Birdie see, Birdie do: Zebra finch observational learning in three cognitive domains with discussion of cerebellar involvement

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ABSTRACT

Observational learning is a cognitive ability that allows individuals to acquire information or skills through watching others. Examples of observational learning can be seen in all major vertebrate groups and some invertebrates. Observational learning may confer a selective advantage to animals due to improvements in decision-making and increased behavioral flexibility. While studies of animals’ observational learning capabilities and the types of information acquired have been examined in many species, multiple types of observational learning have rarely been examined in non-rodent species in controlled laboratory experiments. Additionally, only recently have the neural mechanisms that support observational learning been examined. I sought to expand our understanding of avian observational learning and explore the role of the cerebellum in information acquisition. Using zebra finches I tested three types of observational learning (stimulus enhancement, observational conditioning, and imitation). I found that female zebra finches selected males based on observations of the traits of the females paired with the males but not based on observation of simply whether the male was associated with a female. Zebra finches were found to be capable of learning about the threat value of a stimulus by witnessing conspecifics undergoing tone-shock fear conditioning. However, I found no evidence that spatial information could be acquired via observation. I worked toward the goal of determining a role of the avian cerebellum in fear conditioning. Lesions of the lateral cerebellar nuclei did not interfere with fear conditioning. As humans and rodents are capable of all of these types of observational learning and have cerebellar involvement in fear conditioning, these findings illustrate a lack of conservation in observational learning and the role of the
cerebellum in specific tasks across vertebrate classes. The ecological relevance of the type of
information required for survival and reproduction has likely driven the evolution of
observational learning in vertebrates as zebra finch ecology makes it unlikely that acquiring
spatial information from conspecifics would affect fitness. Conservation of cerebellar
contributions to fear conditioning may be conserved but the specific circuits involved may differ.
DEDICATION

This dissertation is dedicated to my family, who for years would ask me when I was going to stop being a professional student and get a real job. Well, at least I’m accomplishing one of those things.
**LIST OF ABBREVIATIONS AND SYMBOLS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>CB</td>
<td>Cerebellum or cerebellar</td>
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<tr>
<td>CBl</td>
<td>Lateral cerebellar nuclei</td>
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<tr>
<td>CF</td>
<td>Wild type coloration female zebra finch</td>
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<td>CR</td>
<td>Conditioned response</td>
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<tr>
<td>CS</td>
<td>Conditioned stimulus</td>
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<td>DEM</td>
<td>Day escape maze</td>
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<tr>
<td>dlPFC</td>
<td>Dorsolateral prefrontal cortex</td>
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<td>EPC</td>
<td>Extra-pair copulations</td>
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<td>Exp</td>
<td>Experiment</td>
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<td>FC</td>
<td>Fear conditioning</td>
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<td>LTD</td>
<td>Long-term depression</td>
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<td>MCC</td>
<td>Mate choice copying</td>
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<td>MNS</td>
<td>Mirror neuron system</td>
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<td>Abbreviation</td>
<td>Description</td>
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<td>--------------------------------------------------</td>
</tr>
<tr>
<td>MQB</td>
<td>Mate quality bias</td>
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<tr>
<td>MWM</td>
<td>Morris water maze</td>
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<tr>
<td>PM</td>
<td>Preferred male</td>
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<tr>
<td>PVC</td>
<td>Polyvinyl chloride</td>
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<tr>
<td>rTMS</td>
<td>Repetitive transcranial magnetic stimulation</td>
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<tr>
<td>SE</td>
<td>Standard error</td>
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<tr>
<td>UR</td>
<td>Unconditioned response</td>
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<tr>
<td>US</td>
<td>Unconditioned stimulus</td>
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<tr>
<td>WF</td>
<td>White morph female zebra finch</td>
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ACKNOWLEDGMENTS

It takes an enormous amount of time and energy to write a PhD dissertation. Whether the writing is spread over five years, five months or five weeks makes little difference in the amount of blood, sweat, tears and shingles that goes into its completion. I know these acknowledgments will be little read, which is good because it provides me the freedom to thank all of the people who have assisted me throughout my degree in the least direct way I possibly can.

First, I would like to thank Dr. Lainy Day. Without her deciding to take a chance on a visiting prospective student who had taken several years away from science and pretty much had just played with parrots for her Masters, I would never have gotten to this point. At the conclusion of this dissertation, you get to decide whether your decision proved to be a stroke of genius or, to use the vernacular of our times, an EPIC FAIL (please let it be the former). Throughout my tenure here, she taught me several significant lessons, the most important of which is never give up on yourself or your science. She has improved me not just as a scientist but an educator as well. Thank you for being my biggest academic supporter and not letting my biggest detractor (myself) get in the way too much.

I also want to express my sincere thanks to Dr. Richard Buchholz, or as he’s known in more casual conversations, Dr. B. Dr. B played many roles throughout my academic stay here from his first as my interim committee chair, as an armchair therapist and homesickness cure when I missed the North and thought I had made a mistake moving here, and a sounding board for my experimental design ideas. You have been so kind and generous to me from the
beginning, tolerating my inability to spell your name correctly several times (although I swear I copied and pasted it from the website), listening to my endless diatribes, and in general weathering the numerous other assaults to your sanity that I have delivered over the past five years with a patience and understanding that only you are capable of possessing. Thank you for all the technical, physical and intellectual contributions you have made to this work.

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To the Day lab, with all its members past and present, I have benefitted from all your advice and assistance more than you will ever know. I want to particularly thank Willow Lindsay, who provided me unconditional love, advice and support throughout all the issues with my experiments and who partied hard with me when all else failed; Jack Pemment, who introduced me to the fine art of coffee making, the therapeutic benefits of cake decorating, and was always there to listen to my insanity (and then assure me I’m not a psychopath since he’s an expert on the subject); and finally to my extraordinary minions, James Roberts and Natey Webb, both of whom were always there to assist on any task or experiment (no matter how senseless my ideas were) without complaint (well, almost without any complaints). James and Natey, you both
are amazing and will go on to do great things in the future. I will forever be proud to say “I knew them when…”

Outside of the Day Lab but still within the wonderful Shoemaker universe, I want to thank the most wonderful Senior Lab Technician, Lance Sullivan. Since just about day one, you have assisted me with so many different crazy tasks, from spray painting little metal cages to construction of Rube Goldberg-type apparatuses to just being there to hand me Kleenex and give me some of the best hugs of my life. I want you to know that you are and will forever be the most useful man I have ever been fortunate enough to meet.

