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The Lasting Effects of Perceived Predation Risk on the Avian Brain and Behaviour

Lauren E. Witterick
The University of Western Ontario

Supervisor
Dr. Liana Zanette
The University of Western Ontario

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Abstract

Predators affect prey populations not only through direct killing, but also through perceived predation risk – the 'fear' of predators. Responding to predation risk is critical for prey survival, however perceived predation risk can have lasting effects ranging from individual changes in neurobiology up to population level effects. I manipulated perceived predation risk using auditory playbacks of predators or non-predators in wild caught black-capped chickadees *(Poecile atricapillus)* in acoustic isolation and wild caught brown-headed cowbirds (*Molothrus ater)* in large outdoor aviaries. I found changes in dendritic morphology and inhibited neurogenesis in response to increased perceived predation risk lasting at least one week. I also found changes in both escape behaviour and in the response to a conspecific alarm call. My research shows that perceived predation risk has long-lasting effects on both the brain and behaviour, with applications for both ecologists and biomedical researchers.

Keywords

Predator-prey ecology, fear, perceived predation risk, post-traumatic stress disorder, nucleus taeniae of the amygdala, hippocampus, caudal nidopallium, dendritic morphology, ΔFosB, doublecortin, neurogenesis, *Poecile atricapillus, Molothrus ater*

Co-Authorship Statement

Julia Hrynkiewicz will be the second co-author on the manuscript that will be published from Chapter 3 of this thesis. Julia assisted with the predation risk manipulation and data collection in the field. Julia scored the escape behaviour data and conducted a series of immunohistochemistry for her undergraduate honours thesis.

Dr. Scott MacDougall-Shackleton will be the third co-author on the manuscript that will be published from this thesis. Scott provided guidance with regards to my surgeries, immunohistochemistry protocols, and the microscopy required to obtain my results.

Dr. Craig Bailey will be the fourth co-author on the manuscript that will be published from this thesis. Craig provided his expertise with regards to the Golgi-Cox staining and processing, and the neuron tracing to analyze dendritic morphology. Craig also provided access to the microscope system used for neuron tracing.

Dr. Michael Clinchy will be the fifth co-author on the manuscript that will be published from this thesis. Mike provided guidance on the overall experimental design, experimental site set-up and the statistical analysis.

Dr. Liana Zanette will be the sixth co-author on the manuscript that will be published from this thesis. Liana provided a great deal of input in the development of my experimental design, as well as feedback on my data analysis and the writing of this thesis. Additionally, she provided the experimental site (aviaries), NSERC funding to support this research, and the ethics approval for animal use.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

BCCH	Black-capped chickadee
BHCO	Brown-headed cowbird
CDK-5	Cyclin-dependent kinase-5
DCX	Doublecortin Antibody
Hp	Hippocampus
M	Mesopallium
NC	Caudal Nidopallium
PTSD	Post traumatic stress disorder
TnA	Nucleus taeniae of the amygdala

List of Appendices

Chapter 1

1 General Introduction

1.1 *Predator-Prey Interactions*

Predator-prey dynamics traditionally focused on the impact of predators based solely on how many prey they could capture and kill (Taylor 1984, Abrams 2000, Vandermeer et al. 2001), as predation represents the ultimate, negative effect on individual fitness since dead prey do not reproduce (Kavaliers and Choleris 2001, Boonstra 2013). However, most predators encounter more prey than they kill, as limited capture success gives many prey the opportunity to escape from the jaws of death (Vermeij 1982). For example, under severe winter conditions, recently reintroduced wolves *(Canis lupus)* in Yellowstone were only successful in killing elk (*Cervus elaphus)* in 26% of predation attempts, killing only 3% of the elk they chased (Mech et al. 2001). Additionally, the Merlin (*Falco columbarius*) predation success rate peaks at 26% of attempts across a variety of hunting strategies and observed populations (Page and Whitacre 1975, Buchanan et al. 1988). Few species reach a predation success rate of 90%, with success rates that high generally restricted to predation on prey in vulnerable sizes classes (Vermeij 1982). Given the low success rates, many individuals will survive predation attempts, learning and adapting from the experience.

1.2 *Anti-predator response*

In order to survive, all animals must be able to recognize predation risk and respond accordingly. In high predation environments, individuals may use adaptive avoidance to alter their behaviour and minimize the potential for predation (Lima and

Dill 1990, Lima and Bednekoff 1999). This behaviour can either be reactive to predation or predictive based on cues of predator presence, and may include spending more time under cover, increased vigilance, or changes to feeding behaviour, habitat selection, or escape behaviour (Lima and Dill 1990, Lima 1998, Steiner 2007, Walters et al. 2017). For example, increased predation pressure from reintroduced wolves led elk in Yellowstone to shift to conifer dominated feeding areas rather than their preferred grasslands, altering the habitat selection in elk (Creel et al. 2005). These behavioural changes from increased predation risk may also be accompanied by physiological changes, such as increased production of corticosteroid hormones (Boonstra et al. 1998, Clinchy et al. 2011b). Increased corticosteroid hormones from predator presence can lead to demographic consequences, as high levels of corticosteroid hormones have been connected with smaller litters in snowshoe hares (*Lepus americanus)* (Sheriff et al. 2009). Although these changes in behaviour and physiology can have lasting effects on populations, they traditionally had been thought to be acute and reversible changes, with the assumption that behaviour and physiological condition would return to baseline once the stress had been removed (Cannon 1915, Sapolsky 2004). However, elevated corticosteroid hormones from increased predation risk can also induce lasting physical changes in prey species.

For some species, the presence of predators or predator cues can induce permanent morphological changes. For example, the presence of predators can induce a larger body phenotype in tadpoles *(Rana piricia, Rana sylvatica)*, with a cost of reduced swimming performance (Kishida and Nishimura 2004, Middlemis Maher et al. 2013). Additionally, *Daphnia* have been shown to grow larger helmets and longer tail spines in a

high predator environment (Krueger and Dodson 1981, Dodson 1989). While these changes in behaviour, physiology, and morphology can help individuals avoid predation, they can also incur costs with repeated challenges.

The impact of predators extends past direct consumption alone, as the non-consumptive effects of predation risk can have a greater impact on populations than consumption. The presence of predators has been shown to have a greater effect on prey demography, growth, maturation, and density than consumption, with the effects especially pronounced when prey experience limited resources and increased competition (Preisser et al. 2005, Bolnick and Preisser 2005). These non-lethal effects of predators can extend up to the population and community level when individuals undergo repeated challenges (Lima 1998, Creel and Christianson 2008, Cresswell et al. 2010).

The net effect of these anti-predator responses can change prey population demographics, as the costs associated with avoiding predation can affect reproduction and offspring survival. For example, exposing song sparrows (*Melospiza melodia*) to increased perceived predation risk led to a 40% reduction in offspring survival (Zanette et al. 2011). Perceived predation risk can also lead to cascading effects, with changes in perceived predation risk in a mesocarnivore affecting species at three different levels of the food chain (Suraci et al. 2016). These demographic consequences can have epigenetic actions extending across generations, leading to important ecosystem implications.

1.3 *Predation Risk and the Brain*

Studying how perceived predation risk affects the brain will expand our understanding of the underlying causes of ecological changes associated with increased predation risk, as changes in the brain can impact the individual long after the threat of predation has passed. Predation stress has been shown to have long lasting effects on prey species, based on a range of different measures. Prey learn from previous predation experience, and the memory of a predation event or exposure to predator cues can lead to lasting changes in prey behaviour. For example, ringed salamanders (*Ambystoma annulatum*) exposed to predator cues as embryos showed significant behavioural changes post-hatching, as they were both less active and spent more time under vegetation (Mathis et al. 2008). Additionally, when predator cues were associated with higher risk, wood frog tadpoles (*Rana sylvatica)* retained the predator-related information longer (Ferrari et al. 2010). Retaining cues related to previous predation experience is important for future survival, and can be seen not only in behaviour, but also in lasting changes in the brain. For example, predator exposure has been shown to induce changes in the dendritic morphology of the brain of lab rats, through alterations to dendritic length, branching, and number of spines (Baran et al. 2005, Mitra et al. 2009, Adamec et al. 2012). Predation stress has also been shown to inhibit brain cell proliferation in rats and fish (Tanapat et al. 2001, Falconer and Galea 2003, Dunlap et al. 2016). Finally, increased perceived predation risk has been shown to increase immediate early gene activated protein production in the brains of rats and birds in response to predator cues (Staples et al. 2009, Hobbs 2015). These changes in the brain can be long lasting, and continue to impact individuals long after the immediate threat of predation has been

removed. Understanding the neural mechanisms behind predation stress can provide us with a greater understanding of the underlying causes of changes in behaviour and physiology.

There are three brain regions implicated in the stress response mediating fear and anxiety: the amygdala, the hippocampus, and the medial prefrontal cortex. The amygdala is the region most implicated in fear, and is of particular importance in learned fear (Gross and Canteras 2012). The amygdala is thought to play a crucial role in fear processing, through the development and expression of conditioned fear, detecting aversive environmental stimuli and responding accordingly (Davis 1992, Janak and Tye 2015). The hippocampus is important for spatial memory, interacting with the amygdala to integrate environmental context to predator cues (Gross and Canteras 2012). Finally, the medial prefrontal cortex is though to influence the expression of fear conditioning through interactions with the amygdala (Gross and Canteras 2012), in addition to controlling the emotional response (Steimer 2002).

1.4 *Predation Risk and Post Traumatic Stress Disorder*

Understanding the effects of perceived predation risk is useful not only for studying predator-prey dynamics, but also for modelling the effects of life threatening traumatic stress in humans. Experiencing, witnessing, or repeated exposure to life threatening traumatic events can lead to the development of post traumatic stress disorder (PTSD) in humans (American Psychiatric Association 2013). Symptoms can include intrusive memories, changes in emotional reactions, and avoidance behaviour, with diagnosis possible after symptoms are present for at least one month (American Psychiatric Association 2013). Individuals with PTSD may also have an increased

propensity for drug abuse and shifts in response to non-stress related stimuli (American Psychiatric Association 2013). The estimated lifetime prevalence for PTSD in humans is 7.8%, with woman more than twice as likely as men to develop PTSD, and onset most commonly associated with combat stress in men and sexual violence in women (Kessler et al. 1995).

In humans, long term changes in neurobiology have been associated with PTSD. The three brain regions thought to be involved in the human stress response are the amygdala, the hippocampus, and the medial prefrontal cortex (Bremner et al. 1999, Nutt and Malizia 2004, Shin et al. 2006). For example, in humans diagnosed with PTSD, negative trauma related stimuli induced an increased response in the amygdala (Protopopescu et al. 2005), suggesting that this region is sensitive to stress. Additionally, those diagnosed with PTSD showed a significant reduction in hippocampal volume when compared to healthy individuals (Gurvits et al. 1996, Bremner et al. 1997). Combat veterans also showed decreased blood flow in the medial prefrontal cortex in response to traumatic images and sounds (Bremner et al. 1999). Animal models are often used to study the etiology of PTSD, to gain a better understanding of the neurobiological mechanisms leading to changes seen in those diagnosed with PTSD. By focusing on the causes of PTSD rather than just studying the symptoms and the behavioural response, therapies can be targeted to reversing the effects of the traumatic event or mitigating the impact that these lasting changes have on individual behaviour.

Perceived predation risk presents an excellent stimulus to use in animal models for studying the effects of PTSD, which can be induced by life threatening traumatic events. Controlled manipulations of predation risk on animal models offer many

advantages for the study of PTSD for both ecologists and biomedical researchers (Cohen et al. 2012, 2014), with the extreme stress of predation mimicking the circumstances leading to PTSD in humans (Clinchy et al. 2011a). Animal models have shown behavioural changes consistent with the increased anxiety present in those with PTSD in response to increased perceived predation risk (Adamec & Shallow, 1993). Additionally, lab studies using animal models have shown long lasting changes in neuron morphology and protein expression in response to increased perceived predation risk (Adamec et al., 2012; Mitra et al., 2009; Staples et al., 2009). Animal models using perceived predation risk are advantageous in studying the effects of PTSD because the traumatic event induces no physical pain, while resulting in similar physiological and behavioural abnormalities seen in those diagnosed with PTSD (Cohen et al. 2012, 2014, Clinchy et al. 2013, Zoladz and Diamond 2016).

1.5 *Measuring Fear in Wild Animals*

Wild caught animals provide an excellent model to assess how perceived predation risk affects the brain and behaviour, as they have spent their entire life escaping predators so their response would more closely mimic the response in free-living animals than a laboratory raised animal. Wild animals would have learned to assess predator cues in order to survive, habituating to non-threatening cues of predation risk, although the extent of their previous predation experience is unknown. For example, Fiddler Crabs (*Uca vomeris*) exposed to dummy predators showed the ability to habituate to the presence of the predator over time, but also adjusted their behaviour when the predator location was altered and the apparent risk level changed (Hemmi and Merkle 2009). Exposing naïve lab raised animals to predators leads to potential overestimation of

predation risk, as they would not have learned to evaluate the extent of predation risk through previous experience with observational learning. Naïve animals can also have significant differences in their anti-predator response in comparison to experienced individuals (Brown and Warburton 1999). Using wild caught animals to assess the effects of perceived predation risk on the brain and behaviour would provide the most ecologically relevant view of the natural response that could be tested in a captive environment.

When measuring the effects of perceived predation risk on the brain, little is known about the effects on wild animals, particularly for non-mammalian species. Very few studies have investigated the effects of perceived predation risk on the avian brain and the networks processing predator induced fear. Increased activation has been found in the avian brain in response to fearful stimuli in captive, wild caught animals, (Marzluff et al. 2012, Cross et al. 2013, Hobbs 2015), however the effects of perceived predation risk on the brain have never been tested in a semi-natural or free-living environment. It would be expected that encounters with predators or predator cues would be perceived as life threatening by free-living prey, and lead to similar long lasting changes in the brain to those seen in humans with PTSD and in rodents (Clinchy et al. 2011a, Boonstra 2013, Cohen et al. 2014). When quantifying the long term effects of fear in the brain of wild animals, it would be expected that they have prior experience with predation risk and would be functioning at a higher baseline level of risk than predator naïve lab raised animals. Therefore any significant increases in a measure of perceived predation risk, whether on behaviour, physiology, or neurobiology, in wild animals

would represent a meaningful effect of fear (Clinchy et al. 2011a, Boonstra 2013, Cohen et al. 2014).

Three brain regions have been suggested as part of the network that processes predator induced fear in the avian brain: the nucleus taeniae of the amygdala (TnA), the hippocampus (Hp), and the caudal nidopallium (NC) (Cross et al. 2013, Hobbs 2015). The TnA is the avian homologue to the mammalian medial amygdala (Yamamoto et al. 2005), which plays a crucial role in fear processing (Davis 1992, Gross and Canteras 2012). It is suggested that this region acts as a switchboard, conveying information about threatening stimuli in the environment to other parts of the brain, with increased activation shown in response to both predator presence and cues (Marzluff et al. 2012, Cross et al. 2013, Hobbs 2015).

The avian Hp is homologous to the mammalian Hp, although its role in processing predation risk is not as well defined as the TnA (Colombo and Broadbent 2000, Bingman et al. 2003, Cross et al. 2013). The Hp plays a role in many processes involving learning and memory, including encoding and retrieval of fear memory and the processing of spatial information (Colombo and Broadbent 2000, Bingman et al. 2003, Gross and Canteras 2012). Increased activation has been shown using both positron emission tomography and immediate early gene activation in response to threatening stimuli, including predator cues and dead conspecifics (Cross et al. 2013, Hobbs 2015).

The role of the avian NC, analogous to the mammalian prefrontal cortex, is also not as well defined as the TnA. The avian NC is proposed to be involved in executive functions and processing information to generate behaviour (Herold et al. 2011).

Additionally, the increased activation has been found in the NC in response to viewing a predator (Cross et al. 2013).

1.6 *Quantifying the Effects of Fear in the Brain*

In order to asses the effects of perceived predation risk on the brain, it is necessary to quantify the extent of any changes that occur. Little is known about the effects on wild caught animals, particularly for a non-mammalian species. By adapting measures used to assess changes in the brain used in mammals, changes in brain activation and neural structure can be measured in a semi-natural environment. This allows us to assess how laboratory methods for studying the brain translate to the field, which could be beneficial for future comparison with biologically meaningful effects on reproduction (Clinchy et al. 2011a). In particular, I will focus on three different markers for assessing long lasting changes in the avian brain: ΔFosB, dendritic morphology, and neurogenesis.

One method which has been used to study the lasting effects of perceived predation risk in the brain is through changes to ΔFosB (Staples et al. 2009, Hobbs 2015). ΔFosB is a protein splice variant of FosB, a transcription factor produced rapidly and transiently in response to stress (Nestler et al. 2001). ΔFosB is a relatively long-lived molecule that accumulates in the brain following the breakdown of FosB, but can no longer be detected 1-2 months post stimulus withdrawal (Nestler et al. 2001). Changes in ΔFosB activation evident after seven days are representative of changes seen in PTSD, and are considered long lasting when individual lifespan is considered (Staples et al. 2009, Cohen et al. 2012). Perceived predation risk increased ΔFosB activation in the TnA and the HP seven days post treatment in black-capped chickadees under controlled

laboratory manipulations (Hobbs 2015), however it has not been studied in birds manipulated in semi-natural conditions.

The lasting effects of perceived predation risk can also be studied through changes in dendritic morphology. Dendrites are the component of neurons that are specialized to receive signals (Kulkarni and Firestein 2012). Stress induces structural plasticity, modifying the amount of connectivity between neurons to regulate excitatory neurotransmission through growth or retraction of spines, producing immediate and long term changes in synaptic function (Leuner and Shors 2013). Changes to dendritic morphology can be measured by examining changes to length, number of branches, or number of spines in the brain area of interest. Although linked to ΔFosB activation (Nestler et al. 2001, Ruffle 2014), changes to dendritic morphology can be longer lasting and potentially permanent, with changes still evident after behavioural extinction (Maroun et al. 2013) and 8 weeks post exposure (Juarez-Mendez et al. 2006). These lasting changes are thought to maintain traces of the fear response for future reactivation after behavioural extinction (Pignataro and Ammassari-Teule 2015). Previous studies have shown changes in dendritic morphology from perceived predation risk in rats over a week after the traumatic event (Baran et al. 2005, Mitra et al. 2009, Adamec et al. 2012), however this has yet to be tested in an avian species.

A third measure to look at the lasting effects of perceived predation risk is through neurogenesis, the generation of new neurons in the brain. Neurogenesis generally consists of three distinct phases: cell proliferation, neuronal differentiation, and maturation into functional neurons (Christie and Cameron 2006). Neurogenesis is thought to be involved in brain remodeling, allowing new memories to form and old

memories to break down and be removed (Frankland et al. 2013, Mongiat and Schinder 2014). High levels of neurogenesis has been shown to disrupt established hippocampus-dependent memories, leading to decreased memory retention (Akers et al. 2014). Neurogenesis is also highly variable depending on the environment, as experiences such as learning, environmental enrichment, exercise, and stress, can affect the rate of neurogenesis (Deng et al. 2010, Schoenfeld and Gould 2012, Egeland et al. 2015).

One particular stressor that has been shown to affect neurogenesis is predation risk, with multiple different cues of predation leading to decreased neurogenesis. For example, rats exposed to fox odor cues showed decreased cell proliferation in the dentate gyrus (Tanapat et al. 2001, Falconer and Galea 2003). Additionally, living in an environment with a naturally high predation risk led to decreased cell proliferation in electric fish (*Brachyhypopomus occidentalis*) when compared to those in a low predation pressure environment (Dunlap et al. 2016). However, this was a correlational study looking at natural variation in predation risk, with little known about how these wild caught animals would respond to a controlled predation risk manipulation.

In order to quantify differences in neurogenesis, we can quantify the number of neurons expressing doublecortin (DCX). DCX is a microtubule-associated protein associated with migrating immature neurons. It is a reliable marker to visualize and quantify neurogenesis, detectable for at least 60 days in newborn neurons, with some neurons expressing DCX for at least one year (Couillard-Despres et al. 2005, Vellema et al. 2014). Additionally, DCX has the advantage over bromodeoxyuridine (BrdU), another common marker of neurogenesis, because it does not require in vivo labelling of neurons through injections (Couillard-Despres et al. 2005). Using DCX allows us to

minimize handling the research animals, preventing additional stress that could be confound the experiment. Finally, there is evidence for co-labelling between Egr-1, an immediate early gene product, and DCX (Vellema et al. 2014). Egr-1 has been shown to be elevated in response to increased perceived predation risk (Hobbs 2015), so it would be expected that if there was a link between these measures that we would also see changes in DCX with increased perceived predation risk.

1.7 *Research Objectives*

My research aims to answer the question of how chronic perceived predation risk continues to affect the avian brain even after the stimulus is removed. My first objective is to determine if perceived predation risk induces lasting changes to the dendritic morphology of the brain. My second objective is to test for lasting changes in brain activation from perceived predation risk in a semi-natural environment, allowing birds to be protected from direct predation while still exposed to their natural environmental variation and predator cues. I hypothesize that under increased perceived predation risk, birds will show long lasting changes in brain activation and dendritic morphology. I predicted that I would see increased brain activation in both the Hp and the TnA, dendritic retraction in the Hp and dendritic extension in the TnA. I also hypothesize that any changes we see in the brain will translate to long lasting changes in anti-predator behaviour.

In Chapter 2 my objective was to assess the effects of chronic perceived predation on the dendritic morphology and behaviour in Black-capped chickadees (*Poecile atricapillus*), tested in acoustic isolation in the lab. In Chapter 3, I expanded this objective and assessed the effects of chronic perceived predation risk on the brain

activation, neurogenesis, dendritic morphology, and behaviour in Brown-headed cowbirds (*Molothrus ater*) living in semi-natural outdoor aviaries. In Chapter 4, I discuss the broader ecological and biomedical significance of my findings, and how they can expand our knowledge of the effects of perceived predation risk on the brain.

1.8 *Study Species*

I used two different study species in my experiments, in order to expand on previous research. For Chapter 2, my study species is the Black-capped chickadee (*Poecile atricapillus;* hereafter referred to as chickadees). In Chapter 3, my study species is the Brown-headed cowbird (*Molothrus ater*; hereafter referred to as cowbirds).

Black-capped chickadees are a well-known and easily recognizable bird across North America. Chickadees are found throughout the majority of Canada and the northern two-thirds of the United States (all material reviewed from Smith, 1991 unless stated otherwise). They are year-round residents, making them an ideal research subject as they are accessible for study throughout the year. Chickadees average 10-14 grams and are distinguished by their dark cap and bib; white cheeks; and dark back, wings, and tail. Chickadees feed on a variety of insect and plant species, and cache food for future use.

Chickadees have a complex vocal repertoire and use a variety of different vocalizations to communicate. They have at least 15 different vocalizations, signalling territories, feeding, reproductive availability, or predator presence. Chickadees will use the high zee call to alert other members of the flock that there is an immediate predation threat. The high zee is primarily given by males in response to avian and mammalian

predators. It conveys such a strong message that many other species of small birds will also freeze or take cover upon hearing this call.

Chickadees live in non-breeding flocks during the fall and winter, shifting to monogamous, territorial breeding pairs in the spring and summer. They nest in cavities in stumps and rotting branches, with females responsible for nest building and the incubation of eggs. Males and females share feeding duties when the young have hatched. The chickadees used in my experiment were all resident to London, in the area surrounding the University of Western Ontario. Chickadees are known to adapt readily to captivity, and did well in the semi-natural aviaries where they were housed on campus.

Brown-headed cowbirds are found across North America, ranging from northern Mexico up to Southern Canada (all material reviewed from Ortega, 1998 unless stated otherwise). Cowbirds are migrants, travelling between wintering and breeding grounds. Males are distinguished by their glossy back plumage and brown head while females have brown plumage with fine light streaking. Cowbirds are ground foragers, feeding primarily on insects and seeds.

Cowbirds flock together in groups throughout the year. As an obligate brood parasite, they lay their eggs in other birds' nests and are known to parasitize at least 220 different host species (Friedmann and Kiff 1985). This minimal investment in parental care allows for assessment of the effects of perceived predation risk during the breeding season with minimal impact on behaviour resulting from parental care responsibilities. The cowbirds used in this experiment were all captured during migration at a banding

station in Southern Ontario. They were group housed in semi-natural aviaries, to mimic their natural environment as closely as possible.

1.9 *References*

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Chapter 2

2 Assessing the effects of perceived predation risk on dendritic morphology and behaviour

2.1 *Introduction*

Predators can affect prey through both direct predation (e.g. killing and eating prey) and through indirect effects on prey species. All living organisms face the threat of predation, although responses differ across taxa. Understanding how prey perceive predation risk is essential to our knowledge of predator-prey ecology, as lasting changes in behaviour are often first signalled by a change in the brain. By expanding our knowledge of how a life threatening event like predation affects the brain, this can aid our understanding of how life-threatening situations can impact the human brain and affect human health.

In order to survive, animals must be able to perceive and respond to predation risk. Anti-predator responses vary across taxa, but can generally be broken down into changes in physiology, morphology, and behaviour. In high risk environments, behavioural changes to minimize predation risk can include spending more time under cover, increasing vigilance, or changing feeding behaviour, habitat selection, or escape behaviour (Lima and Dill 1990, Lima 1998, Steiner 2007, Walters et al. 2017). When facing increased predation risk, animals may also decrease the frequency of movement and reduce spontaneous activity levels to minimize the potential for detection by predators (Lima and Dill 1990, Lima 1998). These behavioural changes may be accompanied by physiological changes, such as increased production of corticosteroid hormones (Boonstra et al. 1998, Clinchy et al. 2011b), or morphological changes to avoid

gape limited predators (Krueger and Dodson 1981, Dodson 1989, Kishida and Nishimura 2004). When exposed to repeated challenges, the net effect of these anti-predator responses can change prey population demographics, as anti-predator costs affect reproduction and offspring survival (Creel and Christianson 2008, Zanette et al. 2011).

