each treatment group. The treatment groups include the control group with tails left intact, the short-term recovery (1 d) from tail amputation group, and the long-term recovery (18 d) from amputation. B) Density of proliferating cells within the diencephalon (midbrain), specifically within the periventricular zone (PVZ) for each treatment group.

Experiment 2: Social Environment and Predator Induced Changes in Cell Proliferation

Within each experimental group, there was a significant effect of region ($F(2,36) = 3.752$, $P = 0.0331$) and treatment ($F(2,18) = 8.371$, $P = 0.0011$) on cell proliferation, but there no was no significant interaction between brain region and treatment ($F(6,36) = 0.6347$, $P = 0.7016$). This indicates the three telencephalic brain regions responded similarly to treatment (Figure 3).

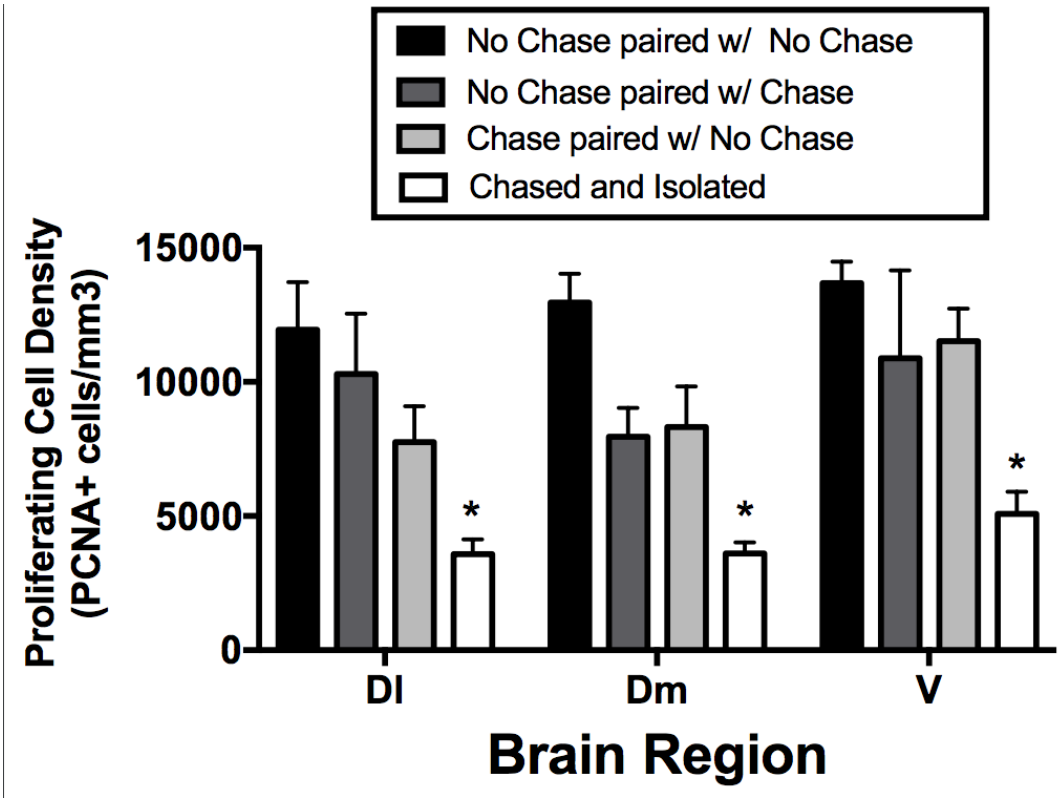


Figure 3. Proliferating cell density within three regions of the forebrain, the dorsolateral telencephalon (DL), the dorsomedial telencephalon (DM), and the ventral telencephalon (V) This figure demonstrates that there is not a regional specific difference in proliferating cell density within the forebrain in each treatment group. The error bars indicate standard error.

Using a Tukey's multiple comparisons test post hoc, the results showed that cell density in the chase isolated group was significantly less than in all other groups (chase isolated vs. no chase paired with no chase: $P = 0.0006$; chase isolated vs. chase paired with no chase:

P=0.0486; chase isolated vs. no chase paired with chase: P = 0.0198), but no other significant differences were seen between other treatment groups (Figure 4).

