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### **Social buffering of brain cell proliferation and behavioral responses to tail injury in weakly electric fish, *Apteronotus leptorhynchus***

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SOCIAL BUFFERING OF BRAIN CELL PROLIFERATION AND BEHAVIORAL RESPONSES TO TAIL INJURY IN  
WEAKLY ELECTRIC FISH, *APTERONOTUS LEPTORHYNCHUS*

BY

MARGARITA M. VERGARA

A THESIS SUBMITTED TO  
THE FACULTY OF THE DEPARTMENT OF BIOLOGY  
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SOCIAL BUFFERING OF BRAIN CELL PROLIFERATION AND BEHAVIORAL RESPONSES TO  
TAIL INJURY IN WEAKLY ELECTRIC FISH, *APTERONOTUS LEPTORHYNCHUS*

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## Abstract

Social interactions can mitigate the damaging effects of threatening stimuli, a phenomenon termed ‘social buffering’. In two different forms of social buffering, social interactions reduce stress-induced decreases in brain cell proliferation and enhance recovery from somatic injury. However, the positive effects of social interactions on the brain cell proliferation response to somatic injury have not been extensively examined. Here, I investigated the social buffering of the brain cell proliferation response to tail injury in an electric fish, *Apteronotus leptorhynchus*. I ask three major questions: 1) Does social interaction mitigate the decrease in brain cell proliferation caused by simulated predatory tail injury?; 2) Does the timing of social interaction relative to injury alter this social buffering response?; and 3) Does tail injury modify affiliation with a non-injured social partner? I mimicked predatory injury through experimental tail amputation, exposed fish to paired interactions that varied in timing, duration, and recovery period, and measured cell proliferation (PCNA+ cell density) in the forebrain and midbrain. I also measured social affiliation based on the position of fish in retreat sites located near or distant to a stimulus fish. Social interaction either before or after tail amputation mitigated the negative effects of tail injury on brain cell proliferation. This buffering effect was specific to the forebrain and occurred after short-term (1 d) or long-term (7 d) recovery periods following tail amputation. However, social interaction both before (4 d) and after (7 d) tail amputation produced an even greater buffering effect in localized regions of the forebrain and midbrain. Similarly, fish exposed to social interaction both before and after tail amputation sought close affiliation with non-injured stimulus fish, but this effect did not occur in fish exposed to social interaction only after injury. Thus, despite the social buffering response on brain cell proliferation, it remains unclear whether fish modify their affiliation behavior in response to tail injury.

## Introduction

### *The Study of Social Buffering*

Social interactions have important influences on both physiological and behavioral functions in most animals. For example, in humans, social connectedness is positively correlated with immune function and cardiovascular health, whereas social isolation can act as a psychosocial stressor, inducing a risk for depression and anxiety-related disorders (Lieberwirth and Wang, 2012). Among other animals, social interactions can promote the production of new neurons (neurogenesis), and grouping behaviors can provide protection from environmental threats (Kikusui et al., 2006; Holmes, 2016). In addition, a few mammalian models of stress and cutaneous wounds have demonstrated that living with a social partner reduces stress following injury and enhances wound healing relative to living alone (DeVries et al., 2007). However, the positive effects of social interaction on both the neurogenic and behavioral responses to body injury have not been extensively examined. Here, I investigate the relationship between social interaction, simulated predatory injury, and neurogenesis in weakly electric fish, *Apteronotus leptorhynchus*.

In highly gregarious animals, the presence of conspecifics ameliorates the neural or behavioral responses to a perceived threat— a phenomenon termed “social buffering” (Kiyokawa, 2018; Kikusui et al., 2006). In 1955, Davitz and Mason first proposed a social buffering effect after discovering evidence that the presence of a non-fearful rat reduced the strength of the fearful response (immobility) experienced by other rats in a novel open field. More than 30 years later, Wiener et al. (1987) described the first case of social buffering in non-human primates after examining the positive effects of social interaction on the stress response of infant squirrel monkeys to brief disruptions in mother-infant bonding. Their

results showed that exposure to familiar colony members downregulated the plasma cortisol levels of infant monkeys after they were separated from their mothers (cited in Kikusui et al., 2006). Following these revolutionary studies, social buffering phenomena have been further documented in a broad range of species, including sheep (Lyons et al., 1993), guinea pigs (Hennessy et al., 2008), common marmosets (Galvao-Coelho et al., 2012; cited in Kiyokawa, 2018), and zebrafish (Faustino et al., 2017).

Furthermore, several studies have noted that social buffering effects can also be influenced by the familiarity and number of social partners. In adult squirrel monkeys, the presence of multiple familiar companions, rather than a single familiar partner, reduces stress after exposure to fear-conditioned stimuli (Stanton et al., 1985). Moreover, studies in rats have shown that a familiar partner is more effective than an unfamiliar partner at reducing behavioral responses to stress (Terranova et al., 1999) and that social buffering effects are further enhanced by an increasing number of partners (Kiyokawa et al., 2018). However, other controversial studies in rats and zebrafish have documented that social buffering is independent of partner familiarity (Cirulli et al., 1996) and group size (Faustino et al., 2017). Thus, when examining social buffering, it is important to understand that its effects may vary according to the species involved, their social history, and the social conditions to which they are subjected during experimental threat exposure (Kikusui et al., 2006).

Social buffering has been commonly examined in two types of experimental conditions based on the presence or absence of a partner during threat exposure. In “housing-type” social buffering, the subject is first exposed to a threatening stimulus while isolated and co-housed with a conspecific (Kiyokawa, 2018). In “exposure-type” social buffering, the subject is exposed to a threatening stimulus in the presence of a conspecific (Kiyokawa,

2018). Most studies to date have been conducted in the laboratory and have focused on exposure-type social buffering (Kiyokawa, 2018). However, examining the effects of social interaction at the time of exposure to threat ignores another potential social influence in real-world settings. In natural populations, individuals are usually not identified as “social” or “isolated” until after their exposure to injury (Venna et al., 2014); therefore, it is also advantageous to examine the effects of social interactions in the recovery following adverse situations. In support of this argument, Venna et al. (2014) showed that mice housed with a healthy partner after stroke showed improved functional recovery and increased neurogenesis compared with mice housed alone or mice housed with a stroke partner. In contrast, a few studies on amphibians and mammals have indicated that social interactions prior to threat exposure can also improve behavioral recovery from aversive events by increasing individual risk-taking behavior and activity (Urszán et al., 2015) or lowering individual fear responses (Siviy, 2008). Together, these studies show that social interactions can buffer behavioral responses to threat and neurogenic responses to brain injury, but the timing mechanism and overall effect of social interactions on the behavioral and neurogenic responses to injury outside the brain (somatic injury) are still unclear.

### *Social Buffering in Threat Perception and Behavioral Recovery*

Animals can use information provided by others to assess the presence of threat in the environment and alter their behavior accordingly (Oliveira and Faustino, 2017). Specifically, alarm responses to environmental threat can be influenced by three different social phenomena: social buffering, social transmission (contagion), and social facilitation (Oliveira and Faustino, 2017). In social transmission, individuals use social cues such as alarm calls (e.g. in birds and mammals) to signal the presence of a threat and trigger an alarm response,



even when they have not perceived a threat directly. Social facilitation occurs when the behavior of a group influences the decision made by an individual based on direct information indicating the presence or absence of a threat. Here, I focus on cues used in the context of social buffering— when the presence of calm conspecifics in the environment contradicts the threat detected by an individual and thereby diminishes its alarm response (Oliveira and Faustino, 2017). Specifically, I house injured electric fish with intact (“calm”) conspecifics to investigate the social buffering of the brain cell proliferation and behavioral responses to experimental tail amputation, mimicking predatory tail injury in the field.

Research in mammals has suggested that social buffering of behavioral responses to threat exposure correlates with increases in social affiliation with conspecifics. Taylor (1981) found that rats exposed to a stressful noise in groups showed less freezing behavior relative to isolated rats, and stressed rats were also more likely to interact with another rat in a T-maze compared to non-stressed rats. This increase in social affiliation was observed in rats that had been reared either individually or in groups, indicating that stressed animals may choose to interact with conspecifics to possibly mitigate the negative effects of threat exposure (Kikusui et al., 2006). Similar effects have been recorded in human subjects who show increased attraction for a partner during the shared stressful experience of receiving moderately painful electric shocks (Latané et al., 1966). In this thesis, I investigate whether individual electric fish, *A. leptorhynchus*, seek affiliation with a single social partner as a potential behavioral response to minimize the inhibitory effects of experimental tail injury on brain cell proliferation (Dunlap et al., 2017).

Overall, I seek to answer three different questions of social buffering in *A. leptorhynchus*: 1) Does the presence of conspecifics mitigate the negative effect of simulated

predator-induced tail injury on brain cell proliferation?; 2) Does the timing of social interaction relative to injury influence the social buffering of cell proliferation?; and 3) Does the proliferative response to social interaction in injured fish correlate with choices in social affiliation?

### *Social Buffering of Stress-induced Decreases in Neurogenesis*

Until the groundbreaking work by Joseph Altman in the 1960s, the birth of new neurons in the brain of adult vertebrates was considered impossible (Holmes, 2016). This process, known as adult neurogenesis, has received major attention in recent decades due to its potential therapeutic role in treating neurological deficits (Holmes, 2016). It was first documented in oscine songbirds (Nottebohm, 2002), and it is now clear that the adult mammalian brain includes highly neurogenic niches, such as the subventricular zone/olfactory bulb system and the dentate gyrus of the hippocampus (Holmes, 2016), that are known to respond to social buffering stimuli.

The presence of conspecifics buffers against decreases in neurogenesis caused by social isolation and other non-social stressors (Holmes, 2016). Social buffering acts by suppressing stress-induced increases in glucocorticoids resulting from the activation of the hypothalamic-pituitary-adrenal (HPA) axis —the major neuroendocrine system controlling stress (Holmes, 2016; Kiyokawa, 2018). In particular, social buffering reverses stress-induced decreases in neurogenesis by possibly restoring the levels of brain-derived neurotrophic factor (BDNF) or nerve growth factor (NGF) in the hippocampus of adult mammalian brains (Tzeng et al., 2013). For example, Tzeng et al. (2018) showed that, compared to solitary mice, paired mice exposed to a stressor avoided stress-induced decreases in neurogenesis in the hippocampal dentate gyrus by stimulating local BDNF

expression. Similarly, positive relationships and stable social networks can lower the activity of the HPA axis in response to a novel environment or other behavioral and physiological responses to fearful stimuli (Hennessy et al., 2009; Kiyokawa, 2018). Terranova et al. (1999), for instance, revealed that pairing juvenile rats with a same-sex conspecific during exposure to a novel environment significantly suppressed their HPA axis response relative to isolated individuals confronted with novelty. Furthermore, while most studies on the neurophysiological mechanisms of social buffering have focused on mammals, Edgar et al. (2015) reported the first findings in birds after observing that the presence of avian mothers can reduce their chicks' stress response to an aversive air puff.

Social buffering also modifies the neural activity of other brain regions associated with stress responses (the hypothalamic paraventricular nucleus or PVN) and fear conditioning (the central and lateral amygdala) [Kikusui et al., 2006; Kiyokawa et al., 2007]. In a study on sheep (da Costa et al. 2004), the visual presentation of familiar sheep facial pictures to socially isolated sheep significantly lowered the expression of c-Fos— a marker of neural activity— in the hypothalamic PVN and central amygdala. Simultaneously, these social stimuli reduced their behavioral (activity and protest vocalizations), autonomic (heart rate), and endocrine (cortisol and adrenaline) stress responses. As a more direct measure of neural activity, Fuzzo et al. (2015) used neurophysiological methods to measure the local electrical activity in the lateral amygdala of male rats during social buffering. They showed that, compared to isolated rats, paired rats exposed to an aversive conditioned stimulus had decreased auditory evoked field potentials, gamma oscillations, and high-frequency oscillations in the lateral amygdala. Moreover, these neural responses correlated positively with reduced duration of freezing behavior (fear response) in social rats. Thus, this study

indicated that the social buffering of conditioned fear responses could occur by suppressing the fear-induced activation of the lateral amygdala.

Neurogenesis involves the processes of brain cell proliferation and neuronal differentiation (Ohnuma and Harris, 2003). This thesis centers on brain cell proliferation, which refers to the birth of brain cells (Ohnuma and Harris, 2003). Brain cell proliferation is often enhanced in enriched social conditions or inhibited by environmental stressors like predatory stimuli (Dunlap, 2016a). Here, I examine the influence of social buffering on brain regions that are likely associated with neurogenic and behavioral responses to social exposure and predatory stimuli in weakly electric fish, *A. leptorhynchus*.

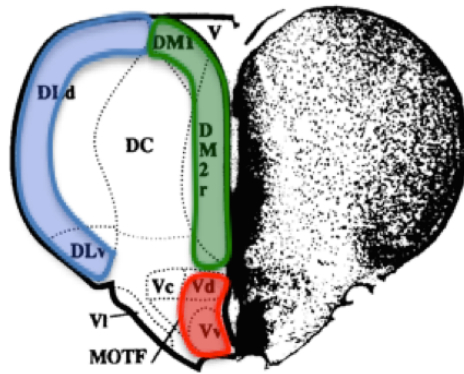
#### *Social Buffering of Predation (Behavioral and Neural Responses in Mammals)*

Animals are naturally exposed to the risk of predator-induced injury and, in turn, predators can modify the fear expression (Siviy, 2008), social behavior (Bowen et al., 2013), and cellular processes that shape the brain structure of their prey (Walsh et al., 2016; Dunlap et al., 2017). Nonetheless, several studies in mammals have demonstrated that social buffering can ameliorate both behavioral and neural responses to simulated predatory stimuli. Siviy (2008) revealed that rats exposed to predator odor in the presence of an unfamiliar partner were less fearful than those tested alone, which extends earlier findings by Davitz and Mason (1955) showing the occurrence of social buffering when the fear-eliciting stimulus is a predator. Moreover, Siviy (2008) also examined the effects of early social experience on the fearful response of rats to predator odor. In this case, rats were allowed to play repeatedly before being isolated and exposed to a worn cat collar. The results showed that rats that had previously played before exposure to cat odor did not differ significantly in their fearful

response compared with those that lacked prior social interaction. Therefore, this study suggested that the timing of social interaction relative to predatory exposure plays an important role in the social buffering of behavioral responses to threat.

Furthermore, Bowen et al. (2013) assessed the behavioral correlates of the social buffering of predation in brown rats (*Rattus norvegicus*). Rats exposed to cat odor in groups of four showed reduced inhibition of grooming, locomotor activity, and stimulus contacts compared with those exposed to cat odor while isolated. This behavioral response indicated that the social buffering of predation might be associated with a “many-eyes effect,” as individuals benefit from the vigilance of others in the group. When relying on a group for detecting predators, individuals can reduce their own vigilance effort and increase their time spent in non-threat related tasks essential for survival (Bowen et al., 2013).

Bowen et al. (2013) also investigated the neural mechanisms underlying this social buffering of predation. Compared to isolated rats, rats that were socially exposed to cat odor had lower neural activity (reduced c-Fos expression) in hypothalamic and limbic regions associated with stress and defensive responses to predatory threat: the medial caudate putamen (CPuM), lateral preoptic nucleus (LPO), lateral amygdala (LA<sub>mg</sub>), dorsomedial periaqueductal grey (DMPAG), and lateral habenula (LHb). The LHb regulates the motor aspects of the defensive response to threat by connecting the DMPAG to limbic regions, such as the LPO, and to parts of the basal ganglia, such as the CPuM (Bowen et al., 2013). Finally, these results are particularly relevant to this thesis because social buffering influenced brain structures that represent the mammalian homologs to specific regions of the forebrain in teleost fish (Figure 1).