In addition to changes in the behavioural response when a predator is present, prey will also learn cues associated with predation risk, retaining the memory of cues associated with the predation event. For example, ring salamanders *(Ambystoma annulatum)* exposed to predator cues as embryos showed significant behavioural changes post-hatching, as they were both less active and spent more time under vegetation (Mathis et al. 2008). Additionally, prey have been shown to retain the memory of predator-related information longer when they had previously been associated with higher risk cues (Ferrari et al. 2010). Prey species can also learn to recognize both visual and acoustic social cues alerting predator presence, with socially acquired predator avoidance found in fish, birds, eutherians, and marsupials (Griffin 2004). Black-capped chickadees, in particular, will use a high zee call to alert other members of their flock to an immediate predation threat (Smith 1991). This call is given primarily by males in response to avian and mammalian predators, and conveys such a strong message of risk that many other small bird species will freeze or take cover in response to the high zee call (Smith 1991).

In order to better understand the underlying causes of these ecological changes associated with increased predation risk, it is beneficial to study how perceived predation risk affects the brain (Clinchy et al. 2011a, 2013). Wild caught animals present an excellent model to assess the how perceived predation risk affects the brain, as they likely

have some previous predation experience. When quantifying fear in the brain of wild animals, it would be expected that they have prior experience with predation risk, rather than predator exposure as a novel stimulus, and would be functioning at a higher baseline level of risk than predator naïve lab raised animals since they are likely to have experience judging cues of predation risk. Therefore any significant increases in a measure of perceived predation risk, whether on behaviour, physiology, or neurobiology, in wild animals would represent a meaningful effect of fear (Clinchy et al. 2011a, Boonstra 2013, Cohen et al. 2014).

Two of the brain regions thought to be involved in fear learning and memory are the amygdala and the hippocampus. The region most implicated in fear learning is the amygdala (Gross and Canteras 2012). The amygdala is thought to play a crucial role in the development and expression of conditioned fear, and in the detection of aversive environmental stimuli and responding accordingly (Davis 1992, Janak and Tye 2015). The hippocampus interacts with the amygdala to integrate environmental context to predator cues and plays an important role in learning and memory (Gross and Canteras 2012). In the avian brain, two brain regions that have been suggested as part of the network processing perceived predation risk are the nucleus taeniae of the amygdala (TnA), and the hippocampus (Hp) (Cross et al. 2013, Hobbs 2015). The avian homologue to the medial amygdala is the TnA, which plays a crucial role in fear processing (Davis 1992, Gross and Canteras 2012). The TnA is thought to be the centre of the avian fear network, conveying information about threatening environmental stimuli to other parts of the brain. Increased activation has been round in the TnA in response to predation stress (Marzluff et al. 2012, Cross et al. 2013, Hobbs 2015). The role of the

avian Hp, homologue to the mammalian Hp, is not as well defined as the TnA (Colombo and Broadbent 2000, Cross et al. 2013). The Hp is responsible for the encoding and retrieval of fear memory and the processing of spatial information (Colombo and Broadbent 2000, Gross and Canteras 2012).

Understanding the neural mechanisms behind a life-threatening event like a predation attempt is beneficial not only for studying predator-prey ecology, but also for modelling how these traumatic events affect the human brain. In humans, exposure to a life-threatening traumatic event can lead to the development of post traumatic stress disorder (PTSD) (American Psychiatric Association 2013). Long term changes in human neurobiology have been associated with PTSD, with the amygdala, hippocampus, and medial prefrontal cortex thought to be involved in the human stress response (Shin et al. 2006, Bremner et al. 2008). Animal models are often used to study the etiology of PTSD, in order to understand the neurobiological mechanisms behind the symptoms.

Controlled manipulations of perceived predation risk presents an excellent animal model for PTSD, offering many advantages for both ecologists and biomedical researchers (Cohen et al. 2012, 2014) using predation risk to mimic the circumstances leading to PTSD in humans (Clinchy et al. 2011a). PTSD research has traditionally focused on the treating the symptoms of the disease, rather than understanding the cause. Using animal models allows researchers to control the conditions leading to the onset of PTSD symptoms, to better understand the neurological mechanisms leading to this condition. Animal models have shown behavioural changes consistent with human PTSD symptoms, and lab studies have shown long lasting changes in neuron morphology and protein expression in response to increased perceived predation risk (Adamec and

Shallow 1993, Mitra et al. 2009, Staples et al. 2009, Adamec et al. 2012). Additionally, using perceived predation risk to model PTSD allows for the manipulation of a life threatening traumatic event without inducing physical pain, while still resulting in similar physiological and behavioural abnormalities (Clinchy et al., 2013; Cohen et al., 2012, 2014; Zoladz & Diamond, 2016). One major criticism of using perceived predation risk is whether lab rats with no previous predation experience would show the same response to predation stress as their free living counterparts, which can be mitigated through the use of wild caught animals (Clinchy et al. 2011a).

In order to quantify the brain response to increased perceived predation risk, I looked at changes in dendritic morphology. Dendrites are the component of neurons specialized to receive signals (Kulkarni and Firestein 2012). Stress induces structural plasticity, modifying the connectivity between neurons to regulate excitatory neurotransmission and producing immediate and long lasting changes in synaptic function that can be measured as changes in length, branching, or number of spines, with changes differing between acute and chronic stimuli (Leuner and Shors 2013). Previous studies have shown changes in dendritic morphology from perceived predation risk in rats, with reduced length and branching in the Hp and increased branching in the basolateral amygdala (Baran et al. 2005, Mitra et al. 2009, Adamec et al. 2012), however these changes have never been assessed in a wild caught animal. Previous work has shown increased ΔFosB, a protein splice variant of the immediate early gene FosB, in the TnA and Hp of wild caught black-capped chickadees one week after increased perceived predation risk (Hobbs 2015). Overexpression of ΔFosB increases expression of cyclin-dependent kinase-5 (CDK-5), which is one of the pathways that have been shown to

induce changes in dendritic morphology (Chen et al. 2000, Nestler et al. 2001, Norrholm et al. 2003, Ruffle 2014). Given the high levels of ΔFosB evident in both the Hp and the TnA under the same experimental conditions, it would be expected that we would also see structural plasticity in these regions. Finally, both males and females were assessed, to account for any sex differences in the response to increased predation risk.

My study aimed to investigate the long term behavioural and neurological changes in black-capped chickadees (*Poecile atricapillus*) in response to increased perceived predation risk. I used playbacks of predator and non-predator species to manipulate perceived predation risk (following Zanette et al. 2011), then looked at the behavioural response to a conspecific alarm call and the effect on dendritic morphology seven days later.

I predicted that I would see long lasting changes in behaviour in response to the alarm call, with those exposed to increased predation risk showing a greater response if they retained the memory or became sensitized to the predation risk from the previous week. I also predicted that I would see changes in the length, branching, and number of spines in birds exposed to the greater perceived predation risk, with the TnA showing increased dendritic length and branching and the Hp showing reduced dendritic length and branching (Leuner and Shors 2013).

2.2 *Methods*

2.2.1 *Overview*

I used wild caught black-capped chickadees (*Poecile atricapillus*; hereafter referred to as chickadees) to study the long term effects of perceived predation risk manipulated through auditory playbacks. I used auditory playbacks of species known as predators or non-predators of chickadees to simulate chronic predation risk over two days, following Hobbs (2015). I looked at long lasting behavioural changes in response to a conspecific alarm call and the effect of perceived predation risk on dendritic morphology in brain regions thought to be involved in processing the fear circuitry of the brain to look for lasting effects of perceived predation risk.

2.2.2 *Predation Risk Manipulation*

Between January and March 2016, I captured 15 chickadees (nine male; six female; all after hatch year) using seed baited potter traps from multiple sites at Western University, London, Ontario. Upon capture, chickadees were weighed and sex was estimated based on wing chord (males >60mm; females <60mm; confirmed with post mortem laparotomy). I housed chickadees in mixed sex groups of four to six in outdoor aviaries with *ad libitum* access to Mazuri small bird diet, black oil sunflower seeds, striped sunflower seeds, mealworms, and water. Chickadees were captured at least seven days prior to the start of manipulations in order to acclimate to captivity.

Chickadees were randomly assigned to either the predator or non-predator treatment while maintaining a balanced sex ratio between the treatments. For the predation risk manipulation, chickadees were transferred to a new cage within individual sound-attenuating acoustic chambers. Each chamber was outfitted with a Hipstreet mp3

player, a set of speakers, and a Logitech webcam. The chambers were setup so that the mp3 player and webcam could be operated for playbacks, recording, and monitoring without opening the chamber and disturbing the birds. The chambers operated on a natural light cycle (11.5h light: 12.5h dark) and chickadees had access to food and water *ad libitum* throughout the manipulation.

Seven species known to prey on chickadees were used (Table 1), with predator and non-predator species matched for maximum amplitude and frequency (Hobbs 2015). All calls were obtained from the Macaulay Library Database (Cornell University Lab of Ornithology, Ithaca, New York, USA) and the Xeno-Canto foundation (www.xeno-canto.org). I used a sound pressure metre at the centre of the cage at perch height to measure the sound level to 74dB. During the playback periods, chickadees were exposed to 5 minutes of calls every hour. Calls were broadcast at randomly selected intervals with each species used one to four times every two hours (depending on call length), and different exemplars used for each instance to help prevent habituation.

Individual birds were transferred to individual acoustic isolation chambers 24 hours before the manipulation began to acclimate to the new environment. The predation risk manipulation ran for 48 hours, with playbacks running 12 hours each day during daylight hours. Following playbacks, the chickadees were returned to their semi-natural home aviary for seven days, after which I conducted a behavioural assay. Immediately following the behavioural assay, birds were transferred to a post-mortem room where they were euthanized by isoflurane overdose.

Table 2.1: List of species used in the auditory playbacks for the chickadees. Predator and non-predator species were matched based on their acoustic call characteristics (frequency and maximum amplitude).

Predators	**Non-Predators**
Cooper's hawk, *(Accipiter cooperii)*	Song sparrow *(Melospiza melodia)*
American crow *(Corvus brachyrhynchos)*	Mallard *(Anas platyrhynchos)*
Red-tailed hawk *(Buteo jamaicensis)*	Blue jay *(Cyanocitta cristata)*
Barred owl *(Strix varia)*	Northern leopard frog *(Lithobates pipiens)*
Sharp-shinned hawk *(Accipiter striatus)*	Hairy woodpecker *(Picoides villosus)*
Northern saw-whet owl *(Aegolius acadicus)*	Wood frog *(Lithobates sylvaticus*)
Merlin *(Falco columbarius)*	Downy woodpecker *(Picoides pubescens)*

2.2.3 *Behavioural Assay*

The behavioural assay took place in the acoustic isolation chamber and consisted of 15 minutes of novel cage exploration followed by 15 minutes of exposure to chickadee high zee calls. The novel cage exploration also allowed for an assessment of baseline behaviour in the chamber before any additional stimuli were present. The setup of the acoustic isolation chamber was identical to the predation manipulation with the exception of the cage, which was substituted with a larger cage (38.5cm x 35cm x 36.5cm) containing only black oil sunflower seeds and water. The behavioural assay was recorded using the webcam inside the acoustic isolation chamber for later analysis. The high zee

playlist consisted of vocalizations from three different individuals, with each call playing for 5s followed by 45s of silence (following Hobbs, 2015). This 60s playlist was repeated 15 times at 74dB.

Behaviour was scored by an observer blind to experimental treatment using an ethogram developed to assess chickadee behaviour, looking at consumption, movement, aggressive, resting, and immobile behaviours (Appendix 1). Every change in location was recorded in the first 10 minutes of each video to assess exploratory behaviour. Additionally, behaviour was scored continuously for 1 minute prior to and for the 1 minute of the high zee playback to look at individual differences in response the alarm.

2.2.4 *Brain Processing*

All brains were processed following Louth et al. (2017). Brains were removed immediately and incubated in Golgi-Cox Solution (1% potassium dichromate, 0.8% potassium chromate, and 1% mercuric chloride in water) in the dark for 25 days, then transferred to 30% sucrose for 48h until saturated and frozen at -80°C for long term storage. Brains were returned to 30% sucrose 24h before processing. Brains were sliced at 500μm coronally in 30% sucrose using a vibrotome (Leica VT10005), starting from the back of the brain and ending once the anterior commissure had been sliced, and left in 6% sucrose overnight. Sections were processed in 2% paraformaldehyde for 15 minutes, 2.7% NH_4OH for 15 minutes and fixed in Kodak Fixitive A for 25 minutes, before being mounted on slides, dehydrated in ethanol, cleared in citrisolv and coverslipped (see Appendix C for staining).

2.2.5 *Neuron Tracing*

In each brain region of interest, 4 pyramidal neurons were selected for tracing. Each neuron was selected from a difference hemisphere, covering a minimum of two slices and four hemispheres within the region of interest. In the Hp, these were within the first three slices on the caudal side of the anterior commissure, and in the TnA they were selected from the two slices with the TnA clearly visible. Three-dimensional image stacks were captured for each neuron, with images spaces 1μm apart in the z-plane at 30X magnification with an Olympus BX53 microscope. Neurons were traced using Neurolucida software (MicroBrightField, Williston, VT, USA) and Neurolucida Explorer (MBF Bioscience, Williston, VT, USA) software was used to extract data on morphological characteristics and perform the Sholl analysis (Sholl 1953). To be selected for the analysis, neurons had to be fully contained within the slice, with no breaks in dendritic branches or obtrusions (such as the cell body of another neuron). I captured all images and traced all neurons without knowing which treatment the individual belonged to, to avoid bias in the results. Spines were counted over a 10μm length of dendrite at three different locations on the longest branch of the apical dendrite and the longest basal dendrite. Counting for the proximal spines started approximately 10μm from the cell body, distal spines were counted starting approximately 10μm from the end of the dendrite, and medial spines were counted at the approximate centre point between the proximal and distal measurements.

2.2.6 *Statistical Analysis*

For the behavioural assay, I calculated the difference between before and during the high zee playback for each individual behaviour. I compared the number of

occurrences and the time spent for each behaviour using a non-parametric Mann-Whitney U Test.

For the general dendritic morphology, I used a two-way ANOVA with treatment and sex as fixed factors. Sex was included as a fixed factor to account for potential sex effects, as mammalian research on dendritic morphology is often biased towards males. To analyze spine counts, I used a repeated measures ANOVA, with location as my repeated measures factor and treatment and sex as fixed factors.

For the Sholl analysis, I used a two-way repeated measures ANOVA with treatment and sex as fixed factors and the distance from the cell body as the repeated measures factor, followed by a Tukey HSD post-hoc test to look for differences between treatments at each radial distance. Prior to analysis with, all data were Box-Cox transformed to meet the assumption of homogeneity of variances. All analyses were conducted using Statistica (Version 13.0.04, Dell Inc.). I present the median and interquartile range for behaviour and the non-transformed means ± SE for clarity in the brain analyses.

2.3 *Results:*

2.3.1 *Behaviour Assay*

The behavioural assay showed significant, long lasting changes in anti-predator behaviour in response to a conspecific alarm call. Specifically, chickadees exposed to predators showed a significant decrease in the number of location movements they made around the cage (Fig 2.1; n=15, p=0.015) and the time they spent on these location movements (n=15, p=0.024) when comparing before to during the high zee playback.

Additionally, chickadees spent more time immobile (Fig 2.2; n=15, p=0.0065) during the high zee playback if they had been in the predator treatment one week earlier. There were no sex differences for any of the behaviours assessed (p>0.1).

2.3.2 *Nucleus taeniae of the amygdala (TnA)*

I found a trend towards increased total dendritic length (Fig 2.3; Treatment: $F_{1,11}$=3.58, p=0.085) in the predator treatment. I also found a trend towards increased branching in the basal dendrites of the TnA (Fig 2.4; Treatment: $F_{1,11}$=3.76, p=0.078) with increased predation risk, however this same difference was not found in the apical dendrites of the TnA (Treatment: $F_{1,11}$=0.26, p=0.620). The Sholl analysis revealed a significant treatment effect on the distribution of dendritic length (Fig2.5; Treatment*Distance: $F_{9,99}$=3.28, p=0.0015), with increased predation risk leading to clusters of increased dendritic length. I found no other significant treatment effects, sex effects, or interactions for the number of dendrites or number of dendritic spines (all p-values > 0.1) for the total dendritic material, basal dendrites or apical dendrite. The Sholl analysis revealed no treatment effect, sex effect or interaction for the branching, branch endings, or intersections with the Sholl rings (p>0.1).

2.3.3 *Hippocampus (Hp)*

In the hippocampus (Hp), the Sholl analysis revealed differences in dendritic complexity, with both increased perceived predation risk showing clusters of increased intersections with the Sholl rings (Fig 2.6; Treatment*Sex*Distance: $F_{8,88}$=2.56, p=0.015) and increased branching (Treatment*Distance: $F_{6,66}$=2.29, p=0.045; Fig 2.7; Treatment*Sex*Distance: $F_{6,66}$=4.63, p=0.00056), with the differences appearing to be driven by the females. I found no treatment effects, sex effects, or interactions for the

number of branches, number of dendrites, the total length of the dendrites, or the number of dendritic spines ($p>0.1$). Additionally, the Sholl analysis revealed no treatment effects, sex effects, or interactions for the complexity of the length or number of branch endings ($p>0.1$).

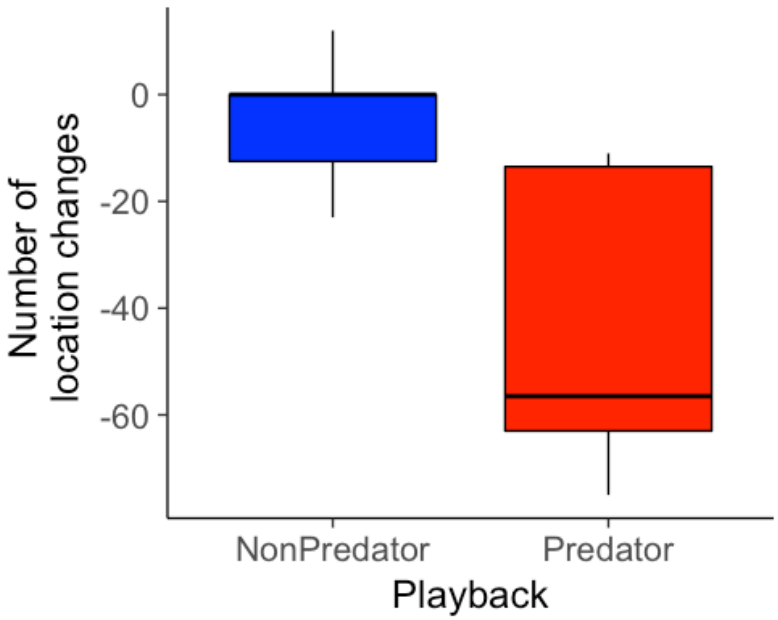


Figure 2.1: The number of location changes within the cage made by chickadees significantly differed between treatments in the first minute of exposure to the high zee alarm call compared to pre-exposure in chickadees: n=15, U= 6.5, p=0.015.

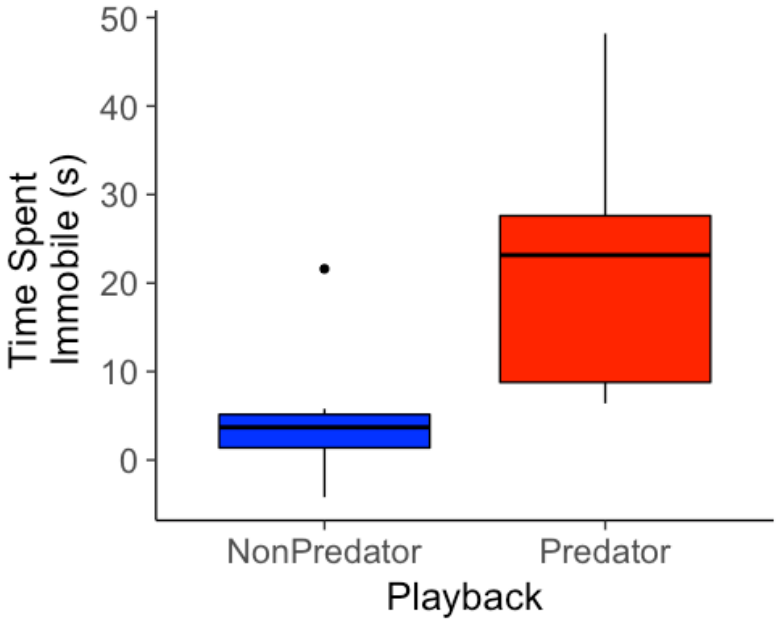


Figure 2.2: Time spent immobile significantly differed between playback treatments in the first minute of exposure to a high zee alarm call: n=15, U= 4, p=0.0065.

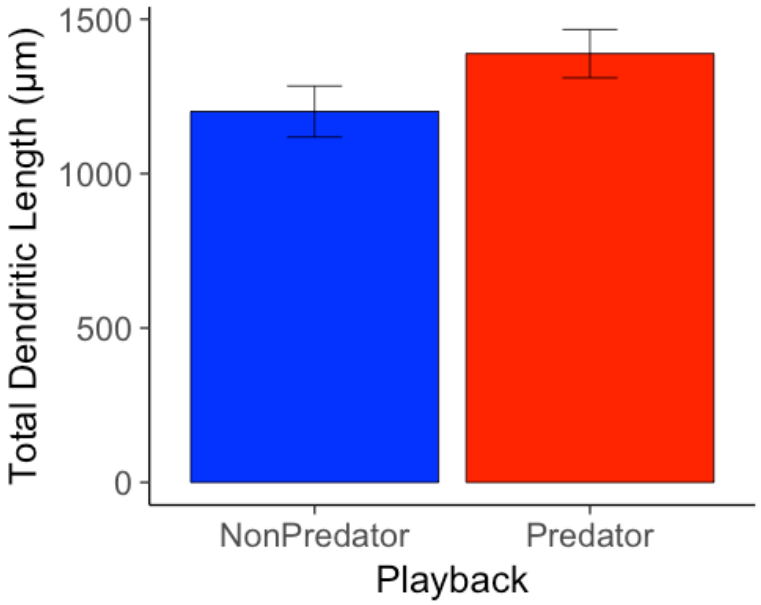


Figure 2.3: Total dendritic length in the nucleus taeniae of the amygdala (TnA) showed a trend to increased length in response to predator playbacks in chickadees: Treatment: $F_{1,11}$=3.58, p=0.085, n=15 (both sexes included).

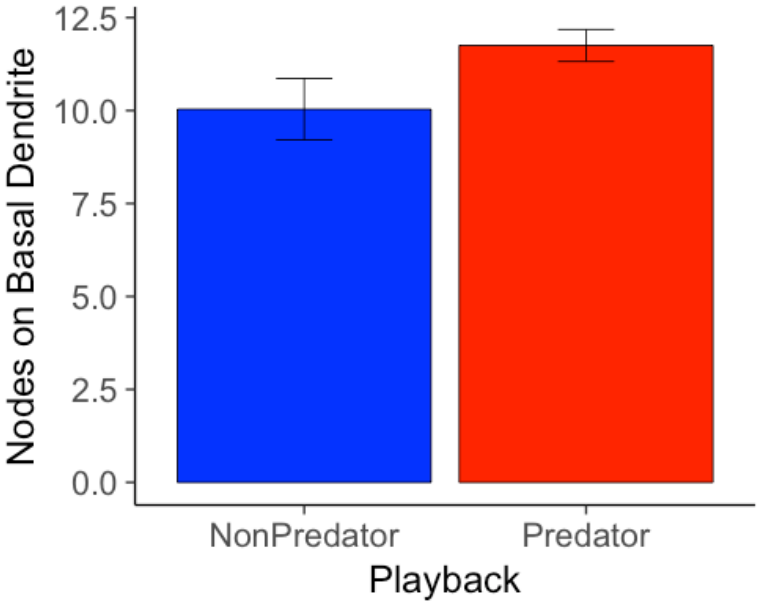


Figure 2.4: The basal dendrites of the nucleus taeniae of the amygdala (TnA) showed a trend toward increased branching in response to predator playbacks in chickadees: Treatment: F1,11=3.76, p=0.078, n=15 (both sexes included).