I would like to be serious for at least one paragraph, and thank those who have been a constant source of love, support, and inspiration for me. To my friends who are several hundred or now even several thousand miles away: Jason Bruck and Kara Fitzgerald. Despite having to tolerate me for many, many years, you two have still always provided me with so much friendship and love. I know having friends like you makes me truly blessed. And, to my friends I acquired here in Oxford, Tim Colston and the rest of the Colston clan (Katya, Maddison, and Harper Reid), Ehlana Stell, Matt Gaylord, Bryan Cage, Edward Hanlon and Michael Clear (who, while only being a recent addition to the list, has become very important…perhaps too much so…in a short period of time). All of you have assisted me in so many ways and have done so much for me that putting it to mere words seems utterly insufficient. Let us just say that all of you have invested your time, your energy, and probably a good portion of your sanity into
helping me through this ordeal, and I know that I could not have done any of this without each and every one of you.

And with that being said, you’ve also been a constant source of distraction (and I mean endless distraction). I’m going to spread the blame for taking so long to finish, and you are all in my crosshairs. I love you all exceedingly!

All of this blame-spreading reminds me of the classic tardy award acceptance speech from the cinematic masterpiece, Clueless. Here it is with slight alterations to fit my own experiences. “I would like to say this: Tardiness is not something you can do on your own. Many, many people contributed to my tardiness. I would like to thank my dogs, Kaylie, Ivan and Carys, for being the world’s best cuddlers and most needy canines who make leaving the house nearly impossible, the City of Oxford for their near constant road construction projects and changes to traffic patterns that create massive traffic jams, and last but not least, the wonderful crew from Starbucks who spend hours making the world’s best whipped cream and raspberry mochas, without which I might never be tardy.”
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CHAPTER 1: INTRODUCTION

Observational learning is learning acquired by observing the behavior of another individual. Learning is generally accepted to have occurred when the observer’s behavior changes due to the information gained from the behavior of a demonstrator or model. Observational learning is a type of social learning and observers can use any sensory modality (e.g. olfaction, audition, written or spoken words in humans) to “observe” the demonstrator. It has been proposed that observational learning is a vital process in animals because it bridges the gap between species-specific (innate) behaviors and operant (trial and error) learning [1].

Largely innate behaviors can be advantageous as they are highly reliable and do not depend on reinforcement or a learning process. However, they lack a high degree of plasticity and may become obsolete in a changing environment [1]. Conversely, learning requires an individual to be exposed to a stimulus and experience the consequences of interacting with this stimulus in order to learn an appropriate behavioral response. This can be extremely time-consuming and may lead to disastrous consequences, for example death or sickness from eating a novel food that is toxic or becoming a victim of predation [2-7]. Observational learning can provide more flexibility than innate behaviors and allow an individual to avoid many of the negative consequences associated with learning [1].

Observational learning can still be costly (both in time and energy), and may still be error prone if the information acquired is outmoded in a changing environment [1, 8, 9]. However, natural selection should counterbalance poor models by selecting against individuals not
engaging in the most appropriate behaviors in the current environment. Thus, doing what others do should typically lead to positive outcomes [1, 10]. The advantages of acquiring relevant real-time information, avoiding cost, and increasing flexibility may explain the prevalence of observational learning in animal species and its emergence in early development in most species [11].

Although extensive research has examined the capability for and limitations of observational learning in particular species (for reviews, see [5, 8, 12-14]), only in the past two decades has the examination of the neural mechanisms supporting this type of learning been performed and only for a limited number of species [13, 15-21]. A majority of these studies focus on the mirror neuron system (MNS) [16, 22-24]. The MNS is a group of mammalian brain regions containing neurons that respond both when an individual makes active movements and when the individual observes the same action conducted by a demonstrator, "mirroring" the behavior [22, 25]. The MNS has only been decisively and extensively demonstrated in primates; although some evidence may support an analogous MNS-type system in non-primates including a passerine bird species and the laboratory rat [26]. Early theories proposed that the MNS might underlie unique primate abilities for observational learning, particularly that allowing action imitation [16]. However, macaque monkeys, the model organism for testing the function of the MNS system, do not imitate demonstrators [27]. This implies that the MNS, at least in the macaque, is not sufficient for expression of action imitation [28].

Comparing the large number of species capable of observational learning to the limited number that possess an MNS and considering the fact that the only species conclusively shown to possess a MNS does not demonstrate observational action imitation, I
must propose that another brain region contributes to observational learning. This region could be the cerebellum (CB) or cerebellar analogs in non-vertebrates [29, 30]. CB function was first examined by Pierre Flourens in 1825. He discovered that CB ablations resulted in impaired motor coordination [31, 32]. As a result, the CB had been viewed mainly as a postural control area. More recent studies have indicated cerebellar involvement in numerous cognitive functions including language, spatial navigation, working memory, and implicit and explicit learning and memory [33-35], and, more importantly, in observational learning in rats and humans [18, 19, 21, 36]. In the observational learning studies in rats and humans, it was shown that ablation in rats or deactivation, via transcranial magnetic stimulation, in humans severely impairs the ability to acquire information from a demonstrator in spatial or procedural imitation tasks [19, 35, 37]. Furthermore, the structure and function of the CB is highly conserved [29, 38] and observational learning is widely distributed in the animal kingdom [for review, see 12]. Thus, I propose that observational learning of various types exists in Aves, that a role for the CB in observational learning of procedural tasks is conserved in Aves and mammals, and that the CB would also be involved in the ability to perform observational learning of other types of tasks.

Specifically, for my dissertation, I first tested the capabilities of a passerine bird, the zebra finch (*Taeniopygia guttata*), to perform three types of learning. I then tested the ability of observers to acquire this learned information from demonstrators. The type of observational learning in three tasks varied; requiring either stimulus enhancement, observational conditioning or mindful imitation, as will be defined shortly. Additionally, I worked toward the goal of determining a role of the CB in observational learning in those tasks that were acquired via observational learning.
The importance of my work is that I devised ways to empirically test the different types of observational learning in an avian species via tasks that make cross-species comparisons possible. By examining which types of observational learning a species is capable of and the types of information that are acquired by different species, the adaptive significance of observational learning and its evolution may be determined. Additionally, I began an investigation into brain regions that may underlie observational learning in a bird. My studies make important contributions to the study of animal cognition and have applications to the study of human behavior.