**Figure 1. Transverse section of the forebrain of weakly electric fish, *Apteronotus leptorhynchus* (Maler et al., 1991).** Blue designates the dorsolateral telencephalon, homologous to the mammalian hippocampus. Green designates the dorsomedial telencephalon, homologous to the mammalian amygdala. Red designates the ventral telencephalon, homologous to the mammalian basal ganglia.

#### *Social Buffering of Somatic Injury (Studies in Mammals)*

Extensive research in mammals describes the positive effects of social interactions on neural and behavioral responses to predatory stimuli, but little is known about these effects in relation to body (somatic) injury. Only a few experimental models and clinical studies have provided support for a positive relationship between social interactions and recovery from somatic injury. Detillion et al. (2004) adapted a mouse model of wounding and stress in Siberian hamsters (*Phodopus sungorus*) to determine if social relationships could facilitate the wound healing process. Their results showed that paired hamsters had reduced stress and improved wound healing compared with socially isolated individuals. Moreover, Mitchell et al. (2014) reported that injured athletes who received social support in their recovery period benefited from diminished behavioral responses to sports injury (e.g. restlessness and isolation) as opposed to those who lacked social support. Taken together, these studies suggest that social interactions can promote wound healing and buffer both physiological and behavioral responses to somatic injury.

This thesis does not examine the direct effects of social interaction in recovery from injury. Instead, I examine the effect of social buffering on brain cell proliferation and behavioral responses to simulated predatory tail amputation in *A. leptorhynchus*. My main goal is to analyze brain cell proliferation and social affiliation at different time points relative to amputation to answer the following questions: 1) Does social interaction mitigate the decrease in brain cell proliferation caused by tail amputation? 2) Does *pre-* or *post-* amputation social interaction buffer the brain cell proliferation response to predatory stimuli? 3) Does tail amputation increase social affiliation with a healthy (non-injured) social partner? Answers to these questions will allow me to expand our understanding of the interaction between social buffering, somatic injury, and predation.

### *Social Interaction and Social Buffering in Fish: Neural and Behavioral Correlates*

Compared to mammals, fish have significantly higher rates of brain cell proliferation and neurogenesis during adulthood (Dunlap, 2016b). For example, the brain of weakly electric fish, *A. leptorhynchus*, can generate new cells at rates from 10 to 100 times greater than the brain of rodents (cited in Dunlap, 2016b). In contrast to mammals, most fish are ectotherms that grow continuously during adulthood, and some species have a capacity for sex change and somatic regeneration, all of which can cause extensive changes in cell proliferation (Dunlap, 2016b). This intrinsic dynamic physiology and morphology allow fish to respond to their surroundings through processes that influence brain cell proliferation (e.g. changes in body temperature and growth rate) but are not linked to specific behavioral responses to environmental conditions (Dunlap, 2016b). Therefore, it is essential to distinguish specific effects in behaviorally important brain regions from global changes on

cell proliferation across the entire brain or body (Dunlap, 2016b).

Specific brain regions regulating social behavior in fish have received attention in recent years. For instance, Cabrera-Álvarez et al. (2017) examined the effects of social exposure on the brains of wild-type guppies (*Poecilia reticulata*). They investigated the shoal-elicited activation of four brain regions involved in processing social information and reward: the preoptic area (POA), the dorsal part of the ventral telencephalon (Vd), the ventral part of the ventral telencephalon (Vv), and the supracommissural part of the ventral pallium (Vs). These brain regions form part of the social decision-making network (SDMN)— a network of brain nuclei that modulates social behavior and is well conserved across different vertebrate taxa (Cabrera-Álvarez et al., 2017). They showed that only the POA of the forebrain had increased neuronal activation in fish exposed to a large shoal relative to isolated controls. This finding in fish suggested that the POA might help modulate social behavior across all vertebrates, in addition to its well-documented conserved role in sexual behavior.

By contrast, social interactions can also have negative effects on the brain and behavior of teleost fish. In salmonid fish such as the rainbow trout (*Oncorhynchus mykiss*), social interactions induce stress and promote aggressive responses that have a major influence on brain cell proliferation (Sørensen et al., 2007). Sørensen et al. (2012) reported that dyadic (or paired) interactions in rainbow trout significantly decreased forebrain cell proliferation in socially subordinate fish relative to isolated fish. The reduction in brain cell proliferation in socially subordinate fish was only correlated with the frequency of aggressive interactions received by each subject 1 d post-social interaction and hierarchy formation. This study revealed that the timing of social interaction relative to social hierarchy formation



might influence the neural responses to social stimuli in certain species of fish. In addition, behavioral and physiological analyses provided evidence of chronic stress in subordinate fish, including reduced feeding behavior and increased plasma cortisol levels.

While the neural and behavioral responses to *social interaction* in fish have received recent examination, the neural and behavioral mechanisms underlying *social buffering* in fish have been poorly investigated. Nearly all cases reporting social buffering in fish come from studies in zebrafish (*Danio rerio*). Faustino et al. (2017) revealed the occurrence of social buffering in zebrafish by showing that individuals exposed to an aversive stimulus (alarm substance) in the presence of both olfactory (shoal water) and visual (sight of shoal) conspecific cues exhibited a lower fear (freezing) response than those exposed to alarm substance during isolation. When tested separately, visual cues were more effective than olfactory cues in decreasing the fear response during long-term exposure to threat, indicating that the sight, rather than the smell, of conspecifics is better at inducing social buffering. Moreover, the neural activity patterns underlying this social buffering effect paralleled the co-activation of forebrain regions implicated in mammalian social buffering: the dorsomedial telencephalon (Dm, homologue of the mammalian pallial amygdala), the ventral nucleus of the ventral telencephalon (Vv, homologue of the mammalian nucleus accumbens and septum), and the preoptic area (POA, homologue of the mammalian preoptic area/paraventricular nucleus). Based on the relationship between forebrain activation and social stimuli in fish (Cabrera-Álvarez et al., 2017), part of this thesis examines cell proliferation in specific regions of the forebrain of *A. leptorhynchus*, including the dorsomedial telencephalon and ventral telencephalon.

Similar to recent studies in zebrafish (Faustino et al., 2017), one aim of my research

is to determine whether proliferative neural responses underlying a potential social buffering effect coincide with behavioral (social affiliation) responses in injured *A. leptorhynchus*.

Here, I first examine social affiliation in *intact* electric fish to determine whether individuals tend to seek or avoid social interaction in the absence of predatory stimuli. My purpose is to compare social affiliation between intact fish and injured fish (with amputated tails) living with a healthy (intact) social partner. By doing so, I evaluate whether injury increases social affiliation with a healthy social partner and whether this behavioral response correlates to the brain cell proliferation response to social interaction in injured electric fish.

#### *Weakly Electric Fish as Models for Studying the Social Regulation of Neurogenesis*

Weakly electric fish are good models for investigating the relationship between the social environment and neurogenesis (Dunlap et al., 2013a). Electric fish have a relatively simple neural circuitry, such that the activity of brain regions regulating communication signals is directly connected to the behavioral output of the whole fish (Dunlap et al., 2013a). The neurons of the pre-pacemaker nucleus of the midbrain, for example, are only two synapses removed from the cells that generate electric signals, which facilitates the study of the behavioral relevance of neurogenesis in this region (Dunlap et al., 2013a). Another benefit of working with electric fish is that electrocommunication signals can be easily manipulated and replayed in the laboratory (Dunlap et al., 2013a). Thus, electric fish are practical for examining the social regulation of adult neurogenesis (Dunlap et al., 2013a). In this thesis, I use weakly electric fish, *A. leptorhynchus*, to quantify the relationship between social buffering and cell proliferation in specific regions of the forebrain and midbrain in response to simulated predatory injury.

### *Social Interaction Increases Midbrain Cell Proliferation in Weakly Electric Fish*

Previous studies in weakly electric fish, *Brachyhypopomus gauderio*, showed that midbrain brain cell proliferation is greater in fish living in natural habitats than in those living isolated in captivity and even in those living socially in semi-natural laboratory conditions (Dunlap et al., 2011). Dunlap et al. (2011) indicated that social interaction in *B. gauderio* had a regionally specific effect on cell proliferation only in the breeding season and only in midbrain regions involved in electrocommunication: the periventricular zone (PVZ) adjacent to the pre-pacemaker nucleus (PPn). Consequently, these results suggested that increased brain cell proliferation might be related to either seasonal changes in electrocommunication behavior or to social enhancement of cell proliferation in associated brain regions (Dunlap et al., 2011, Dunlap et al., 2013a).

In a closely related species of electric fish, *A. leptorhynchus*, social interaction also increases midbrain cell proliferation in a regionally specific manner. Dunlap et al. (2006) showed that pairing fish for 7 d after social isolation increased both cell addition (cell proliferation and survival) in the PVZ and the production of chirps— an electrocommunication signal used in aggression and controlled by the PPn. Furthermore, Dunlap et al. (2013b) found that although fish paired for 7 d had higher rates of cell addition than isolated fish, fish paired for 14 d had rates of cell addition similar to those of isolated fish. However, exposing fish to novel social partners reversed this habituation and further enhanced cell addition. Together, these results suggested that brain cell proliferation is affected more by social novelty than by the presence of social stimuli and that the *duration* of social interaction is an important factor influencing the neurogenic response to social stimuli in weakly electric fish.

### *Predation Decreases Forebrain Cell Proliferation in Weakly Electric Fish*

The forebrain of teleost fish is a major focus of research because, unlike the midbrain, it contains regions that are likely involved in coordinating behavioral responses to predation (Dunlap et al., 2016). Specifically, this thesis examines brain cell proliferation in three regions of the forebrain of weakly electric fish: the dorsolateral telencephalon, dorsomedial telencephalon, and ventral telencephalon (see Figure 1). These forebrain regions are particularly relevant because of their homology to mammalian brain structures: the hippocampus, the amygdala, and the basal ganglia, respectively (Dunlap et al., 2016).

Two regions of the forebrain that may participate in the social buffering of predation in weakly electric fish are the dorsomedial telencephalon and ventral telencephalon. In teleost fish, the dorsomedial telencephalon functions in regulating conditioned avoidance responses, whereas the ventral telencephalon aids in selecting motor actions and evaluating their outcome (Dunlap et al., 2016). More importantly, the dorsomedial telencephalon (homologue of the mammalian amygdala) and ventral telencephalon (homologue of the mammalian basal ganglia) are homologous to forebrain regions that participate in the neural activity pattern of mammalian social buffering (e.g. Bowen et al., 2013) and, most likely, in the neural mechanisms of social buffering in teleost fish (Faustino et al., 2017). Thus, using fish to detect increased cell proliferation in forebrain regions that are homologous to those in mammals may support the presence of a conserved neural mechanism of social buffering across vertebrates (Faustino et al., 2017).

Previous studies in weakly electric fish, *Brachyhypopomus occidentalis*, showed that fish living in natural streams with a high abundance of predatory catfish (*Rhamdia quelen*) had lower forebrain cell proliferation than those living in streams with low predation pressure

(Dunlap et al., 2016a). At the same time, fish living in streams with high *Rhambia* density had a higher incidence of tail injury, and fish with injured tails also had lower forebrain cell proliferation than those with intact tails (Dunlap et al., 2016a). This field study suggested a correlation between predator exposure, tail injury, and forebrain cell proliferation, but it was still uncertain whether interactions with predators or tail regeneration caused the inhibition of cell proliferation (Dunlap et al., 2017; Lasky, 2017).

In a later study in electric fish, both *B. gauderio* and *A. leptorhynchus*, Dunlap et al. (2017) showed that experimental tail amputation decreased forebrain cell proliferation in *captive* fish in a fashion that resembled the effect of predatory tail injury in *free-living* electric fish, *B. occidentalis* (Dunlap et al., 2016a). They hypothesized that tail regeneration was responsible for the decrease in brain cell proliferation observed in the recovery period after amputation. However, Lasky (2017) demonstrated that tail amputation, rather than subsequent tail regeneration, causes the greatest decline in brain cell proliferation in *A. leptorhynchus*. Fish that experienced a short-term (1 d) recovery period had ~85-95% lower density of proliferating cells relative to intact fish, whereas fish that experienced a long-term (18 d) recovery period and, in turn, higher tail regeneration, had only ~50% decline (Lasky, 2017).

### *Experimental Questions*

In this thesis, I investigate the occurrence of social buffering of brain cell proliferation and behavioral responses to predatory stimuli in *A. leptorhynchus*. To study these potential buffering effects, I manipulate both the *timing* of social interaction relative to tail amputation and the *duration* of social interaction. I also examine social affiliation in

*intact* electric fish and whether it varies according to the sex of the social partner. Overall, this thesis aims to determine: 1) whether the presence of conspecifics mitigates the decrease in brain cell proliferation caused by tail amputation; 2) whether it is *pre-* or *post-*amputation social interaction that possibly buffers the brain cell proliferation response to predatory stimuli; 3) whether fish naturally seek or avoid social interaction in the absence of simulated predatory tail amputation; and 4) whether the social buffering of brain cell proliferation in injured fish correlates with increased affiliation to a non-injured social partner.

## Materials and Methods

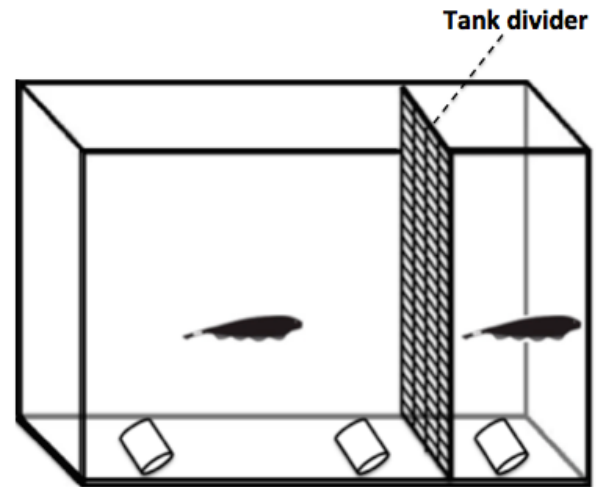
### *Subject Animals and Housing Conditions*

Brown ghost knifefish (*A. leptorhynchus*) were obtained from commercial distributors and housed in individual 38-L aquaria that formed part of two 1235-L water filtration systems. Water pH was 6.9, water conductivity was 300-400  $\mu\text{S}/\text{cm}$ , and water temperature was  $27 \pm 1^\circ\text{C}$ . Fish were kept under a 12-h light/12-h dark cycle with lights off at approximately 1800 h. The fish were fed frozen bloodworms and brine shrimp every 2 d and were acclimated in these conditions for at least 2 d before experimental testing. Sex was determined based on the electric organ discharge (EOD) frequency of each fish (males:  $\geq 880$  Hz and females:  $\leq 830$  Hz).

### *Social affiliation*

Fish were exposed to social stimuli by pairing individual focal fish with a single social partner (stimulus fish) that was placed on the opposite side of a tank divider in a 38-L aquarium (50.3 x 26.0 x 31.3 cm) (Figure 2).