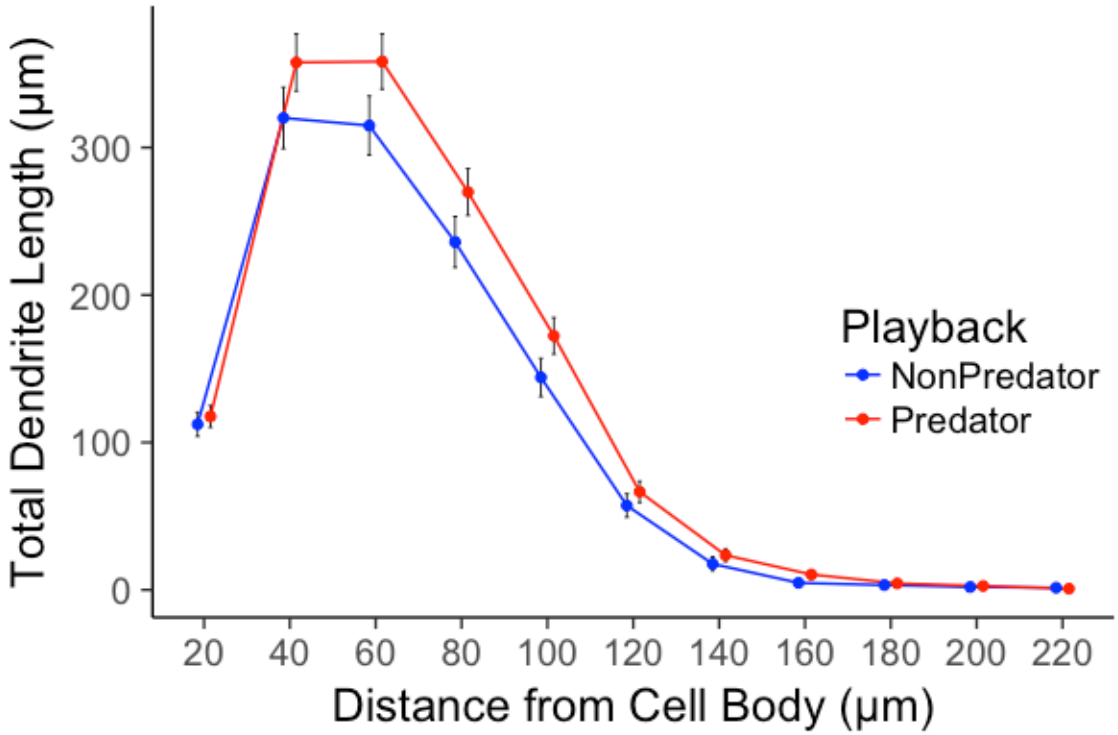
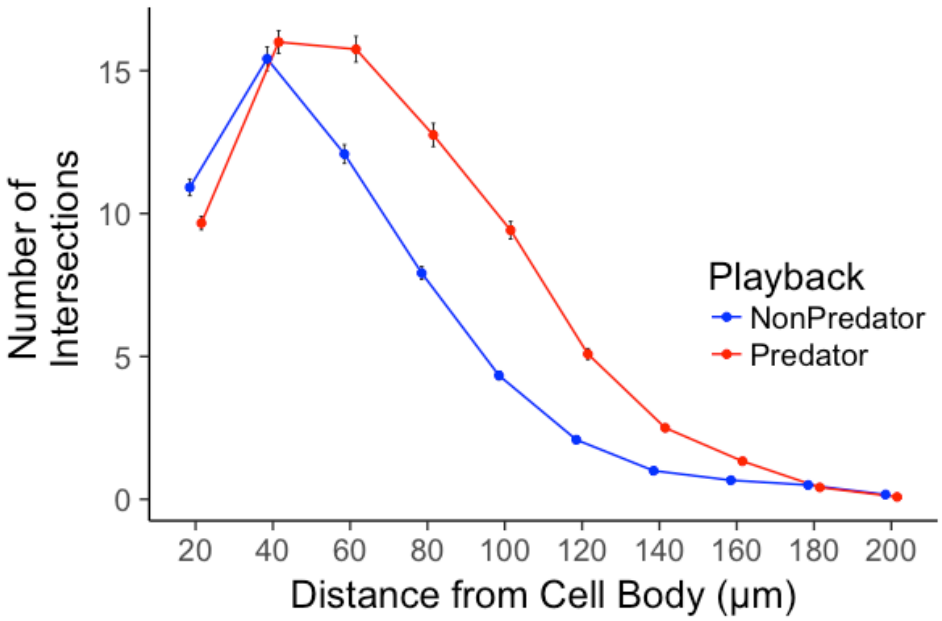


Figure 2.5: The dendritic length at each distance from the centre of the cell body on all chickadee dendrites in the nucleus taeniae of the amygdala; Treatment*Distance: $F_{9,99}=3.28$, $p=0.0015$.

A)



B)

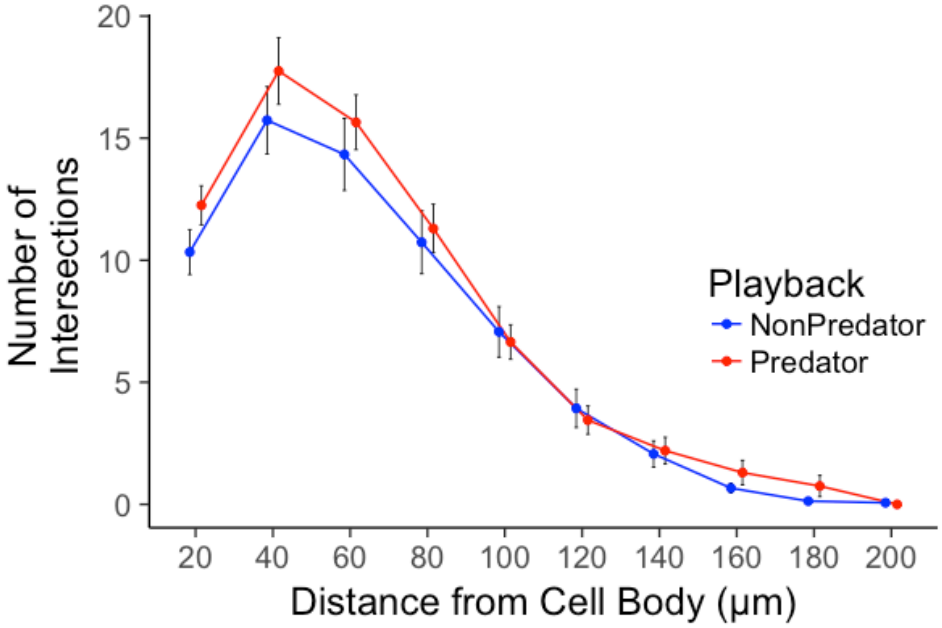
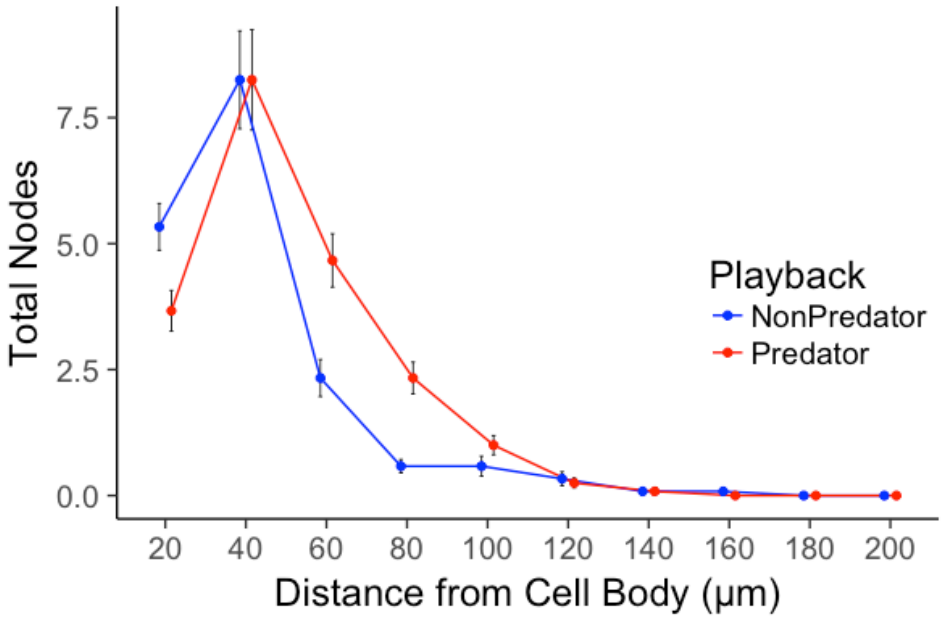


Figure 2.6: The number of intersections with Sholl rings at each distance from the centre of the cell body on all dendrites in the chickadee hippocampus in A) females and B) males; Treatment*Sex*Distance: $F_{8,88}=2.56$, $p=0.015$

A)



B)

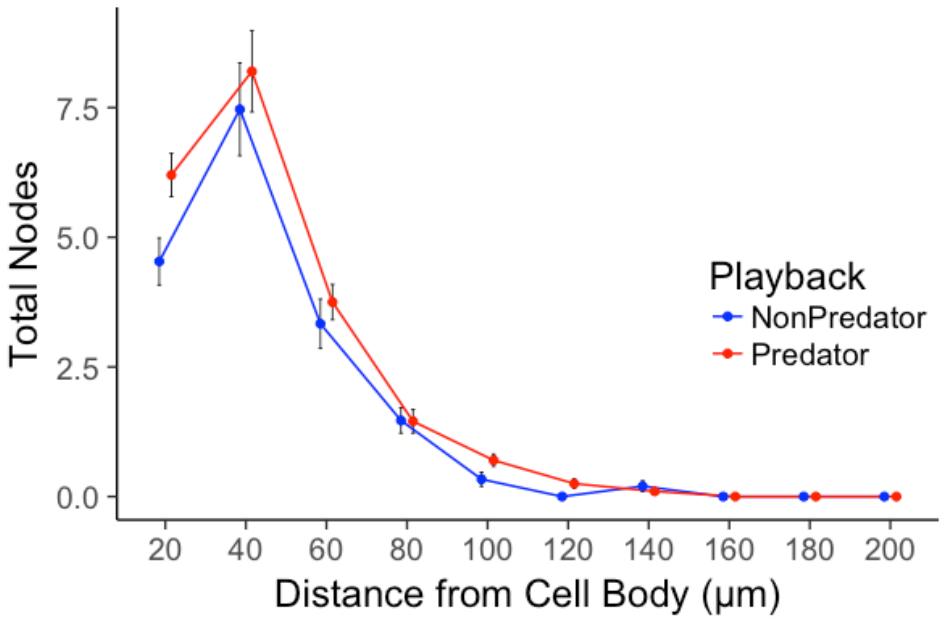


Figure 2.7: The number of branches at each distance from the centre of the cell body on all dendrites in the chickadee hippocampus in A) females and B) males; Treatment*Sex*Distance: $F_{6,66}=4.63$, $p=0.00056$.

2.4 *Discussion*

The experimental results demonstrate that manipulating the level of perceived predation risk prey are exposed to has long lasting effects on both behaviour and neurobiology. Exposure to high levels of perceived predation risk led to long lasting changes in anti-predator behaviour as chickadees made fewer location changes (Figure 2.1) and spent more time immobile (Figure 2.2) in response to a conspecific alarm call one week after the predation risk manipulation. Decreasing movements is a common prey response to increased predation risk, in order to minimize the potential for detection by predators (Lima and Dill 1990, Lima 1998). This provides evidence that behavioural changes in response to increased predation risk are long lasting, and can be maintained even after the increased risk has been removed. Additionally, my results present lasting changes in the dendritic morphology of two brain regions associated with processing predation risk, identifying quantifiable and long lasting changes in neurobiology in response to increased perceived predation risk.

Finding long lasting changes in both the brain morphology and behaviour in a wild caught species has strong implications for both ecology and biomedical research. These wild caught individuals had likely already experienced predation attempts prior to this study, unlike predator-naïve lab-raised study organisms. With previous predation experience, these animals would likely have learned to distinguish between predator cues that require immediate attention and those that do not pose an immediate threat. This increase in the baseline level of predation risk seen in wild animals suggests that any changes seen in response to my predation risk manipulation were in addition to any pre-existing effects prior to capture. Given the likelihood for previous predation experience,

the changes in dendritic morphology I have found may provide a better representation of the brain response to traumatic events leading to PTSD in humans. This study shows similar lasting changes in dendritic morphology in the avian brain to those seen in laboratory studies on mammals, showing evidence that these changes are comparable across multiple taxa and suggesting an adaptive advantage to retaining a memory of traumatic situations for future survival.

In the TnA, these results follow the same pattern as seen in previous mammalian research, with increased predation risk leading to increased length and branching (Vyas et al. 2002, 2004, Leuner and Shors 2013). These long lasting changes in the amygdala, in combination with previous research showing increased ΔFosB activation support the proposed function of the TnA as the fear processing centre of the avian brain (Cohen & Goff, 1978; Hobbs, 2015). Additionally, stress induced changes in the mammalian basolateral amygdala has been shown to be persistent, with lasting changes in the brain and behaviour after a 21-day recovery period (Vyas et al. 2004). This suggests that these changes could persist and continue to affect behaviour past the seven day period tested.

In the Hp, the changes in the pattern of length and branching do not follow the same trend as seen in the mammalian literature. In mammals, the Hp dendrites have been shown to retract in length and branching in response to stress (Sousa et al. 2000, Vyas et al. 2002, Baran et al. 2005, Christian et al. 2011, Leuner and Shors 2013). In my experiment, the length and branching did not change overall, but distribution of branching changed as evident in the Sholl analysis (Figures 2.6 and 2.7). Additionally, where there were variations between the treatments, the length and number of branches were higher in those exposed to increased perceived predation risk. This variation could

be due to using a different taxa, as most research looking at the effects of perceived predation risk has been conducted on laboratory mammals. In humans, variation has been shown in these same regions, as those with PTSD showed an increased functional response in the amygdala in response to trauma related stimuli (Protopopescu et al. 2005), and increased hippocampal volume in comparison to healthy individuals (Gurvits et al. 1996, Bremner et al. 1997). When looking at other brain regions, comparative analysis has shown there are distinct differences in the arrangement of neurons in the visual cortex when comparing mammals and passerines (Chand et al. 2013). Given that structural plasticity can alter the connectivity between neurons, a different neural arrangement between taxa may lead to baseline differences in connectivity and a variation in the structural changes in response to stress. In particular, it is impossible to know the baseline dendritic morphology given the finite nature of the individuals in the experiment. Finally, chickadees are known to undergo seasonal hippocampal plasticity, as the Hp plays an important role in spatial memory and food caching behaviour (Sherry and Vaccarino 1989, Sherry and Hoshooley 2010). Given that the chickadees used in this experiment were wild caught and housed in semi-natural outdoor aviaries, it is possible that their brains would respond differently than a mammal living under laboratory conditions.

These changes in dendritic morphology are consistent with previous work showing increased ΔFosB in the Hp and the TnA in chickadees one week after increased perceived predation risk (Hobbs 2015). Overexpression of ΔFosB increases expression of cyclin-dependent kinase-5 (CDK-5), which has been shown to induce changes in dendritic morphology (Chen et al. 2000, Nestler et al. 2001, Norrholm et al. 2003, Ruffle

2014). Given the high levels of ΔFosB evident in both the Hp and the TnA under the same experimental conditions, it would be expected that we would see structural plasticity in these regions and could continue to see changes past the seven day period tested. With both the Hp and the TnA showing elevated ΔFosB, it would be expected that both regions would show similar effects on dendritic morphology, in this case increasing in both length and branching in the TnA, and altering the length and branching patterns in the Hp.

When looking at the behavioural changes, the behavioural changes in freezing and number of movements in response to the conspecific alarm call was a common reaction to the threat of predation. When predation risk is high, reducing activity levels is a common method of avoiding detecting by predators (Lima and Dill 1990, Lima 1998). Given that there was no refuge available, when exposed to an immediate threat reducing activity would likely have been the best response to minimize predation risk. The increased time spent immobile and reduced number of movements in those with prior exposure to predation suggest that they did retain the memory of that predation experience, and that it continued to affect their anti-predator behaviour even after a period of low risk. Given the changes we can see in the dendritic morphology in the TnA and the Hp and the increased ΔFosB activation in the TnA and Hp (Hobbs 2015), it is likely that these changes in behaviour are linked to these long lasting changes in the brain. These behaviours are also consistent with those symptomatic of PTSD in humans, such as hypervigilance and a marked reaction to external cues that resemble an aspect of the traumatic event (American Psychiatric Association 2013). This behavioural consistency provides further support for the use of perceived predation risk in animal

models for PTSD, and suggests that the changes in dendritic morphology seen in this experiment could be occurring in those with PTSD.

This study is likely to represent meaningful effects of perceived predation risk on the brain and behaviour due to the use of an auditory predator cue alone. Many mammalian studies looking at the effects of perceived predation risk on dendritic morphology use exposure to a live predator as the stimulus (Baran et al. 2005, Diamond et al. 2006, Mitra et al. 2009, Adamec et al. 2012). Response to predation threat has been shown to change depending on the cue used, with live predators eliciting stronger and longer lasting behavioural responses than predator odour, as sound generally represents a more definitive indicator of predator presence than odour (Adamec et al. 1998, Wiedenmayer 2004). The fact that we see a neural response to an auditory cue alone shows the importance of these cues for prey fitness, and in conjunction with the lasting behavioural response suggests that retaining a memory of previous predation experience is beneficial for future fitness.

This study has provided new insight into the long lasting effects of perceived predation risk on behaviour and dendritic morphology, showing that increased perceived predation risk leads to lasting changes in both the TnA and the Hp as well as the response to a conspecific alarm cue. However, there are still many questions that warrant further investigation. First would be to investigate whether we can find similar effects to the changes in dendritic morphology measured in this study and the increased ΔFosB shown in previous work (Hobbs 2015) if we conducted a similar test in a semi-natural environment. Previous work looking at the effects of perceived predation risk on the brain has always been assessed in a laboratory environment, and it would be interesting

to see if we can detect a similar lasting signature of predation risk even in the presence of natural environmental variation and predation risk. Additionally, it would be interesting to see if there is any difference in the neural response to perceived predation risk during the mating and brood rearing period. Increased perceived predation risk during brood rearing is known to impact offspring production and survival (Zanette et al. 2011), and it understanding the neural response in both the parents and offspring during this critical period could provide new insight into the mechanisms behind these demographic consequences.

Providing evidence for long lasting changes in behaviour and dendritic morphology in a wild caught, non-mammalian species in response to perceived predation risk, a common stimuli used in the study of PTSD, provides ecological validation for animal models of PTSD. Using wild caught birds and the cues from their natural predators, I have modelled a scenario that is closer to that of free living animals and could better mimic the conditions leading to PTSD in humans. Humans encountering a PTSD inducing traumatic event have likely experienced other stressful events in their life, just like my wild caught animals would likely have a baseline level of experience avoiding predation. Although the use of wild animals in biomedical models is not common, it provides a new tool for validating animal models and better understanding the etiology of PTSD. I have shown that predation risk can induce changes lasting at least one week in dendritic morphology in wild animals comparable to previous laboratory studies, suggesting that these changes represent a meaningful effect of increased predation risk rather than simply an effect of predator naiveté. Finally, this gives a better understanding of the neurological mechanisms involved in processing

predation risk, and provides a tool for comparing lasting changes in the brain with changes in behaviour and physiology in wild animals.

In this study, I have identified long lasting changes in behaviour and dendritic morphology in the TnA and the Hp in wild-caught chickadees. This provides further evidence that the effects of perceived predation risk continue long after the fearful stimuli has been removed, and could lead to lasting effects on an individual's fitness if these changes impair foraging or mating. Impairments in foraging and mating can lead to demographic effects if individuals undergo repeated challenges, with effects evident in both parents and offspring (Boonstra et al. 1998, Creel et al. 2007, Zanette et al. 2011). This study connects changes in the brain and behaviour, providing support for future research into the role of perceived predation risk on the neurobiology of free-living animals.

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Chapter 3

3 Assessing the effects of predation risk in a semi-natural environment

3.1 *Introduction:*

Predators can affect prey populations both directly, through prey consumption, and indirectly, through non-consumptive effects such as inducing prey defences. Research has traditionally focused on direct consumption, looking at the impacts of predation based on how many prey they could capture and kill (Taylor 1984, Abrams 2000, Vandermeer et al. 2001). Often overlooked were the non-consumptive effects of predators, which have been shown to have an equal or greater effect on prey species than direct consumption alone (Preisser et al. 2005, Bolnick and Preisser 2005). In order to minimize predation risk, individuals may alter their behaviour, physiology, or morphology. Behavioural changes in response to increased predation risk may include spending more time under cover, increasing vigilance, or changing feeding behaviour, habitat selection, or escape behaviour (Lima and Dill 1990, Lima 1998, Steiner 2007, Walters et al. 2017). Physiological changes, such as increased production of corticosteroid hormones (Boonstra et al. 1998, Clinchy et al. 2011b), or morphological changes, such as variation in body size to avoid gape limited predators (Krueger and Dodson 1981, Dodson 1989, Kishida and Nishimura 2004), may also accompany these behavioural changes. When exposed to repeated challenges, increased predation risk can also lead to demographic consequences or trophic cascades (Creel et al. 2007, Zanette et al. 2011, Suraci et al. 2016).

One behaviour that is particularly important in avoiding predation is the ability to escape from threatening situations. In order to avoid predation, individuals in high risk environments may alter their habitat selection, particularly for feeding grounds, in favour of areas that are less conspicuous and have more opportunities for refuge (Lima and Dill 1990, Creel et al. 2005). Prey species may also alter their flight initiation distance with varying predation risk, weighing the benefits of avoiding predation with the costs of flight and any lost resources (Lima and Dill 1990, Cooper 2006, Díaz et al. 2013). Additionally, providing prey species with adequate space to flee or avoid predation risk is an important factor in assessing anti-predator behaviour in a captive environment, to avoid any response measured being attributed to the unnatural effects of captivity (Clinchy et al. 2011a).

In order to better understand the changes associated with increased perceived predation risk, it is important to understand the neural mechanisms involved in the perception of predation risk (Clinchy et al. 2011a, 2013). Understanding how predation risk affects the brain can help us to understand the varying behavioural responses to predation risk, and how prey retain the information related to predator cues for future survival. In particular, using wild caught animals to assess the effects of perceived predation risk presents an excellent model, as wild animals likely have previous experience with predation risk and would respond more closely to their free-living counterparts than laboratory raised animals. Additionally, studying how perceived predation risk affects wild caught animals in a semi-natural environment rather than a laboratory can give us greater insight into the natural behaviours and associated neural mechanisms in the response to perceived predation risk. Allowing prey to perform

natural anti-predator behaviours, such as escape and seeking refuge, and experiencing natural variations in the level of predation risk can give greater insight into the neural and behavioural response that would be expected of free-living animals (Clinchy et al. 2011a, Cohen et al. 2014).

Understanding the neural mechanisms behind perceived predation risk can give us new insight into both predator-prey ecology and post traumatic stress disorder (PTSD). Perceived predation risk presents an excellent stimulus to use in animal models to mimic the life threatening traumatic events which can induce PTSD in humans, with the advantage of controlled manipulations to further understand the etiology of PTSD (Clinchy et al. 2011a, Cohen et al. 2012, 2014). In humans, PTSD diagnosis requires symptoms be present for at least one month after a traumatic event (American Psychiatric Association, 2013), which is often translated to one week in the life span of a small mammal or bird (Cohen et al., 2012). Using perceived predation risk provides an advantage over other stressors used in animal models of PTSD, such as foot shock, because it allows researcher to manipulate a life threatening traumatic event without inducing any physical pain (Clinchy et al., 2013; Cohen et al., 2012, 2014; Zoladz & Diamond, 2016). Using wild caught animals in a semi-natural environment to study PTSD can also mitigate one of the major criticisms of animal models, that lab rats in cages may not show the same neurological and behavioural responses as their free-living counterparts (Clinchy et al. 2011a).

In humans, long term changes in neurobiology associated with PTSD have been found in the amygdala, hippocampus, and medial prefrontal cortex, three regions thought to be involved in the human stress response (Shin et al. 2006, Bremner et al. 2008). In

the avian brain, three corresponding brain regions have been proposed as part of the network that processing perceived predation risk: the nucleus taeniae of the amygdala (TnA), the hippocampus (HP), and the caudal nidopallium (NC) (Cross et al. 2013, Hobbs 2015). The TnA is the avian homologue to the mammalian medial amygdala and plays a crucial role in fear processing (Davis 1992, Reiner et al. 2005, Gross and Canteras 2012). The TnA is suggested to convey information about predation stress to other regions of the brain, and has shown increased activation in response to predation stress (Marzluff et al. 2012, Cross et al. 2013, Hobbs 2015). The Hp is homologous to the mammalian Hp, playing a role in many processes including the encoding and retrieval of fear memory, and the processing of spatial information (Colombo and Broadbent 2000, Bingman et al. 2003, Gross and Canteras 2012). Threatening stimuli, such as predator cues and dead conspecifics, have been shown to induce increased activation in the Hp (Cross et al. 2013, Hobbs 2015). The NC, an analogue of the mammalian prefrontal cortex, is proposed to be involved in executive functions and processing information to generate behaviour (Herold et al. 2011), and has shown increased activation in response to viewing a predator (Cross et al. 2013).

In order to better understand how perceived predation risk affects the brain, it is necessary to quantify any changes that occur. Three methods that can be used to quantify effects of perceived predation risk on the brain are: ΔFosB activation, neurogenesis, and dendritic morphology. ΔFosB is a protein splice variant of FosB, a transient transcription factor that is produced rapidly in response to stress, peaking after approximately 6 hours (Nestler et al. 2001). ΔFosB accumulates in the brain following the breakdown of FosB, with detection possible for up to 1-2 months post stimulus (Nestler et al. 2001).

Increased ΔFosB activation has been shown in both birds and mammals in response to increased perceived predation risk, however this has yet to be tested outside of a controlled laboratory environment (Staples et al. 2009, Hobbs 2015).

A second method to quantify the effects of perceived predation risk on the brain is through changes in neurogenesis. Neurogenesis is proposed to be involved in brain remodeling, allowing old memories to be forgotten and new memories to be formed (Frankland et al. 2013, Mongiat and Schinder 2014). Increased neurogenesis has been shown to decrease memory retention and disrupt established hippocampus-dependent memories (Akers et al. 2014), and is highly variable depending on environmental cues (Deng et al. 2010, Schoenfeld and Gould 2012, Egeland et al. 2015). Predator cues have been shown to inhibit neurogenesis in both rats and fish, but this has yet to be tested through a controlled manipulation in a semi-natural environment (Tanapat et al. 2001, Dunlap et al. 2016).

The effects of perceived predation risk on the brain can also be assessed through changes in dendritic morphology. Dendrites are the components of neurons specialized to receive signals (Kulkarni and Firestein 2012). Connectivity can be modified through stress induced plasticity, producing immediate and lasting changes in synaptic function (Leuner and Shors 2013). Changes in dendritic morphology have been linked to changes in ΔFosB activation (Nestler et al. 2001, Ruffle 2014), but are generally longer lasting and potentially permanent (Juarez-Mendez et al. 2006). Previous laboratory studies have shown changes in dendritic morphology from perceived predation risk in rats (Adamec et al., 2012; Baran et al., 2005; Mitra et al., 2009, Chapter 2), however this has yet to be tested in an avian species or a semi-natural environment.