I. Principles and Types of Observational Learning

Learning in animals is commonly divided into two broad categories, direct and observational learning, which are further divided into numerous subcategories. Direct learning categories are based on the type of learned association made directly by the individual [39]. In contrast, observational learning categories are based on what type of information has been acquired from a demonstrator [40].

Observational learning theory is derived from the research and proposals made by Albert Bandura. Bandura studied the cognitive and information-processing capabilities necessary to learn through observation [41-45]. There are three core concepts in Bandura’s social learning theory: 1. people (animals) can learn via observation of another individual; 2. learning does not always result in a behavioral change; and, 3. internal mental states of the observer are an essential component of this process [42, 44, 45]. He also discovered that numerous factors influence the efficacy of observational learning and specific steps necessary for learning to occur. First, the observer must be attentive to the demonstrator. Any distractions will negatively affect the learning process. Second, the observer must retain the information acquired. Third, the
observer must be capable of performing the behavior. Finally, motivation to imitate the behavior must be present or, although the information was learned, the behavior will not be displayed.

There are six categories of observational learning that are differentiated according to the role the demonstrator plays in generating the matching behavior in the observer [46]. In local enhancement, the demonstrator’s behavior increases the probability the observers will attend to or interact with the same stimulus. Stimulus enhancement, although similar, results in the observer's interaction with any stimuli of the same physical type (e.g. color, smell, shape). In observational conditioning, the demonstrator’s behavior acts as a unconditioned stimulus (US) eliciting a matched conditioned response (CR) in the observer. The demonstrator’s behavior may also act as a discriminative stimulus as in match-dependent behavior, or as a model for a non-goal directed process (copying) or a goal-directed process (imitation).

Imitation is distinct from the other non-imitative observational learning categories. In non-imitative observational learning, the animal is only learning about the environment (e.g. what foods are palpable, how to avoid a predator, etc.). In contrast, during imitation, the animal is learning about the behavior (e.g. the underlying purpose, the exact motor patterns) by observing others [47]. Imitation is divided into three forms: kinesthetic, symbolic and mindful [48]. Kinesthetic imitation involves matching body movements and postures to those of the demonstrator. Symbolic imitation involves individuals making a mental representation of a past observed behavior for replication in the future when the demonstrator is no longer present. During mindful imitation, the individuals must recognize and encode the demonstrator’s behavior and intentions so they can reproduce the behavior and achieve the same goal as the demonstrator.
II. Observational Learning across Species

Observational learning in nonhuman animals has been investigated for over a hundred years. Observational learning has been found in animals as diverse as cephalopods, insects, fish, herptiles, birds, rodents, cetaceans, and primates. Observational learning may affect numerous biologically significant decisions made by animals and animals may gain a survival advantage from learning information from a demonstrator [13]. Since solitary species have minimal contact between individuals and thus little opportunity for observational learning to occur, one might posit selection for observational learning would not occur in asocial species [1, 47]. Yet, observational learning has been clearly demonstrated in several non-social species including the red-footed tortoise [49], common octopus [50], golden hamster [51] and several non-colonial insects [52]. This implies that observational learning is a highly conserved cognitive process and is not contingent on social group dynamics. Studies into observational learning are so prolific that for the purposes of this dissertation, I provide only a few examples illustrating this phenomenon across taxa (with a primary focus on avian species). The diversity of the types of information being learned is highlighted.

Invertebrates

The first publication of observational learning of which I am aware is Darwin’s bee studies [53], and since that time, observational learning has been examined has been studied and shown in cephalopods and arthropods. For example, when the common octopus is presented with two objects of different colors, they are more likely to attack the one they previously witnessed a conspecific attacking – a clear example of stimulus enhancement [50]. Several species of hymenoptera use the presence of a conspecific to identify feeding sites or novel food sources [54-61]. These are examples of local enhancement observational learning. Foraging preferences
can also be acquired via stimulus enhancement. If allowed to view demonstrators preferentially foraging on green “nectar reward” flowers while avoiding orange “no nectar reward” flowers, naïve common eastern bumblebee workers will exhibit a significant preference for green unlike non-observer control bees [62]. In addition to foraging behaviors, predator avoidance may also be acquired via observational learning in insects. Damselfly larvae can gain the ability to recognize and respond to predation based on conspecific and heterospecific cues [63]. While olfactory cues from a pike predator do not elicit any response, when pike cues are combined with chemical cues from injured conspecifics, the larvae reduce feeding activity and movement. If the same individuals are subsequently exposed to just the olfactory cues from the pike, they will again change their behavior and become less active [63]. This may be an example of observational conditioning whereby the UR (reduced activity to be less conspicuous) to an US (the olfactory cues from injured conspecifics) become paired with a previous neutral stimulus (the pike cues) resulting in the pike’s presence eliciting the reduced activity [46, 47, 64].

_Fishes_

Fish species use observational learning in numerous ways. Blue-head wrasse learn mating sites by observation [65], and juvenile French grunts learn resting locations and migration paths along the reefs [66]. Additionally, various species of fish learn to avoid a neutral stimulus that has been paired with an alarm substance secreted by the skin of an injured conspecific. This observationally learned and exhibited response to a previously neutral stimulus can then serve as a model to naïve individuals and induce a fear response in these individuals leading to observational conditioning [67, 68]. Mate-choice copying has been demonstrated in several fish species and has shown that females in several species, after observing a male interacting with or
mating with another female, will alter their preference for that male or males with similar characteristics (stimulus enhancement) [69-72].

**Herptiles**

Amphibians and reptile species have been largely ignored in studies of observational learning. However, a recent study in the red-footed tortoise supports observational learning in reptiles [49]. Given a detour problem where one of two fences blocked a food reward, tortoises that first watched a conspecific correctly navigate the course not only made the detour correctly but also utilized the same correct route as the demonstrator. In contrast, all non-observer tortoises failed to reach the food reward, even after numerous trials. In another example, blue spiny lizards learned food preferences from desert iguana. Both these species are principally insectivores and only the iguana will eat vegetation naturally. However, when housed with an iguana and only provided with lettuce, the blue spiny lizards not only ate the lettuce they watched their cagemates eat, but also adopted the same postures and motor patterns for consumption. Even when mealworms were provided, 75% of the blue spiny lizards continued eating the lettuce [73].