Prior to experimental testing, all fish were anaesthetized (0.06% of 2-phenoxyethanol in aquarium water) to record their EOD frequency (sex), body mass, and body length from the tip of the snout to the end of the tail. The EOD frequency values of each fish were recorded using a multimeter and were standardized to  $28^\circ\text{C}$  using a  $Q_{10}$  of 1.62 (Dunlap et al., 2000).



**Figure 2. Social affiliation experimental setup.** The focal compartment (with a focal fish) and stimulus compartment (with a social partner) were physically separated by a tank divider. To measure social affiliation, focal fish were analyzed for their tube-seeking preference for a front tube (FT), near the stimulus compartment, or a back tube (BT), distant to the stimulus fish.

The experimental setup consisted of tanks divided into focal compartments (containing a focal fish) and stimulus compartments (containing a social partner;). Tank dividers were built using plastic grids (1.5 x 1.5 cm) covered by a custom window screen (2 x 2 mm) to prevent physical contact between the fish while still allowing the full flow of electric and chemical signals. All fish were provided with PVC shelter tubes (length: 5.0-5.5 cm; diameter: 4.8 cm). Each focal compartment contained a “front tube” (near the stimulus compartment) and a “back tube” (distant to the stimulus compartment). Front tubes were placed from 3-3.5 cm from the tank divider, back tubes were placed from 27.5-28.5 cm from the tank divider, and both tubes were placed from 19.0-20.0 cm from each other. The divider itself was placed from 8.5-9.5 cm from the front of the tank (Figure 2).

Because fish always seek shelter in tubes during the day, social affiliation was measured based on the position of the fish’s retreat sites (i.e. tubes). Individual social partners were placed inside the stimulus compartment prior to relocating individual focal fish to their corresponding compartment. Once moved to their compartment, focal fish had access to the front or back tube placed near or distant to the stimulus fish, respectively. Social affiliation was measured by recording the position of each focal fish in the front or back tube. Tube-seeking preference was recorded three times a day at 2-4 h intervals for a total duration of 6-7 d. All observations were conducted during the day between 0700 and 1800 h.

### *Experimental Tail Amputation*

To simulate predator-induced tail injury in the field, the tails of focal fish were experimentally amputated. Prior to tail amputation, all fish were anaesthetized (0.06% of 2-phenoxyethanol in aquarium water) to measure their body mass and body length. Experimental tail amputation was designed to mimic the mean degree of tail loss (20% of



total body length) in wild-caught electric fish, *B. occidentalis* (Tran, 2014). Tails were amputated using a scalpel to remove the caudal 20% of the total body length. Fish were returned to their home aquaria for a short-term recovery period of 1 d (Experiment 1) or a long-term recovery period of 7 d (Experiment 3, 4, & 5). This was similar to the amputation procedure used in Dunlap et al., 2017.

### *Brain Dissection and Freezing*

Fish were anaesthetized (0.075% of 2-phenoxyethanol in aquarium water), and their brains were dissected and fixed in paraformaldehyde (4% in PBS) for 80 min at 4°C. Brains were rinsed in PBS (2×30 min) and cryoprotected overnight in sucrose solution (25%) at 4°C. The next day, brains were quickly rinsed again with a single wash of PBS and frozen in cold isopentane at -80 °C. Brains were sectioned (30 µm) using a freezing microtome. Sections were mounted on frosted microscope slides and kept at -20°C until immunolabeling.

### *Immunohistochemistry*

To identify proliferating cells, an immunohistochemical procedure was conducted to label cells for the expression of proliferating cell nuclear antigen (PCNA). Brain sections were treated with HCl (2N) for 30 min at 37 °C and washed in borate buffer solution (1M, pH 8.5, 2×10 min) at room temperature. The slides were placed in PBS for 1 h and transferred to a humidity chamber. Blocking solution (5% donkey serum, 0.3% Triton X-100 in PBS) was applied to all sections for 1 h. The primary antibody (mouse anti-PCNA, F-2, Santa Cruz Biotechnology, Santa Cruz, CA, USA, 1:400 dilution in blocking solution,) was applied and left overnight at room temperature in the dark. The next day, the slides were rinsed in PBS (3×20 min) to remove any traces of primary antibody. The secondary antibody

(donkey anti-mouse IgG, Jackson ImmunoResearch, West Grove, PA, USA, 1:300 dilution in PBS was applied and left in the dark for 2 h. The slides were rinsed in PBS (3×20 min), coverslipped, and remained refrigerated until visualization.

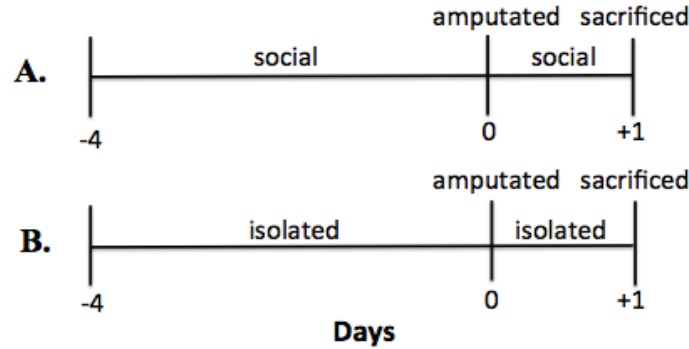
### *PCNA Quantification*

Brain tissue was examined under a Nikon E600 epifluorescence scope at 200X to quantify the density of proliferating brain cells. The density of PCNA-labeled cells was estimated in specific brain regions, which were identified using the brain atlas of *Apteronotus leptorhynchus* (Maler et al., 1991). The number of PCNA+ cells was counted unilaterally in a 100µm-band at the periphery of three regions of the forebrain (the dorsolateral, dorsomedial, and ventral telencephalon) at axial levels corresponding to sections 30-36 in the *Apteronotus* brain atlas. In the midbrain, PCNA+ cells were counted in a 100 µm-band surrounding the periventricular zone (PVZ) at two axial regions, the region adjacent to the pre-pacemaker nucleus (PVZ 1; sections 17-19 in the *Apteronotus* brain atlas) and an adjacent posterior region (PVZ 2; sections 14-16). The areas of each brain region were estimated using NIH ImageJ (v. 4.0). The density (PCNA+ cells/ mm<sup>3</sup>) of proliferating cells was calculated by dividing the cell counts by the area of each region and the section thickness (30 µm).

### *Experiment 1: Social Buffering of the Brain Cell Proliferation Response to Tail Amputation*

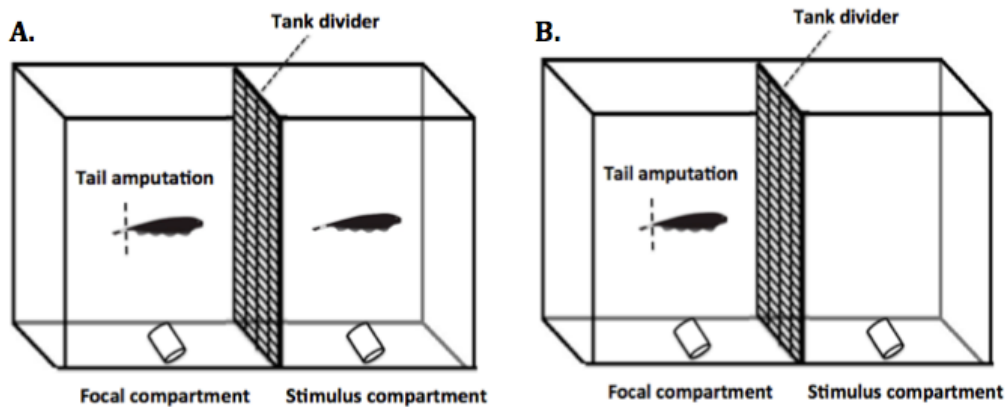
Prior to experimental tail amputation, all fish remained intact in either paired or isolated conditions for 4 d. Isolated fish were relocated to new tanks to ensure that all fish were exposed to environmental novelty. Focal fish were divided into two treatment groups: a) paired fish with experimental tail amputation and b) isolated fish with experimental tail amputation. Same-sex pairings were made between fish with similar EOD frequencies (within 25 Hz) and similar body masses (within 1 g). Fish were returned to their home

aquaria for a short-term recovery period of 1 d. This amputation procedure was identical to that previously reported in Dunlap et al., 2017. All focal fish were sacrificed 1 d post-amputation for PCNA quantification.



**Figure 3. Timeline for experiment 1.** Conditions experienced by focal fish in each treatment group: A) paired amputated condition and B) isolated amputated condition. Fish remained intact in either paired or isolated conditions for 4 d (day -4) prior to tail amputation (day 0). On day 0, fish from both groups remained either paired or isolated and had a recovery period of 1 d before being sacrificed (day +1) for brain dissection and analysis.

The experimental setup consisted of tanks divided into focal and stimulus compartments of equal dimensions (25.2 cm). Tank dividers were placed from 14.5-15.5 cm from the front of each aquarium such that they roughly separated each tank in half. Each compartment contained a single PVC tube that was placed from 7.0-8.0 cm from the tank divider. In tanks with *isolated* focal fish, stimulus compartments remained empty (a single tube with no fish).



**Figure 4. Social buffering experimental setup.** The focal compartment (with focal fish) and the stimulus compartment (with social partner or empty) were equally separated by a tank divider. A) Paired, amputated focal fish. B) Isolated, amputated focal fish.

**Table 1. Mean values  $\pm$  SEM for paired and isolated conditions in *Apteronotus leptorhynchus* with experimental tail amputation.** EOD frequency (Hz), body mass (g), body length (cm), and cut tail length (mm) were recorded in all fish. Stimulus fish remained intact and their body length was not recorded. Paired fish (focal fish plus stimulus fish) had similar mean EOD frequencies (within 19 Hz) and mean body masses (within 0.47 g) to those of isolated fish. Paired focal fish consisted of a 1:1 ratio of males to females.

<b>Paired, Amputated Condition</b>				
Treatment Group (N)	EOD frequency (Hz)	Body Mass (g)	Body Length (cm)	Cut Tail Length (mm)
Focal Fish (6)				
Males (3)	904 $\pm$ 54	3.66 $\pm$ 0.21	11.4 $\pm$ 0.2	19.3 $\pm$ 0.3
Females (3)	810 $\pm$ 7	4.72 $\pm$ 0.21	13.1 $\pm$ 0.5	23.0 $\pm$ 3.1
Stimulus Fish (6)				
Males (3)	910 $\pm$ 71	4.18 $\pm$ 0.27	-	intact
Females (3)	797 $\pm$ 3	4.54 $\pm$ 0.43	-	intact
<b>Isolated, Amputated Condition</b>				
Focal Fish (7)				
Males (4)	948 $\pm$ 20	3.92 $\pm$ 0.70	12.3 $\pm$ 0.7	21.0 $\pm$ 2.0
Females (3)	799 $\pm$ 8	3.69 $\pm$ 0.75	11.9 $\pm$ 0.7	21.0 $\pm$ 1.7

Prior to experimental testing, EOD frequency (range: 790-1052 Hz) and body mass (range: 2.19-5.84 g) were recorded in all fish. Body length (range: 10.4-14.2 cm) was only recorded in focal fish. During experimental testing, the tails of all focal fish were amputated to remove the caudal 20% of their body length (range: 18-27 mm). All data for the fish used in each treatment group are presented as means  $\pm$  SEM in Table 1.

#### *Experiment 2: Social Affiliation in Intact Electric Fish*

Prior to social pairing, all fish were relocated to new tanks to ensure that both paired fish and isolated controls were exposed to environmental novelty. Fish remained isolated for either 2 d (in same-sex pairing conditions) or 5 d (in opposite-sex pairing conditions) in the focal compartment of each aquarium with the option of seeking shelter in a “front” tube placed near a tank divider or a “back tube” placed distant to the divider (see Figure 2).

The social affiliation (tube-seeking preference) of each focal fish was tested as

described above for a total duration of 6 d. Focal fish were exposed to one of three different social contexts: a) a stimulus fish of the same sex, b) a stimulus fish of the opposite sex, or c) an empty stimulus compartment (isolated controls). Fish with similar EOD frequencies (within 12-36 Hz) were paired for the same-sex treatment group, whereas fish with different EOD frequencies ( $\geq 125$  Hz difference) were paired for the opposite-sex treatment group. In both sex treatment groups, fish in each pair had similar body masses (within 1-2 g). In the same-sex treatment group, paired fish had similar mean EOD frequencies (within 19 Hz) and mean body masses (within 0.73 g) to those of isolated fish. In the opposite-sex treatment group, paired fish also had similar *mean* EOD frequencies (within 21 Hz) and *mean* body masses (within 1.25 g) to those of isolated fish, but fish in each pair had *different* EOD frequencies (within 126-251 Hz difference).

Focal fish in the same-sex pairing condition were selected such that roughly half of the fish had previously shown a preference for either the “front” or “back” tube in the *pre*-pairing phase of the experiment (2 d of isolation). Focal fish in the opposite-sex condition consisted of fish that had been previously used as isolated controls in the same-sex pairing condition. In addition, focal fish were paired in an attempt to obtain an almost 1:1 ratio of males to females in both sex treatment groups. All data for EOD frequency (range: 749-1048 Hz), body mass (range: 3.53-10.69 g), and body length (range: 12.0-16.5 cm) are presented as means  $\pm$  SEM in Table 2.

The electric field strength experienced by each focal fish at both tube positions was estimated according to the body mass of its social partner. When a focal fish was paired with a small stimulus fish (3.64 g), the range of the electric field strength was 0.01-0.67 mV/cm, measured from the back (distant to the stimulus fish) to the front tube position (near the

**Table 2. Mean values  $\pm$  SEM for same-sex and opposite-sex pairing conditions in intact *Apteronotus leptorhynchus*.** EOD frequency (Hz), body mass (g), and body length (cm) were recorded in all fish. Same-sex pairs had similar EOD frequencies (within 12-36 Hz), whereas opposite-sex pairs had different EOD frequencies ( $\geq 125$  Hz difference). Social pairs (focal fish plus stimulus fish) had similar mean EOD frequencies (within 7 Hz) and mean body masses (within 1.13 g) to those of isolated fish in either sex condition. Paired focal fish consisted of an almost 1:1 ratio of males to females.

**Same-Sex Pairing Condition**

Treatment Group (N)	EOD frequency (Hz)	Body Mass (g)	Body Length (cm)
<b>Isolated Focal Fish</b>			
Males (9)	995 $\pm$ 22	6.21 $\pm$ 0.62	14.2 $\pm$ 0.4
Females (5)	827 $\pm$ 15	7.10 $\pm$ 1.01	14.6 $\pm$ 0.6
<b>Paired Focal Fish</b>			
Males (5)	1014 $\pm$ 22	6.37 $\pm$ 0.41	14.5 $\pm$ 0.5
Females (4)	816 $\pm$ 17	5.49 $\pm$ 0.58	14.9 $\pm$ 0.4
<b>Stimulus Fish</b>			
Males (5)	989 $\pm$ 16	6.38 $\pm$ 0.48	14.6 $\pm$ 0.5
Females (4)	799 $\pm$ 13	4.84 $\pm$ 0.46	13.2 $\pm$ 0.4

**Opposite-Sex Pairing Condition**

<b>Isolated Focal Fish</b>			
Males (8)	962 $\pm$ 20	7.04 $\pm$ 0.54	14.7 $\pm$ 0.3
Females (5)	788 $\pm$ 18	6.53 $\pm$ 1.13	15.2 $\pm$ 0.4
<b>Paired Focal Fish</b>			
Males (5)	956 $\pm$ 7	5.11 $\pm$ 0.38	14.0 $\pm$ 0.6
Females (4)	773 $\pm$ 19	6.20 $\pm$ 0.61	14.2 $\pm$ 0.4
<b>Stimulus Fish</b>			
Males (4)	980 $\pm$ 27	6.25 $\pm$ 0.60	14.9 $\pm$ 0.5
Females (5)	790 $\pm$ 10	5.09 $\pm$ 0.44	12.8 $\pm$ 0.3

stimulus fish), respectively. When paired with a large stimulus fish (7.16 g) and with an intermediate-sized stimulus fish (4.68 g), focal fish experienced electric field strengths from 0.2-2.2 mV/cm and from 0.08-0.5 mV/cm, respectively.