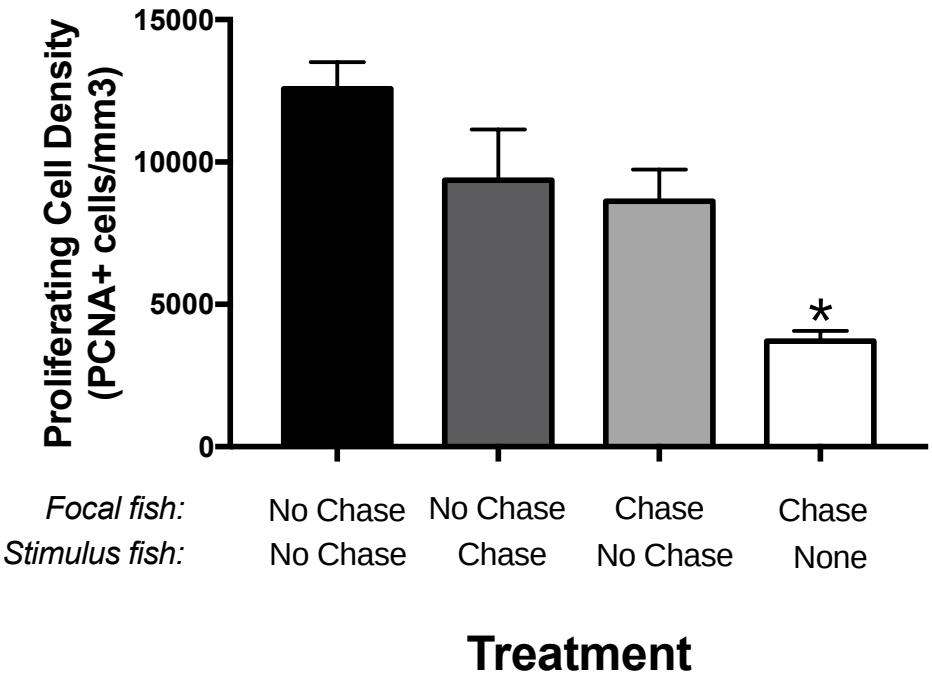


Figure 4. Proliferating cell density within the forebrain for each treatment group The focal fish refers to the fish for which the data are being graphed and the stimulus fish is the condition of the partner that it had while living in the tank. The isolated chase group has no stimulus fish. The error bars indicate standard error. Asterisk indicates significant difference from all other groups.

Experiment 3:Simulated Predation and Spatial Learning Observation

Simulated predation in the form of chasing did not change spatial learning abilities. There was no effect of treatment ($F(1,144) = 2.715$, $P = 0.1016$), no effect of time ($F(7,144) = 1.721$, $P = 0.1085$), and no interactive effect ($F(7,144) = 0.4255$, $P = 0.8851$). In addition, there was no significant difference between the control fish and the fish that underwent simulated predation in the latency to find the hole when the hole was switched post treatment (Figure 5).