**Mammals**

Observational learning is well known in mammals [18, 19, 35, 37, 42, 44, 45, 48, 74-131]. The various types that have been shown are local enhancement [76-78, 80, 88, 90-93, 95, 98-100, 103, 104], stimulus enhancement [107, 108], matched dependent [80, 128, 130], observational conditioning [81, 82, 87, 111, 112, 121], copying [85, 114, 115] and imitation [84, 118, 123, 124, 127, 131]. One order of mammal that relies heavily on observational learning is Cetacea. In the wild, several dolphin species have been observed relaying information about how to obtain prey to naive or inexperienced dolphins. Killer whale mothers will modify their
stranding behavior (a behavior used to capture seal pups on a breeding beach) in the presence of naïve juveniles, suggesting they are providing opportunities for the juveniles to view various stranding techniques to obtain seal pups [96, 97]. Atlantic spotted dolphin mothers engage in similar behaviors [132], chasing their fish prey for longer durations and making more referential body pointing movements in the presence of juveniles. In addition to motor imitation, this group is capable of vocal learning and imitation – imitating the sounds of conspecifics as well as other species [101, 102, 106, 122, 133, 134].

Aves

Observational learning in birds has been demonstrated in at least sixteen families representing seven orders [135]. Observational learning studies conducted in the field have been mainly focused on foraging behavior – where, what and how to eat. A classic example was described by Fisher and Hinde in the late 1940s [136, 137]; blue tits in Britain were observed opening the silver tops of milk bottles to skim the cream settled on top of the milk. This behavior spread throughout Britain’s blue tit population much quicker than expected by trial and error learning. Initially researchers believed imitation of the behavior was occurring as naïve animals viewed their conspecific opening the tops; however, subsequent laboratory studies have shown that the acquisition of this behavior is based on stimulus or local enhancement [138].

For numerous avian species, observational learning is important in learning feeding site locations [139, 140], edible food items [141-144], prey hunting techniques [145, 146], food item manipulation [135, 147], and tool use [148, 149]. Learning may begin early in the bird’s development, using parents as role models, and may continue into adulthood by learning from conspecifics [150]. Most observationally learned behaviors in Aves are thought to be the result
of stimulus or local enhancement, and not true imitation (the copying of motor patterns) [12]; however, New Caledonian crows may imitate tool manufacture and use (an example of mindful imitation). Most tool use appears to be acquired via trial and error learning, but imitation of parents seems to influence the behavior as well [148, 151] leading to distinct tool designs among geographically separated populations without any obvious ecological constraint [149].

Observational learning may be involved in migration. In some species, inexperienced birds may be guided by older, more experienced conspecifics along migration routes to appropriate seasonal habitats. Using observational learning, humans have been able to successful train naïve, hand-reared birds to use certain migration routes using a microlight aircraft as a demonstrator [152]. Nest building techniques do not appear to be acquired via observation in large number of species studied [153-155]. However, some evidence of observational learning is seen in construction techniques used by male bowerbirds to build their courtship arenas, and “dialects” of building types are seen across populations [156].

Observational learning is used in recognition of predators [6, 157-159] and brood parasites [160, 161]. Alarm calls are fairly stereotyped within a species, but observational learning may be involved in teaching what response is most appropriate to a specific alarm call [6, 159, 161]. Additionally, observational learning may be responsible for the identification of new predator types. American crows were captured by researchers wearing a particular “dangerous” face mask and then housed in captivity and fed by researchers wearing different “neutral” face masks for one month before release. After release, the crows would use harsh vocalizations to scold and mob individuals wearing the "dangerous" mask. This effect not only persisted for years after release, but actually multiplied over the two year study as birds that had not experienced capture also began displaying threat responses toward “dangerous” masked
individuals [157, 158]. This suggests that observational learning or at least social facilitation may be used for the identification and recognition of a specific individual as a threat.

Observational learning also appears to be extremely important in avian mate choice, primarily via sexual imprinting early in development [162]. An abundance of evidence from captive studies, and some support from field experiments, shows that sexual imprinting as a juvenile may last the duration of the bird’s life [163-169]. The importance of sexual imprinting may vary by species [170]. After maturity, birds may use public information acquired by observing the mate choices of conspecifics to make mate choices [171]. Costs and benefits of this copying may differ between the sexes [172]. Although compelling evidence for mate choice copying exists in several species of polygynous birds, the results are more inconclusive for monogamous bird species [173].

There have been two forms of imitative learning heavily investigated in avian species: vocal learning and motor imitation. Vocal learning has been demonstrated in three avian groups: psittacines (parrots), hummingbirds, and oscine songbirds [174-178]. Vocal learning occurs in two stages: the sensory learning stage in which the bird listens and memorizes the spectral and temporal qualities of a song or sound; and, the sensorimotor learning stage in which the bird begins vocalizing and practicing the song or song until it matches the memorized template [133, 176, 179, 180]. Some species maintain the ability to acquire new songs throughout their lifetimes while others are limited to acquisition only during specific critical periods [133, 177, 178]. A few species not only mimic the sounds of their species, but can imitate the vocalizations of other birds, human speech and environmental noises [181]. While most experiments on vocal learning have been performed in the laboratory setting, evidence for song and sound imitation is found in wild populations [181]. Much like the tool usage of the New Caledonian crows, distinct song
dialects may form in specific geographical populations due to songs being passed from older tutors to juveniles [182-185].

Numerous studies on motor imitation have examined whether birds who watch demonstrators engaged in completing a task have a savings in time to learn the task compared to birds that did not watch a demonstrator. Studies conducted on several avian species, mainly concentrating on manipulating and removing obstacles to access rewards, have shown that the learning curve of observers is much faster than that of non-observers [186-189]. Motor imitation does not always require a learning period after observation. Pigeons viewing a demonstrator depressing a treadle either by foot or by beak pecking to obtain a food reward were found to use the same motor tactic when given access to the treadle without any additional training [189].

This brief survey of observational learning in the different taxa clearly demonstrates the highly conserved nature of this type of learning and the diversity of information that may be transmitted from demonstrators to observers. By extension, the brain region that would underlie observational learning should be a region, as previously mentioned, that is highly conserved across taxa and could be responsible for tying together sensory representations of self and other, currently bodily states and movements, and action plans.