*Experiment 3: Social Affiliation in Injured Electric Fish*

Similar to experiment 2, all fish were relocated into new tanks and remained isolated for 4 d prior to social pairing and/or experimental tail amputation. Focal fish were divided into three treatment groups: a) fish paired *after* experimental tail amputation, b) fish isolated both *before and after* experimental tail amputation, and c) paired fish with *intact* tails. Same-sex pairings were made according to the EOD frequency and body mass criteria used in

experiment 1. Tail amputation was conducted as described above to mimic predator-induced tail loss in the field. Social affiliation was tested using the same setup as in experiment 2 (see Figure 2) for a total of 7 d post-amputation or intact condition. This experiment was repeated twice, which I have classified as experiment 3A and experiment 3B. In Table 3, I have listed all data as means  $\pm$  SEM for the fish used in experiment 3A: their EOD frequency (range: 736-1038 Hz), body mass (range: 2.44-10.16 g), body length (range: 10.5-17.4 cm), and cut tail length (range: 12.0-28.0 mm). I will later present this same kind of data for the fish used in experiment 3B (see Table 5).

**Table 3. Mean values  $\pm$  SEM for experiment 3A: post-amputation social interaction, pre- and post-amputation social isolation, and paired intact condition in *Apteronotus leptorhynchus*.** EOD frequency (Hz), body mass (g), body length (cm), and cut tail length (mm) were recorded in all fish. Stimulus fish remained intact in all conditions. Fish in all three groups had similar mean EOD frequencies (within 44 Hz) and mean body masses (within 0.75 g). Paired focal fish consisted of a 1:1 ratio of males to females.

<b>Post-Amputation Social Interaction</b>				
Treatment Group (N)	EOD frequency (Hz)	Body Mass (g)	Body Length (cm)	Cut Tail Length (mm)
Focal Fish (6)				
Males (3)	908 $\pm$ 32	6.01 $\pm$ 1.90	12.6 $\pm$ 0.6	18.0 $\pm$ 1.2
Females (3)	775 $\pm$ 13	5.77 $\pm$ 1.05	14.1 $\pm$ 1.4	18.7 $\pm$ 4.7
Stimulus Fish (6)				
Males (3)	902 $\pm$ 27	6.66 $\pm$ 1.66	14.6 $\pm$ 1.5	intact
Females (3)	764 $\pm$ 19	6.26 $\pm$ 1.12	14.7 $\pm$ 0.6	intact
<b>Pre- and Post-Amputation Social Isolation</b>				
Focal Fish (7)				
Males (3)	968 $\pm$ 52	4.22 $\pm$ 1.47	12.9 $\pm$ 0.8	18.7 $\pm$ 1.2
Females (4)	794 $\pm$ 23	6.64 $\pm$ 1.68	13.2 $\pm$ 1.4	21.3 $\pm$ 3.6
<b>Paired, Intact Condition</b>				
Focal Fish (6)				
Males (3)	919 $\pm$ 13	5.85 $\pm$ 1.67	13.9 $\pm$ 0.9	intact
Females (3)	812 $\pm$ 24	6.39 $\pm$ 1.90	14.2 $\pm$ 0.7	intact
Stimulus Fish (6)				
Males (3)	934 $\pm$ 11	6.02 $\pm$ 1.26	14.6 $\pm$ 1.0	intact
Females (3)	823 $\pm$ 23	6.44 $\pm$ 1.57	13.9 $\pm$ 1.9	intact

#### *Experiment 4: Pre-Amputation Social Interaction, Brain Cell Proliferation, and Behavior*

Prior to experimental testing, EOD frequency (range: 767-976 Hz), body mass (range:

3.01-8.63 g), and body length (range: 11.3-16.7 cm) were measured in all fish (see Table 4).

Focal fish were divided into three treatment groups: a) fish paired *before* experimental tail amputation, b) fish paired both *before and after* experimental tail amputation, and c) isolated fish with experimental tail amputation. Same-sex pairings were made as in experiments 1 & 3. Tail amputation was conducted to remove the caudal 20% of a fish's body length (range: 11.3-16.7 mm).

**Table 4: Mean values  $\pm$  SEM for pre-amputation social interaction, pre- and post-amputation social interaction, and isolated amputated condition in *Apteronotus leptorhynchus*.** EOD frequency (Hz), body mass (g), body length (cm), and cut tail length (mm) were recorded in all fish. Stimulus fish remained intact in all conditions. Fish in all three groups had similar mean EOD frequencies (within 9 Hz) and mean body masses (within 0.55 g). Paired focal fish consisted roughly of a 1:1 ratio of males to females.

**Pre-Amputation Social Interaction**

Treatment Group (N)	EOD frequency (Hz)	Body Mass (g)	Body Length (cm)	Cut Tail Length (mm)
Focal Fish (8)				
Males (4)	927 $\pm$ 20	7.19 $\pm$ 0.79	15.4 $\pm$ 0.5	29.0 $\pm$ 1.8
Females (4)	781 $\pm$ 8	4.78 $\pm$ 0.69	12.7 $\pm$ 1.2	22.3 $\pm$ 4.1
Stimulus Fish (8)				
Males (4)	912 $\pm$ 18	6.73 $\pm$ 0.64	15.6 $\pm$ 0.6	intact
Females (4)	781 $\pm$ 9	4.61 $\pm$ 0.67	12.9 $\pm$ 0.3	intact

**Pre- and Post-Amputation Social Interaction**

Focal Fish (5)				
Males (3)	894 $\pm$ 16	5.80 $\pm$ 0.87	13.2 $\pm$ 1.0	19.0 $\pm$ 7.6
Females (2)	823 $\pm$ 27	7.23 $\pm$ 1.38	12.8 $\pm$ 1.4	17.0 $\pm$ 7.0
Stimulus Fish (5)				
Males (3)	885 $\pm$ 11	5.24 $\pm$ 0.62	13.8 $\pm$ 1.3	intact
Females (2)	811 $\pm$ 42	7.23 $\pm$ 1.30	14.7 $\pm$ 2.0	intact

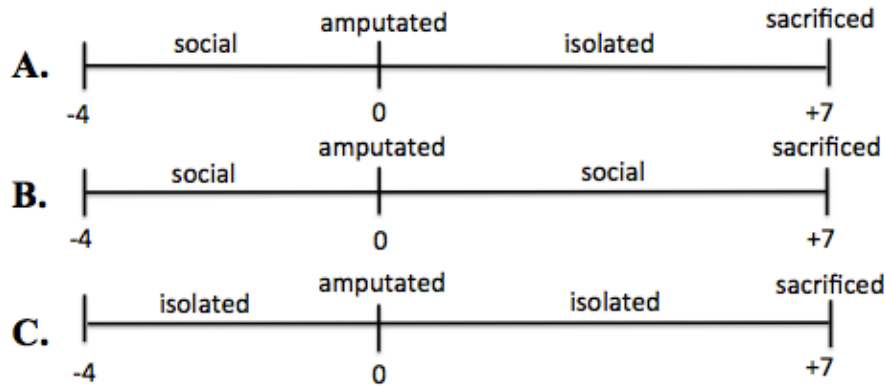
**Isolated, Amputated Condition**

Focal Fish (5)				
Males (2)	948 $\pm$ 32	6.06 $\pm$ 0.42	13.7 $\pm$ 0.1	19.5 $\pm$ 4.5
Females (3)	770 $\pm$ 21	5.84 $\pm$ 0.69	13.5 $\pm$ 1.1	18.7 $\pm$ 4.7

In contrast to experiment 1, focal fish were allowed to have a longer recovery period (7 d) following tail amputation rather than a short-term recovery period (1 d). The social affiliation (tube-seeking preference) of each focal fish was tested for 7 d after tail amputation



using the same tank setup as in experiments 1 & 3 (see Figure 2). After treatment, focal fish were sacrificed and their brains were dissected and fixed for immunohistochemistry and PCNA quantification as described above.

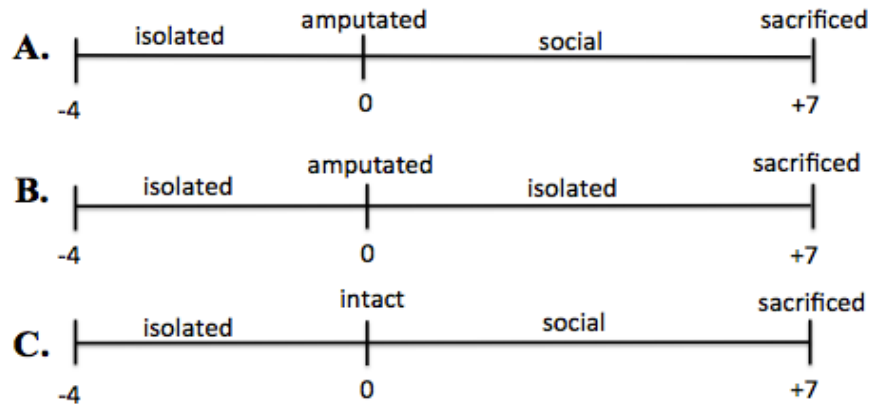


**Figure 5. Timeline for experiment 4.** Conditions experienced by focal fish in each treatment group: A) pre-amputation social interaction, B) pre- and post-amputation social interaction, and C) isolated amputated condition. Fish remained intact in either paired or isolated conditions for 4 d (day -4) prior to tail amputation (day 0). On day 0, fish from group A were isolated, and fish from all groups had a recovery period of 7 d before being sacrificed (day +7) for brain dissection and analysis.

#### *Experiment 5: Post-Amputation Social Interaction and Brain Cell Proliferation*

Prior to experimental testing, EOD frequency (range: 748-1028 Hz), body mass (range: 1.80-10.69 g), and body length (range: 8.4-16.5 cm) were measured in all fish (see Table 5). Focal fish were then divided into three treatment groups: a) fish paired *after* experimental tail amputation, b) fish *isolated* both *before and after* experimental tail amputation, and c) paired fish with *intact* tails. Fish in these treatment groups were simultaneously used to test social affiliation in experiment 3B (see Table 5). Same-sex pairings were made as in earlier experiments. Tail amputation was conducted to remove the caudal 20% of a fish's body length (range: 9.0-34.0 mm). In contrast to experiment 4, all fish remained *isolated* for 4 d prior to tail amputation, and not all focal fish had their tails

amputated. However, just as in experiment 4, focal fish had a recovery period of 7 d before being sacrificed for brain dissection and analysis. This was the same timeline used in experiment 3 to test social affiliation.



**Figure 6. Timeline for experiment 3 & experiment 5.** Schematic representation of the conditions experienced by focal fish in each treatment group: A) post-amputation social interaction, B) pre- and post-amputation social isolation, and C) paired intact condition. Fish remained isolated for 4 d (day -4) prior to tail amputation or intact condition (day 0). On day 0, fish from groups A and C were paired, and all fish had a recovery period of 7 d before being sacrificed (day +7) for brain dissection and analysis.

**Table 5. Mean values  $\pm$  SEM for experiment 3B & experiment 5: post-amputation social interaction, pre- and post-amputation social isolation, and paired intact condition in *Apteronotus leptorhynchus*.** EOD frequency (Hz), body mass (g), body length (cm), and cut tail length (mm) were recorded in all fish. Stimulus fish remained intact in all conditions. Fish in all three groups had similar mean EOD frequencies (within 33 Hz) and mean body masses (within 1.08 g). Paired focal fish consisted roughly of a 1:1 ratio of males to females.

<b>Post-Amputation Social Interaction</b>				
Treatment Group (N)	EOD frequency (Hz)	Body Mass (g)	Body Length (cm)	Cut Tail Length (mm)
Focal Fish (10)				
Males (5)	948 $\pm$ 23	6.49 $\pm$ 0.58	14.2 $\pm$ 0.4	23.2 $\pm$ 2.4
Females (5)	799 $\pm$ 12	6.18 $\pm$ 0.37	14.8 $\pm$ 0.4	24.8 $\pm$ 3.4
Stimulus Fish (10)				
Males (5)	951 $\pm$ 23	6.49 $\pm$ 0.57	14.8 $\pm$ 0.5	intact
Females (5)	785 $\pm$ 11	5.61 $\pm$ 0.31	12.8 $\pm$ 0.2	intact
<b>Pre- and Post Amputation Social Isolation</b>				
Focal Fish (7)				
Males (3)	949 $\pm$ 51	7.36 $\pm$ 1.26	15.1 $\pm$ 0.9	26.3 $\pm$ 2.8
Females (4)	821 $\pm$ 15	5.09 $\pm$ 1.99	12.1 $\pm$ 1.7	17.0 $\pm$ 3.5
<b>Paired, Intact Condition</b>				
Focal Fish (7)				
Males (4)	962 $\pm$ 8	5.78 $\pm$ 0.73	14.2 $\pm$ 0.5	intact
Females (3)	773 $\pm$ 18	5.88 $\pm$ 1.13	14.3 $\pm$ 0.5	intact
Stimulus Fish (7)				
Males (4)	973 $\pm$ 14	5.83 $\pm$ 1.09	14.1 $\pm$ 0.5	intact
Females (3)	773 $\pm$ 1	5.24 $\pm$ 1.13	13.3 $\pm$ 1.5	intact

### *Statistical Analysis*

Separate statistical tests were conducted for neural and behavioral data. Brain cell proliferation was analyzed in Experiments 1, 4, & 5. Social affiliation was analyzed in Experiments 2, 3, & 4.  $P < 0.05$  was considered statistically significant.

#### *1) Brain cell proliferation*

Experiment 1: The effect of tail amputation on brain cell proliferation was determined using two-way repeated measures ANOVA with treatment (paired vs. isolated) as the independent variable, brain region (dorsolateral, dorsomedial, or ventral telencephalon) as the repeated measure, and density of PCNA+ cells as the dependent variable. All statistical analysis was conducted with Prism 7.0 software. All data are expressed as means  $\pm$  SEM.

Experiment 4: Two-way repeated measures ANOVA was used. Treatment (pre- and post-amputation social vs. isolated or pre-amputation social vs. isolated) was the independent variable, brain region was the repeated measure, and the density of PCNA+ cells was the dependent variable.

Experiment 5: Data were analyzed using the same procedure described above. In this case, treatment (paired vs. isolated or amputated vs. intact) was the independent variable.