In this study, I looked at the effects of perceived predation risk on the brain and behaviour of brown-headed cowbirds (*Molothrus ater*) in a semi-natural environment, as previous experiments with this species showed behavioural changes in response to increased predation risk (Cheng 2016, Walters et al., 2017). In particular, I assessed both the immediate and the long term impact of perceived predation risk on escape behaviour, and to look for lasting changes in ΔFosB activation, neurogenesis, and dendritic morphology in the brain. I expected that we would see immediate changes in cowbird escape behaviour, as previously shown by Walters et al. (2017). Looking at ΔFosB, I expected that there would be increased ΔFosB activation in the TnA and the Hp in response to increased perceived predation risk, similar to previous laboratory studies in both birds and rodents (Staples et al. 2009, Hobbs 2015). For neurogenesis, I expected that there would be inhibited neurogenesis in the TnA and the Hp, as inhibitions in neurogenesis have been shown in response to increased perceived predation risk in both rats and fish (Tanapat et al. 2001, Dunlap et al. 2016). Finally, for dendritic morphology, I expected that we would see changes to the length, branching, and number of spines in the TnA and the Hp, as previous mammalian research has shown changes in the Hp and the amygdala in response to increased predation risk (Baran et al. 2005, Mitra et al. 2009, Adamec et al. 2012). Here, I report that increased perceived predation risk led to alterations in escape behaviour in comparison to a non-predator control. Additionally, I found long lasting effects of increased perceived predation risk in the TnA and the Hp on both neurogenesis and dendritic morphology in both sexes. Finally, there was no lasting changes in ΔFosB in the TnA and the NC, and Hp response differed between the two rounds of the experiment.

3.2 *Methods:*

I used wild caught brown-headed cowbirds (*Molothrus ater;* hereafter referred to as cowbirds*)* to study the long term effects of perceived predation risk manipulated through auditory playbacks. Cowbirds were exposed to either predator or non-predator playbacks, each consisting of multiple species over 10 days. I looked at the effect of increased perceived predation risk on escape behaviour and on the protein splice variant ΔFosB, dendritic morphology, and neurogenesis to study lasting changes in brain regions thought to be involved in processing predation risk from changes in perceived predation risk.

3.2.1 *Animal Housing*

Between April 4 and April 26, 2016, I captured 112 cowbirds at Ruthven Park Banding Station, Cayuga, Ontario as they returned from migration. Each bird was given a unique combination of colour bands for individual identification. Upon capture, seven male and seven female cowbirds were housed in each of four large outdoor aviaries (3.66 m x 9.14 m x 18.29 m) at the Environmental Sciences Western Field Station. The remaining birds were housed in the same sized groups in large cowbird traps on site, acoustically and visually isolated from the experimental aviaries until the second round of the experiment. Each aviary was equipped with perches, trees, grass, and an A-frame shelter, providing a semi-natural environment while protecting birds from predation. Adjacent aviaries were separated by an opaque barrier, visually isolating the groups. The aviaries pairs were separated by 150m, separating groups both visually and acoustically. Cowbirds had *ad libitum* access to a modified Bronx Zoo diet for omnivorous birds

(Travers et al. 2010) and water, in addition to foraging for naturally present food in the aviaries.

3.2.2 *Predation Risk Manipulation*

To manipulate predation risk, cowbirds were simultaneously exposed to auditory playbacks and taxidermic mounts of predator and non-predator species between May 9 and June 25 2016. Cowbirds were given a minimum of two weeks from the time of capture to the beginning of the experiment and a minimum of one week in their assigned experimental aviary to acclimate before the playbacks began. I conducted two rounds of the experiment, where each treatment was conducted in each of the aviary pairs, to account for any differences in the micro-climate or surrounding environment between the aviary locations. Each predation risk manipulation risk manipulation consisted of 10 days of exposure to playbacks and taxidermic mounts of predator or non-predator species, followed by 7 days without treatments to look for lasting effects. After 7 days in the aviary without any treatment, 10 individuals from each treatment (five males, five females) were sacrificed. Birds in the second round of the experiment were also given a minimum of seven days to acclimate to the aviaries before the treatments began. To prevent sound contamination, adjacent aviaries received the same treatments at the same time. Aviaries 1A and 1B received the non-predator treatment first, while aviaries 3A and 3B received the predator treatment first. The start date for the aviary pairs was staggered by three days (i.e. Aviary 3A and 3B began on May 9, while aviary 1A and 1B began on May 12) to allow for behavioural observations to be taken on the same day of the experiment and at the same time of day for each treatment. To avoid habituation to the

treatments, playbacks and taxidermic mounts were presented on days 1-4 and days 7-10 (following Cheng, 2016; Walters et al., 2017)

Table 3.1: List of species used in the auditory playbacks for the brown headed cowbirds. Predator and non-predator species were matched based on their acoustic call characteristics (peak frequency, maximum frequency, minimum frequency, and frequency range).

Time	Predators	Non-Predators
Day	Sharp-shinned hawk (*Accipiter striatus*)	Killdeer (*Charadrius vociferous*)
Day	Cooper's hawk (*Accipiter cooperii*)	Northern flicker (*Colaptes auratus*)
Day	Red-shouldered hawk (*Buteo lineatus*)	American robin (*Turdus migratorius*)
Day	Red-tailed hawk (*Buteo jamaicensis*)	Yellow-rumped warbler (*Dendroica coronate*)
Day	American kestrel (*Falco sparverius*)	Cedar waxwing (*Bombycilla cedrorum*)
Night	Eastern screech owl (*Megascops kennicottii*)	Common loon (*Gavia immer*)
Night	Northern saw-whet owl (*Aegolius arcadius*)	Wood frog (*Rana sylvatica*)
Night	Barred owl (*Strix varia*)	Northern leopard frog (*Lithobates pipiens*)

Cowbirds were randomly assigned to either the predator or non-predator treatment, while maintaining a balanced sex ratio between the treatments. For the predation risk manipulation, each aviary was equipped with two playback units housed in weatherproof boxes. Each playback unit contained a pair of speakers (Logitech Z130 Speakers) and an MP3 player (Hipstreet 4GB MP3 Player). The playback units were mounted 2.4m high, placed at least 12m apart from each other and moved to a new location within the aviary every two days to prevent habituation. Sounds of predator or non-predator species were broadcast at 80dB from 1m away with a call-to-silence ratio of

1:1.5 during the day and 1:2.3 during the night to simulate temporal variations in predation risk (following (Cheng 2016, Walters et al. 2017)). Eight different species were used for each playback treatment, with each species matched to the appropriate time of day to simulate the natural variation in predation risk (Table 3.1). Playbacks were also randomized between the playback units so that only one unit was broadcasting within each aviary at any given time.

On days 1-4 and days 7-10, taxidermic mounts were also presented to the birds. Two species of taxidermic mounts selected for each treatment, matched for size and stance between the treatments (Table 3.2). Two different mounts were presented to each aviary each day that the playbacks were broadcast, with the first at a randomized time between 1100h and1400h and the second at a randomized time between 1400h and 1700h. The location of the mounts was changed daily to minimize habituation. Mounts were covered with an opaque box, and revealed to the birds for 5 minutes for each presentation.

Table 3.2: List of taxidermic mounts used in the predation risk manipulation for brown-headed cowbirds. Species were matched for size and stance between treatments.

Predator	***Non-Predator***
Red-shouldered hawk	Northern pintail
(Buteo lineatus*)*	(*Anas acuta)*
Cooper's hawk	Northern flicker
(Accipiter cooperii*)*	(*Colaptes auratus)*

3.2.3 *Assaying take-off behaviour*

Anti-predator behaviour was assayed by measuring take-off behaviour on treatment day 5 and 6 and post-treatment day 6 (following Walters et al 2016). We used a specially designed apparatus to measure the speed and angle of as birds initiate take-off.

The apparatus consisted of two parallel vertical $1m^2$ walls 45cm apart, attached to a $1m^2$ base. The back wall was painted white with a mounted feeder. The front wall was a transparent acrylic sheet marked with a 2.54cm grid to provide a scale for measuring the vertical and horizontal displacement during flight. When a bird landed on a perch to feed, a researcher hidden behind a blind outside the aviary would record the bird ID and pull a string, releasing a spring loaded flag to initiate take-off.

Take-off behaviour was recorded using digital video recorders (Swann DVR4-3425, 30 frames/s) positioned perpendicular to each flight apparatus. A second camera recorded the feeder to confirm individual bird ID. Vertical and horizontal displacements (to the nearest 1.27cm) were measured for the first six frames (0.2s) of each take off event using the center of the head as the reference point for calculations (following Walters et al. 2016). Take-off behaviour was assayed for differences in angle (°) and speed (m/s) both during the treatment period and 6 days post treatment to look for lasting effects on behaviour.

3.2.4 *Brain Processing*

After seven days in the aviary without any treatments, birds to be euthanized were randomly assigned to be processed for immunohistochemistry or dendritic morphology. Birds assigned to immunohistochemistry were euthanized using an overdose of isoflurane followed by a transcardial perfusion with 0.1M phosphate buffered saline (PBS) (pH 7.4) and 4% paraformaldehyde. Brains were removed and left in paraformaldehyde for a minimum of 24h, followed by sucrose for 48h until saturated, and then frozen at -80° for long term storage. I used a cryostat at -20°C to section brain slices into 40μm coronal

slices. I started collecting slices when the anterior commissure was no longer visible, collecting every slice for Nissl and three series of immunohistochemistry. Nissl slices were stained to locate regions of interest in the brain. I carried out immunohistochemistry to label ΔFosB (FosB (102) rabbit IgG, sc-48, Santa Cruz Biotechnology) and doublecortin (DCX (C-18) goat IgG, sc-8066, Santa Cruz Biotechnology) according to standard IEG protocol, with the primary anti-body at a concentration of 1:500 and 1:250 in 0.3% phosphate-buffered saline with triton (PBS/T), respectively. Sections were then labelled with a secondary antibody (goat anti-rabbit for ΔFosB and horse anti-goat for DCX, Santa Cruz Biotechnology) and visualized with diaminobenzidine solution (see Appendix D-I for staining).

Birds assigned to dendritic morphology were processed following Louth et al., (2017). Brains were removed immediately and incubated in Golgi-Cox Solution (1% potassium dichromate, 0.8% potassium chromate, and 1% mercuric chloride in water) in the dark for 25 days, then transferred to 30% sucrose for 48h until saturated and frozen at -80°C for long term storage. Brains were returned to 30% sucrose 24h before processing. Brains were sliced at 500μm in 30% sucrose using a vibrotome (Leica VT10005), and left in 6% sucrose overnight. Sections were processed in 2% paraformaldehyde for 15 minutes, 2.7% NH_4OH for 15 minutes and fixed in Kodak Fixitive A for 25 minutes, before being mounted on slides, dehydrated in ethanol, cleared in citrisolv and coverslipped.

3.2.5 *Quantifying ΔFosB*

I quantified immunoreactivity in the TnA, Hp, and NC for each slice clearly labelled in the region of interest. I also quantified immunoreactivity in a control region, the mesopallium (M), to compare to the regions of interest and ensure that I was not quantifying "background" expression (following Hobbs, 2015).

I used a Leica CTR6500 microscope and Leica Application Suite software to capture a z-stack image to cover the depth of each region in each slice, using the 10X (TnA, Hp, Control) and 5X (NC) objective lenses centred over the region of interest for 16 individuals. I calibrated ImageJ (NIH) to the image measurement, and measured the area in mm^2 of the region of interest in each slice quantified. I then converted the image from colour to 16-bit black and white, subtracted the background and enhanced the contract. I used the thresholding tool within ImageJ to convert ΔFosB positive nuclei to black against a white background. To quantify ΔFosB, I used the count function within Image J to measure positive cells/mm^2 in each slice in each brain region of interest. All images were collected and counted without knowledge of the treatment groups to avoid bias in the results.

3.2.6 *Quantifying DCX*

I used a Leica CTR6500 microscope and Leica Application Suite software to capture a z-stack image of each hemisphere in five slices for each region, using the 40X objective lenses. In the Hp, three photographs were taken in each hemisphere for each slice, covering the lateral, medial, and ventral Hp. In the TnA, two photographs were taken for each slice, covering the lateral and ventral TnA. In ImageJ, I then converted the

image from colour to 16-bit black and white, subtracted the background and enhanced the contract. I used the thresholding tool within ImageJ to convert DCX positive cells and fibres to black against a white background and quantified the percent coverage of DCX positive cells.

3.2.7 *Neuron Tracing*

In each of the Hp and the TnA, four pyramidal neurons were selected and traced for 12 individuals according to the protocol outlined in Chapter 2.

3.2.8 *Statistical Analysis*

For escape behaviour, take-off angle (°) and speed (m/s) were analyzed using a two-factor ANOVA, with treatment and sex as fixed factors. For each individual, only the first take-off event was included in the calculations.

For ΔFosB, I averaged the ΔFosB positive cell densities for all slices clearly stained and quantifiable per brain region per individual to give one data point per individual for the TNA, Hp, and NC (following Hobbs 2015). I then compared the ΔFosB positive cells/mm2 for each brain region with a three-factor ANOVA, with treatment, sex, and experimental round as my fixed factors. This was followed by a Tukey HSD test to determine any differences within factors.

For doublecortin activation, the percent cover of DCX positive cells and fibres was averaged across each individual for each brain region to give one data point per individual for the TNA, Hp, and NC. I then compared the DCX percent cover for each brain region with a two-factor ANOVA, with treatment and sex as my fixed factors.

I used a two-way ANOVA with treatment and sex as fixed factors for the general dendritic morphology. Sex was included as a fixed factor to account for potential sex effects, as previous research has shown sex differences cowbirds in the behavioural response to perceived predation risk (Cheng 2016, Walters et al. 2017). To analyze spine counts, I used a repeated measures ANOVA, with location as my repeated measures factor and treatment and sex as fixed factors.

I used a two-way repeated measures ANOVA for the Sholl analysis (Sholl 1953), with treatment and sex as fixed factors and the distance from the cell body as the repeated measures factor, followed by a Tukey HSD post-hoc test to look for differences between treatments at each radial distance. Prior to analysis with, all data were Box-Cox transformed to meet the assumption of homogeneity of variances. All analyses were conducted using Statistica (Version 13.0.04, Dell Inc.). I present non-transformed means ± SE for clarity.

3.3 *Results:*

3.3.1 *Behavioural Analysis*

When in a high predation risk environment, the cowbirds altered their escape behaviour to take off at a steeper angle (Fig 3.1; Treatment: $F_{1,56}$= 6.68, p=0.012), with no effect of sex (Sex: F1,56=0.90, p=0.346; Treatment*Sex: $F_{1,56}$=0.19, p=0.668). Cowbirds also took off at a slower speed (Fig 3.2; Treatment, $F_{1,56}$ = 4.49, p = 0.039) in the high predation risk environment, with no effect of sex (Sex: $F_{1,56}$ = 0.026, p = 0.87; Treatment * Sex $F_{1,56}$ = 0.68, p = 0.412). This suggests a trade-off in escape behaviour between angle and speed. Indeed, we found no significant changes differences in

mechanical energy between the predator (2.55 ± 0.115 J/kg) and non-predator treatments (2.53 ± 0.094 J/kg; $F_{1,56} = 0.86$, $p = 0.771$), regardless of sex (Sex: $F_{1,56} = 0.72$, $p = 0.399$; Treatment * Sex: $F_{1,56} = 1.05$, $p = 0.31$). These behavioural changes were not long lasting, however, as one week post playbacks no difference was found in either take-off angle (Fig 3.1; Treatment: $F_{1,45}=0.23$, $p=0.632$) or take-off speed (Fig 3.2; Treatment: $F_{1,45}=0.19$, $p=0.663$).

3.3.2 *Nucleus taeniae of the amygdala (TnA)*

The nucleus taeniae of the amygdala (TnA) showed inhibitions in neurogenesis and alterations in the dendritic morphology, with no long lasting difference in ΔFosB activation. There was a significant reduction in DCX positive cells in the TnA of birds exposed to increased perceived predation risk (Fig 3.3; Treatment: $F_{1,14}=179.7$, $p<0.0001$), regardless of sex (Sex: $F_{1,14}=0.18$, $p=0.679$; Treatment*Sex: $F_{1,14}=0.09$, $p=0.770$). Overall the length of the dendrites in the TnA was altered by increased predation risk, with females showing increased dendritic length and males decreasing in dendritic length. This marginally significant treatment by sex interaction for total length (Fig 3.6; Treatment*Sex: $F_{1,8}=4.95$, $p=0.057$) is supported by the Sholl analysis, showing significant treatment by sex by radius interactions for the distribution of length across the neuron (Treatment*Sex*Distance: $F_{10,80}=4.23$, $p=0.000099$) and the number of intersections with the Sholl rings (Fig 3.7; Treatment*Sex*Distance: $F9,72=2.45$, $p=0.017$). There was also trend towards a sex effect on the number of dendrites in the TnA, with females (6.67 ± 0.40) showing a greater number of dendrites than males (5.71 ± 0.18; Sex: $F_{1,8} = 3.95$, $p=0.082$), with no effect of treatment (Treatment: $F_{1,8}=1.26$, $p=0.294$; Treatment*Sex: $F_{1,8}=0.60$, $p=0.46$). There was no treatment effect, sex effect,

interaction, or Sholl effect revealed for number of branches or number of branch endings ($p > 0.05$). In contrast, there was no significant treatment effect on ΔFosB activation in the TnA (Fig 3.4; Treatment: $F_{1,8}=0.078$, $p=0.786$), with activation consistent between sexes (Sex: $F_{1,8}=0.85$, $p=0.382$; Treatment*Sex: $F_{1,8}=0.0003$, $p=0.986$) and experimental rounds (Round: $F_{1,8}=0.02$, $p=0.89$; Treatment*Round: $F_{1,8}=0.63$, $p=0.452$).

3.3.3 *Hippocampus (Hp)*

The hippocampus (Hp) showed inhibitions in neurogenesis, with no long lasting difference in ΔFosB activation. There was a significant reduction in DCX positive cells (Fig 3.3; Treatment: $F_{1,14}=91.0$, $p<0.0001$) in birds exposed to increased perceived predation risk. This inhibition of DCX positive cells was consistent across the sexes (Sex: $F_{1,14}=0.058$, $p=0.820$; Treatment*Sex: $F_{1,14}=1.88$, $p=0.192$). There were also significant treatment by sex interactions for both the overall dendritic morphology and the patterns revealed in the Sholl analysis. For the general dendritic morphology in the Hp, there was a significant treatment by sex interaction on the total length (Fig 3.8; Treatment*Sex: $F_{1,8}=5.67$, $p=0.044$), branching (Fig 3.8; Treatment*Sex: $F_{1,8}=7.25$, $p=0.027$), and branch endings (Treatment*Sex: $F_{1,8}=9.25$, $p=0.016$). In each of these measures females increased with increased predation risk, while males showed dendritic retraction with increasing predation risk. The Sholl analysis revealed a significant treatment by sex by radius interaction for intersections with the Sholl rings (Treatment*Sex*Distance: $F_{9,72}=6.56$, $p= 0.0000009$) and the distribution of dendritic length (Treatment*Sex*Distance: $F_{10,80}=8.16$, $p= 0.000000006$). The Sholl analysis also showed a significant treatment by radius interaction for branching (Treatment*Distance: $F_{8,64}=2.55$, $p=0.018$) and a significant sex by radius interaction for branch endings

(Sex*Distance: $F_{10,80}$=2.34, p=0.018). In contrast, there was no significant treatment effect on ΔFosB activation (Treatment: $F_{1,8}$=0.046, p=0.836). There was, however, a significant treatment by round interaction for ΔFosB activation in the Hp (Fig 3.5; Treatment*Round: $F_{1,8}$=5.86, p=0.042), which seems to be driven by a trend towards variation in the response in the non-predator treatment (Non-predator Round1:Non-predator Round 2; Tukey HSD: p= 0.092).

3.3.4 *Caudal Nidopallium (NC)*

In contrast to the TnA and Hp, the caudal nidopallium (NC) showed no long lasting effects from increased perceived predation risk. There was no significant effect of the playback treatment on DCX positive cells (Fig 3.3; Treatment: $F_{1,14}$=1.36, p=0.263) or on ΔFosB activation (Fig 3.4; Treatment: $F_{1,8}$=0.45, p=0.522). This was consistent across sexes for both DCX (Sex: $F_{1,14}$=0.17, p= 0.688; Treatment*Sex: $F_{1,14}$=0.44, p= 0.518) and ΔFosB (Sex: $F_{1,8}$=0.20, p=0.665; Treatment*Sex: $F_{1,8}$=0.55, p=0.48).

3.3.5 *Mesopallium (M) (Control)*

In contrast to the TnA, Hp, and NC, there was a significant treatment effect (Fig 3.4; Treatment: $F_{1,8}$=6.53, p=0.034) and treatment by sex interaction (Treatment*Sex: $F_{1,8}$=5.68, p=0.044) on ΔFosB activation in the M control region. This seems to be driven by the males, as a Tukey HSD post-hoc test revealed a significant difference between the treatments for males (Tukey HSD - Predator Male:Non-Predator Male: p=0.033).

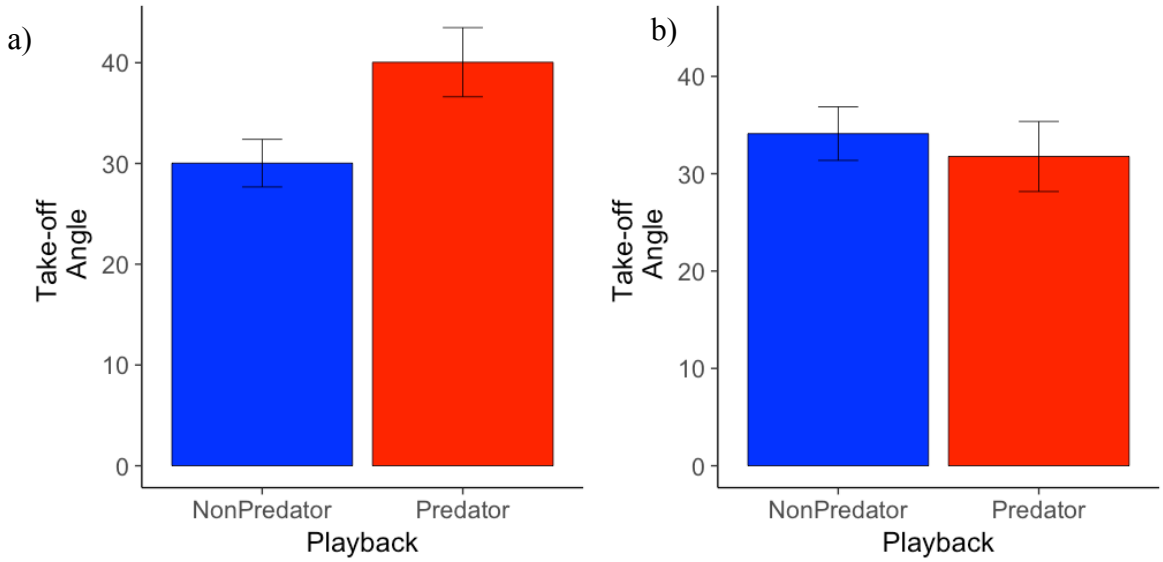


Figure 3.1: The take-off angle a) during the playback treatments (Treatment: $F_{1,56}$= 6.68, p=0.012) and b) one week after the playback treatments for cowbirds (Treatment: $F_{1,45}$=0.23, p=0.632).

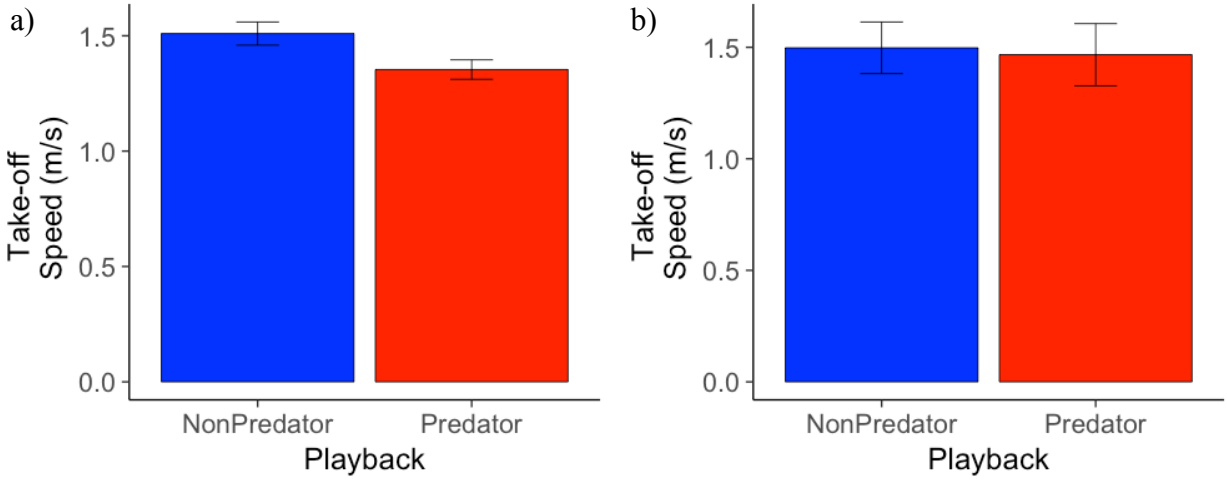


Figure 3.2: The take-off speed (m/s) a) during the playback treatments (Treatment, $F_{1,56}$ = 4.49, p = 0.039) and b) one week after the playback treatments for cowbirds(Treatment: $F_{1,45}$=0.19, p=0.663).