III. The Cerebellum – Anatomy, Microcircuitry and Comparison across Vertebrates

One of most conserved brain areas across vertebrate taxa is the cerebellum (CB). All of the ~50,000 extant species of vertebrates, possessing hundreds of different mechanical designs and sensory systems, have a CB with similar cerebellar circuitry [38]. The CB’s conservation indicates its importance in coordinating multi-appendage motor movements, and may be indicative of its importance in aspects of cognition, especially observational learning. The CB, or
“little brain”, is located in the hindbrain. Although it is roughly 10% of total brain volume, it contains between 50-70% of the neurons in the brain [190]. Early research concluded CB damage lead to deficits in the motor coordination and posture [31, 32]. Because of these studies and the CB’s extensive outputs to motor regions, the CB was viewed as strictly a motor structure for over a century. In the late 1960s, a novel theory proposed the CB may have a role in learning motor skills, especially those important for movement and posture [29]. Since then, experimenters, using several approaches at the molecular, cellular, and behavioral levels, have tested for a role of the CB in learning and memory processes. These studies have provided evidence that the CB functions in several types of learning and plays a role in the consolidation and maintenance of different types of memories including motor learning [191], classically-conditioned eye-blink response [192-195], long-term habituation [196, 197], spatial learning [35], recognition memory [198, 199], reading [200], rhyming [201, 202], speech/language production [203], and discrimination learning [34].

Two major components comprise the CB: the cerebellar nuclei and the cerebellar cortex [204]. The cell layers of the CB connect similarly throughout the CB and the CB is relatively simple in comparison to cerebral connections [204]. Input connections may be separated into two groups: mossy fiber inputs and climbing fiber inputs. Mossy fibers project from the pontine nuclei, the reticular formation, the vestibular nuclei and the spinal cord via excitatory (glutamate) projections onto the cerebellar nuclei and the granule cells within the cerebellar cortex [204]. The granule cells project toward the cortical surface and bifurcate in the molecular layer where each collateral, called parallel fibers, moves in opposite directions running parallel to the folia and make excitatory synapses with the Purkinje cells that project perpendicular to the folia.
Thousands of parallel fibers may synapse with each Purkinje cell and have summate to activate the cell [204].

Climbing fibers arise solely from the inferior olivary nuclei of the medulla. These fibers synapse with the cerebellar nuclei and directly with the Purkinje cells causing a powerful excitement of the cell [204]. There is only one major climbing fiber input per Purkinje cell and each fiber only synapses with 1-10 Purkinje cells. It is thought that the inferior olivary nucleus plays a role in motor error detection and that when an error is detected, the powerful activation of the Purkinje cells, through the climbing fibers, inhibits the cerebellar nuclei and terminates the undesired component of the action [38].

Learning in the CB appears to result from the plasticity of the synapse between the parallel fiber and the Purkinje cell [34]. When a Purkinje cell is excited by a climbing fiber, all synapses along the Purkinje’s dendrites that were recently excited by the parallel fibers undergoes long-term depression (LTD). If the climbing fiber functions to convey an error in signal, then LTD corrects the problem by inhibiting the synapses involved in the error creation and each synapse can be adjusted during learning to shape the correct cerebellar output [34]. The deep cerebellar nuclei are the sole output structures of the CB. Therefore, the lesioning of these nuclei is somewhat equivalent to the removal of the whole CB. In mammals, the lateral “dentate” nuclei receive inputs from the lateral hemisphere and cerebellar afferents that carry information from the cortex. These nuclei project to the contralateral red nucleus and ventrolateral thalamic nucleus (which in turn continues to the cerebral cortex) [204]. This system, collectively called the cerebrocerebellum, is believed to be responsible for all CB-facilitated learning, e.g. procedural learning [205]. The cerebellar nuclei in birds appear to be homologous to those in humans and maintain analogous, if not homologous, functional subunits though their
morphology and connectivity [206] but vary somewhat with only three, instead of four, recognizable cerebellar nuclei in most birds [207].

IV. Cerebellar Involvement in Learning

While the CB plays a prominent role in postural control, the CB is now more often recognized to play a role in feed-forward control (correction of error in a sequence based on predicted outcome of current environmental and internal state) of a variety of functions including various purely cognitive functions that do not necessitate motor output such as learning to differentially respond to different stimuli (discrimination learning) [34], recognizing familiar stimuli (recognition memory) [198, 199], and long-term habituation [196, 197]. For the purpose of this dissertation, I will review the involvement of the CB only in tasks similar to those I conducted (additional reviews are located within Chapters 3 and 4).

A number of studies in fish [208], birds [209-211], rats [18-21, 37, 212], and primates (human and non-human) [18, 19, 36, 194, 200, 203, 213-216] have demonstrated that the CB has an essential role in certain types of learning for example, classical-conditioning of a fear response and procedural components of spatial navigation [19, 21, 33-37, 217, 218]. When motor learning is required, the CB appears to aid in the acquisition of new procedures [37]. Pharmacological inactivation by of the CB using tetrodotoxin or lidocaine has been shown to disrupt learning complex goal-directed behaviors and lesioning of the CB impedes motor sequence learning, but not conditional visuomotor learning (i.e. learning to associate stimuli with responses, recall the associations, and adapt them to different behavioral contexts) or spatial working memory (i.e. the ability to remember the location in which something is perceived and recall a series of visited locations) [18-21, 35-37, 212, 219]. However, CB inactivation following acquisition does not appear to hinder the performance of learned sequences, thus, the CB is
involved in learning motor sequences rather than simply performing actions. In human studies, CB activation appears during “motor thoughts” where the subject is instructed to imagine performing an action without generating overt movements [19]. There is also activation when an individual observes a demonstrator performing a goal-directed motor behavior (imitation) or non-goal-directed motor behavior (copying) to be copied by the observer, but not when watching meaningless actions not to be imitated [19]. Moreover, researchers have demonstrated that CB lesions in rats impair the learning of new procedures – both when learning is direct or via observation [37].

V. Brain Regions for Observational Learning

All forms of learning require neural networks for successful acquisition, retention, and recall of information, but not every part of the network is required for all three processes. Since observational learning is conserved and prevalent across all vertebrate taxa, it is reasonable to theorize the brain regions involved, especially in acquisition, should be conserved across vertebrates. In addition, the region(s) should be able to support learning, be connected to brain regions necessary for motor output, and have some involvement in sensory perception. The reason for this is that observational learning is more than just observing the actions of another; it mandates that the observer generate an image of his or herself performing that same action and realize the goal of the behaviors [19].