#### *2) Social affiliation*

Experiment 2: The independent and potentially interactive effects of treatment (paired vs. isolated) and day on the social affiliation of intact focal fish for a same-sex or opposite-sex stimulus fish were examined using generalized linear mixed models (GLMM; lme4 package) in the statistical software R. Separate models were constructed for same-sex and opposite-sex social affiliation data. Social affiliation was modeled with a binomial error distribution and logit link. Treatment and day were modeled as fixed effects, and an

interaction between these effects was included in both models. These models also included individual fish as a random effect. Fitted versus Pearson's residual plots were used to assess model fits. To test the significance of fixed effects, these terms were dropped and nested models were compared using likelihood ratio tests. Random effects were retained in all model comparisons.

Experiment 3: Data were analyzed using the same procedure described above. Separate models were constructed for the data obtained in experiments 3A & 3B. In both cases, treatment (paired amputated, paired intact, or isolated amputated conditions) and day were modeled as fixed effects and the interaction between these effects was included.

Experiment 4: Data were also analyzed as described above. In this case, treatment (pre- and post-amputation social interaction, pre-amputation social interaction, or isolated amputated condition) and day were modeled as fixed effects.

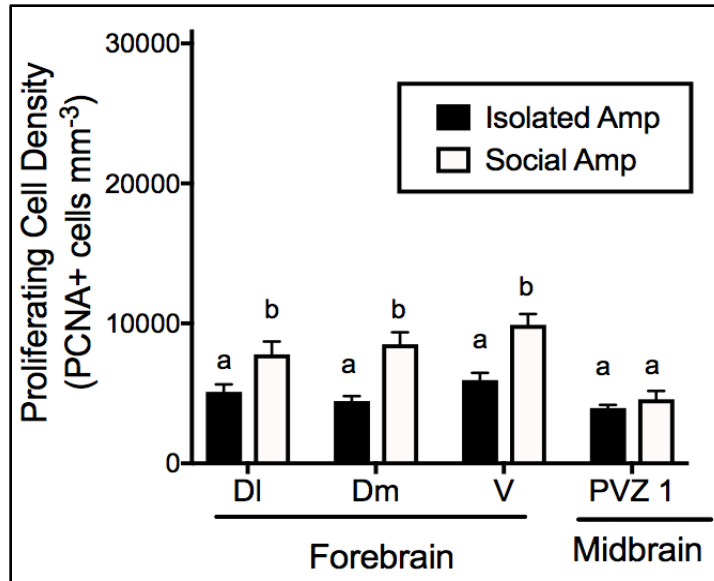
## Results

### *Experiment 1: Social Buffering of the Brain Cell Proliferation Response to Tail Amputation*

I investigated whether the presence of a social partner (stimulus fish) mitigates the decrease in brain cell proliferation following tail amputation in *A. leptorhynchus*. Specifically, I examined the effects of both pre-amputation and post-amputation social interaction on this social buffering response. All fish were given a short-term recovery of 1 d post-amputation, as this is the period in which *A. leptorhynchus* experiences the greatest reduction in brain cell proliferation (Dunlap et al., 2017). Fish living with a social partner both before (4 d) and shortly after (1 d) tail amputation had a significantly higher density of proliferating cells than amputated fish living in isolation (Table 6 and Figure 7). This social buffering effect occurred in all three examined forebrain regions, the dorsolateral (Dl), dorsomedial (Dm), and ventral telencephalon (V), but did not occur in the midbrain, indicating that the effect was regionally specific (Table 6 and Figure 7). When including both the forebrain and the midbrain in the analysis, a significant treatment x region interaction was observed (Table 6).

**Table 6. ANOVA results for experiment 1.** Two-way ANOVA results showing the effects of brain region, social treatment, and the interaction of brain region with social treatment on brain cell proliferation.

Effect	d.f.	F	P
brain region	3	19.4	<0.005
treatment	1	23.7	< 0.001
brain region x treatment	3	5.4	<0.05

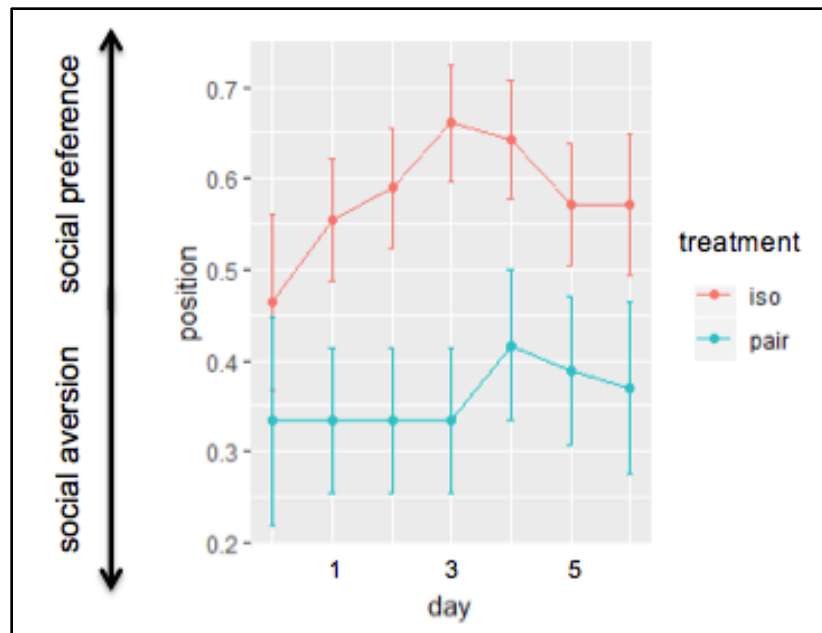


**Figure 7. Brain cell proliferation within the forebrain and midbrain.** Proliferating cell density (PCNA+ cells/mm<sup>3</sup>) was measured in three forebrain regions, the dorsolateral (DI), dorsomedial (Dm), and ventral (V) telencephalon, and in one midbrain region, the periventricular zone region adjacent to the pre-pacemaker nucleus (PVZ 1). This graph shows that the effects of social buffering occurred globally across all three regions of the forebrain but did not occur in the midbrain. Different letters (a or b) indicate significant differences between treatment groups. Error bars indicate standard error.

## *Experiment 2: Social Affiliation in Intact Electric Fish*

### *A. Same-Sex Pairing Condition*

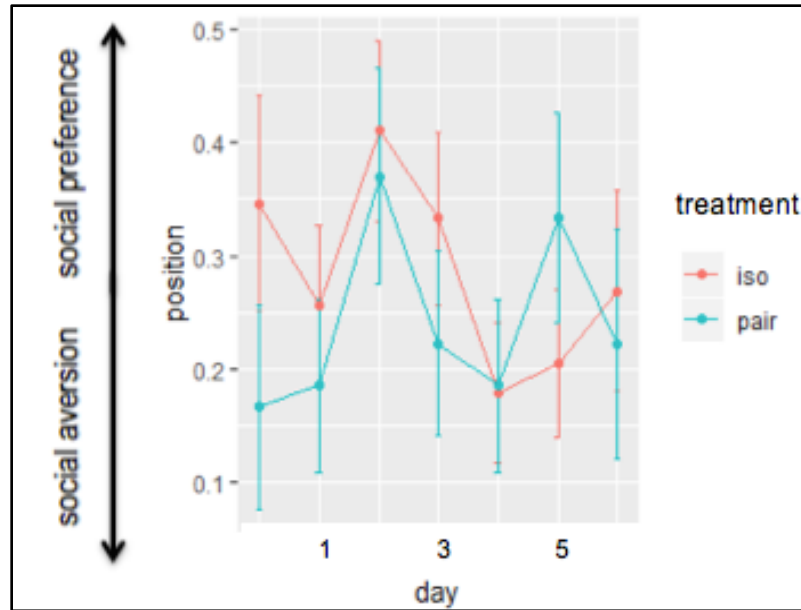
I investigated whether intact *A. leptorhynchus* naturally showed a preference or aversion to a same-sex or opposite-sex social partner. When paired with a same-sex partner, paired focal fish tended to avoid the stimulus compartment (showed a greater preference for the “back tube”) relative to isolated focal fish after 1 d of social interaction. In contrast to paired fish, isolated fish were mostly located near the stimulus compartment over the course of the experiment, suggesting that paired fish were actively choosing to avoid their social partner (Figure 7). However, there was still no significant effect of treatment (isolated vs. paired) ( $P = 0.193$ ) or day ( $P = 0.241$ ) on social affiliation and no significant treatment x day interaction ( $\chi^2 = 0.074$ ,  $P = 0.785$ ).



**Figure 7. Tube-seeking (position) preference in same-sex paired fish and isolated fish.** All fish remained isolated for 2 d before behavioral testing. On day 0, focal fish (n= 9) were paired with a same-sex stimulus fish for 6 d, while control fish (n= 14) remained isolated. Data on tube-seeking preference were transformed as “1” if fish were positioned near the stimulus compartment (inside the front tube) or as “0” if fish were positioned distant to the stimulus compartment (inside the back tube). In experiment 2, fish paired with a same-sex partner showed a greater preference for the back tube, indicating a tendency for social avoidance (positions <0.5). However, isolated fish showed a greater preference for the front tube (positions >0.5).

### *B. Opposite-Sex Pairing Condition*

When paired with an opposite-sex social partner, paired focal fish showed a similar trend of avoiding the stimulus compartment as isolated focal fish (Figure 8). Thus, no significant effect of treatment (isolated vs. paired) ( $P= 0.171$ ) on social affiliation was found, but the effect of day ( $P= 0.057$ ) on social affiliation and the treatment x day interaction were not significant either ( $\chi^2= 3.311$ ,  $P= 0.069$ ).

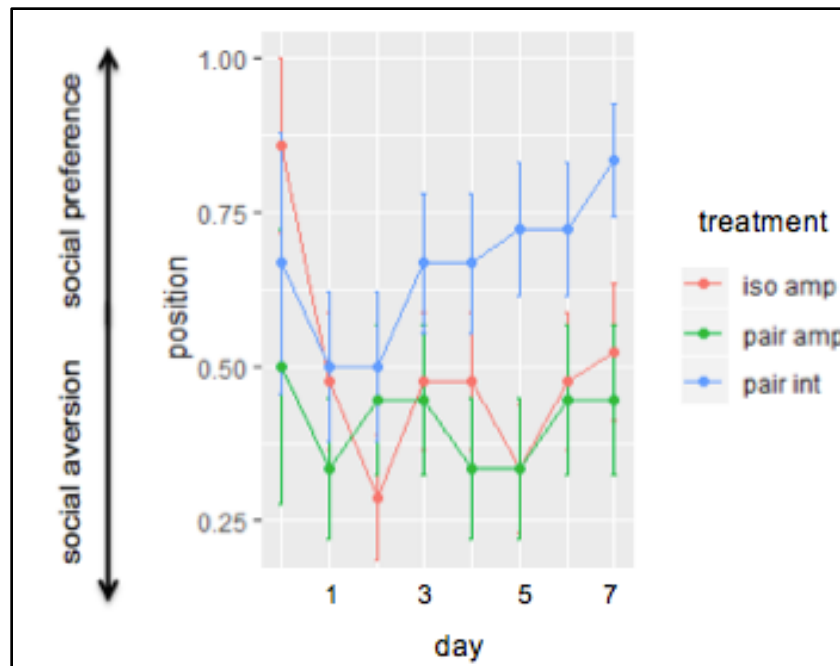


**Figure 8. Tube-seeking (position) preference in opposite-sex paired fish and isolated fish.** All fish remained isolated for 5 d before behavioral testing. On day 0, focal fish (n= 9) were paired with an opposite-sex stimulus fish for 6 d, while control fish (n= 13) remained isolated. As shown above, both paired fish and isolated fish showed a greater preference for the back tube, indicating a similar trend of avoidance toward the stimulus compartment (positions <0.5).

### *Experiment 3: Social Affiliation in Injured Electric Fish*

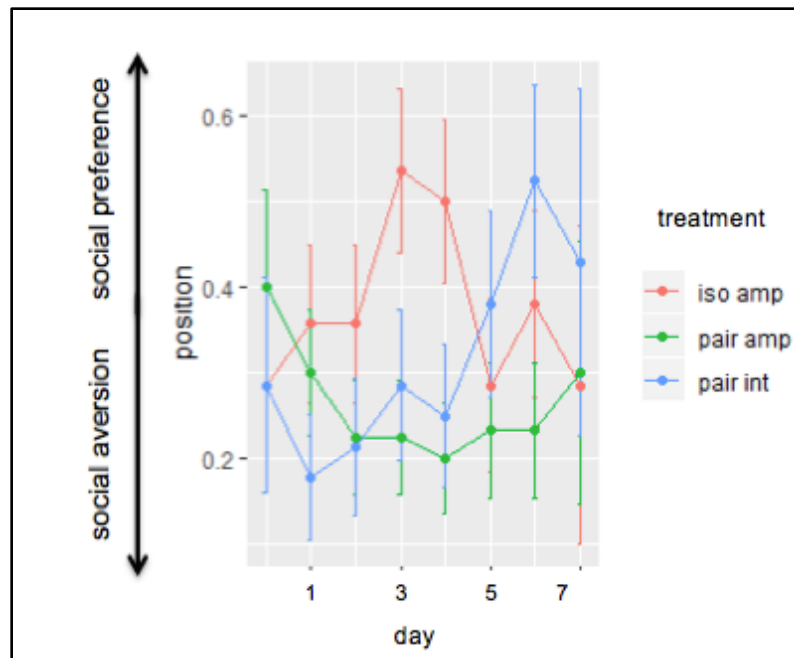
I investigated whether injury could increase social affiliation in *A. leptorhynchus* paired with a healthy (non-injured) partner of the same sex. In both experiments, focal fish were only paired *after* experimental tail amputation for a total duration of 7 d. In experiment 3A, paired intact fish seemed to be attracted to the stimulus fish, but amputated fish, both paired and isolated, seemed to mostly avoid the stimulus compartment (Figure 9). However, the interaction between treatment x day was not significant ( $\chi^2 = 5.101$   $P = 0.078$ ).





**Figure 9. Tube-seeking (position) preference in amputated fish and intact fish.** All fish remained isolated and intact for at least 4 d before behavioral testing. On day 0, focal fish were either tail-amputated (n= 6), paired (n= 7), or both paired and tail-amputated (n= 7). Social affiliation was recorded for a total duration of 7 d. As shown above, paired intact fish seemed to be attracted to their stimulus fish (positions >0.5), but amputated fish seemed to mostly avoid the stimulus compartment (positions <0.5) whether paired or isolated.

In experiment 3B, both paired amputated fish and paired intact fish seemed to avoid their social partners. However, paired amputated fish seemed to continuously increase their social *aversion*, while paired intact fish seemed to continuously increase their social *preference*. In addition, isolated amputated fish mostly avoided the stimulus compartment and were only slightly attracted to it on day 3 post-amputation. Overall, fish in all treatment groups tended to avoid the stimulus compartment (Figure 10). There was a significant treatment x day interaction ( $\chi^2 = 15.479$ ,  $P < 0.001$ ).



**Figure 10. Tube-seeking (position) preference in isolated amputated fish, post-amputation social fish, and paired intact fish.** All fish remained isolated and intact for 4 d before behavioral testing. On day 0, focal fish were either tail-amputated ( $n=7$ ), paired ( $n=7$ ), or both paired and tail-amputated ( $n=10$ ). Social affiliation was recorded for a total duration of 7 d. As shown above, paired fish seemed to avoid their stimulus fish (positions  $<0.5$ ) whether amputated or intact. Similarly, isolated fish seemed to mostly avoid the stimulus compartment, with only a slight attraction to it on day 3 post-amputation (position  $>0.5$ ).