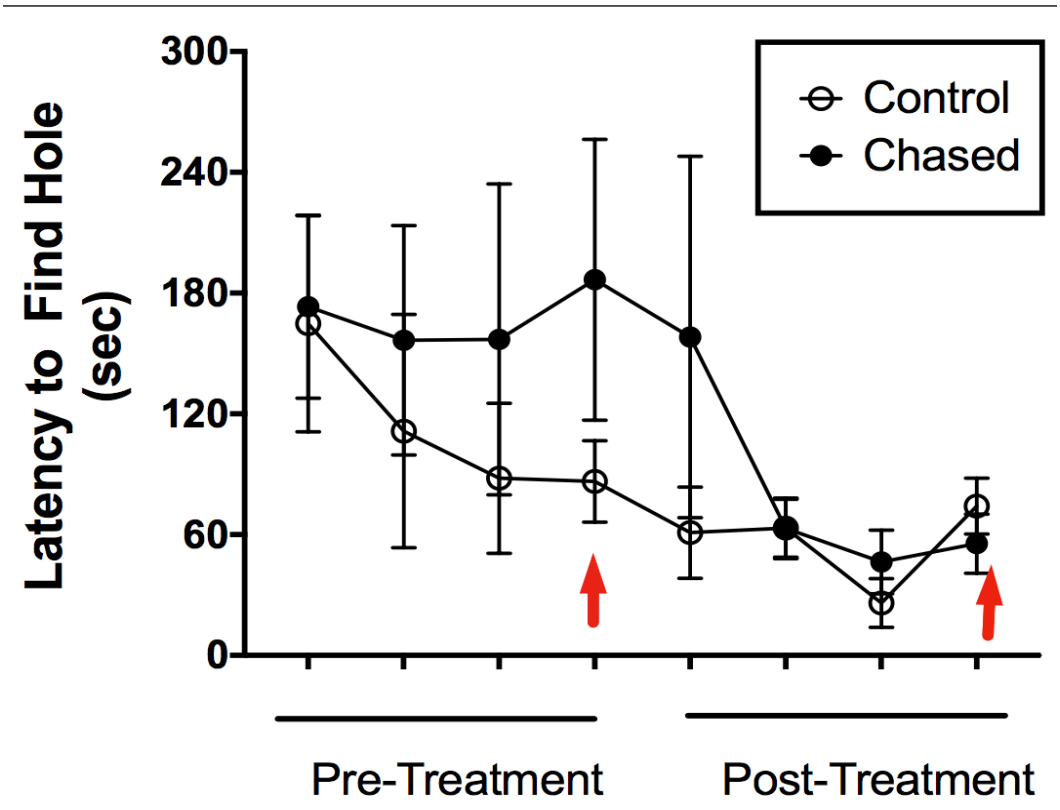


Figure 5. Latency to find the hole in control fish and fish that have undergone simulated predation before and after simulated predation treatment The first three data points for both the control and chased fish show the average latency to find the hole on the first day of the experiment, before any simulated predation occurred. The fourth data points show the latency to find the hole after the hole was switched from the original location. The fifth through seventh data points for each treatment group show the latency to find the hole after 7 d of treatment. The eighth data point shows the latency to find the hole after the hole was moved from its original location. The arrows indicate when the hole has been moved from its original location. The error bars represent standard error.

Discussion

Experiment 1: Tail Amputation and Brain Cell Proliferation

We found that *Apteronotus leptorhynchus* showed a significant decrease in brain cell proliferation after the short term recovery compared to fish that had not been amputated and fish with amputation and long term recovery. In earlier studies, Dunlap et al. (2017) determined that in another species of weakly electric fish, *Brachyhypopomus occidentalis*, brain cell proliferation decreased in response to tail amputation. They hypothesized that the decrease in brain cell proliferation following tail amputation is due to the regenerative processes of the tail, since tail regeneration is higher during the recovery period than immediately after amputation. This hypothesis predicts that the long-term recovery group would show the greatest decrease in brain cell proliferation (Dunlap, 2016). However, my results indicate that the acute stress of tail amputation causes the greatest decrease in brain cell proliferation, rather than the regenerative processes in the tail. I found that the fish that had a 1 d recovery period showed ~85-95% lower proliferating cell density compared to intact fish, while fish that had an 18 d recovery period had ~50% decrease. Moreover, I observed the decrease in brain cell proliferation both across the telencephalon, and within the diencephalon in the fish that were allowed a 1 d recovery, while I only saw a decrease across the telencephalon, and not within the diencephalon in fish that were allowed an 18 d recovery period. Thus, the duration of the recovery period influences both the quantity and distribution of cell proliferation.

Experiment 2: Social Environment and Predator-Induced Changes in Cell Proliferation

Forebrain cell proliferation in *B. occidentalis* correlates negatively with exposure to predation both within the lab and in the wild (Dunlap et al., 2016; Dunlap et al., in press).