Despite the above requirements, the majority of studies investigating which brain regions are responsible for facilitating observational learning have revolved around the mirror neuron system (MNS), a collection of brain regions found almost exclusively in primates [16] that do not met the requirements necessary to play a primary role in observational learning. Neurons in this system have similar responses to watching a behavior being conducted as they do when the
individual is engaged in the behavior and are called “mirror neurons.” Activities of mirror neurons in the monkeys’ premotor cortex appear to be associated with goal-oriented movements (e.g. picking up an object), and not just simple movements (e.g. just contracting the hand into a grasp). Mirror neurons in the inferior parietal lobe appear to play a more complex role and may allow for understanding of the observed action behavior (i.e. they code for the goal) [16].

Studies using human subjects have shown the presence of a MNS in regions that are homologous to the areas within the monkey MNS. Iacoboni et al. [102] examined brain activity in human subjects while they passively watched or actively imitated (which involved observation and performance) a particular sequence or temporal pattern of finger movements being demonstrated by a human hand. Results showed the pars opercularis of the left inferior frontal cortex (an area within Broca’s area) and the rostral posterior parietal cortex contained neurons with mirror properties [23].

While evidence points to the MNS as the brain center for observational learning and action meaning, there are researchers who vehemently argue against their significance and even their existence [28, 220-224]. In humans, there are significant differences in neuronal pattern firing within the MNS if the motor act is executed first then observed (a condition which violates mirror neuron criteria). This led the authors to conclude that human mirror neurons do not exist, at least to the degree which was previously thought [220]. In addition, mirror neuron and MNS brain regions identified in primates are not present in other groups of vertebrates, and evidence for mirror neurons in homologous brain regions is minimal. There is some evidence supporting the existence of neurons that act like mirror neurons in some rodent species and an oscine bird species [15, 26] but they do not appear in brain regions homologous to those considered to be a part of the MNS in primates. Thus, a MNS may not be critical for observational learning, a
phenomenon that exists across taxa. Instead, the MNS may be involved in recall of already performed behaviors (e.g. grasping objects, facial expressions, gestures, etc.) but not in the learning of motor sequences that may be later imitated [220]. The brain region that would underlie observational learning should be a region, as previously mentioned, that is conserved across taxa and that could be responsible for tying together representations of self and other, current bodily states, and action plans.

The region that best fits these requirements and, therefore, may be responsible for the acquisition of observationally-learned information is the CB, one of the most conserved brain regions across vertebrates and which has analogues in several invertebrates studies [29]. A study investigating the neural unpinning of learning a sequential visuospatial task via observation yields support that the CB is a region involved in observational learning [20]. In a sequential visuospatial task, the observer must acquire both the sequence of items and the procedural rules of how to perform the task correctly. In a human study, demonstrators were positioned in front of a touch screen that had a grid of squares on the screen. One block was darkened, and starting from that square, the demonstrators had to touch adjacent squares to determine the rules of the task (e.g. first step in the sequence is horizontal, second is vertical, etc.) and ultimately acquire the correct sequence via corrective feedback. Observer subjects viewed actors detecting the correct sequence. The subject then had to perform the task by producing the old sequence they viewed and by producing a novel sequence (starting from a new darkened block) based on the rules learned during the observation. Reproducing the old sequence required knowledge of the observed sequence whereas the new sequence required the utilization of procedural competencies linked to the rules. Just prior to the observation or to the execution period, subjects received low-frequency repetitive transcranial magnetic stimulation (rTMS) on the cerebellum or
on the dorsolateral prefrontal cortex (dPFC). rTMS causes over-stimulation of the neurons in a target area thereby deactivating them during treatment and for several minutes afterward. This allowed the researchers to distinguish the effects of regional brain deactivation on both the observation and the actual execution of the task. These brain regions were chosen because past evidence indicated they have distinctly different competencies - the CB in the acquisition of procedural components and the prefrontal cortex in declarative components of a task and visuospatial working memory.

It was discovered that when observational learning followed rTMS on the left lateral CB, deficits were present in detecting the new sequence but not in replicating the observed sequence. rTMS on the right dPFC caused the impairments to be reversed [20]. These impairments on the task showed that without a fully functional CB, the human subject was able to learn the procedural rules observationally, but was unable to gain knowledge of the observed sequence (and then imitate the motor pattern); and, without a fully functional dPFC, they were able to observationally acquire the motor sequence but not learn the procedural rules behind the sequence. These results support the theory that the CB involved in the acquisition phase of observational learning. The results also support a role for the dPFC, a part of the MNS, in observational learning. The interplay may be that the CB acquires the appropriate procedural competencies for the task while prefrontal regions provide flexibility among already stored solutions of the task since it appears to be the site of procedural rules consolidation [21].

Similarly, it has been shown that suspending rats in small observer chambers over the Morris Water Maze (MWM) and allowing them to watch 200 trials performed by a companion rat significantly improves learning of the task [18-20, 37]. However, if a hemicerebellectomy is performed on observers prior to viewing the demonstrators, this effect is lost. In contrast, if a
hemicerebellectomy is performed after the viewing the demonstrators, the rats’ procedural abilities were comparable to unlesioned observers [37]. These results indicate that complex spatial information is acquired by the rats, but only if the cerebellum is intact, and indicates the CB is necessary for the acquisition of mindful or kinesthetic imitation in the rat.

Furthermore, by using the reliable sequential strategies used to find the target platform in the Morris water maze (MWM), researchers were able to investigate whether the acquisition of procedural skills have an organizational structure that may be dissected into simpler units and whether these units can be singularly acquired without the observation of preceding steps or whether the complete procedural sequences is required [212]. When a rat is placed in the MWM, it consistently exhibits different strategies in a set order when learning the maze [225]. First, the rat will engage in peripheral circling which is an instinctive strategy and does not require any learning. Next, the rat utilizes extended searching where it swims through the pool not just around the edge. As learning progresses, restricted searching comes into play as the rat only searches the quadrant in which the platform is located. When learning is completed and spatial memory is consolidated, finding without searching occurs with the rat swimming directly to the platform with absolutely no searching behaviors [226]. These strategies are always acquired from least to most effective in a procedural chain sequence. Since the steps are dependent on CB control [18-21, 35-37], it is possible to block the acquisition of new strategies while retaining any previously acquired strategies. Rats were allowed to observe the swimming patterns and behaviors associated with just one of the above strategies developed by their conspecifics and were lesioned post-observation. When the observers were placed in the maze, they did not copy the exact swimming trajectories of the demonstrator, but copied the strategy employed by the demonstrator and did not progress in the learning sequence past the observed point [212]. These
results indicate that single behavioral units may be acquired separately without the necessity of seeing the whole chain sequence, and further supports the CB as the facilitator of mindful or kinesthetic imitation.