#### *Experiment 4: Pre-Amputation Social Interaction, Brain Cell Proliferation, and Behavior*

##### *Brain Cell Proliferation Results*

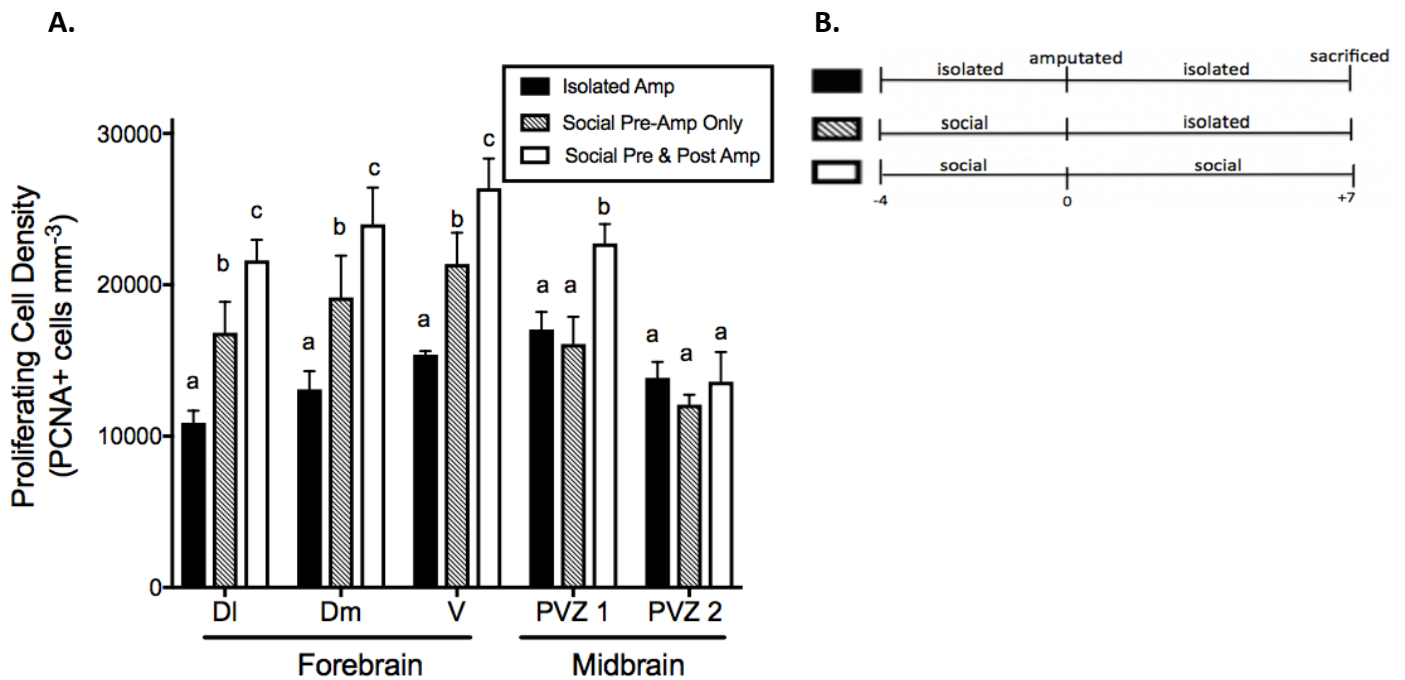
I investigated the exclusive effects of *pre*-amputation social interaction in the buffering response. Specifically, I examined both the brain cell proliferation response and the social affiliation of fish subjected to experimental tail amputation. In contrast to experiment 1, focal fish were allowed to have a longer recovery period (7 d) following tail amputation rather than a short-term recovery period (1 d). The choice of this recovery period was based on previous studies in *A. leptorhynchus* suggesting that the positive effects of social interaction on the neural response to tail amputation act slower than the negative effects of tail amputation on brain cell proliferation (Dunlap *et al.*, 2017). Moreover, it is important to

consider that any changes in brain cell proliferation following somatic injury in fish may be confounded by increased levels of cell proliferation in their tails caused by regeneration in the long-term recovery period (18 d) (Dunlap, 2016). Thus, the purpose of using an intermediate recovery period (7 d) was to lengthen the influence of social interaction on the brain while eliminating some of the brain changes that may result from tail regeneration rather than from the amputation process *per se* (Dunlap, 2016).

Fish paired only before (4 d) tail amputation showed significantly higher density of proliferating cells than isolated amputated fish but significantly lower density of proliferating cells than fish paired both before (4 d) and after (7 d) tail amputation (Table 7 and Figure 11). Because pre-amputation social fish had proliferating cell densities between those of isolated amputated fish and pre- and post-amputation social fish (Figure 11), this result indicated that *pre*-amputation social interaction buffered but did not totally reverse the brain cell proliferation response to tail amputation. This buffering effect occurred globally across all three examined forebrain regions (dorsolateral, dorsomedial, and ventral telencephalon). However, *pre- and post*-amputation social interaction exerted an even greater buffering effect than *pre*-amputation social interaction alone on the forebrain cell proliferation response to tail amputation.

Interestingly, in the midbrain periventricular zone region adjacent to the pre-pacemaker nucleus (PVZ 1), which is important for social communication in electric fish, there was no significant difference in proliferating cell density between *pre*-amputation social fish and isolated amputated fish. In the region distant to the pre-pacemaker nucleus (PVZ 2), brain cell proliferation was not altered by tail amputation and/or social interaction (Figure 11). However, both *pre*-amputation social fish and isolated amputated fish had

significantly lower proliferating cell density than *pre- and post-*amputation social fish in the PVZ 1 (Table 7 and Figure 11). This indicated that social interaction both before *and* after tail amputation may be required for a buffering response in the midbrain. Thus, the forebrain and midbrain differed in their social buffering effects, and the midbrain showed regional differences in the negative effects of tail injury with continuous social interaction. When including both the forebrain and the midbrain in the analysis, a significant treatment x region interaction was observed (Table 7).



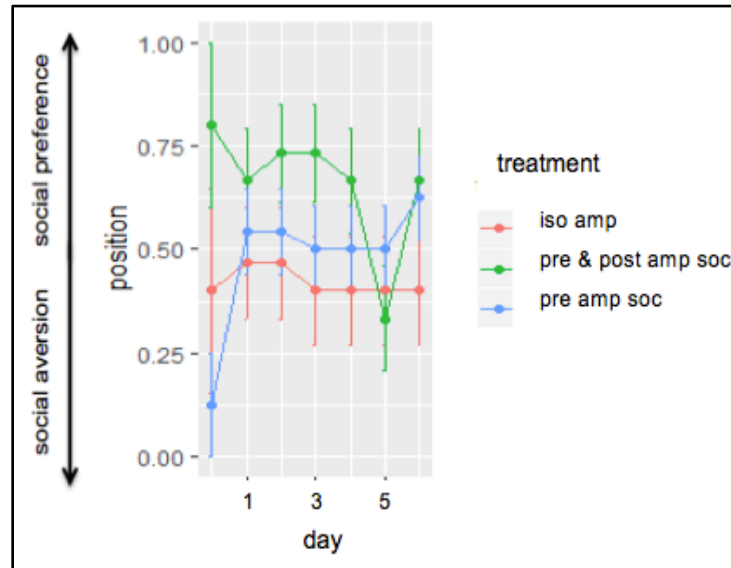
**Figure 11. Density of proliferating cells within the forebrain and midbrain.** A) Proliferating cell density (PCNA+ cells/mm<sup>3</sup>) was calculated in three forebrain regions, the dorsolateral (DI), dorsomedial (Dm), and ventral (V) telencephalon, and in two regions of the midbrain periventricular zone, located adjacent to (PVZ 1) or distant to (PVZ 2) the pre-pacemaker nucleus. This graph shows that social buffering in the forebrain was caused by: a) *pre-*amputation social interaction in the DI, Dm, and V regions and by b) *pre- and post-*amputation social interaction in the DI and Dm regions. In the midbrain PVZ 1, social buffering was only induced by *pre- and post-*amputation social interaction. No social buffering effect was evident in the midbrain PVZ 2. Different letters (a, b, or c) indicate significant differences between treatment groups. Error bars indicate standard error. B) This scheme shows the timeline of the experimental conditions experienced by focal fish in each treatment group.

**Table 7. ANOVA results for experiment 4.** Two-way ANOVA results showing the effects of brain region, social treatment, and the interaction of brain region with social treatment on brain cell proliferation.

Effect	d.f.	<i>F</i>	<i>P</i>
brain region	4	13.6	= 0.004
treatment	2	7.9	< 0.0005
brain region x treatment	8	3.3	= 0.005

### *Social Affiliation Results*

Fish living with a social partner only before (4 d) tail amputation were either slightly attracted to the stimulus compartment or randomly chose their tube, but fish living with a social partner both before (4 d) and after (7 d) amputation were strongly attracted to the stimulus compartment. In contrast, isolated amputated fish avoided the stimulus compartment (Figure 12). There was a significant treatment x day interaction ( $\chi^2 = 7.837$ ,  $P < 0.02$ ).



**Figure 12. Tube-seeking (position) preference in isolated amputated fish, pre- & post-amputation social fish, and pre-amputation social fish.** Fish were either paired or isolated for 4 d before behavioral testing. On day 0, all focal fish were tail-amputated and divided into three groups: isolated amputated ( $n = 5$ ), pre- and post-amputation paired ( $n = 5$ ), and only pre-amputation paired ( $n = 8$ ). Social affiliation was recorded for a total duration of 6 d. As shown above, fish paired only before tail amputation were slightly attracted to their stimulus fish (positions  $>0.5$ ) or randomly chose their tube (positions  $=0.5$ ), while fish paired both before and after tail amputation were mostly attracted to their stimulus fish (positions  $>0.5$ ) except on day 5. In contrast, isolated fish constantly avoided the stimulus compartment (positions  $<0.5$ ).

### *Experiment 5: Post-Amputation Social Interaction and Brain Cell Proliferation*

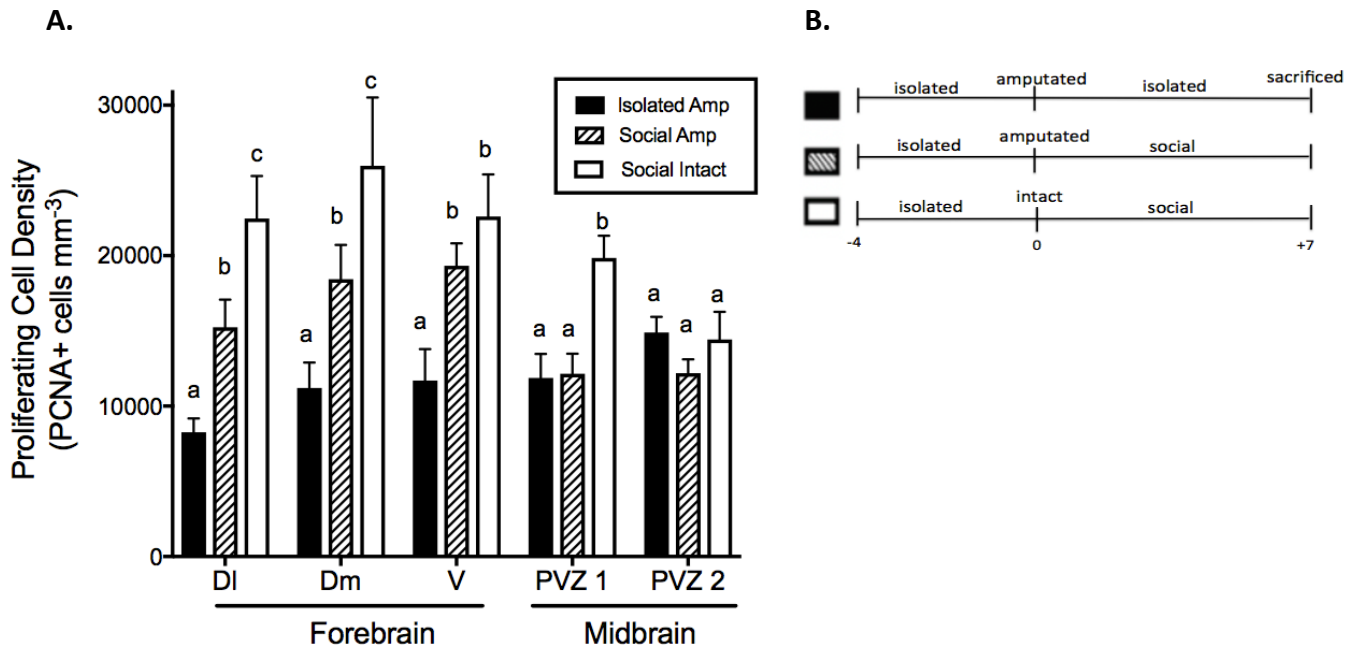
I investigated the exclusive effects of *post*-amputation social interaction on brain cell proliferation in *A. leptorhynchus*. In all three examined regions of the forebrain, the dorsolateral (Dl), dorsomedial (Dm), and ventral telencephalon (V), isolated amputated fish had significantly lower (~50%) proliferating cell density than intact fish paired (7 d) with a social partner. However, fish paired *post*-amputation (7 d) had significantly higher density of cell proliferation than isolated amputated fish (Table 8 and Figure 13), indicating that *post*-amputation social interaction buffers the brain cell proliferation response to tail amputation. This buffering effect occurred globally across all forebrain regions (Figure 13). In two of these regions (Dl and Dm), fish paired *post*-amputation still had lower density of cell proliferation than paired intact fish, but in the V, *post*-amputation social interaction completely reversed the negative effect of tail amputation on brain cell proliferation (Table 8 and Figure 13).

In the two examined midbrain regions (PVZ 1 & PVZ 2), there was no significant difference in brain cell proliferation between fish paired *post*-amputation and isolated amputated fish (Figure 13), indicating that social buffering did not occur in the midbrain. Cell proliferation in the periventricular zone region distant to the pre-pacemaker nucleus (PVZ 2) was not affected by either tail amputation or social interaction (Figure 13). Not surprisingly, in the midbrain periventricular zone region known to be important in social communication (PVZ 1), paired intact fish had significantly higher density of cell proliferation than both fish paired *post*-amputation and isolated amputated fish (Table 8 and Figure 13). Thus, social buffering effects were specific to the forebrain as a whole, and the

midbrain showed regional differences in the positive effects of social interaction. When including both the forebrain and the midbrain in the analysis, a significant treatment x region interaction was observed (Table 8).

**Table 8. ANOVA results for experiment 5.** Two-way ANOVA results showing the effects of brain region, social treatment, and the interaction of brain region with social treatment on brain cell proliferation.

Effect	d.f.	F	P
brain region	4	4.4	<0.005
treatment	2	11.3	< 0.0005
brain region x treatment	8	3.0	<0.005



**Figure 13. Density of proliferating cells within the forebrain and midbrain.** A) Proliferating cell density (PCNA+ cells/mm<sup>3</sup>) was calculated in three forebrain regions, the dorsolateral (DI), dorsomedial (Dm), and ventral (V) telencephalon, and in two regions of the midbrain periventricular zone, located adjacent to (PVZ 1) or distant to (PVZ 2) the pre-pacemaker nucleus. This graph shows that post-amputation social interaction buffered the cell proliferation response to tail amputation across all three examined forebrain regions (DI, Dm, and V). This effect was specific to the forebrain and did not occur in the midbrain. Different letters (a, b, or c) indicate significant differences between treatment groups. Error bars indicate standard error. B) This scheme shows the experimental timeline of the conditions experienced by focal fish in each treatment group.