The effects of predation on brain cell proliferation have been observed both when the fish experience tail injury from predators, as well as when they simply live among abundant predators but have no injury (Dunlap et al., 2017; Dunlap et al., 2016). In contrast, in *A. leptorhynchus*, brain cell proliferation is enhanced in fish living in a paired social environment (Dunlap et al., 2016). This increased cell addition has been attributed to electrocommunication, because the increase in cell proliferation is observed in the part of the midbrain region that is closely associated with electrocommunication (Dunlap et al., 2013). A study on rats showed that the increased brain cell proliferation caused by social interaction mitigated the effects of stress (Mitra and Sapolsky, 2008). One objective of my thesis was to determine whether the positive effects of social interaction similarly mitigate the negative effects of predator stimuli.

All treatment groups with paired fish showed no significant difference in proliferating cell density. Comparing the paired non-chased group with the non-chased paired with chased group shows that living around a chased partner is no different than living around a non-chased partner. Thus, when paired, the fish do not experience any direct or indirect effects of predators. However isolated, chased fish had cell proliferation rates significantly lower than all paired fish. Comparing the chased fish living with and without a partner shows that living with an unstressed partner abolishes the negative effect of chasing. Thus they experience an indirect positive effect. These data suggest that social interaction mitigates the deleterious effects of predator stimuli on brain cell proliferation. However, due to the small sample size, additional trials of this experiment are needed to further validate my results and possibly discover more nuanced differences between the paired treatment groups.

The phenomenon of social interaction mitigating the deleterious effects of stressors, such as predator stimuli, on brain cell proliferation has been noted in other studies. Cherng et al. (2010) used an intense mixed stress paradigm, which included stressors such as foot shocks on adult male mice. When a mouse was exposed to this paradigm while in the presence of a familiar or unfamiliar male mouse, the stress effects on neurogenesis were prevented (Cherng et al., 2010). Conversely, in another study on rodents, the absence of social interaction negated or delayed the positive effects of exercise on neurogenesis (Stranahan et al., 2006).

In a review of social regulation of adult neurogenesis, Holmes (2016) developed a hypothesis for such "social buffering." He posited that social buffering prevents the actions of glucocorticoids that are stress induced, thereby preventing associated decreases in Brain Derived Neurotrophic Factor and Nerve Growth Factor in the dentate gyrus of adult mice (Holmes, 2016). The concept of social buffering may explain the lack of a decrease in brain cell proliferation in fish that are socially paired while under the stress of predator stimuli.

Experiment 3: Simulated Predation and Spatial Learning Observation

The effect of stress on cognition has been studied in other animals, such as rats (Conrad, 2010). Chronic stress alters hippocampal structure, and rats that have undergone chronic stress have impaired spatial memory on a learning maze (Conrad, 2010). Similarly a tropical poeciliid fish (*Brachyrhaphis episcopi*) from a high predation environment demonstrates decreased cognitive abilities as compared to conspecifics from a low predation environment (Brown and Braithwaite, 2005). These differences in cognitive ability are drastic as the fish from high predation sites solved spatial learning tasks at about half the speed of fish from a low predator environment (Brown and Braithwaite, 2005). In previous

studies in the Dunlap lab, we determined that the predator stimuli (i.e., chasing) caused a decrease in brain cell proliferation, specifically within the dorsolateral telencephalon, which is homologous to the mammalian hippocampus, the part of the brain that is used for learning. I hypothesized that brain cell proliferation would influence the spatial learning ability of the weakly electric fish *Apteronotus leptorhynchus*, and I predicted that chased fish would require more time to learn the new hole position. However, compared to control fish, the fish that experienced predator stimuli over 7 d prior to the learning task showed no significant decrement in the latency to find the hole, and ultimately locating shelter. Although it has been determined that simulated predation in the form of chasing decreases brain cell proliferation within the dorsolateral telencephalon, this study provided no evidence that chasing affects the spatial learning behavior of the fish.

Future Research

Based on the tail amputation study and the spatial learning study, future research could address how predator induced injury affects the spatial learning ability of weakly electric fish. Previous studies (Dunlap et al., in press) showed that non-injurious predation stimuli, like chasing, does not increase cortisol levels but does decrease brain cell proliferation, while injurious predation stimuli, like tail amputation, caused an increase in cortisol levels and a decrease in brain cell proliferation. The preliminary results of my study using non-injurious predation stimuli showed no effect on the spatial learning abilities of the fish. Brandão et al. (2015) found that cichlids (*Cichlasoma paranaense*) it was found that fish in isolation (an environment that increases the fish's cortisol level) exhibited a lower ability to learn the correct route on a spatial learning task compared to fish living socially. To determine whether the increase in cortisol is what is affecting spatial learning abilities rather

than the decrease in brain cell proliferation, fish that have undergone tail amputation could be given the same learning task that was used in this thesis.