VI. The Zebra Finch

I conducted my experiments using a common laboratory bird, the estrildid zebra finch (*Taeniopygia guttata*) within the passerine order. Zebra finches are highly social birds, nesting in large charms of twenty to a thousand birds. They are sexually dimorphic in plumage coloration and behavior. Only the males sing. Female choice is the predominant mode of sexual selection but male choice occurs. Once a pair-bond has been established, they remain socially (but not sexually) monogamous for life [227, 228].

The zebra finch is an appropriate model organism for investigating observational learning for two reasons. Firstly, zebra finches are the model organism for studying a rare type of observational learning, vocal learning [180, 229]. During song learning, young males acquire a song that is similar to, but not an exact copy of, the tutor’s song [229]. Within the song acquisition pathway neurons with mirror neuron-like properties were found [15]. These neurons appear to respond to hearing and performing the same song. However, these neurons are not responsible for the acquisition of learning. Activation does not occur during acquisition or post-learning, and therefore, these neurons do not fit the exact definition of mirror neurons. Recently, it has been shown that a portion of vocal learning is supported by the cerebellum [209, 211]. Secondly, observationally learning foraging [230, 231] and mate selection [14, 173, 232-235] has been demonstrated in this species.
CHAPTER 2: STIMULUS ENHANCEMENT LEARNING IN THE FEMALE ZEBRA FINCH AND ITS INFLUENCE ON MATE PREFERENCES

FOREWORD

My first study involved an investigation of stimulus enhancement learning abilities in the female zebra finch by examining the acquisition and utilization of public information pertaining to male quality during mate selection. I predicted that observation of female demonstrators interacting with males would influence the formation and expression of mate preferences in the observer depending on certain environmental conditions. The results of this study will be submitted to Proceedings of the Royal Society of London B.

ABSTRACT

Mate selection is open to change based on public information acquired by the observation of another individual’s mate choices (non-independent mate choice). Two types of non-independent mate choice have been proposed: mate choice copying (MCC) and mate quality bias (MQB). MQB should be the predominant form of choice copying in species with assortative mating wherein the pair members are of similar intrinsic quality. Presumably a copying female should re-assess her initial mate preference if there is a mismatch between the quality of that male and his female associate. In two experiments, I investigated MCC and MQB in the female zebra finch (Taeniopygia guttata) by conducting pre- and post-observational mate preference trials. Females did not alter their male preference after viewing him interacting with a randomly chosen female, suggesting that MCC does not influence mate choice. However, in the MQB
experiments females significantly altered their preference in favor of a previously non-preferred male after viewing their preferred male with an inferior female phenotype. I conclude that female finch preference is influenced by the conspecific associations of her prospective mate. This form of public information use in mate copying is consistent with MQB-based methods of mate choice found in other monogamous species.

1. BACKGROUND

Independent mate choice occurs when an individual selects a mate based solely on the suitor’s morphological, olfactory, and/or acoustic display signals. Non-independent mate choice occurs when social environment and observational learning can influence the formation and expression of mate preferences. In many taxa the mate preference criteria seen during independent mate choice can be over-ridden when females are able to observe, and copy, the mate choice decisions of conspecifics [72, 171, 236-238]. Thus mate choice copying (MCC) is when a female preferentially selects a male previously seen in proximity to or actively mating with other females. MCC has been convincingly demonstrated in numerous species of fish [see review: [239], several species of birds [black grouse: [240]; Japanese quail: [241]; sage grouse: [242], fallow deer [see [237] and variably in humans [e.g. [243, 244].

There are two main hypotheses offered to explain the adaptive benefits of this type of non-independent mate choice: reduced cost of sampling and increased discrimination. The first hypothesis posits that by investigating the mate choices of female conspecifics (called ‘models’), an observing female can obtain a superior mate while avoiding potential costs (e.g. lost foraging time, increased predation risk, etc.) associated with searching for and deciding on a mate [171]. The second explanation holds that observing females use the information gained through surveillance to refine their personal assessments of the traits associated with male quality, thus
improving their own mate selection [245]. In summary, copying may be adaptive because it decreases costs and/or because it increases benefits associated with mate choice. Therefore the adaptive functions of copying are likely to differ between species, but possibly also within species if individual females differ in the benefits that they seek from being choosy [171].

When observing the interaction between the model female and her prospective mate, the observing female gains information about both parties, not just the displaying male. If the function of mate copying is to refine an observer’s ability to detect the “best” male, theoretically the quality of the model female with whom he associates is a reflection of the male’s self-assessment of his own quality. By eavesdropping on the communication between the courting pair, the observer is able to improve her mate assessment beyond the information available to her by simply assessing the male’s display characteristics. Vakirtzis and Roberts [245-247] call this form of copying mate quality bias (MQB). If observing females are refining their independent assessments of male traits, then inferior model females should have more negative influence on the mate choices of the observer than model females of higher quality. An influence of the traits of model females on observer female choice has been demonstrated in several species, including humans [120], guppies [71, 248], and the sailfin molly [249]. Some of the traits found influential in fish are the age and size of the model females [69, 71, 248, 249] and in humans, traits such as attractiveness of the model female and character (pleasant versus unpleasant) determine if the model’s choice will influence a choosing female [79, 120, 250].

The majority of non-independent mate choice research has been focused on promiscuous and polygynous species, wherein mutual mate choice between the sexes is less likely. In polygynous species with little or no paternal care, males maximize individual fitness by copulating with as many mates as possible [72, 237, 240]. In this situation, a male’s quantitative
sexual success (i.e. the number of successful matings) is a trait with meaningful variation and can be used by observing females to determine male quality. Thus, polygynous mating systems should favor the evolution of MCC by females. In socially monogamous mating systems with paternal care, on the other hand, there is almost no skew in male mating success and therefore, copying females would not find quantitation of a male’s association with different females to be informative [245]. Instead, MQB should be expected because, due to mutual choice of both the female and the male, there should exist a positive correlation between a male’s quality and the quality of the females that associate with him. In other words, the most desirable females should attempt to pair with the most desirable males. This correlation could be exploited by the observer female to enhance her mate quality discrimination [245].