## Discussion

### *Overview*

The main goal of this thesis was to determine whether social interaction mitigates the decrease in brain cell proliferation caused by simulated predatory tail injury in weakly electric fish, *Apteronotus leptorhynchus*. This buffering effect was observed in experiment 1 in fish paired both before (4 d) and after (1 d) tail amputation for a total of 5 d. In experiments 4 & 5, I examined whether it was pre-amputation or post-amputation social interaction that buffered this neural response. Experiment 4 showed that fish paired for 4 d before tail amputation had higher brain cell proliferation than isolated amputated fish. However, fish paired both before (4 d) and after (7 d) tail amputation for a total of 11 d had higher brain cell proliferation than fish paired only before tail amputation. Thus, these results suggested that pre-amputation social interaction buffers the brain cell proliferation response to injury but that pre- and post-amputation social interaction elicits a greater buffering response. Experiment 5 showed that fish paired for 7 d after tail amputation had higher brain cell proliferation than isolated amputated fish, indicating that post-amputation social interaction also induces a buffering effect on brain cell proliferation. This neural evidence for social buffering in injured fish led to behavioral studies aimed to determine whether intact fish naturally seek or avoid social interaction and whether injury increases social affiliation with an intact partner. Experiment 2 showed that intact fish generally avoid social interaction, but experiments 3A & 3B showed that post-injury social interaction does not reverse this social aversion. Finally, experiment 4 showed that fish paired both before and after tail amputation were attracted to their social partner, indicating that pre- and post-injury social interaction may be better than post-injury social interaction at enhancing social affiliation.



## *Neural Responses*

### *a) Social Buffering of the Brain Cell Proliferation Response to Tail Injury*

In experiment 1, social interaction both before and shortly after (1 d) tail amputation increased brain cell proliferation in amputated *Apteronotus leptorhynchus*. In earlier studies, Dunlap et al. (2016, 2017) showed that forebrain cell proliferation in weakly electric fish, *B. occidentalis* and *A. leptorhynchus*, decreases with exposure to predatory stimuli both in the field and in the lab. This effect was observed both when fish are naturally exposed to high predation pressure and when experimentally exposed to tail amputation or simulated predator chase (Dunlap et al., 2016; Dunlap et al., 2017). By contrast, in *A. leptorhynchus*, cell proliferation in the midbrain increases in fish living with a social partner (Dunlap et al., 2006). This increased brain cell proliferation has been correlated with electrocommunication, as it only occurs in a specific midbrain region known to regulate electrocommunication (chirping) behavior in electric fish (Dunlap et al., 2006; Dunlap et al., 2013). Similarly, many studies in mammals have shown that neurogenesis can increase with social interaction and that social interaction can buffer decreases in neurogenesis caused by social isolation or non-social stressors (Holmes, 2016).

Most studies on social buffering of neural responses in mammals focus on forebrain regions homologous to specific regions in the fish forebrain and examine neural activity with a c-Fos marker (e.g. da Costa et al., 2004; Bowen et al., 2013) or direct measures (e.g. Fuzzo et al., 2015). By contrast, the present study and some studies (e.g. Tzeng et al., 2013, 2018) examine the social buffering of neurogenesis per se. Specifically, I examined the occurrence of social buffering of one stage of adult neurogenesis, brain cell proliferation, in weakly electric fish, *A. leptorhynchus*, exposed to simulated predatory injury under different social

environments. My major goal was to investigate whether the presence of conspecifics could mitigate the decrease in brain cell proliferation caused by experimental tail amputation.

A recent study by Faustino et al. (2017) provided the first evidence of social buffering in fish. They found that the presence of conspecific cues significantly reduced the fear response (freezing behavior) of zebrafish (*Danio rerio*) exposed to an aversive stimulus. Interestingly, this social buffering effect coincided with the co-activation of forebrain regions known to function in mammalian social buffering (Faustino et al., 2017). Based on this relationship between forebrain activation and social buffering in both fish and mammals (Faustino et al., 2017; da Costa et al., 2004; Fuzzo et al., 2015), I examined cell proliferation in three forebrain regions, the dorsolateral (DL), dorsomedial (Dm), and ventral (V) telencephalon, that represent the homologues of particular forebrain regions in mammals and help coordinate the behavioral response to predators in fish. In addition, I examined cell proliferation in the midbrain periventricular zone region (PVZ 1) that is influenced by social stimuli in *A. leptorhynchus* (Dunlap et al., 2006; Dunlap et al., 2013; Dunlap & Chung, 2013).

The results of experiment 1 showed that *A. leptorhynchus* living with a social partner both before and shortly after (1 d) tail amputation had significantly higher brain cell proliferation than injured fish living in isolation. This result provides the first evidence of social buffering in a species of *electric* fish. Furthermore, previous studies on intact *A. leptorhynchus* showed that 1 d of social interaction is not enough to increase brain cell proliferation in any region of the midbrain PVZ (Dunlap et al., 2006). This suggests that the 4 d of social interaction before tail amputation are required to elicit a social buffering response in the short-term (1 d) recovery period from tail amputation. It also demonstrates

that social interaction both before and after tail amputation can elicit a buffering effect during this recovery period (1 d) known for showing the greatest decline in brain cell proliferation in *A. leptorhynchus* (Dunlap et al., 2017).

The effect of social buffering was specific to the forebrain and did not occur in the midbrain. Within the forebrain, all three examined regions (Dl, Dm, and V) significantly responded to social buffering; that is, all forebrain regions showed significantly higher brain cell proliferation in paired amputated fish than in isolated amputated fish. This result agrees well with a study by Faustino et al. (2017) showing that social buffering in zebrafish induces a pattern of co-activation of forebrain regions that are involved in mammalian social buffering, including both the Dm and V. Thus, studies in both zebrafish (*Danio rerio*) and weakly electric fish (*Apteronotus leptorhynchus*) have now demonstrated that neural processes underlying social buffering occur globally across the forebrain rather than in localized brain regions. This effect of social buffering in forebrain regions that are homologous to those involved in the same phenomenon in mammals suggests the existence of a conserved neural mechanism of social buffering across vertebrates (Faustino et al., 2017).

#### *b) Pre-Amputation Social Interaction and Brain Cell Proliferation*

In experiment 4, fish were exposed to social interaction before tail amputation and were immediately isolated to determine whether *pre*-amputation social interaction could mitigate the deleterious effects of tail amputation on brain cell proliferation. This contrasts with experiment 1, in which I showed that pairing fish both before and after tail amputation mitigated the decrease in brain cell proliferation caused by tail amputation. Thus, experiment 1 used an exposure-type condition in which focal fish were paired *during* exposure to a

threatening stimulus (Kiyokawa, 2018), while experiment 4 used a “*pre-exposure-type*” condition in which fish were paired only *before* exposure to a threatening stimulus (tail amputation).

Moreover, in contrast to experiment 1, fish in experiment 4 were allowed to have a longer recovery period (7 d) after tail amputation. The rationale for this extended recovery period came from previous studies in *A. leptorhynchus* suggesting that the increase in brain cell proliferation caused by social interaction takes longer than the decrease in brain cell proliferation caused by experimental tail amputation (Dunlap et al., 2006, Dunlap et al., 2017). However, while brain cell proliferation in *A. leptorhynchus* decreases drastically in the short-term (1 d) post-amputation, it partially rebounds in the long-term (18 d) post-amputation (Dunlap et al., 2017; Dunlap, 2016). Thus, in experiment 4, an intermediate recovery period (7 d) was used to lengthen the positive effects of social interaction on brain cell proliferation while eliminating any changes in cell proliferation resulting from tail regeneration rather than from tail amputation *per se* (Dunlap, 2016).

In experiment 4, fish paired only before tail amputation had significantly higher brain cell proliferation than isolated amputated fish, indicating that *pre-amputation* social interaction can buffer the decrease in brain cell proliferation due to tail amputation in *A. leptorhynchus*. This result also demonstrates that *pre-injury* social interaction is effective at inducing social buffering even at day 7 after tail amputation in *A. leptorhynchus*. Similar to experiment 1, the buffering effects of *pre-amputation* social interaction occurred globally across all three examined forebrain regions (Dl, Dm, and V) and did not occur in the midbrain PVZ 1. In contrast to experiment 1, cell proliferation was also examined in a control midbrain region (PVZ 2) that is unaffected by social stimuli in *A. leptorhynchus*. As

expected, there was no effect of social interaction on cell proliferation in the PVZ 2, as well as no effect of tail amputation on the midbrain (Dunlap et al., 2017).

Notably, fish paired both before and after tail amputation showed significantly higher levels of both forebrain and midbrain cell proliferation than fish paired only before tail amputation. In the forebrain, this effect occurred globally across all three examined regions, the dorsolateral (DI), dorsomedial (Dm), and ventral (V) telencephalon. In the midbrain, this effect was specific to the periventricular zone region (PVZ 1) that controls electrocommunication in electric fish (Dunlap et al., 2006). These results suggested that both pre- and post-amputation elicits a greater buffering response than only pre-amputation social interaction in the forebrain cell proliferation response to tail amputation. However, the results also indicated that social interaction both before and after tail amputation might be necessary for the social buffering of *midbrain* cell proliferation in *A. leptorhynchus*.

It is important to note that the effects of social buffering were *not* observed in the midbrain PVZ 1 when fish paired both before and after tail amputation were only given a short-term (1 d) recovery period (experiment 1). Thus, this new evidence of social buffering in the midbrain not only highlighted the importance of the timing of social interaction relative to injury in *A. leptorhynchus* but also the importance of both a pre-injury (4 d) social period (experiments 1 & 2) and an intermediate (7 d) recovery period (experiment 2) in allowing sufficient time for the influence of social interaction on the brain. However, this also introduces a new question: Is it the *timing* of social interaction relative to tail amputation or the *duration* of social interaction that really elicits this social buffering of brain cell proliferation? Fish in experiment 1 were exposed to social interaction for a total of 5 d, while fish in experiment 4 were exposed to social interaction for a total of 4 d (pre-amputation

treatment group) or 11 d (pre- and post-amputation treatment group). Thus, it is possible that the increase in brain cell proliferation in fish paired both before and after tail amputation compared with fish paired only before tail amputation is simply a result of the extended period of social exposure rather than the different timing of social exposure relative to tail amputation. Indeed, in rats, it has been shown that extending the duration of social interaction increases the social buffering effects of conditioned fear responses (Kiyokawa et al., 2016). In *Apteronotus*, future studies manipulating the timing of social interaction while controlling both the duration of social pairing and the recovery period after tail amputation are necessary to accurately distinguish the effects of pre-injury vs. pre- and post-injury social interaction on brain cell proliferation.

### *c) Post-Amputation Social Interaction and Brain Cell Proliferation*

Having observed the buffering effects caused by both pre- and post-amputation social interaction (experiment 1) and only pre-amputation social interaction (experiment 4), I investigated the exclusive effects of *post*-amputation social interaction on the brain cell proliferation response to tail amputation in *A. leptorhynchus* (experiment 5). In contrast to experiment 1, experiment 5 used a “housing-type” experimental condition in which fish were first exposed to a threatening stimulus (tail amputation) *while isolated* and co-housed with a conspecific (Kiyokawa, 2018). However, just as in experiment 4, fish in experiment 5 were given an intermediate recovery period (7 d) after tail amputation to allow sufficient time for the beneficial effects of social interaction on brain cell proliferation.

The results of experiment 5 showed that fish paired after tail amputation had significantly *higher* proliferating cell density than isolated amputated fish, indicating that post-amputation social interaction buffers the negative effect of tail amputation. When comparing

all treatment groups, fish paired after tail amputation had proliferating cell densities between those of isolated amputated fish and paired intact fish in two forebrain regions (Dl and Dm). However, in one forebrain region (V), there was no significant difference in proliferating cell density between fish paired after tail amputation and paired intact fish, indicating that post-amputation social interaction completely reverses the negative effect of tail amputation in this region.

As fish can respond to environmental stimuli through many different cellular processes (Dunlap et *al.*, 2016), it is conceivable that paired amputated fish are actually increasing brain cell proliferation as part of a non-specific, global change in cell proliferation. For example, paired amputated fish may choose to proliferate brain cells at the expense of repairing their tail injury (tail regeneration), while isolated amputated fish may simply show lower levels of brain cell proliferation because they divert their energy for the process of tail regeneration rather than brain cell proliferation. However, previous studies (Ragazzi and Dunlap, unpublished data) discovered that social pairing does not change the rate of tail regeneration or overall body growth in *A. leptorhynchus*, suggesting that social interaction does not simply increase the overall levels of cell proliferation in the body. Thus, experiment 5 showed that *post*-amputation social interaction indeed buffers the decrease in brain cell proliferation caused by tail amputation in *A. leptorhynchus*. This result agrees well with a study by Venna et *al.* (2014) showing that mice living with a healthy partner *after* having a stroke had higher neurogenic rates than those living isolated. Together, studies in mammals and in weakly electric fish have now provided evidence that social interactions cannot only buffer the neurogenic response to brain injury but also the neurogenic response to injury outside the brain (somatic injury).

Furthermore, just as in experiments 1 & 4, the buffering effect of *post*-amputation social interaction on brain cell proliferation occurred globally across all three examined forebrain regions (Dl, Dm, and V) and did not occur in the midbrain (PVZ 1 and PVZ 2). Thus, when compared to isolated amputated treatment groups, *pre*-amputation, *post*-amputation, and both *pre- and post*-amputation social interaction seem to share a conserved neural mechanism of social buffering that involves the forebrain as a whole. The occurrence of this specific neural response in electric fish extends the abovementioned findings in zebrafish showing that social buffering induces a pattern of co-activation of *forebrain* regions, which includes the dorsomedial (Dm) and ventral telencephalon (V) as well as another region, the pre-optic area (POA). Similarly, a previous study in wild-type guppies showed that social interaction increases neuronal activation in the POA of the forebrain relative to living in isolation (Cabrera-Álvarez et al., 2017). Thus, due to the involvement of the POA on the neurogenic responses to both social interaction and social buffering in fish, future studies in *A.leptorhynchus* should also examine the effects of social buffering in the cell proliferation rate of the POA.

The buffering effect of *post*-amputation social interaction on brain cell proliferation re-introduces the need to differentiate the influence of the *timing* of social interaction relative to injury from the *duration* of social interaction on evoking this buffering response. While fish in experiments 4 & 5 experienced the same recovery period post-amputation (7 d), they differed in both their timing and duration of social pairing. Fish in experiment 4 were paired for 4 d *before* tail amputation, while fish in experiment 5 were paired for 7 d *after* tail amputation and were expected to show increased cell proliferation in the midbrain PVZ 1 (Dunlap et al., 2006). Although *pre*-amputation vs. *post*-amputation social treatments were



not directly compared in the same experiment, they both showed a similar buffering response in the forebrain, and this response occurred despite differences in the duration of social interaction. This suggests that social interaction either before or after tail amputation is equally effective in buffering the decrease in brain cell proliferation in *A. leptorhynchus*. By contrast, when fish were paired both before and after tail amputation (experiment 4), their forebrain cell proliferation was significantly higher than when exposed to either pre- or post-amputation social interaction alone. Such additional benefits of continuous social interaction (both pre- and post-threat) have also been reported in the neural activation (c-Fos expression) and fear (freezing) behavior of rats exposed to aversive auditory stimuli (Kiyokawa et al., 2007).