Another valuable project would be to quantify the times that fish return to the original location of the hole when running the spatial learning task. While measuring the latency to find the hole, I noticed that chased fish were more likely to return to the original position of the hole. This is consistent with research by Geoffrey Keane at Trinity College. In his thesis, he found that predator stimuli increased learning speed for a spatial task, which was almost identical to the one in my experiment, but the hole was never relocated. He hypothesized that this increase in learning speed may be due to increased motivation to find shelter or to the preservation of neural pathways that have previously encoded the pathway to shelter (Keane 2016). Recording the return to the location of the original hole may provide further insight into the way in which the decrease in brain cell proliferation affects behavior, and the process by which it affects the spatial learning ability of the fish.

In addition to looking at the behavior of returning to the hole, future studies could develop ways to record and quantify different behavioral responses to predator stimuli. When performing the simulated predation, I observed that each fish reacted differently immediately after experiencing the predator stimulus of chasing. For example, one fish moved to one side of the tank and made circles against the wall of the tank throughout the chasing procedure. By keeping track of the variety of reactions of the fish to predator stimuli, it can be determined if their behavioral response affects their spatial learning abilities in different ways. This also may allow us to determine if fish that respond in an extreme manner differ in spatial learning abilities after subjected to predator stress.

Conclusion

The effects of predator stimuli, both injurious and non-injurious, and social interaction on brain cell proliferation and spatial learning were evaluated through three separate experiments. In the first experiment, I concluded that the action of amputating the tail of *A. leptorhynchus* causes a significant decrease in brain cell proliferation. A drastic decrease in the density of proliferating cells was observed in the brain of the fish in the short-term recovery (1 d) as compared to the brains of the fish that were allowed a long-term recovery (18 d) and the intact fish. In the second experiment, I found that social interaction might mitigate the negative effects of predator stimuli on brain cell proliferation. Finally, in the third experiment, I found that chasing, a non-injurious predator stimulus, does not affect the spatial learning abilities of *A. leptorhynchus*.

Literature Cited

Altman J and Das GD. 1965. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. Journal of Comparative Neurology. 124(3): 319-35.

Amrein I, Dechmann DKN, Winter Y, and Lipp HP. 2007. Absent or low rate of adult neurogenesis in the hippocampus of bats (Chiroptera). PloS. 2(5), e455.

Brandão ML, Braithwaite VA, and Gonçalves-de-Freitas E. 2015. Isolation impairs cognition in a social fish. Applied Animal Behavioral Science. 171: 204-10.

Brown C and Braithwaite VA. 2005. Effects of predation pressure on the cognitive ability of the poeciliid *Brachyraphis episcopi*. Behavioral Ecology. 16(2): 482-87.

Conrad, CD. 2010. A critical review of chronic stress effects on spatial learning and memory. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 34(5): 742-55.

Cherng CG, Lin PS, Chuang JY, Chang WT, Lee YS, Kao GS, Lai YT, and Yu L. 2010. Presence of conspecifics and their odor impregnated objects reverse stress decreased neurogenesis in mouse dentate gyrus. Journal of Neurochemistry. 112: 1138-46.

Dranovsky A and Hen R. 2006. Hippocampal neurogenesis: regulation by stress and antidepressants. Biological Psychiatry. 59: 1136-43.

Dunlap KD. 2016. Fish neurogenesis in context: assessing environmental influences on brain plasticity within a highly labile physiology and morphology. Brain, Behavior and Evolution. 87(3): 156-66.

Dunlap KD, Chung M, and Castellano JF. 2013. Influence of long term social interaction on chirping behavior, steroid levels, and neurogenesis in weakly electric fish. Journal of Experimental Biology. 216: 2434-41.