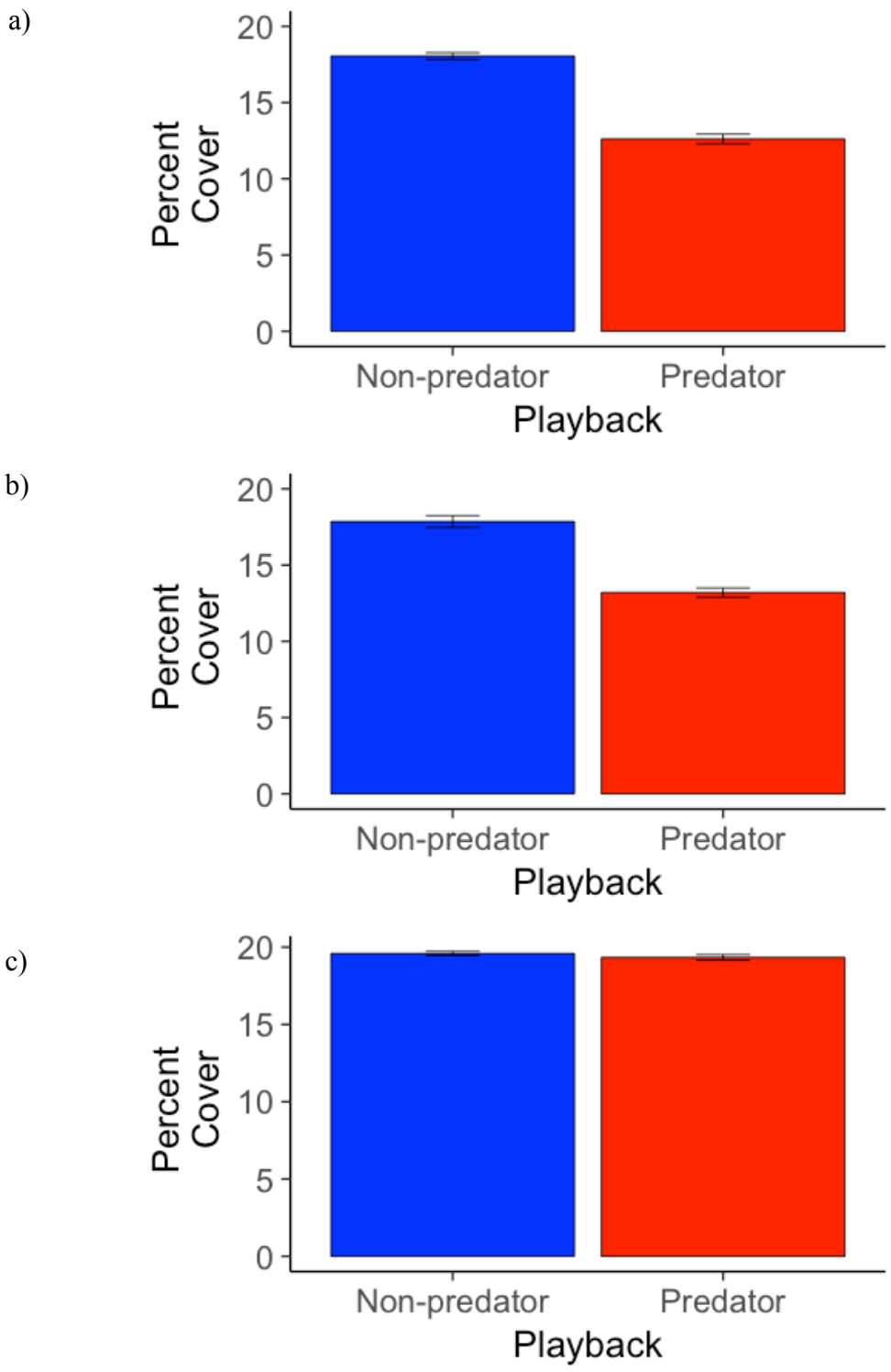
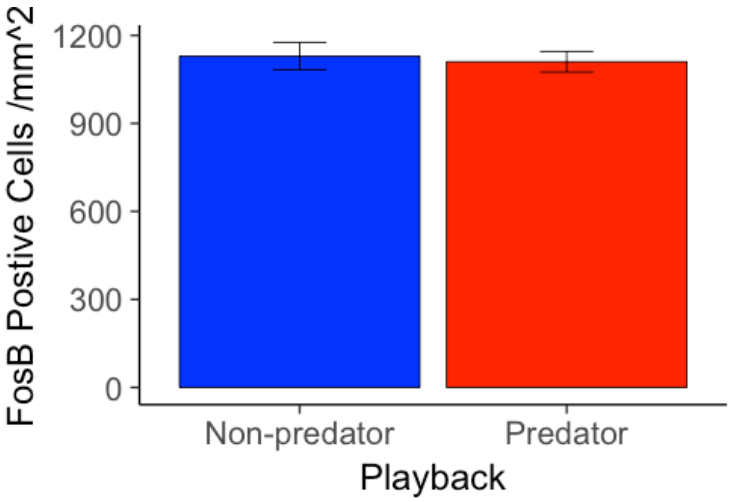
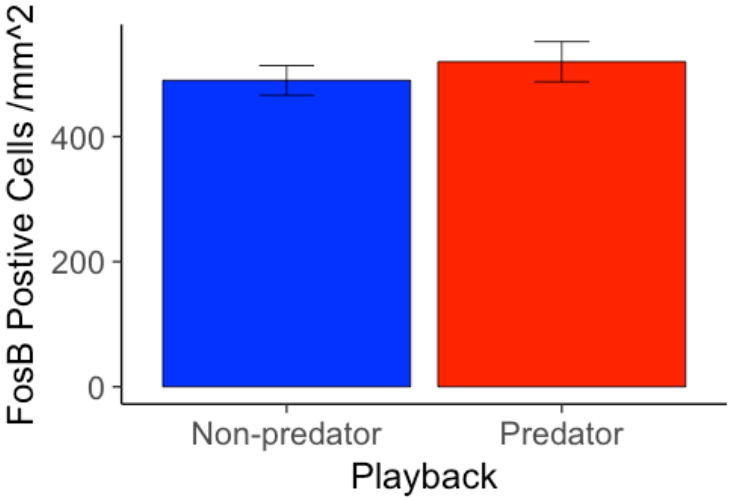


Figure 3.3: The percentage of brain slices covered by DCX positive cells in the a) TnA (Treatment: $F_{1,14}$=179.7, p<0.0001), b) Hp (Treatment: $F_{1,14}$=91.0, p<0.0001), and the NC (Treatment: $F_{1,14}$=1.36, p=0.263) in cowbirds.

a)



b)



c)

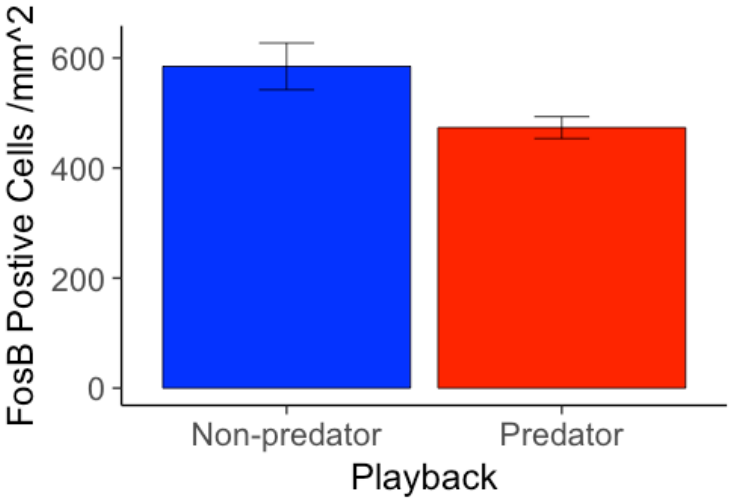


Figure 3.4: The density of FosB positive cells in the a) TnA (Treatment: $F_{1,8}$=0.078, p=0.786), b) NC (Treatment: $F_{1,8}$=0.45, p=0.522), and c) M (control; Treatment: $F_{1,8}$=6.53, p=0.034) in cowbirds.

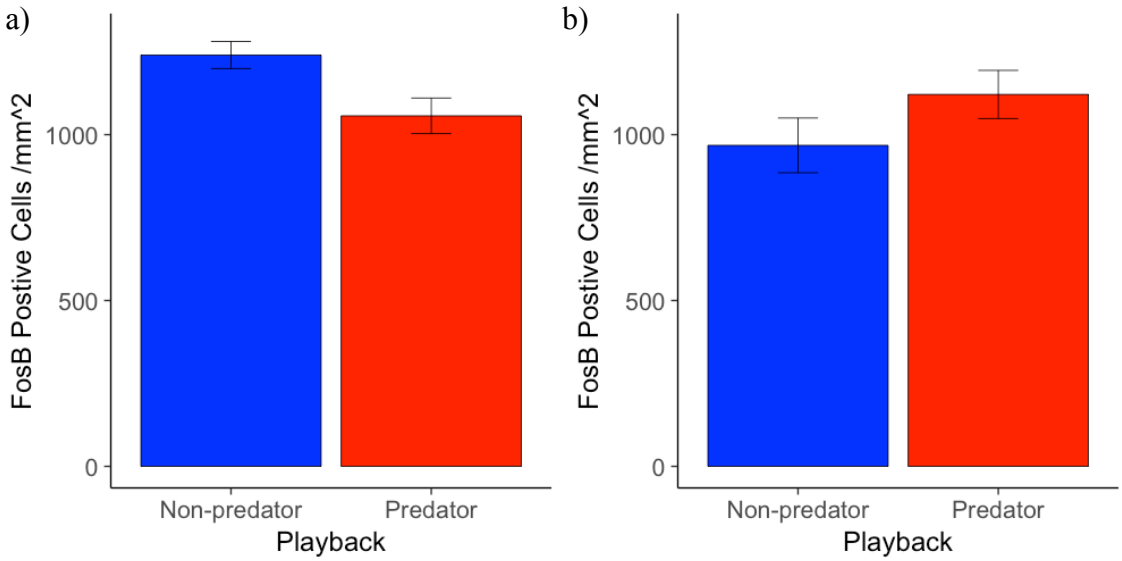


Figure 3.5: The density of FosB positive cells in the Hp for a) experimental round 1 and b) experimental round 2 (Treatment*Round: $F_{1,8}$=5.86, p=0.042) in cowbirds.

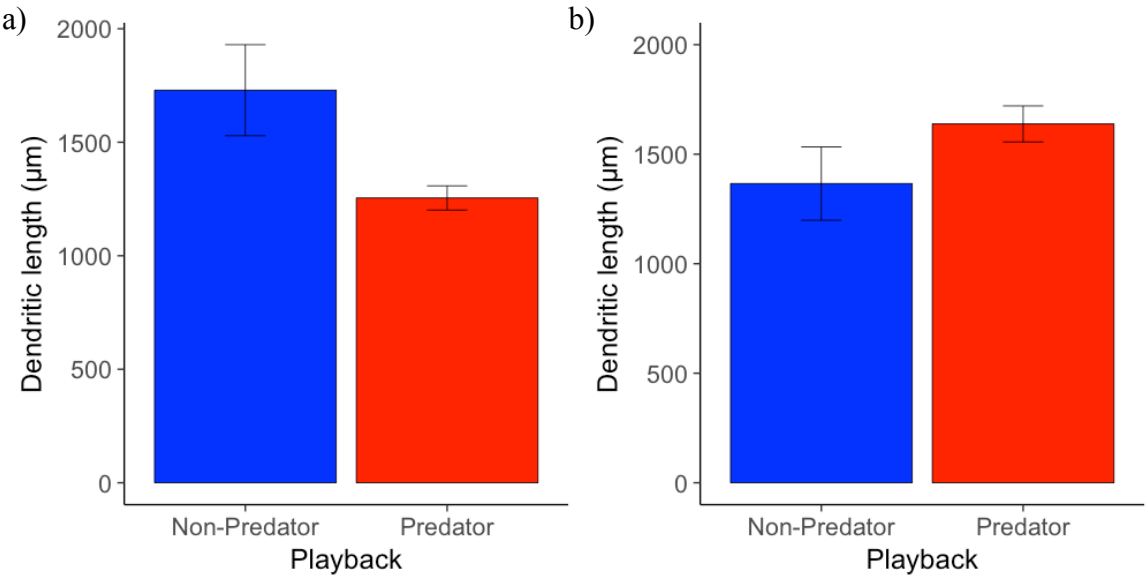


Figure 3.6: The total dendritic length in the TnA for a) male and b) female (Treatment*Sex: $F_{1,8}$=4.95, p=0.057) cowbirds.

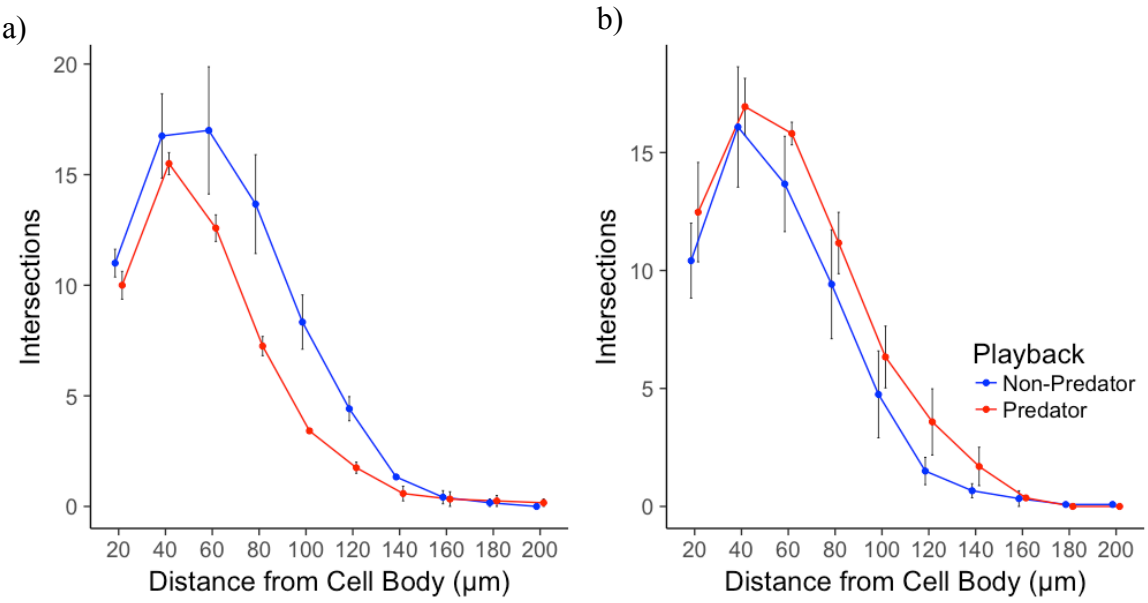


Figure 3.7: The number of intersections with the Sholl rings at each radius from the cell body for a) male and b) females(Treatment*Sex*Distance: $F_{9,72}$=2.45, p=0.017) cowbirds.

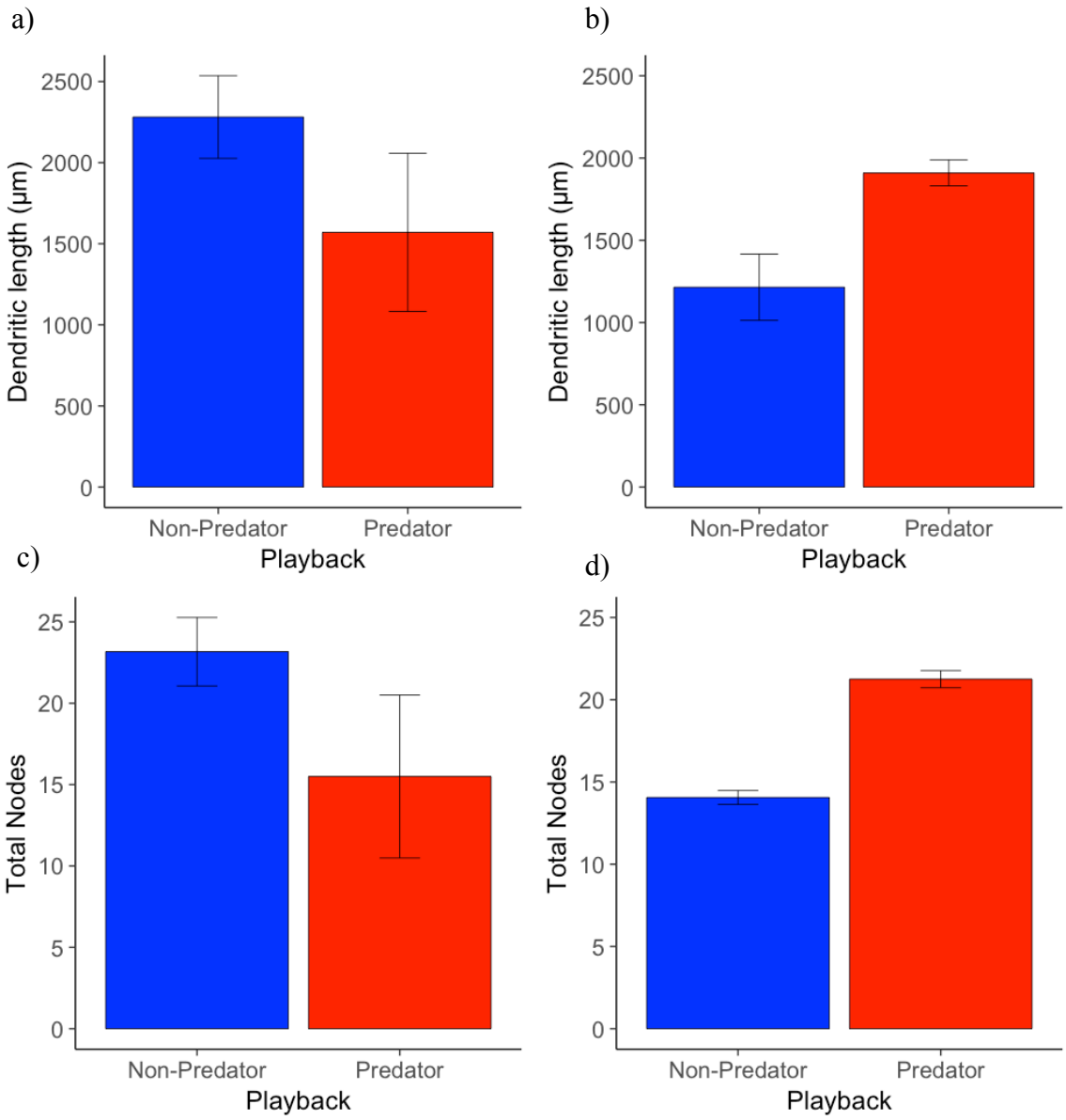


Figure 3.8: The total dendritic length in the cowbird Hp for a) males and b) females (Treatment*Sex: $F_{1,8}$=5.67, p=0.044) and the total number of branch points in the Hp for c) males and d) females (Treatment*Sex: $F_{1,8}$=7.25, p=0.027).

3.4 *Discussion*

The experimental results demonstrate that manipulating the level of perceived predation risk prey are exposed to can have long lasting effects on brain morphology. Exposure to high levels of perceived predation risk led to changes in escape behaviour, as cowbirds initiated flight at a steeper angle and slower speed in the predator treatment. Additionally, we found lasting effects of perceived predation risk on neurogenesis and dendritic morphology in regions associated with processing predation risk that persisted for at least seven days after the manipulated threat was removed. This provides evidence that there are long lasting changes in prey neurobiology, and that these changes are still evident when predation risk is manipulated in a semi-natural environment.

When looking at behaviour, we found that increased perceived predation risk led to transient changes in flight take-off behaviour. We found the same behavioural trade-off of cowbirds taking off at a steeper angle and slower speeds that was previously shown by Walters et al. (2017). It has been suggested that a steeper take-off angle aids prey in evading a predators line of attack and out-climbing the predator, which can reduce the likelihood of capture (Howland 1974, Lind et al. 2002, Walters et al. 2017). This also shows that the experimental protocol used here produces replicable results, as we found the same pattern of high predation risk leading to a steeper take-off angle and lower take-off speed as seen in previous research (Walters et al. 2017). Additionally, the lack of a treatment effect six days after the playbacks ended suggests that changes to escape behaviour are transient, and will return to baseline conditions once the threat has been removed. This is consistent with previous work, as the repeated measures design used by Walters et al. (2017) showed that individuals who had been exposed to the predator

treatment first showed no significant difference in their behaviour in the non-predator treatment 12 days after the end of the predator playbacks compared to those who experienced the non-predator treatment first.

In both the TnA and the Hp, we found a lasting inhibition in neurogenesis in cowbirds exposed to increased perceived predation risk. This is likely to retain the memory of the stressful situation, as it has been suggested that increased neurogenesis promotes the formation of new memories and the forgetting of old information (Frankland et al. 2013, Mongiat and Schinder 2014). Increased neurogenesis has been shown to disrupt established memories in rats (Akers et al. 2014), suggesting that the inhibition in neurogenesis here promotes retaining the memory of the high predation risk situation. Previous work has shown prey do retain information related to predator cues, with cues associated with higher risk retained longer (Ferrari et al. 2010), supporting the idea that prey retain the memory of high risk situations. Additionally, this result is consistent with previous work showing inhibited neurogenesis with high predation risk in both rats and fish (Tanapat et al. 2001, Dunlap et al. 2016).

When looking at ΔFosB, there was variation in the response between the TnA and the Hp. In the TnA, there was no significant treatment effect of predation risk on ΔFosB activation. This is likely due to the influence of external cues in the semi-natural environment, as previous work has shown significantly increased ΔFosB in the TnA in response to high perceived predation risk in the lab (Hobbs 2015). One potential factor that can also increase ΔFosB is sex, as previous work with rats has shown increased c-fos, a closely related protein, in the medial amygdala in response to sexual activity

(Erskine 1993). Additionally, males rats exposed to estrous females displayed a reduced aversion, decreased behavioural stress response, and a weakened hormonal stress response to predator odour (Kavaliers et al. 2001). Given that previous work has shown that female cowbirds in the non-predator treatment were significantly more responsive to male displays (Cheng 2016), it is likely that these birds were also engaging in more sexual arousal. Given that both increased sexual activity and increased predation risk could potentially be contributing to the increase in ΔFosB, these factors could potentially cancel out any quantifiable treatment effect for this protein. In the Hp, there was a significant effect treatment by round interaction, driven by a trend towards a round effect in the non-predator treatment. Given that these changes were driven by those in the non-predator treatment, this round effect would likely have been driven by an external cue influencing the cowbirds brain and behaviour in the semi-natural environment. The results for ΔFosB activation suggest that while this is an extremely useful tool for studying brain activation in the lab, there may be too many potential stimuli for this to be used as an indicator of perceived predation risk in the field. ΔFosB is thought to increase sensitivity in the reward circuitry of the brain, exerting anti-depressant-like behavioural responses to help individuals cope in times of stress (Nestler 2008), and is often implicated in addiction studies (Reviewed in Nestler, 2001, 2004, 2008). While ΔFosB has been shown to increase in response to increased perceived predation risk (Staples et al. 2009, Hobbs 2015), this has previously been shown only in a controlled laboratory environment. Given the strong treatment response seen in both neurogenesis and dendritic morphology, these results suggest that there were too many external stimuli in the semi-natural environment that could also affect ΔFosB for it to be used as a tool for

detecting the long lasting signature of perceived predation risk.

For dendritic morphology, both the TnA and the Hp showed significant treatment by sex interactions. This was evident for total dendritic length in the TnA and for total length, branching, and branch endings in the Hp. In every case, females in the predator treatment increased compared to those in the non-predator treatment, while males in the predator treatment decreased for each measure in comparison to the non-predator controls. This variation in response to perceived predation risk is likely due sex differences in the processing of fearful information. Sex differences have been found in rodents in the recall of fear conditioning and extinction, in particular when exposed to chronic stress (Baran et al. 2009). Research has shown both sex and seasonal differences in the hippocampal volume in brown-headed cowbirds (Sherry et al. 1993, Clayton et al. 1997). For females, having a relatively larger Hp is thought to be important for finding host nests (Sherry et al. 1993). If increased dendritic material in the Hp aids female cowbirds in remembering the risk associated with the high predation pressure location, it could be beneficial for the survival of their offspring. Previous work using the same species with the same experimental protocol also has shown that males displayed less to the less receptive females in a high predation risk environment, but showed no difference in their displays towards other males (Cheng 2016). By reducing the length, branching, and branch endings in a high predation risk environment, males could be reducing the connectivity in the Hp to better ignore the threatening information and maintain the behaviours required to protect their status within the social hierarchy. Overall, these changes in dendritic morphology suggest that increased perceived predation risk can lead to long lasting changes in both the TnA and the Hp, supporting their proposed importance

in the avian fear network (Cross et al. 2013, Hobbs 2015).

In the NC, we can see no long lasting effects in either DCX or ΔFosB activation. This is consistent with previous work in the lab showing no long lasting effects of increased perceived predation risk on ΔFosB (Hobbs 2015). In contrast, perceived predation risk has been shown to induce short term changes in activation in the NC (Cross et al. 2013, Hobbs 2015), which is consistent with the suggestion that the NC is responsible processing information to generate behaviour (Herold et al. 2011). It is likely that this region is involved with the processing of fearful information, but these results and previous work looking at ΔFosB in black-capped chickadees (*Poecile atricapillus*) (Hobbs 2015) suggests that the NC does not retain a lasting signature of this information in the long term.

The mesopallium control region showed a significant treatment effect in males, with those in the non-predator treatment showing increased ΔFosB activation. It is likely that this is linked to a behavioural response in the males, as previous research has shown male zebra finches to increase in c-fos, another transcription factor in the Fos family, in response to exposure to females for a first attempt at courtship (Sadananda and Bischof 2002). This is consistent with previous behavioural changes seen in cowbirds under the same experimental protocol, who made fewer courtship displays to females when under high perceived predation risk (Cheng 2016). Given that this same region has previously been used as a control to ensure that changes in ΔFosB activation were limited to regions involved in processing fear information (Hobbs 2015), it is likely that this result is evidence of ΔFosB activation involved in mediating behavioural changes rather than a

direct response to processing perceived predation risk.

With dendritic morphology, the sex differences seen in the response in both the TnA and the Hp emphasize the importance of considering both sexes when looking at the behavioural and neural response to perceived predation risk, particularly during the breeding season. Previous work looking at the effects of increased perceived predation risk on dendritic morphology has been focused on males (Baran et al. 2005, Mitra et al. 2009, Adamec et al. 2012), leaving a knowledge gap in how this increased risk affects females. Additionally, given the sex differences seen in courtship behaviour and escape ability (Cheng 2016, Walters et al. 2017), it is not surprising that this species showed sex differences in the dendritic morphology as well. By considering both sexes, these results can give us a greater understanding of cowbird neurobiology and a potential contributing factor for the sex differences shown in the behavioural response to increased perceived predation risk.