Lastly, it is not possible to completely rule out the presence of differences in the buffering effects elicited by pre-amputation and post-amputation social interaction in *A. leptorhynchus*. Other studies in rats have shown that the timing of social interaction relative to the stressor exposure modifies neural activation in specific forebrain regions (Kiyokawa et al., 2007). Thus, further studies in *A. leptorhynchus* should compare the effects of *post*-amputation vs. *pre*-amputation social interaction on brain cell proliferation while controlling the duration of social pairing and the recovery period.

### *Behavioral Responses*

#### *a) Social Affiliation in Intact Electric Fish*

In experiment 2, I examined social affiliation in intact *A. leptorhynchus* to answer two main questions: a) Do intact fish naturally show a preference or aversion to social interaction? b) Does social affiliation in intact fish vary according to the sex of their partner? The results indicated that paired intact fish generally avoid social interaction regardless of

having a same-sex or an opposite-sex partner (stimulus fish). This suggests that fish inherently avoid social interaction in the absence of simulated predatory tail amputation. Nonetheless, in both same-sex and opposite-sex pairing conditions, no significant treatment (paired vs. isolated) x day interaction was found. Moreover, in contrast to the seemingly different behavior of isolated fish vs. same-sex paired fish throughout the entire experiment, the independent effect of treatment was not significant either. I suspect that this pattern might have resulted from a few individual fish that showed a consistent preference for either the front tube or the back tube and thus drove the treatment effect that is visible in the plot (Figure 8). Previous behavioral studies in *A. leptorhynchus* have used a greater number of focal fish presented with a social partner (n= 15; Zupanc et al., 2006) or simulated EOD signals from a stimulus fish (n=30; Zupanc & Maler, 1993), relative to the number of focal fish exposed to dyadic interactions in the present study (n= 9). Thus, future studies on social affiliation in *A. leptorhynchus* may benefit from using a larger sample size that could help overcome the observed biases.

#### *b) Social Affiliation in Injured Electric Fish*

After observing the occurrence of social buffering of brain cell proliferation in *injured* fish (experiment 1) and the trend of social aversion in *intact* fish (experiment 2), I sought to answer the following question: Does injury increase social affiliation with a healthy (non-injured) social partner? Behavioral testing was conducted in separate replicate experiments (experiments 3A & 3B), but these experiments yielded different results. In experiment 3A, paired intact fish were mostly attracted to their stimulus fish, while amputated fish similarly avoided the stimulus compartment after 1 d of treatment whether paired or isolated. In experiment 3B, paired fish, whether intact or amputated, avoided the

stimulus fish, but paired amputated fish showed a greater trend of social avoidance compared to paired intact fish. In both experiments, isolated amputated fish generally avoided the stimulus compartment, but their behavior was still highly inconsistent. A significant treatment x day interaction was only found in experiment 3B, indicating that the effect of treatment on social behavior depended on time.

Furthermore, the results in experiment 3A suggesting that paired intact fish tend to be attracted to their stimulus fish did not agree well with those in experiment 2 suggesting that paired intact fish tend to avoid their stimulus fish regardless of their sex. Overall, neither experiment 3A nor experiment 3B suggested that experimental tail injury in *A. leptorhynchus* increases social affiliation. This would have been suggested if paired amputated fish had shown a significantly greater social affiliation relative to paired intact fish. However, in both experiments, paired amputated fish showed little affiliation toward their social partner.

Finally, since tube-seeking preference was only collected *after* social interaction/tail amputation, this raises another question: Do fish naturally prefer their tubes to be located in a particular part of the tank prior to the experimental runs and is this preference altered by the trials? Data collected prior to experiment 3B indicated that fish naturally prefer to seek shelter in the back tube, with the exception of a few individual fish that consistently preferred the front tube. Nonetheless, in agreement with the trend of social aversion observed in all treatment groups, the same fish that had preferred the front tube *before* the experimental run preferred the back tube *after* exposure to social interaction and/or tail amputation. This reinforces the idea that intact *A. leptorhynchus* generally avoid social interaction (experiment 2) and that injury does not stimulate fish to seek closer affiliation with a healthy social partner (experiment 3A & 3B). However, it is still unclear why paired intact fish in

experiment 3A seemed attracted to their social partners and whether this seeming attraction was influenced by the proportion of individual fish that intrinsically preferred the front tube prior to the experiment.

*c) Social Affiliation in Pre-amputation and Pre- and Post-amputation Social Electric Fish*

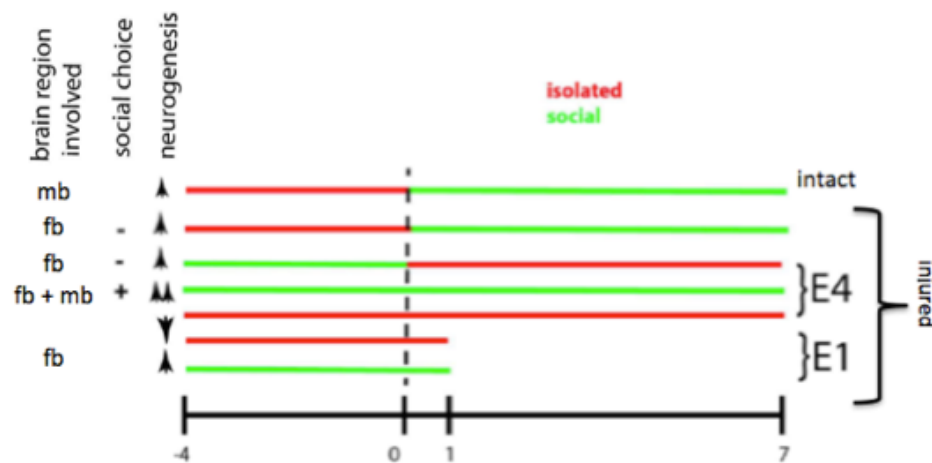
In experiment 4, I measured both social affiliation and brain cell proliferation in injured *A. leptorhynchus* to answer the following question: Does the social buffering of brain cell proliferation caused by pre-amputation alone and both pre- and post-amputation social interaction correlate with changes in social affiliation? Specifically, I examined whether fish paired both before and after tail amputation showed an increase in social affiliation compared with fish paired only before tail amputation. In this case, both treatment groups started as paired intact fish and diverged into an isolated amputated group (in pre-amputation social fish) and a paired amputated group (in pre- and post-amputation social fish) following tail amputation. Thus, if no difference in social affiliation (tube-seeking preference) were found between the two groups, this would suggest that amputated fish do not purposely seek to avoid their social partner *after* being injured, as seen in experiments 3A & 3B.

The results of experiment 4 showed that fish paired both before and after tail amputation were significantly more attracted to the stimulus compartment compared to fish paired only before tail amputation. In this case, fish paired before tail amputation were either slightly attracted to the stimulus compartment or randomly chose their tube, while fish paired both before and after tail amputation were almost constantly attracted to the stimulus fish. This behavioral result was similar to the neural result indicating that *pre- and post-*amputation social interaction evokes a stronger buffering effect than *pre-*amputation social interaction in localized regions of the forebrain (Dl and Dm) and midbrain (PVZ 1; see

Figure 13). In addition, this result agrees well with a study in rats suggesting that social interaction before exposure to predatory stimuli exerts a *minimal* social buffering effect on the behavioral response to threat (Siviy, 2008). In this study, rats that played repeatedly before exposure to a worn cat collar spend *slightly* less time inside a hide box compared with rats exposed to the predator odor without prior social interaction. This effect nonetheless was only subtle and both groups did not significantly differ in their fear response and risk assessment (Siviy, 2008).

In contrast to the social avoidance seen in paired amputated fish in both experiments 3A and 3B, paired amputated fish in experiment 4 seemed to be overall attracted to their social partner. As mentioned before, this increase in social affiliation was particularly noticeable in experiment 4 in fish paired both before and after tail amputation. However, it is important to recall that amputated fish showing little social affiliation in experiments 3A & 3B were only paired after tail amputation. This suggests that *pre- and post-*amputation social interaction might be better than *post-*amputation social interaction at increasing social affiliation in *A. leptorhynchus*. A possible explanation for this is that *post-*amputation social interaction involves a change in both social status (from isolated to paired) and health status (from intact to injured), whereas *pre- and post-*amputation social interaction only involves a change in health status (from intact to injured). Thus, even though *pre-*amputation social interaction alone cannot alter social affiliation, *prior* interaction with a healthy social partner might still be necessary to enhance social affiliation *after* exposure to injury. In this case, it is possible that fish paired both before and after tail amputation simply become habituated to the presence of their social partner; hence, they feel “less threatened” and seek closer affiliation. Lastly, the affiliation behavior of fish paired both before and after tail amputation

also raises another question: Is this potential social habituation causing fish to divert more of their energy for brain cell proliferation and thus responsible for the significant increase in brain cell proliferation in pre-and post-amputation social fish (Figure 11)? However, previous studies showed that continuous exposure (14 d) to a single social partner does not alter brain cell proliferation in paired *intact* fish relative to isolated controls, suggesting that social habituation has little benefit for brain cell proliferation (Dunlap & Chung, 2013). Thus, I believe that social habituation *per se* does not likely explain the observed increase in brain cell proliferation in *injured* fish exposed to continuous social interaction (11 d) both before and after amputation and that this buffering response was indeed induced by the mere presence of a social partner during this period. Further studies varying the periods of continuous social exposure (11 vs. 14 d) would be required to test this hypothesis and possibly discover more explicit differences in both social affiliation and brain cell proliferation in paired amputated treatment groups.



**Figure 14. Summary graph of the effects of social interaction on brain cell proliferation and social affiliation in *Aptereronotus leptorhynchus*.** This graph shows that depending on the specific brain region, social interactions varying in timing and duration can a) have a positive effect on cell proliferation in intact fish (midbrain), b) buffer the negative effects of amputation (forebrain), and c) both positively affect cell proliferation (midbrain) and buffer the negative effects of amputation (forebrain). Evidence of social affiliation was only found in fish exposed to social interaction both before and after tail amputation but not in the pre- or post-amputated phases alone. Fb= forebrain; mb= midbrain; (-) = social aversion; (+) = social preference.

### *Neural and Behavioral Responses*

Unlike fish such as *Danio rerio* that normally associate in shoals both in the field and in the lab (Faustino et al., 2017), *Apteronotus leptorhynchus* normally avoid other conspecifics in the lab but still benefit from social interactions following injury. This appears as a contradiction in terms and raises the following question: Why can social buffering occur in a largely “antisocial” fish? Moreover, if *A. leptorhynchus* normally avoid other conspecifics, what benefit does having one nearby actually produce for the injured fish? A possible answer to these questions must first recall that, from a neurobiological standpoint, paired social interactions are beneficial in *A. leptorhynchus* because they promote brain cell proliferation, as usually happens in the healthiest of conditions. This proliferative response is specific to the midbrain periventricular zone (PVZ) region that regulates the production of electrocommunication signals or “chirps” displayed during agonistic interactions (Dunlap et al., 2006; 2013). Consistent with these results, previous studies have shown that dyadic interactions also increase the production of chirps in *A. leptorhynchus* (Dunlap et al., 2002). This suggests that there is a causal relationship between behavioral and brain cell proliferation responses in electric fish, but it is still unclear whether elevated chirping acts as a stimulus to promote brain cell proliferation or whether elevated brain cell proliferation contributes to socially induced changes in chirping behavior (Dunlap et al., 2013).

From an endocrine and behavioral standpoint, paired social interactions are indeed detrimental in *A. leptorhynchus*. They increase both cortisol levels (Dunlap et al., 2002) and the risk of injury or death during combats (personal observations). However, while often aggressive in dyadic interactions, *A. leptorhynchus* commonly affiliate closely in groups following a stressful event, such as shipping and arrival into the lab. Thus, it is not difficult to

imagine that fish could affiliate closely after the traumatic experience of tail amputation, even in paired conditions. When physically separated as in the present study, fish coexist without risk of injury or death but may still choose to avoid close proximity with other conspecifics as a behavioral strategy to reduce electrosensory interference created when partners' signals interact with their own (Petzold et al., 2018). This might help explain why no evidence of social affiliation was found following tail injury, but there are still some potential interpretations for these negative results, as well as for the occurrence of social buffering of brain cell proliferation. First, it is possible that fish seek closer affiliation with each other during the night, when they increase their locomotor activity and exploratory behavior (Migliaro et al., 2018), and the data collected during the day would have not detected this behavioral change. Second, in *A. leptorhynchus*, social stimuli in the form of conspecific electric signals are sufficient for enhancing brain cell proliferation. It has indeed been demonstrated that the one-way delivery of one fish's EOD to the aquarium of another fish increases PVZ cell proliferation in the fish receiving such stimulus, resembling the levels of cell proliferation in fish living in pairs (Dunlap et al., 2008). In this study, the field strength of the back tube (distant to the stimulus fish) was still well above the threshold of electrosensory detection (Knudsen, 1974). Thus, it is possible that this relatively weaker stimulus was still sufficient to increase brain cell proliferation. Third, dyadic interactions in simplistic laboratory conditions may not be sufficient to stimulate social affiliation as a behavioral strategy to promote recovery from the negative brain response to tail injury. Future studies examining social affiliation in natural populations of injured fish could provide an answer to this issue. Lastly, injury *per se* may simply not enhance social affiliation in *A. leptorhynchus*.



### *Future Research*

This thesis demonstrates that living with an *intact* social partner buffers the decrease in brain cell proliferation caused by experimental tail amputation in *injured* weakly electric fish, *Apteronotus leptorhynchus*. However, while social interaction increases neurogenesis, the health of the social partner is also a critical contributing factor to neurogenic responses. Venna et al. (2014) showed that housing mice with a *healthy* partner after having a stroke significantly enhanced neurogenic rates compared with mice paired with an *unhealthy* (stroke) partner. Thus, future studies in *A. leptorhynchus* could address the following question: Does the injury of a stimulus fish also affect the brain cell proliferation response of a focal fish to tail amputation? Injured focal fish could be paired with an *intact* or *injured* stimulus fish for the same recovery period post-amputation to determine whether significant differences in brain cell proliferation exist between the focal fish in the two groups. Finally, brain cell proliferation could also be quantified in isolated amputated focal fish to determine whether living with an *injured* social partner is better or equivalent to living *isolated*.

## Conclusion

This thesis examined the social buffering of brain cell proliferation and the social affiliation behavior of weakly electric fish, *Apteronotus leptorhynchus*, in response to simulated predatory tail injury. In experiment 1, I found that social interaction buffers the drastic decrease in brain cell proliferation caused in the short-term (1 d) recovery period following tail amputation. In experiments 4 & 5, I found that social interaction either before or after tail amputation mitigates the decrease in brain cell proliferation in the long-term (7 d) recovery period following tail amputation. In experiment 4, I found that social interaction both before (4 d) and after (7 d) tail amputation produces an even greater buffering effect on brain cell proliferation. In experiment 3A & 3B, I found that social interaction after tail amputation does not reverse the fish's natural tendency for social aversion, as indicated by intact fish in experiment 2. Finally, in experiment 5, I found that social interaction both before and after tail amputation indeed reverses the fish's tendency for social aversion, suggesting that prior interaction with a healthy social partner might be necessary to stimulate social affiliation following tail amputation.

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