Dunlap KD, Silva AC, and Chung M. 2011. Environmental complexity, seasonality and brain cell proliferation in a weakly electric fish, *Brachyhypopomus gauderio*. Journal of Experimental Biology. 214: 794-805.

Dunlap KD, Silva AC, Smith GT and Zakon HH. 2017. Weakly electric fish: Behavior, neurobiology and neuroendocrinology. In: *Hormones, Brain and Behavior*, D.W.Pfaff, and M. Joels, eds. 3rd edition. Oxford: Academic Press. 69-98.

Dunlap KD, Tran A, Ragazzi MA, Krahe R, and Salazar VL. 2016. Predators inhibit brain cell proliferation in natural populations of electric fish, *Brachyhypopomus occidentalis*. Processdings of the Royal Society B. 283: 20152113.

Dunlap KD, Keane G, Ragazzi M, Lasky E, and Salazar V. In press. Simulated predator stimuli reduce brain cell proliferation in two electric fish species, *Brachyhypopomus gauderio* and *Apteronotus leptorhynchus*. Journal of Experimental Biology.

Gould E, Beylin A, Tanapat P, Reeves A, and Shors TJ. 1999. Learning enhances adult neurogenesis in the hippocampal formation. Nature Neuroscience. 2: 260-65.

Holmes MM. 2016. Social regulation of adult neurogenesis: A comparative approach. Frontiers in Neuroendocrinology. 41: 59-70.

Keane G. 2016. Simulated predation, brain cell proliferation, and spatial learning in weakly electric fish. Unpublished honors thesis. Trinity College, Hartford, Connecticut.

Kempermann G and Gage FH. 1999. Experience-dependent regulation of adult hippocampal neurogenesis: effects of long-term stimulation and stimulus withdrawal. Hippocampus. 9: 321-32.

Kie JG. 1999. Optimal foraging and risk of predation effects on behavior and social structure in ungulates. Journal of Mammalogy. 80(4): 1114-29.

LaDage L. 2015. Environmental change, the stress response, and neurogenesis. Integrative and Comparative Biology. 55(3): 372-83.

Lima SL and Dill LM. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology. 68(4): 619-40.

Lyons DM, Buckmaster PS, Lee AG, Wu C, Mitra R, Duffey LM, Buckmaster CL, Her S, Patel PD, and Schatzberg AF. 2009. Stress coping stimulates hippocampal neurogenesis in adult monkeys. Proceedings of the National Academy of Sciences of the United States of America. 107(33): 14823-14826.

Mitra R and Sapolsky RM. 2008. Effects of enrichment predominate over those of chronic stress on fear-related behavior in male rats. Stress. 12(4): 305-12.

Opendak M and Gould E. 2015. Adult neurogenesis: a substrate for experience dependent change. Trends in Cognitive Sciences. 19(3): 151-61.

Schoenfeld TJ and Gould E. 2012. Stress, stress hormones, and adult neurogenesis. Experimental Neurology. 233: 12-21.

Stranahan AM, Khalil D, and Gould E. 2006. Social isolation delays the positive effects of running on adult neurogenesis. Nature Neuroscience. 9(4): 526-33.

Tanapat P, Hastings NB, Rydel TA, Galea LAM, and Gould E. 2001. Exposure to fox odor inhibits cell proliferation in the hippocampus of adult rats via adrenal hormone-dependent mechanism. Journal of Comparative Neurology. 437:496-504.

Tran, A. 2014. The effects of predation on electric fish signals. Masters Thesis, McGill University, Montreal, Canada

Vaneema AH, de Kloet ER, de Wilde MC, Roelofs AJ, Kawata M, Buwalda B, Neumann ID, Koolhaas JM, and Lucassen PJ. Differential effects of stress on adult hippocampal cell proliferation in low and high aggressive mice. 2007. Journal of Neuroendocrinology. 19: 489-98.

van Praag H, Christie BR, Sejnowski TJ and Gage FH. 1999. Running enhances neurogenesis, learning, and long term potentiation in mice. Proceedings of the National Academy of Sciences of the United States of America. 96(23): 13427-31.