These results can also provide new insight into the ecological relevance of these methods for quantifying changes in the brain for PTSD research. Previous research has suggested perceived predation risk as a useful stimulus to study PTSD (Adamec & Shallow, 1993; Clinchy et al., 2013; Cohen et al., 2012, 2014; Zoladz & Diamond, 2016), however the majority of studies have been conducted on mammals in a laboratory environment. These results show that even in a semi-natural environment, increased perceived predation risk has long lasting effects on both dendritic morphology and neurogenesis. In particular, the changes seen in both dendritic morphology and neurogenesis can be linked to changes in learning and memory that promote retention of cues surrounding the stressful situation (Diamond et al. 2006, Frankland et al. 2013,

Mongiat and Schinder 2014). Given that intrusive memories related to a traumatic event or distress in response to cues resembling an aspect of the traumatic event are common symptoms in humans diagnosed with PTSD (American Psychiatric Association 2013), understanding how a traumatic stimulus like perceived predation risk affects neurogenesis and dendritic morphology could provide valuable insight on the etiology of PTSD. Additionally, this research shows that using wild caught animals to model the conditions leading to PTSD is a valuable tool for to gain a broader understanding of the changes that occur in the brain following a life-threatening traumatic event.

By manipulating perceived predation risk on wild caught individuals in a semi-natural environment, we were able to support the notion that lasting neurological changes are not just a product of predator-naïve individuals or an artificial laboratory environment. These semi-natural conditions better mimic the real life conditions of prey species living in a high predator environment than what could be produced in a lab setting. Additionally, the use of a non-mammalian species to study neurobiological changes previously studied in mammals suggests that these changes could be widespread across a variety of taxa. Furthermore, this study may have underestimated the effects of perceived predation risk on the brain due to the use of a conservative auditory cue and taxidermic mounts. Many previous studies looking at the effects of perceived predation risk on the brain have used live predators as the stimulus (Baran et al. 2005, Diamond et al. 2006, Mitra et al. 2009, Adamec et al. 2012). Given that the cue used can alter the response to predation threat, with live predators eliciting a stronger and longer lasting response than predator odour (Adamec et al. 1998, Wiedenmayer 2004), it would be expected that live predators would elicit a stronger response on both the brain and

behaviour when compared to calls and mounts alone. This suggests that exposure to live predators would lead to a stronger and longer lasting brain response than auditory cues and taxidermic mounts, and that similar neurological changes would be seen in free living animals exposed to live predators.

This study has provided new insight into the long lasting effects of perceived predation risk on the brain, however there are still many questions that warrant investigation. First would be to investigate whether similar lasting effects on neurogenesis and dendritic morphology could be seen in free living animals. Previous research has shown that free living song sparrows exposed to increased perceived predation risk had reduced offspring production and survival (Zanette et al. 2011). Understanding the neurobiological changes occurring during this time could provide valuable insight into the mechanisms contributing to these demographic consequences. Additionally, it would be interesting to see how the brain of developing animals is affected by increased perceived predation risk. Previous research has shown that changing the level perceived predation risk affects parental care, through decreased food provision for young (Fontaine and Martin 2006, Dudeck 2016), and nutritional stress during early development has been shown to impair development in song control regions of the brain (MacDonald et al. 2006). It would be interesting to assess whether increased perceived predation risk during early development has any impact on TnA, Hp, or NC, and how the effects of perceived predation risk on the brain compare between parents and offspring.

In this study we have identified long lasting changes in dendritic morphology and neurogenesis in the TnA and Hp of wild caught cowbirds. This provides further evidence

that the effects of perceived predation risk continue long after the heightened threat has been removed, and that they can have lasting effects on the brain. This study also shows that lasting the effects of perceived predation risk can still be seen in a semi-natural environment, suggesting that similar changes could be seen in free-living animals.

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Chapter 4

4 Discussion

In this thesis, I explored the long lasting effects of perceived predation risk on the avian brain and behaviour. In Chapter 1, I reviewed the effects of perceived predation risk from the population level down to the individual brain. I presented a variety of different tools that can be used to quantify changes in the brain in response to perceived predation risk, highlighting areas that required further exploration with a wild caught species. In Chapter 2, I explored how perceived predation risk affects the brain and behaviour in a controlled laboratory setting, with the focus on long lasting effects that continue even after the predation risk has been removed. I manipulated predation risk in black-capped chickadees using playbacks of predator and non-predator species and measured lasting changes in behaviour and dendritic morphology, allowing me to assess and compare changes in behaviour and neurobiology at a common time point. In Chapter 3, I expanded my study to a semi-natural environment, with brown-headed cowbirds as my study species. I used visual and auditory cues to manipulate perceived predation risk, and assessed changes in escape behaviour (take-off angle and speed) and neurobiology (protein activation, neurogenesis, and dendritic morphology). In this study, I integrated laboratory methods used to assess changes in neurobiology with a wild caught species in a semi-natural environment, to explore how predation risk would affect the brain in a more natural environment. In this final chapter, I will summarize how my work addresses important issues relating to how predation threat impacts the brain and behaviour, and to explore the broader significance of my findings in understanding the long lasting effects of perceived predation risk on prey species.

4.1 *Perceived predation risk has lasting effects on the brain and behaviour*

My results help us to better understand the roles of the avian TnA, Hp, and NC in processing perceived predation risk. The TnA showed lasting changes in dendritic length and inhibited neurogenesis in response to increased perceived predation risk. This supports the proposed role of the TnA as the centre of the avian fear network (Cohen and Goff 1978, Marzluff et al. 2012, Hobbs 2015). Additionally, these results mirror those seen in the mammalian amygdala, which has also shown increased activity and lasting effects in response to threatening stimuli such as predator cues (Vyas et al. 2002, Shin et al. 2006, Mitra et al. 2009, Adamec et al. 2012, Gross and Canteras 2012). In the Hp, I also found lasting changes in dendritic morphology and inhibited neurogenesis. These results reflect the proposed importance of the avian Hp for learning and memory (Colombo and Broadbent 2000, Marzluff et al. 2012, Cross et al. 2013, Hobbs 2015), in addition to supporting the functional similarities with the mammalian hippocampus (Tanapat et al. 2001, Kim and Diamond 2002, Baran et al. 2005, Adamec et al. 2012). In the NC, I found no lasting changes in activation or neurogenesis. This result is consistent with previous work looking the lasting effects of perceived predation risk on the brain (Hobbs 2015). This region has, however, shown short term changes in activation in response to fearful stimuli (Marzluff et al. 2012, Cross et al. 2013, Hobbs 2015), supporting the proposed importance in the avian fear network, but leaving further questions about the duration of activation in this NC.

Behaviourally, my results suggest that certain anti-predator behaviours are longer lasting, and that environmental cues can trigger varying behavioural responses. I found only short term changes in escape behaviour, suggesting that these changes are transient

and that individuals will return to their baseline conditions once the threat has been removed. However, it is evident that prey species will respond to conspecific cues of predation risk, and that previous experience with predation risk affects the behavioural response to these cues. This suggests that they retain a memory of the traumatic situation, and that conspecific alarm cues may trigger that memory if that risk is presented again in future.

My results show that perceived predation risk can produce lasting and quantifiable changes in the avian fear network, in both a laboratory and semi-natural setting, and that these changes can coincide with lasting changes in behaviour. These neurobiological effects may also be linked to other changes in individual biology, with the full extent of the impact of perceived predation risk not yet known. It is clear that the neurological impacts of perceived predation are long lasting, and should be considered both in future study on predator-prey ecology and in conservation. Additionally, it is evident that the neurological response in a semi-natural environment does not always match that seen in the lab, stressing the importance of considering environmental factors in biomedical fear research.

4.2 *Implications for Avian Neurobiology*

My results support the proposed roles of the TnA (Cohen and Goff 1978, Marzluff et al. 2012, Hobbs 2015) and the Hp (Colombo and Broadbent 2000, Cross et al. 2013, Hobbs 2015) in the processing perceived predation risk, and their importance in the avian fear network. Both the TnA and the Hp showed lasting changes in dendritic morphology and inhibited neurogenesis seven days after exposure to increased perceived predation risk, suggesting that these regions are not only important in the perception of

predation risk but also for retaining information about previous predation events. My results also suggest that further research is needed to better understand the role of the NC in the avian fear network, as there was no lasting effect on neurogenesis or ΔFosB activation. These results are consistent with previous work looking at the lasting effects of perceived predation risk (Hobbs 2015), but leave many questions about the extent of this region's involvement in the processing and memory of predation risk. Further research into the duration of activation in the NC, and whether there is any interaction with the lasting changes seen in the TnA and Hp could help us to better understand the role of the region.

Additionally, this research suggests that dendritic morphology and neurogenesis could provide valuable tools for quantifying the neurological impacts of perceived predation risk on the avian brain. With both measures showing effects of increased perceived predation risk in a semi-natural environment, it is likely that these tools would be useful in studying the effect of perceived predation risk on avian neurobiology both in the lab and the field.

4.3 *Implications for biomedical research*

Biomedical research has shown lasting effects on both the brain and behaviour in human and animal models in response to life threatening traumatic events (Adamec and Shallow 1993, Bremner et al. 1999, Shin et al. 2006, Cohen et al. 2012, 2014, Clinchy et al. 2013, Zoladz and Diamond 2016). Animal models are particularly useful in studying the etiology of post traumatic stress disorder (PTSD), as researchers can manipulate the circumstances leading to the onset of PTSD, and assess any changes in neurobiology seen in response to the traumatic stimuli. My experiment addresses one of the major

criticisms of animal models for PTSD, being that laboratory raised animals in cages may not show the same response as their wild counterparts (Clinchy et al. 2013). While it is evident that perceived predation risk can lead to long lasting changes in the brain, the effects seen in laboratory studies do not always persist in the natural environment. For example, in some species aversive stimuli presented in the lab have been shown to induce changes lasting much longer than changes observed in the natural environment (Wiedenmayer 2004). My results show methods used to quantify neurological changes in laboratory mammals elicited similar responses in wild caught animals tested both in the lab and in a semi-natural environment. These wild caught individuals would likely have prior experience with predation risk, suggesting that the responses seen are ecologically meaningful. Additionally, finding lasting changes in a non-mammalian species suggests that these lasting neurological changes seen in response to increased perceived predation risk may be more ubiquitous than previously thought (Sapolsky 2004).

Understanding the neural response to perceived predation risk can also aid biomedical researchers in developing new therapies for disorders like PTSD. Given the sex differences in the cowbird dendritic morphology, this suggests that males and females may respond differently to traumatic stimuli. This suggests that sex may be an important factor to consider when studying potential therapies for PTSD, and that the efficacy of any new treatment must be assessed in both sexes. Additionally, adult neurogenesis is an area of ongoing research, and the extent of adult neurogenesis and its effect on human behaviour has yet to be fully explored (Kheirbek et al. 2012). By identifying these neurological changes occurring in response to increased perceived predation risk, this

could provide new targets for research on drug therapies to help individuals suffering from PTSD.

4.4 *Implications for conservation*

Understanding the impacts of perceived predation risk could provide useful tools for conservation and wildlife management. Previous research has shown that increasing perceived predation risk can impact prey demography and lead to trophic cascades (Zanette et al. 2011, Suraci et al. 2016). Additionally, the presence of predators alone, following the reintroduction of wolves to the Greater Yellowstone Ecosystem, has led to changes in elk habitat selection and fecundity (Creel et al. 2005, 2007). Understanding the neurological mechanisms can help gain a better understanding of how perceived predation risk affects behaviour and demography. It may also be beneficial to look for connections between the changes seen here in the TnA and Hp and the effect on hypothalamic-pituitary-adrenal (HPA) axis, as this stress system is known to interact with the amygdala and hippocampus and can lead to target tissues showing resistance to sex steroids, which could in turn impact reproduction (Tsigos and Chrousos 2002). By connecting these changes in neurobiology with changes in behaviour, we can better understand why behavioural changes are occurring, and the extent to which the memory of a traumatic event remains in the brain. This could also provide valuable insight for conservation managers looking to understand what environmental cues are most stressful or lead to the greatest negative impact on species at risk.

Given the lasting neurological and behavioural changes seen in this experiment, it is clear that prey retain a memory of high predation situations. This provides further support for the importance of predators in the ecosystem, as has been demonstrated by

changes to elk behaviour and demography since wolves were returned to Yellowstone (Creel et al. 2005, 2007, Creel and Christianson 2009). If prey retain a lasting signature of previous predation risk in the brain, they are likely to maintain these behavioural changes even if predators are not constantly present in the area, supporting the importance of protecting small populations of large predators from human disturbance. These results also support the importance of considering fear effects when creating policies for wildlife conservation and management. Given that the indirect effects of predation, such as intimidation, can have greater impacts on prey species than consumption (Preisser et al. 2005, Bolnick and Preisser 2005), it is important to consider the effects of perceived predation risk when altering the predation risk in an ecosystem. This may be of particular importance when capturing animals for relocation or reintroduction to an ecosystem, as a traumatic capture could mimic a predation attempt and lead to unexpected lasting effects on the captured individuals. Whether through the reintroduction of extant predators or increased human activity, it is important for conservation policy to account for the potential indirect effects that predation risk can have on prey neurobiology, behaviour, and demography.

4.5 *Future directions*

My results have expanded our knowledge on the lasting effects of perceived predation risk on prey behaviour, dendritic morphology, and neurogenesis. My results emphasize the importance of the Hp and the TnA in the perception of predation risk, and show that auditory cues can have lasting impacts on behaviour and neurobiology in a semi-natural environment. These results also lay the groundwork for future studies on the impacts of perceived predation risk on prey behaviour and neurobiology.

One area that could use further research would be the NC. My research found no lasting effects of perceived predation risk on neurogenesis or ΔFosB activation in the NC, which is consistent with previous research looking at the long term effects of perceived predation risk on the avian brain (Hobbs 2015). However, the NC has shown changes in short term activation in response to increased perceived predation risk (Hobbs 2015), suggesting that further investigation is needed to gain a better understanding of the role of the NC in processing perceived predation risk and the extent to which this region is changed in response to changes in predation risk.

Another direction worth further investigation is the timescale of these changes in dendritic morphology and neurogenesis, to determine how quickly these changes are evident and whether these changes are still present past the one week period studied. In rats, changes in dendritic morphology response to caffeine administration have been shown to persist for at least 8 weeks (Juarez-Mendez et al. 2006). Future study into the lasting effects of perceived predation risk on dendritic morphology and neurogenesis past this one week time period may help to better understand the neural changes causing persistent PTSD symptoms in humans. PTSD diagnosis in humans requires symptoms to be present for at least one month, however some individuals have maintained symptoms for over 50 years (American Psychiatric Association 2013). Understanding the neural mechanisms behind these symptoms may be beneficial for mitigating symptoms and identifying new therapies for treating PTSD.

My results show that perceived predation risk can have lasting effects on wild caught prey neurobiology in a semi-natural environment. The next step in understanding the lasting neurological effects of perceived predation risk on free-living animals.

Previous work has shown that exposing song sparrows (*Melospiza melodia*) to increased perceived predation risk during the breeding season led to a 40% reduction in offspring survival (Zanette et al. 2011). However little is known about the effects of perceived predation risk on the brain in wild animals, and new research in this area could provide new information about the mechanisms leading to these observed changes in reproduction and offspring survival.

Another area worth further investigation is how perceived predation risk affects the developing brain. Increased perceived predation risk has been shown to reduce parental investment, leading to fewer feeding visits (Zanette et al. 2011, Dudeck 2016). Additionally, nutrient stress during early development has been shown to impair the development of HVC, a song control region in the avian brain (MacDonald et al. 2006). In order to better understand how perceived predation affects prey, it could be beneficial to look at the lasting effects on the developing brain. If increased perceived predation risk did lead to lasting impairments in development it could lead to long lasting impairment in fitness. In song sparrows, song learning is concentrated in during a 40 day period in early development (Marler and Peters 2010). Even if the neurological effects of perceived predation risk were not permanent, if they led to impairments during this critical period for song learning it could lead to lasting impacts on individual fitness. Additionally, it would be interesting to see if the Hp and the TnA showed a similar magnitude of response in the developing brain in comparison to adults, as the perception of the threat associated with perceived predation risk may vary with experience.

Finally, it would be interesting to assess how neurological activation in response to perceived predation risk changes over time, and how this impacts behaviour. One

potential tool to assess this would be positron emission tomography (PET) scans. Previous research has used PET scans to look at activation across the brain in American crows (*Corvus brachyrhynchos*) in response to a variety of threatening stimuli, including a natural predator (Marzluff et al. 2012, Cross et al. 2013). One advantage of using PET is that it is a non-invasive method of assessing changes in brain activation, which would allow for the same individuals to be studied at multiple time points. This could be beneficial for looking at how activation changes if individuals become habituated to certain stimuli, or for gaining a better understanding of what brain regions are activated in response to the social cues (such as a conspecific alarm call) after a threatening event. Finally, the use of PET scans could allow researchers to make more concrete connections between brain activation, behaviour, and physiology, as it would be possible to collect repeated measurements for both at concurrent time scales. By connecting neurological, behavioural, and physiological changes at the same time points for the same individuals, researchers could gain a more holistic view of the effects of perceived predation risk. Additionally, the use of non-invasive methods would allow individuals to be followed over time to gain a better understanding of how the neurological changes associated with perceived predation risk connect with previously observed changes in demography.

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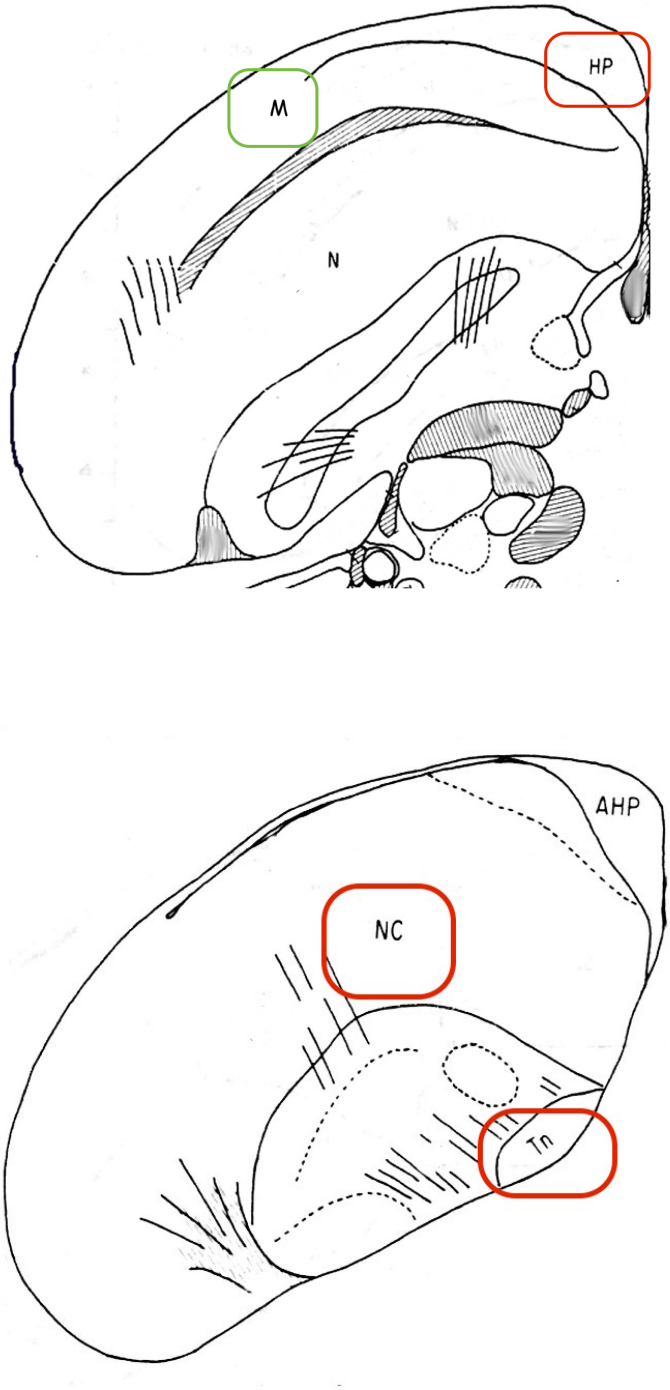
Appendices

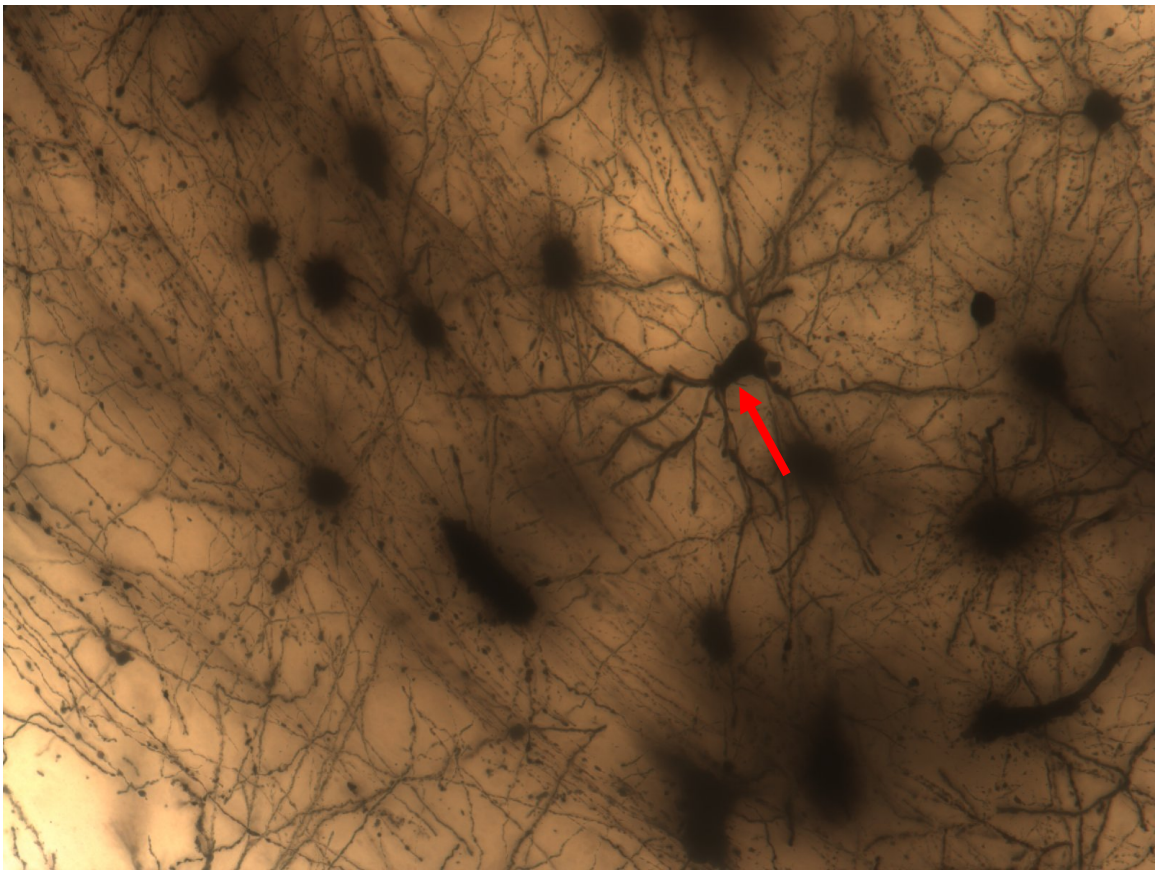
Appendix A: Ethogram for scoring chickadee behaviour.

Category	Behaviour	Description
Consumption	Feeding	Pecking in food cup
	Handling	Use the beak and feet to open seed
	Drinking	Pecking in water cup
Movement	Flying	Moving using wings. Starts when feet are in the air and stops when feet are back on a surface and feathers are folded again
	Landing	Bird stops and takes off again, within a 0.6s timeframe
	Walking	Moving by putting one foot in front of the other. Starts when the first foot is in the air and stops after the last foot is back on a surface
	Hopping	Moving by jumping. Starts when the feet are in the air and stops when the feet are back on a surface and the body is directly over the feet
Resting	Shaking	Wiggling the body from left to right. Starts when the body starts to move and stops when feathers are folded again
	Stretching	Extending at least one limb without changing location
	Preening	Manipulating feathers with the beak or feet or rubbing the beak over a surface
	Puffing Up	Raising feathers, appearing larger
Immobile	Sitting	Staying still with two claws attached to a fixed object for at least 0.6s, with head moving 1-3 times per second
	Looking	Staying still with two claws attached to a fixed object for at least 0.6s, with head moving more than 3 times per second
	Freezing	Staying still with two claws attached to a fixed object for at least 0.6s, with head moving less than 1 time per second and no rapid movement
Aggression	Biting bars	Pecking at the cage bars

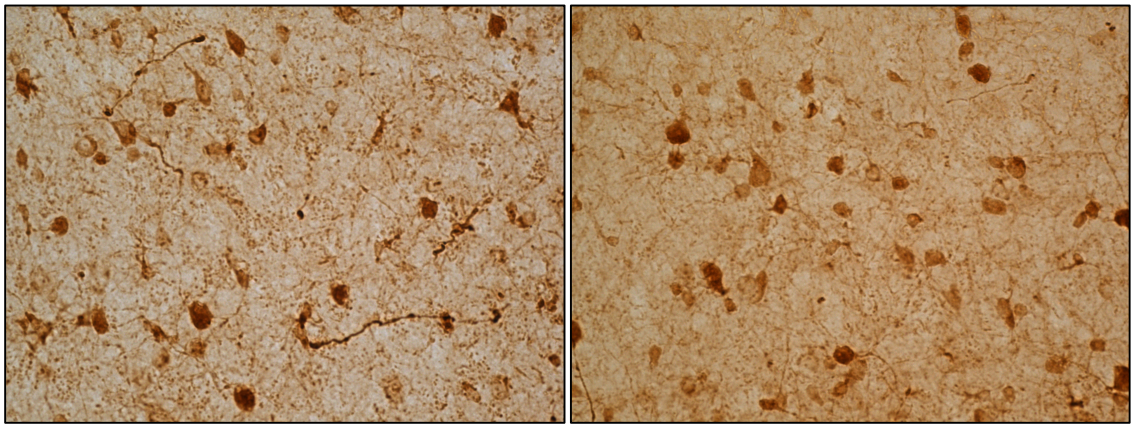
Appendix B: Diagram of the brain regions analyzed: the nucleus taeniae of the amygdala (TnA), the hippocampus (Hp), the caudal nidopallium (NC), and the mesopallium (M) (used as a control region).



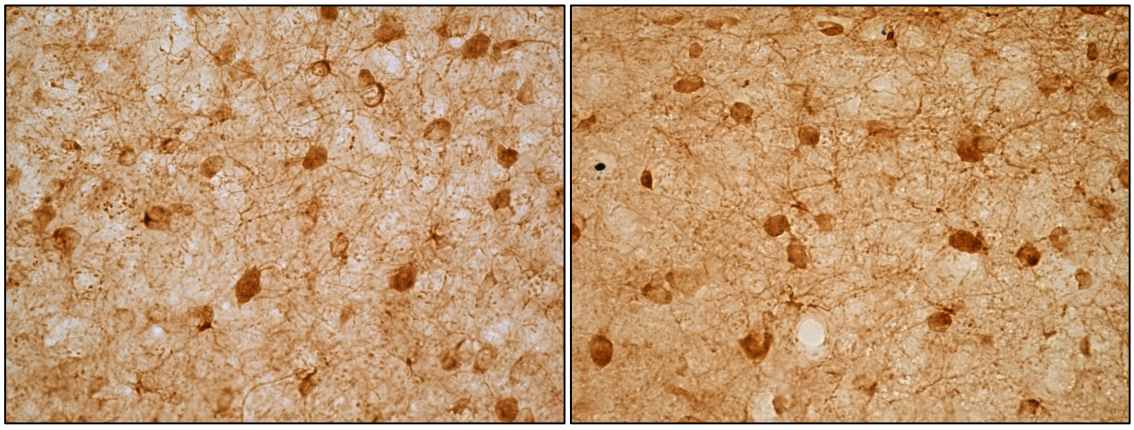
Appendix C: An example of a Golgi-Cox stained pyramidal neuron in a chickadee hippocampus.



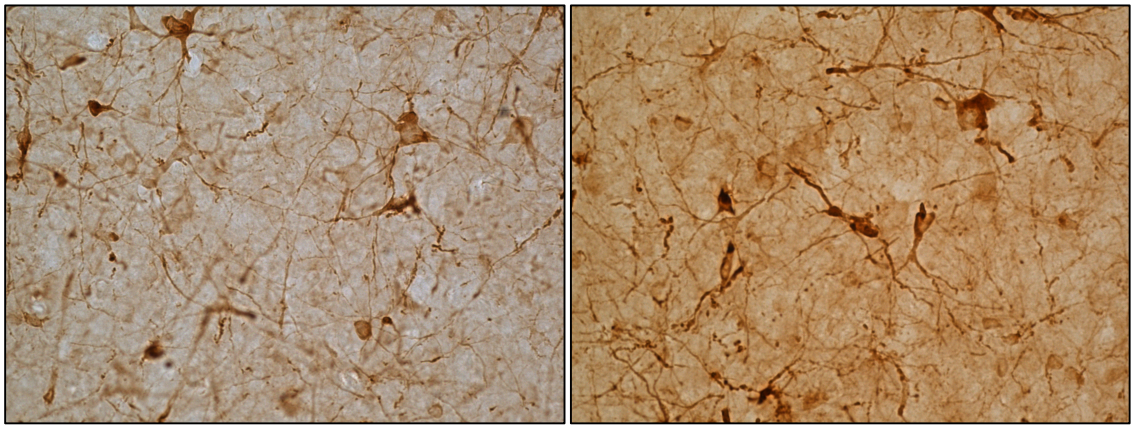
Appendix D: An example of cowbird DCX labelling in the TnA for the predator (left) and non-predator (right) treatments.



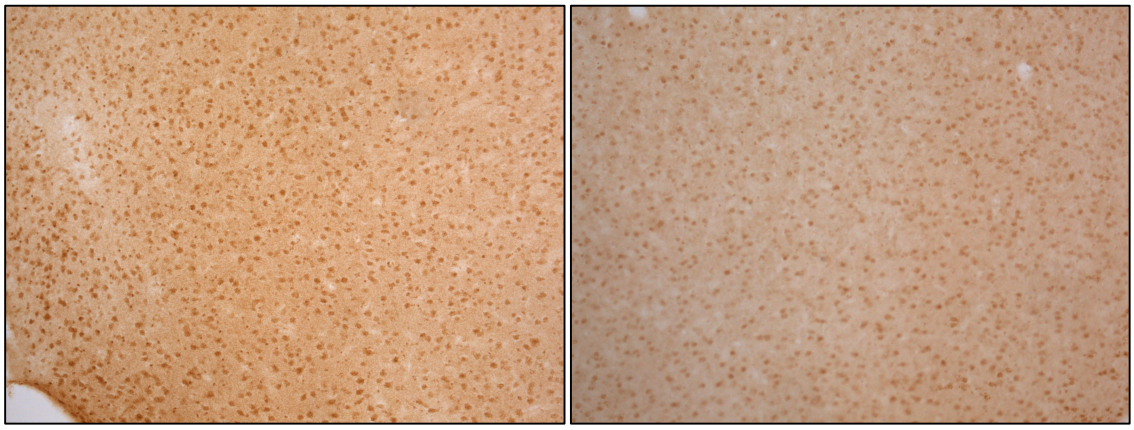
Appendix E: An example of DCX labelling in the Hp for the predator (left) and non-predator (right) treatment.



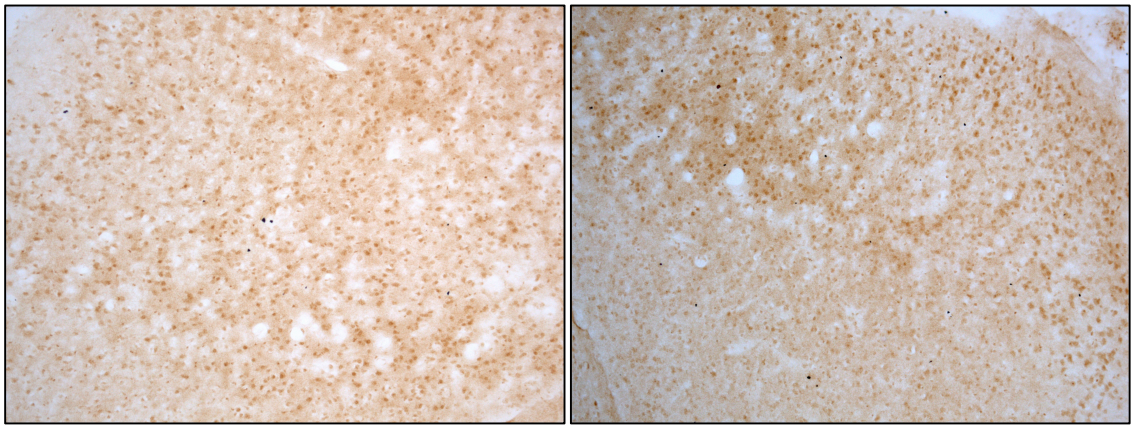
Appendix F: An example of DCX labelling in the NC for the predator (left) and non-predator (right) treatment.



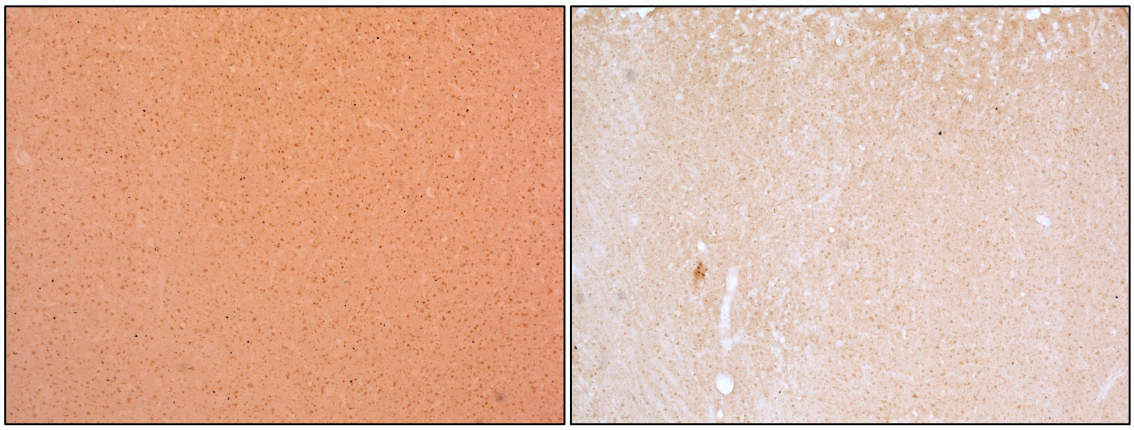
Appendix G: An example of ΔFosB labelling in the TnA for the predator (left) and non-predator (right) treatment.



Appendix H: An example of ΔFosB labelling in the Hp for the predator (left) and non-predator (right) treatment.



Appendix I: An example of ΔFosB labelling in the NC for the predator (left) and non-predator (right) treatment.



Appendix J: Statistical results for black-capped chickadee (BCCH) dendritic morphology measures.

Species	Brain Region	Dendrite	Variable	Measure	p
BCCH	Hp	Total	Nodes	Treatment	0.251
BCCH	Hp	Total	Nodes	Sex	0.945
BCCH	Hp	Total	Nodes	Treatment*Sex	0.925
BCCH	TnA	Total	Nodes	Treatment	0.132
BCCH	TnA	Total	Nodes	Sex	0.870
BCCH	TnA	Total	Nodes	Treatment*Sex	0.460
BCCH	Hp	Total	Ends	Treatment	0.223
BCCH	Hp	Total	Ends	Sex	0.804
BCCH	Hp	Total	Ends	Treatment*Sex	0.871
BCCH	TnA	Total	Ends	Treatment	0.140
BCCH	TnA	Total	Ends	Sex	0.770
BCCH	TnA	Total	Ends	Treatment*Sex	0.285
BCCH	Hp	Total	Length	Treatment	0.217
BCCH	Hp	Total	Length	Sex	0.762
BCCH	Hp	Total	Length	Treatment*Sex	0.389
BCCH	TnA	Total	Length	Treatment	0.085
BCCH	TnA	Total	Length	Sex	0.720
BCCH	TnA	Total	Length	Treatment*Sex	0.209
BCCH	Hp	Total	Quantity	Treatment	0.992
BCCH	Hp	Total	Quantity	Sex	0.606
BCCH	Hp	Total	Quantity	Treatment*Sex	0.429
BCCH	TnA	Total	Quantity	Treatment	0.866
BCCH	TnA	Total	Quantity	Sex	0.294
BCCH	TnA	Total	Quantity	Treatment*Sex	0.393
BCCH	Hp	Apical	Nodes	Treatment	0.311
BCCH	Hp	Apical	Nodes	Sex	0.447
BCCH	Hp	Apical	Nodes	Treatment*Sex	0.782
BCCH	TnA	Apical	Nodes	Treatment	0.620
BCCH	TnA	Apical	Nodes	Sex	0.360
BCCH	TnA	Apical	Nodes	Treatment*Sex	0.771
BCCH	Hp	Apical	Length	Treatment	0.452
BCCH	Hp	Apical	Length	Sex	0.358
BCCH	Hp	Apical	Length	Treatment*Sex	0.917
BCCH	TnA	Apical	Length	Treatment	0.245
BCCH	TnA	Apical	Length	Sex	0.355
BCCH	TnA	Apical	Length	Treatment*Sex	0.262

BCCH	Hp	Apical	Longest Dendrite	Treatment	0.176
BCCH	Hp	Apical	Longest Dendrite	Sex	0.905
BCCH	Hp	Apical	Longest Dendrite	Treatment*Sex	0.969
BCCH	TnA	Apical	Longest Dendrite	Treatment	0.299
BCCH	TnA	Apical	Longest Dendrite	Sex	0.457
BCCH	TnA	Apical	Longest Dendrite	Treatment*Sex	0.049
BCCH	Hp	Apical	Ends	Treatment	0.370
BCCH	Hp	Apical	Ends	Sex	0.370
BCCH	Hp	Apical	Ends	Treatment*Sex	0.971
BCCH	TnA	Apical	Ends	Treatment	0.667
BCCH	TnA	Apical	Ends	Sex	0.388
BCCH	TnA	Apical	Ends	Treatment*Sex	0.731
BCCH	Hp	Basal	Nodes	Treatment	0.339
BCCH	Hp	Basal	Nodes	Sex	0.708
BCCH	Hp	Basal	Nodes	Treatment*Sex	0.968
BCCH	TnA	Basal	Nodes	Treatment	0.078
BCCH	TnA	Basal	Nodes	Sex	0.572
BCCH	TnA	Basal	Nodes	Treatment*Sex	0.407
BCCH	Hp	Basal	Length	Treatment	0.205
BCCH	Hp	Basal	Length	Sex	0.875
BCCH	Hp	Basal	Length	Treatment*Sex	0.260
BCCH	TnA	Basal	Length	Treatment	0.110
BCCH	TnA	Basal	Length	Sex	0.914
BCCH	TnA	Basal	Length	Treatment*Sex	0.324
BCCH	Hp	Basal	Longest Dendrite	Treatment	0.140
BCCH	Hp	Basal	Longest Dendrite	Sex	0.172
BCCH	Hp	Basal	Longest Dendrite	Treatment*Sex	0.158
BCCH	TnA	Basal	Longest Dendrite	Treatment	0.337
BCCH	TnA	Basal	Longest Dendrite	Sex	0.693
BCCH	TnA	Basal	Longest Dendrite	Treatment*Sex	0.817
BCCH	Hp	Basal	Ends	Treatment	0.270

BCCH	Hp	Basal	Ends	Sex	0.857
BCCH	Hp	Basal	Ends	Treatment*Sex	0.818
BCCH	TnA	Basal	Ends	Treatment	0.083
BCCH	TnA	Basal	Ends	Sex	0.287
BCCH	TnA	Basal	Ends	Treatment*Sex	0.214
BCCH	Hp	Basal	Spines	SPINES	0.000
BCCH	Hp	Basal	Spines	SPINES*Treatment	0.235
BCCH	Hp	Basal	Spines	SPINES*Sex	0.250
BCCH	Hp	Basal	Spines	SPINES*Treatment*Sex	0.106
BCCH	TnA	Basal	Spines	SPINES	0.000
BCCH	TnA	Basal	Spines	SPINES*Treatment	0.891
BCCH	TnA	Basal	Spines	SPINES*Sex	0.590
BCCH	TnA	Basal	Spines	SPINES*Treatment*Sex	0.308
BCCH	Hp	Apical	Sholl Intersections	Treatment	0.612
BCCH	Hp	Apical	Sholl Intersections	Sex	0.250
BCCH	Hp	Apical	Sholl Intersections	Treatment*Sex	0.982
BCCH	Hp	Apical	Sholl Intersections	RADIUS	0.000
BCCH	Hp	Apical	Sholl Intersections	RADIUS*Treatment	0.878
BCCH	Hp	Apical	Sholl Intersections	RADIUS*Sex	0.020
BCCH	Hp	Apical	Sholl Intersections	RADIUS*Treatment*Sex	0.261
BCCH	Hp	Basal	Sholl Intersections	Treatment	0.105
BCCH	Hp	Basal	Sholl Intersections	Sex	0.981
BCCH	Hp	Basal	Sholl Intersections	Treatment*Sex	0.358
BCCH	Hp	Basal	Sholl Intersections	RADIUS	0.000
BCCH	Hp	Basal	Sholl Intersections	RADIUS*Treatment	0.139
BCCH	Hp	Basal	Sholl Intersections	RADIUS*Sex	0.941
BCCH	Hp	Basal	Sholl Intersections	RADIUS*Treatment*Sex	0.005
BCCH	Hp	Apical	Sholl Length	Treatment	0.451
BCCH	Hp	Apical	Sholl Length	Sex	0.312
BCCH	Hp	Apical	Sholl Length	Treatment*Sex	0.992

BCCH	Hp	Apical	Sholl Length	RADIUS	0.000
BCCH	Hp	Apical	Sholl Length	RADIUS*Treatment	0.911
BCCH	Hp	Apical	Sholl Length	RADIUS*Sex	0.140
BCCH	Hp	Apical	Sholl Length	RADIUS*Treatment*Sex	0.834
BCCH	Hp	Basal	Sholl Length	Treatment	0.142
BCCH	Hp	Basal	Sholl Length	Sex	0.610
BCCH	Hp	Basal	Sholl Length	Treatment*Sex	0.293
BCCH	Hp	Basal	Sholl Length	RADIUS	0.000
BCCH	Hp	Basal	Sholl Length	RADIUS*Treatment	0.031
BCCH	Hp	Basal	Sholl Length	RADIUS*Sex	1.000
BCCH	Hp	Basal	Sholl Length	RADIUS*Treatment*Sex	0.053
BCCH	Hp	Apical	Sholl Nodes	Treatment	0.396
BCCH	Hp	Apical	Sholl Nodes	Sex	0.414
BCCH	Hp	Apical	Sholl Nodes	Treatment*Sex	0.484
BCCH	Hp	Apical	Sholl Nodes	RADIUS	0.000
BCCH	Hp	Apical	Sholl Nodes	RADIUS*Treatment	0.940
BCCH	Hp	Apical	Sholl Nodes	RADIUS*Sex	0.113
BCCH	Hp	Apical	Sholl Nodes	RADIUS*Treatment*Sex	0.037
BCCH	Hp	Basal	Sholl Nodes	Treatment	0.151
BCCH	Hp	Basal	Sholl Nodes	Sex	0.749
BCCH	Hp	Basal	Sholl Nodes	Treatment*Sex	0.932
BCCH	Hp	Basal	Sholl Nodes	RADIUS	0.000
BCCH	Hp	Basal	Sholl Nodes	RADIUS*Treatment	0.189
BCCH	Hp	Basal	Sholl Nodes	RADIUS*Sex	0.381
BCCH	Hp	Basal	Sholl Nodes	RADIUS*Treatment*Sex	0.001
BCCH	Hp	Apical	Sholl Ends	Treatment	0.370
BCCH	Hp	Apical	Sholl Ends	Sex	0.308
BCCH	Hp	Apical	Sholl Ends	Treatment*Sex	0.915
BCCH	Hp	Apical	Sholl Ends	RADIUS	0.000
BCCH	Hp	Apical	Sholl Ends	RADIUS*Treatment	0.400
BCCH	Hp	Apical	Sholl Ends	RADIUS*Sex	0.762
BCCH	Hp	Apical	Sholl Ends	RADIUS*Treatment*Sex	0.643
BCCH	Hp	Basal	Sholl Ends	Treatment	0.096
BCCH	Hp	Basal	Sholl Ends	Sex	0.727
BCCH	Hp	Basal	Sholl Ends	Treatment*Sex	0.506
BCCH	Hp	Basal	Sholl Ends	RADIUS	0.000
BCCH	Hp	Basal	Sholl Ends	RADIUS*Treatment	0.228
BCCH	Hp	Basal	Sholl Ends	RADIUS*Sex	0.992
BCCH	Hp	Basal	Sholl Ends	RADIUS*Treatment*Sex	0.084
BCCH	Hp	Total	Sholl	Treatment	0.147

BCCH	Hp	Total	Sholl Intersections	Sex	0.787
BCCH	Hp	Total	Sholl Intersections	Treatment*Sex	0.295
BCCH	Hp	Total	Sholl Intersections	RADIUS	0.000
BCCH	Hp	Total	Sholl Intersections	RADIUS*Treatment	0.111
BCCH	Hp	Total	Sholl Intersections	RADIUS*Sex	0.993
BCCH	Hp	Total	Sholl Intersections	RADIUS*Treatment*Sex	0.015
BCCH	Hp	Total	Sholl Length	Treatment	0.172
BCCH	Hp	Total	Sholl Length	Sex	0.784
BCCH	Hp	Total	Sholl Length	Treatment*Sex	0.887
BCCH	Hp	Total	Sholl Length	RADIUS	0.000
BCCH	Hp	Total	Sholl Length	RADIUS*Treatment	0.251
BCCH	Hp	Total	Sholl Length	RADIUS*Sex	0.983
BCCH	Hp	Total	Sholl Length	RADIUS*Treatment*Sex	0.153
BCCH	Hp	Total	Sholl Ends	Treatment	0.155
BCCH	Hp	Total	Sholl Ends	Sex	0.965
BCCH	Hp	Total	Sholl Ends	Treatment*Sex	0.679
BCCH	Hp	Total	Sholl Ends	RADIUS	0.000
BCCH	Hp	Total	Sholl Ends	RADIUS*Treatment	0.806
BCCH	Hp	Total	Sholl Ends	RADIUS*Sex	0.975
BCCH	Hp	Total	Sholl Ends	RADIUS*Treatment*Sex	0.373
BCCH	Hp	Total	Sholl Nodes	Treatment	0.136
BCCH	Hp	Total	Sholl Nodes	Sex	0.875
BCCH	Hp	Total	Sholl Nodes	Treatment*Sex	0.782
BCCH	Hp	Total	Sholl Nodes	RADIUS	0.000
BCCH	Hp	Total	Sholl Nodes	RADIUS*Treatment	0.045
BCCH	Hp	Total	Sholl Nodes	RADIUS*Sex	0.478
BCCH	Hp	Total	Sholl Nodes	RADIUS*Treatment*Sex	0.001
BCCH	TnA	Apical	Sholl Intersections	Treatment	0.241
BCCH	TnA	Apical	Sholl Intersections	Sex	0.550
BCCH	TnA	Apical	Sholl Intersections	Treatment*Sex	0.362
BCCH	TnA	Apical	Sholl Intersections	RADIUS	0.000
BCCH	TnA	Apical	Sholl Intersections	RADIUS*Treatment	0.911

BCCH	TnA	Apical	Sholl Intersections	RADIUS*Sex	0.781
BCCH	TnA	Apical	Sholl Intersections	RADIUS*Treatment*Sex	0.930
BCCH	TnA	Basal	Sholl Intersections	Treatment	0.075
BCCH	TnA	Basal	Sholl Intersections	Sex	0.709
BCCH	TnA	Basal	Sholl Intersections	Treatment*Sex	0.220
BCCH	TnA	Basal	Sholl Intersections	RADIUS	0.000
BCCH	TnA	Basal	Sholl Intersections	RADIUS*Treatment	0.643
BCCH	TnA	Basal	Sholl Intersections	RADIUS*Sex	0.780
BCCH	TnA	Basal	Sholl Intersections	RADIUS*Treatment*Sex	0.966
BCCH	TnA	Apical	Sholl Length	Treatment	0.277
BCCH	TnA	Apical	Sholl Length	Sex	0.401
BCCH	TnA	Apical	Sholl Length	Treatment*Sex	0.306
BCCH	TnA	Apical	Sholl Length	RADIUS	0.000
BCCH	TnA	Apical	Sholl Length	RADIUS*Treatment	0.882
BCCH	TnA	Apical	Sholl Length	RADIUS*Sex	0.929
BCCH	TnA	Apical	Sholl Length	RADIUS*Treatment*Sex	0.937
BCCH	TnA	Basal	Sholl Length	Treatment	0.109
BCCH	TnA	Basal	Sholl Length	Sex	0.920
BCCH	TnA	Basal	Sholl Length	Treatment*Sex	0.328
BCCH	TnA	Basal	Sholl Length	RADIUS	0.000
BCCH	TnA	Basal	Sholl Length	RADIUS*Treatment	0.352
BCCH	TnA	Basal	Sholl Length	RADIUS*Sex	0.960
BCCH	TnA	Basal	Sholl Length	RADIUS*Treatment*Sex	0.968
BCCH	TnA	Apical	Sholl Nodes	Treatment	0.589
BCCH	TnA	Apical	Sholl Nodes	Sex	0.652
BCCH	TnA	Apical	Sholl Nodes	Treatment*Sex	0.769
BCCH	TnA	Apical	Sholl Nodes	RADIUS	0.000
BCCH	TnA	Apical	Sholl Nodes	RADIUS*Treatment	0.999
BCCH	TnA	Apical	Sholl Nodes	RADIUS*Sex	0.320
BCCH	TnA	Apical	Sholl Nodes	RADIUS*Treatment*Sex	0.791
BCCH	TnA	Basal	Sholl Nodes	Treatment	0.111
BCCH	TnA	Basal	Sholl Nodes	Sex	0.360

BCCH	TnA	Basal	Sholl Nodes	Treatment*Sex	0.758
BCCH	TnA	Basal	Sholl Nodes	RADIUS	0.000
BCCH	TnA	Basal	Sholl Nodes	RADIUS*Treatment	0.161
BCCH	TnA	Basal	Sholl Nodes	RADIUS*Sex	0.319
BCCH	TnA	Basal	Sholl Nodes	RADIUS*Treatment*Sex	0.286
BCCH	TnA	Apical	Sholl Ends	Treatment	0.823
BCCH	TnA	Apical	Sholl Ends	Sex	0.711
BCCH	TnA	Apical	Sholl Ends	Treatment*Sex	0.828
BCCH	TnA	Apical	Sholl Ends	RADIUS	0.000
BCCH	TnA	Apical	Sholl Ends	RADIUS*Treatment	0.818
BCCH	TnA	Apical	Sholl Ends	RADIUS*Sex	0.949
BCCH	TnA	Apical	Sholl Ends	RADIUS*Treatment*Sex	0.720
BCCH	TnA	Basal	Sholl Ends	Treatment	0.101
BCCH	TnA	Basal	Sholl Ends	Sex	0.658
BCCH	TnA	Basal	Sholl Ends	Treatment*Sex	0.218
BCCH	TnA	Basal	Sholl Ends	RADIUS	0.000
BCCH	TnA	Basal	Sholl Ends	RADIUS*Treatment	0.978
BCCH	TnA	Basal	Sholl Ends	RADIUS*Sex	0.937
BCCH	TnA	Basal	Sholl Ends	RADIUS*Treatment*Sex	0.850
BCCH	TnA	Total	Sholl Intersections	Treatment	0.089
BCCH	TnA	Total	Sholl Intersections	Sex	0.587
BCCH	TnA	Total	Sholl Intersections	Treatment*Sex	0.154
BCCH	TnA	Total	Sholl Intersections	RADIUS	0.000
BCCH	TnA	Total	Sholl Intersections	RADIUS*Treatment	0.334
BCCH	TnA	Total	Sholl Intersections	RADIUS*Sex	0.891
BCCH	TnA	Total	Sholl Intersections	RADIUS*Treatment*Sex	0.771
BCCH	TnA	Total	Sholl Length	Treatment	0.033
BCCH	TnA	Total	Sholl Length	Sex	0.934
BCCH	TnA	Total	Sholl Length	Treatment*Sex	0.108
BCCH	TnA	Total	Sholl Length	RADIUS	0.000
BCCH	TnA	Total	Sholl Length	RADIUS*Treatment	0.002
BCCH	TnA	Total	Sholl Length	RADIUS*Sex	0.999
BCCH	TnA	Total	Sholl Length	RADIUS*Treatment*Sex	0.586
BCCH	TnA	Total	Sholl Ends	Treatment	0.253
BCCH	TnA	Total	Sholl Ends	Sex	0.748

BCCH	TnA	Total	Sholl Ends	Treatment*Sex	0.439
BCCH	TnA	Total	Sholl Ends	RADIUS	0.000
BCCH	TnA	Total	Sholl Ends	RADIUS*Treatment	0.980
BCCH	TnA	Total	Sholl Ends	RADIUS*Sex	0.803
BCCH	TnA	Total	Sholl Ends	RADIUS*Treatment*Sex	0.890
BCCH	TnA	Total	Sholl Nodes	Treatment	0.210
BCCH	TnA	Total	Sholl Nodes	Sex	0.691
BCCH	TnA	Total	Sholl Nodes	Treatment*Sex	0.733
BCCH	TnA	Total	Sholl Nodes	RADIUS	0.000
BCCH	TnA	Total	Sholl Nodes	RADIUS*Treatment	0.514
BCCH	TnA	Total	Sholl Nodes	RADIUS*Sex	0.210
BCCH	TnA	Total	Sholl Nodes	RADIUS*Treatment*Sex	0.326

Appendix K: : Statistical results for brown-headed cowbird (BHCO) dendritic morphology measures.

Species	Brain Region	Dendrite	Variable	Measure	p
BHCO	Hp	Total	Quantity	Treatment	0.953
BHCO	Hp	Total	Quantity	Sex	0.907
BHCO	Hp	Total	Quantity	Treatment*Sex	0.953
BHCO	Hp	Total	Length	Treatment	0.979
BHCO	Hp	Total	Length	Sex	0.253
BHCO	Hp	Total	Length	Treatment*Sex	0.044
BHCO	Hp	Total	Ends	Treatment	0.961
BHCO	Hp	Total	Ends	Sex	0.643
BHCO	Hp	Total	Ends	Treatment*Sex	0.016
BHCO	Hp	Total	Nodes	Treatment	0.933
BHCO	Hp	Total	Nodes	Sex	0.556
BHCO	Hp	Total	Nodes	Treatment*Sex	0.026
BHCO	TnA	Total	Quantity	Treatment	0.294
BHCO	TnA	Total	Quantity	Sex	0.082
BHCO	TnA	Total	Quantity	Treatment*Sex	0.459
BHCO	TnA	Total	Length	Treatment	0.564
BHCO	TnA	Total	Length	Sex	0.953
BHCO	TnA	Total	Length	Treatment*Sex	0.057
BHCO	TnA	Total	Ends	Treatment	0.800
BHCO	TnA	Total	Ends	Sex	0.733
BHCO	TnA	Total	Ends	Treatment*Sex	0.177
BHCO	TnA	Total	Nodes	Treatment	0.974
BHCO	TnA	Total	Nodes	Sex	0.840
BHCO	TnA	Total	Nodes	Treatment*Sex	0.138
BHCO	Hp	Apical	Longest Dendrite	Treatment	0.692
BHCO	Hp	Apical	Longest Dendrite	Sex	0.097
BHCO	Hp	Apical	Longest Dendrite	Treatment*Sex	0.149
BHCO	Hp	Basal	Longest Dendrite	Treatment	0.427
BHCO	Hp	Basal	Longest Dendrite	Sex	0.985
BHCO	Hp	Basal	Longest Dendrite	Treatment*Sex	0.017
BHCO	Hp	Apical	Nodes	Treatment	0.684

BHCO	Hp	Apical	Nodes	Sex	0.375
BHCO	Hp	Apical	Nodes	Treatment*Sex	0.109
BHCO	Hp	Basal	Nodes	Treatment	0.647
BHCO	Hp	Basal	Nodes	Sex	0.862
BHCO	Hp	Basal	Nodes	Treatment*Sex	0.027
BHCO	Hp	Apical	Ends	Treatment	0.572
BHCO	Hp	Apical	Ends	Sex	0.498
BHCO	Hp	Apical	Ends	Treatment*Sex	0.077
BHCO	Hp	Basal	Ends	Treatment	0.659
BHCO	Hp	Basal	Ends	Sex	0.917
BHCO	Hp	Basal	Ends	Treatment*Sex	0.048
BHCO	Hp	Apical	Total Length	Treatment	0.811
BHCO	Hp	Apical	Total Length	Sex	0.320
BHCO	Hp	Apical	Total Length	Treatment*Sex	0.194
BHCO	Hp	Basal	Total Length	Treatment	0.827
BHCO	Hp	Basal	Total Length	Sex	0.359
BHCO	Hp	Basal	Total Length	Treatment*Sex	0.046
BHCO	Hp	Apical	Mean Length	Treatment	0.811
BHCO	Hp	Apical	Mean Length	Sex	0.320
BHCO	Hp	Apical	Mean Length	Treatment*Sex	0.194
BHCO	Hp	Basal	Mean Length	Treatment	0.773
BHCO	Hp	Basal	Mean Length	Sex	0.528
BHCO	Hp	Basal	Mean Length	Treatment*Sex	0.167
BHCO	TnA	Apical	Longest Dendrite	Treatment	0.692
BHCO	TnA	Apical	Longest Dendrite	Sex	0.097
BHCO	TnA	Apical	Longest Dendrite	Treatment*Sex	0.149
BHCO	TnA	Basal	Longest Dendrite	Treatment	0.427
BHCO	TnA	Basal	Longest Dendrite	Sex	0.985
BHCO	TnA	Basal	Longest Dendrite	Treatment*Sex	0.017
BHCO	TnA	Apical	Nodes	Treatment	0.684
BHCO	TnA	Apical	Nodes	Sex	0.375
BHCO	TnA	Apical	Nodes	Treatment*Sex	0.109
BHCO	TnA	Basal	Nodes	Treatment	0.647
BHCO	TnA	Basal	Nodes	Sex	0.862
BHCO	TnA	Basal	Nodes	Treatment*Sex	0.027
BHCO	TnA	Apical	Ends	Treatment	0.572

BHCO	TnA	Apical	Ends	Sex	0.498
BHCO	TnA	Apical	Ends	Treatment*Sex	0.077
BHCO	TnA	Basal	Ends	Treatment	0.659
BHCO	TnA	Basal	Ends	Sex	0.917
BHCO	TnA	Basal	Ends	Treatment*Sex	0.048
BHCO	TnA	Apical	Total Length	Treatment	0.811
BHCO	TnA	Apical	Total Length	Sex	0.320
BHCO	TnA	Apical	Total Length	Treatment*Sex	0.194
BHCO	TnA	Basal	Total Length	Treatment	0.827
BHCO	TnA	Basal	Total Length	Sex	0.359
BHCO	TnA	Basal	Total Length	Treatment*Sex	0.046
BHCO	TnA	Apical	Mean Length	Treatment	0.811
BHCO	TnA	Apical	Mean Length	Sex	0.320
BHCO	TnA	Apical	Mean Length	Treatment*Sex	0.194
BHCO	TnA	Basal	Mean Length	Treatment	0.773
BHCO	TnA	Basal	Mean Length	Sex	0.528
BHCO	TnA	Basal	Mean Length	Treatment*Sex	0.167
BHCO	Hp	Total	Sholl Intersections	Treatment	0.641
BHCO	Hp	Total	Sholl Intersections	Sex	0.284
BHCO	Hp	Total	Sholl Intersections	Treatment*Sex	0.018
BHCO	Hp	Total	Sholl Intersections	RADIUS	0.000
BHCO	Hp	Total	Sholl Intersections	RADIUS*Treatment	0.717
BHCO	Hp	Total	Sholl Intersections	RADIUS*Sex	0.191
BHCO	Hp	Total	Sholl Intersections	RADIUS*Treatment*Sex	0.000
BHCO	Hp	Total	Sholl Length	Treatment	0.784
BHCO	Hp	Total	Sholl Length	Sex	0.251
BHCO	Hp	Total	Sholl Length	Treatment*Sex	0.020
BHCO	Hp	Total	Sholl Length	RADIUS	0.000
BHCO	Hp	Total	Sholl Length	RADIUS*Treatment	1.000
BHCO	Hp	Total	Sholl Length	RADIUS*Sex	0.070
BHCO	Hp	Total	Sholl Length	RADIUS*Treatment*Sex	0.000
BHCO	Hp	Total	Sholl Nodes	Treatment	0.239
BHCO	Hp	Total	Sholl Nodes	Sex	0.438
BHCO	Hp	Total	Sholl Nodes	Treatment*Sex	0.128
BHCO	Hp	Total	Sholl Nodes	RADIUS	0.000

BHCO	Hp	Total	Sholl Nodes	RADIUS*Treatment	0.018
BHCO	Hp	Total	Sholl Nodes	RADIUS*Sex	0.364
BHCO	Hp	Total	Sholl Nodes	RADIUS*Treatment*Sex	0.319
BHCO	Hp	Total	Ends	Treatment	0.051
BHCO	Hp	Total	Ends	Sex	0.038
BHCO	Hp	Total	Ends	Treatment*Sex	0.000
BHCO	Hp	Total	Ends	RADIUS	0.000
BHCO	Hp	Total	Ends	RADIUS*Treatment	0.495
BHCO	Hp	Total	Ends	RADIUS*Sex	0.018
BHCO	Hp	Total	Ends	RADIUS*Treatment*Sex	0.477
BHCO	TnA	Total	Sholl Intersections	Treatment	0.556
BHCO	TnA	Total	Sholl Intersections	Sex	0.968
BHCO	TnA	Total	Sholl Intersections	Treatment*Sex	0.087
BHCO	TnA	Total	Sholl Intersections	Treatment	0.000
BHCO	TnA	Total	Sholl Intersections	Sex	0.885
BHCO	TnA	Total	Sholl Intersections	Treatment*Sex	1.000
BHCO	TnA	Total	Sholl Intersections	RADIUS	0.017
BHCO	TnA	Total	Sholl Length	RADIUS*Treatment	0.358
BHCO	TnA	Total	Sholl Length	RADIUS*Sex	0.959
BHCO	TnA	Total	Sholl Length	RADIUS*Treatment*Sex	0.072
BHCO	TnA	Total	Sholl Length	Treatment	0.000
BHCO	TnA	Total	Sholl Length	Sex	0.253
BHCO	TnA	Total	Sholl Length	Treatment*Sex	1.000
BHCO	TnA	Total	Sholl Length	RADIUS	0.000
BHCO	TnA	Total	Sholl Nodes	Treatment	0.969
BHCO	TnA	Total	Sholl Nodes	Sex	0.783
BHCO	TnA	Total	Sholl Nodes	Treatment*Sex	0.634
BHCO	TnA	Total	Sholl Nodes	RADIUS	0.000
BHCO	TnA	Total	Sholl Nodes	RADIUS*Treatment	0.998
BHCO	TnA	Total	Sholl Nodes	RADIUS*Sex	1.000
BHCO	TnA	Total	Sholl Nodes	RADIUS*Treatment*Sex	0.999
BHCO	TnA	Total	Sholl Ends	Treatment	0.908
BHCO	TnA	Total	Sholl Ends	Sex	0.718
BHCO	TnA	Total	Sholl Ends	Treatment*Sex	0.471
BHCO	TnA	Total	Sholl Ends	RADIUS	0.000

BHCO	TnA	Total	Sholl Ends	RADIUS*Treatment	0.252
BHCO	TnA	Total	Sholl Ends	RADIUS*Sex	0.980
BHCO	TnA	Total	Sholl Ends	RADIUS*Treatment*Sex	0.092
BHCO	Hp	Apical	Sholl Intersections	Treatment	0.674
BHCO	Hp	Apical	Sholl Intersections	Sex	0.310
BHCO	Hp	Apical	Sholl Intersections	Treatment*Sex	0.101
BHCO	Hp	Apical	Sholl Intersections	RADIUS	0.000
BHCO	Hp	Apical	Sholl Intersections	RADIUS*Treatment	0.781
BHCO	Hp	Apical	Sholl Intersections	RADIUS*Sex	0.344
BHCO	Hp	Apical	Sholl Intersections	RADIUS*Treatment*Sex	0.010
BHCO	Hp	Basal	Sholl Intersections	Treatment	0.950
BHCO	Hp	Basal	Sholl Intersections	Sex	0.332
BHCO	Hp	Basal	Sholl Intersections	Treatment*Sex	0.030
BHCO	Hp	Basal	Sholl Intersections	RADIUS	0.000
BHCO	Hp	Basal	Sholl Intersections	RADIUS*Treatment	0.997
BHCO	Hp	Basal	Sholl Intersections	RADIUS*Sex	0.257
BHCO	Hp	Basal	Sholl Intersections	RADIUS*Treatment*Sex	0.002
BHCO	Hp	Apical	Sholl Length	Treatment	0.646
BHCO	Hp	Apical	Sholl Length	Sex	0.257
BHCO	Hp	Apical	Sholl Length	Treatment*Sex	0.090
BHCO	Hp	Apical	Sholl Length	RADIUS	0.000
BHCO	Hp	Apical	Sholl Length	RADIUS*Treatment	0.773
BHCO	Hp	Apical	Sholl Length	RADIUS*Sex	0.211
BHCO	Hp	Apical	Sholl Length	RADIUS*Treatment*Sex	0.002
BHCO	Hp	Basal	Sholl Length	Treatment	0.906
BHCO	Hp	Basal	Sholl Length	Sex	0.325
BHCO	Hp	Basal	Sholl Length	Treatment*Sex	0.027
BHCO	Hp	Basal	Sholl Length	RADIUS	0.000
BHCO	Hp	Basal	Sholl Length	RADIUS*Treatment	1.000
BHCO	Hp	Basal	Sholl Length	RADIUS*Sex	0.315

BHCO	Hp	Basal	Sholl Length	RADIUS*Treatment*Sex	0.000
BHCO	Hp	Apical	Sholl Nodes	Treatment	0.503
BHCO	Hp	Apical	Sholl Nodes	Sex	0.574
BHCO	Hp	Apical	Sholl Nodes	Treatment*Sex	0.112
BHCO	Hp	Apical	Sholl Nodes	RADIUS	0.000
BHCO	Hp	Apical	Sholl Nodes	RADIUS*Treatment	0.369
BHCO	Hp	Apical	Sholl Nodes	RADIUS*Sex	0.957
BHCO	Hp	Apical	Sholl Nodes	RADIUS*Treatment*Sex	0.152
BHCO	Hp	Basal	Sholl Nodes	Treatment	0.888
BHCO	Hp	Basal	Sholl Nodes	Sex	0.693
BHCO	Hp	Basal	Sholl Nodes	Treatment*Sex	0.036
BHCO	Hp	Basal	Sholl Nodes	RADIUS	0.000
BHCO	Hp	Basal	Sholl Nodes	RADIUS*Treatment	0.812
BHCO	Hp	Basal	Sholl Nodes	RADIUS*Sex	0.119
BHCO	Hp	Basal	Sholl Nodes	RADIUS*Treatment*Sex	0.166
BHCO	Hp	Apical	Sholl Ends	Treatment	0.314
BHCO	Hp	Apical	Sholl Ends	Sex	0.972
BHCO	Hp	Apical	Sholl Ends	Treatment*Sex	0.025
BHCO	Hp	Apical	Sholl Ends	RADIUS	0.003
BHCO	Hp	Apical	Sholl Ends	RADIUS*Treatment	0.568
BHCO	Hp	Apical	Sholl Ends	RADIUS*Sex	0.228
BHCO	Hp	Apical	Sholl Ends	RADIUS*Treatment*Sex	0.201
BHCO	Hp	Basal	Sholl Ends	Treatment	0.776
BHCO	Hp	Basal	Sholl Ends	Sex	0.660
BHCO	Hp	Basal	Sholl Ends	Treatment*Sex	0.005
BHCO	Hp	Basal	Sholl Ends	RADIUS	0.000
BHCO	Hp	Basal	Sholl Ends	RADIUS*Treatment	0.790
BHCO	Hp	Basal	Sholl Ends	RADIUS*Sex	0.197
BHCO	Hp	Basal	Sholl Ends	RADIUS*Treatment*Sex	0.655
BHCO	TnA	Apical	Sholl Intersections	Treatment	0.868
BHCO	TnA	Apical	Sholl Intersections	Sex	0.984
BHCO	TnA	Apical	Sholl Intersections	Treatment*Sex	0.081
BHCO	TnA	Apical	Sholl Intersections	RADIUS	0.000
BHCO	TnA	Apical	Sholl Intersections	RADIUS*Treatment	0.884
BHCO	TnA	Apical	Sholl Intersections	RADIUS*Sex	0.942
BHCO	TnA	Apical	Sholl Intersections	RADIUS*Treatment*Sex	0.218

BHCO	TnA	Basal	Sholl Intersections	Treatment	0.553
BHCO	TnA	Basal	Sholl Intersections	Sex	0.904
BHCO	TnA	Basal	Sholl Intersections	Treatment*Sex	0.081
BHCO	TnA	Basal	Sholl Intersections	RADIUS	0.000
BHCO	TnA	Basal	Sholl Intersections	RADIUS*Treatment	0.761
BHCO	TnA	Basal	Sholl Intersections	RADIUS*Sex	0.977
BHCO	TnA	Basal	Sholl Intersections	RADIUS*Treatment*Sex	0.028
BHCO	TnA	Apical	Sholl Length	Treatment	0.625
BHCO	TnA	Apical	Sholl Length	Sex	0.877
BHCO	TnA	Apical	Sholl Length	Treatment*Sex	0.069
BHCO	TnA	Apical	Sholl Length	RADIUS	0.000
BHCO	TnA	Apical	Sholl Length	RADIUS*Treatment	0.398
BHCO	TnA	Apical	Sholl Length	RADIUS*Sex	0.752
BHCO	TnA	Apical	Sholl Length	RADIUS*Treatment*Sex	0.164
BHCO	TnA	Basal	Sholl Length	Treatment	0.571
BHCO	TnA	Basal	Sholl Length	Sex	0.861
BHCO	TnA	Basal	Sholl Length	Treatment*Sex	0.059
BHCO	TnA	Basal	Sholl Length	RADIUS	0.000
BHCO	TnA	Basal	Sholl Length	RADIUS*Treatment	0.907
BHCO	TnA	Basal	Sholl Length	RADIUS*Sex	0.998
BHCO	TnA	Basal	Sholl Length	RADIUS*Treatment*Sex	0.032
BHCO	TnA	Apical	Sholl Nodes	Treatment	0.858
BHCO	TnA	Apical	Sholl Nodes	Sex	0.480
BHCO	TnA	Apical	Sholl Nodes	Treatment*Sex	0.098
BHCO	TnA	Apical	Sholl Nodes	RADIUS	0.000
BHCO	TnA	Apical	Sholl Nodes	RADIUS*Treatment	0.418
BHCO	TnA	Apical	Sholl Nodes	RADIUS*Sex	0.220
BHCO	TnA	Apical	Sholl Nodes	RADIUS*Treatment*Sex	0.533
BHCO	TnA	Basal	Sholl Nodes	Treatment	0.947
BHCO	TnA	Basal	Sholl Nodes	Sex	0.648
BHCO	TnA	Basal	Sholl Nodes	Treatment*Sex	0.321
BHCO	TnA	Basal	Sholl Nodes	RADIUS	0.000
BHCO	TnA	Basal	Sholl Nodes	RADIUS*Treatment	0.812
BHCO	TnA	Basal	Sholl Nodes	RADIUS*Sex	0.852

BHCO	TnA	Basal	Sholl Nodes	RADIUS*Treatment*Sex	0.576
BHCO	TnA	Apical	Sholl Ends	Treatment	0.973
BHCO	TnA	Apical	Sholl Ends	Sex	0.414
BHCO	TnA	Apical	Sholl Ends	Treatment*Sex	0.127
BHCO	TnA	Apical	Sholl Ends	RADIUS	0.000
BHCO	TnA	Apical	Sholl Ends	RADIUS*Treatment	0.568
BHCO	TnA	Apical	Sholl Ends	RADIUS*Sex	0.941
BHCO	TnA	Apical	Sholl Ends	RADIUS*Treatment*Sex	0.212
BHCO	TnA	Basal	Sholl Ends	Treatment	0.719
BHCO	TnA	Basal	Sholl Ends	Sex	0.988
BHCO	TnA	Basal	Sholl Ends	Treatment*Sex	0.211
BHCO	TnA	Basal	Sholl Ends	RADIUS	0.000
BHCO	TnA	Basal	Sholl Ends	RADIUS*Treatment	0.485
BHCO	TnA	Basal	Sholl Ends	RADIUS*Sex	0.849
BHCO	TnA	Basal	Sholl Ends	RADIUS*Treatment*Sex	0.285
BHCO	Hp	Apical	Spines	SPINES	0.201489235
BHCO	Hp	Apical	Spines	SPINES*Treatment	0.851833705
BHCO	Hp	Apical	Spines	SPINES*Sex	0.550766839
BHCO	Hp	Apical	Spines	SPINES*Treatment*Sex	0.924830269
BHCO	Hp	Basal	Spines	SPINES	0.967618317
BHCO	Hp	Basal	Spines	SPINES*Treatment	0.318365169
BHCO	Hp	Basal	Spines	SPINES*Sex	0.240736736
BHCO	Hp	Basal	Spines	SPINES*Treatment*Sex	0.401213096
BHCO	TnA	Apical	Spines	SPINES	0.752407054
BHCO	TnA	Apical	Spines	SPINES*Treatment	0.661132782
BHCO	TnA	Apical	Spines	SPINES*Sex	0.661132782
BHCO	TnA	Apical	Spines	SPINES*Treatment*Sex	0.356721654
BHCO	TnA	Basal	Spines	SPINES	0.468212458
BHCO	TnA	Basal	Spines	SPINES*Treatment	0.59473228
BHCO	TnA	Basal	Spines	SPINES*Sex	0.767058824
BHCO	TnA	Basal	Spines	SPINES*Treatment*Sex	0.805977921

Appendix L: Ethics approval for animal use.

AUP Number: 2010-024

PI Name: Zanette, Liana

AUP Title: The Effects of Predators and Predator Risk On Prey: From Genes To Ecosystems

Curriculum Vitae

Lauren Elizabeth Witterick
Department of Biology
University of Western Ontario
London, ON N6A 5B7

Education

M.Sc. (Biology) 2017
University of Western Ontario, London, ON

B.Sc. (Honours) (Biology) 2014
Queen's University, Kingston, ON

Research Experience

Graduate Student and Research Assistant 2015-2017
University of Western Ontario
Department of Biology, Liana Zanette, PhD

Fourth Year Honours Thesis 2013-2014
Queen's University
Department of Biology, Shelley Arnott, PhD

Teaching and Supervisory Experience

Volunteer Supervisor 2016
Field assistance and animal care

Graduate Teaching Assistant
Department of Biology, University of Western Ontario, London, ON

BIO 2485B: Environmental Biology	2017
BIO 3442F: Conservation Biology	2016
BIO 3440A: Ecology of Populations	2016
BIO 2485B: Environmental Biology	2016
BIO 3442F: Conservation Biology	2015
BIO 3440A: Ecology of Populations	2015

Awards and Scholarships

Western Graduate Research Scholarship	2015-2017
Mary C. Holdsworth Scholarship	2015-2016
Queen's University Dean's Honour List	2014
Mary C. Holdsworth Scholarship	2010-2013
Queen's University Dean's Honour List	2011
Queen's University Excellence Scholarship	2010

Conferences and Presentations

June 2017. *Fear of predators has long-lasting effects on the brain and behaviour in wild animals.* Animal Behaviour Society Annual Meeting, Toronto, ON.

May 2017. *Fear of predators has long-lasting effects on the brain and behaviour in wild animals.* Ontario Ecology, Ethology, and Evolution Colloquium, Kingston, ON.

March 2017. *Assessing the long term effects of perceived predation risk on the avian brain.* Friday Philosophicals Graduate Seminar Series (Exit Seminar), London, ON.

October 2016. *Assessing the long term effects of perceived predation risk on the avian brain.* Biology Graduate Research Forum, London, ON.

April 2016. *Assessing the morphological and physiological effects of perceived predation risk on the avian brain.* Friday Philosophicals Graduate Seminar Series (Entrance Seminar), London, ON.

March 2014. *Zebra Mussels in the Great Lakes.* Biodiversity Day, Kingston, ON.

October 2013. *Assessing seasonal and inter-annual change in zooplankton community composition.* Undergraduate Thesis Seminar, Kingston, ON.

Professional Memberships

Animal Behaviour Society