Unfortunately, non-independent mate choice is rarely studied in monogamous species. In humans, mate quality bias occurs in socially monogamous populations rather than MCC [120, 251-253]. In studies of mate choice copying in the zebra finch, the only other monogamous non-human species studied so far, no consistent pattern has emerged. Doucet et al. [235] found no evidence that finches copy each other’s mate preferences for a particular male individual. In contrast, Swaddle et al. [173] showed evidence that public information can alter not only the observer female’s preference for a particular male but also her preference for a particular male trait (i.e. leg band color). Neither experiment could be used to distinguish between MCC and MQB because female model characteristics were not examined nor controlled.

The aim of my study was to further investigate mate choice copying in the zebra finch to distinguish between MCC and MQB. Because the zebra finch is socially monogamous I predicted that observer females would assess the quality of a model female when deciding whether to copy her mate choice. I used female zebra finches with white plumage to represent
poor quality model females. There are two reasons why white feathered females are inferior mates for all males: a) white females are not as cryptic under natural conditions and thus presumably are more likely to be depredated, and b) white feathers may also be more susceptible to damage due to physical abrasion, feather mites and microbial digestion [254-256]. At least one other mate choice copying study has used color morphs as copying test subjects [233], but to my knowledge mine is the first to vary systematically the plumage color of the female models. My experimental design involved two dichotomous mate choice experiments. In the first experiment, I tested whether observer females show increased attraction to a non-preferred male after seeing him in association with a model female, while the preferred male remained alone. In the second experiment, I tested whether seeing a preferred male with a low quality model female, while the non-preferred male associated with a higher quality female, decreased the observer’s attraction for the preferred male.

2. METHODS

(a) Subjects and General Housing Conditions

Experimentally and sexually naïve, adult wild type colored female zebra finches (CF) were used as test subjects (N=96). The 30 females used a model females were approximately 6 months older than the test females. Virgin CF choice males (N=24) were observed by test subjects and were approximately the same age as the test females. All males and female models were rebanded to have black leg bands. Birds were housed within their groups (i.e. males, test females and female models) with the females being visually but not acoustically isolated from the males. Seventy-eight test females came from university breeding colony of approximately 200 birds, where CF and white morph zebra finches (WF) are housed together in a ratio of about
20:1 CF:WF. These birds were used for both MCC experiments and the first MQB study. In my second MQB experiment, 18 virgin adult CF test females were purchased from a colony where they never had visual contact with WF zebra finches. These females were housed in similar conditions to the main colony but in a separate room and were habituated for 28 days before testing. Males for the second MQB experiment came from the main colony but were moved into the room with the newly purchased females 2 days before testing. All birds were maintained at an ambient temperature of 21-24°C and on a 14:10 light:dark photoperiod except for the birds from the second MQB study which were kept on a 13:11 light:dark photoperiod to maintain continuity with their former housing conditions. Seed and water for all birds were provided ad libitum.

(b) General Materials and Methods

All testing was conducted in a two-way mate choice chamber (30x45x30cm; see Fig. 2.1). In the choice chamber, the female test bird was placed in the viewing compartment (30x22.5x30cm). Before testing an opaque barrier separated the female and male chambers. During behavioral testing the viewing chamber was divided from the male chambers by a transparent Plexiglas viewing wall. Projecting perpendicularly into the viewing compartment from the center of the viewing wall was a 12cm opaque barrier (so only one male could be viewed by the female at a time). The male compartments (15x22.5x30cm) were separated by an opaque wall and each had a 7.5cm long perch in front of the female viewing window.
**Figure 2.1.** Experimental apparatus and sequence (shown left to right) for female choice tests for mate choice copying (A) and mate quality bias (B) experiments. Pre-Model Test = pre-model preference test, Observ. Period = observational period, Post-Model Test = post-model preference test. Black symbols indicate wild type morphs while gray female symbols indicate the white morph females. MCC control females were not exposed to a model female during the observation period. See text for details on the positioning of model females relative to male quality in the MQB tests.
Test females and males were placed in the preference apparatus and allowed to habituate overnight (approximately 3:10 light:dark hours). During habituation, the males and females were visually but not acoustically separated from each other and provided seed and water. In the morning, the visual barrier between the males and female was removed, and a 20-minute pre-model preference trial was conducted. All test females were assigned at random to a unique dyad of males. Male dyads were viewed by two or three females, with equal number of female viewers from each of the treatment groups and experiments and a counter balance of which experiment was performed first for male dyads across experimental replicates.

We video recorded trials and measured the time females spent in proximity of each male using EthoVision®9XT multi-arena module (Noldus Information Technology, Wageningen, The Netherlands). The measure of preference for a particular male was calculated as the proportion of time spent in proximity to that male (time with male A/(time with male A & B) x 100). Prior research shows that proximity times predict mate choice preference and correlate with copulation preferences in the zebra finch [257-259]. Pre-model preference measures allowed us to randomly assign females to the treatment and control groups that will be explained below. After pre-model trials, the appropriate model females were added to male chambers as per experimental requirements and test females observed these interactions for 4 hours. At the end of observation, quietly in the dark, models were removed and males were swapped between left/right choice chambers to detect female side biases. Any female that showed side, rather than male, preference was eliminated from the analysis. After observation, a 20-minute post-model preference trial, identical to the pre-model trial, was conducted to determine if test females altered their initial preference. The strength of preference-change was calculated as the proportion of time spent with the non-preferred male in the post-model trial minus the proportion of time spent with the
non-preferred male in the pre-model trial. The absolute change was measured by converting
scores to a binary (+/-): spent/did not spend greater than double the amount of time with the non-
preferred male in the post-model trial compared to the pre-model trial. Six test females could be
run at a time. Equal numbers of treatment and control birds were included in each run.

(c) Experiment 1: Mate Quality Bias.

Following pre-model preference trials, each test female was randomly assigned to either:
observe a WF model in the chamber of the preferred male and a CF model in the chamber of the
non-preferred male (WF/PM condition), or the reverse, observe a CF model in the chamber of
the preferred male and a WF model in the chamber of the non-preferred male (CF/PM
ccondition). We first tested CF females bred and housed in the university colony that may have
had exposure to males interacting with WF females in the aviary (WF/ PM: n = 6, CF/PM: n =
6). We then tested the CF females from the private vendor (WF/PM: n = 9, CF/PM: n = 9).

(d) Experiment 2: Mate Choice Copying

Following pre-model preference trials, each test female was randomly assigned to either:
observer a CF model in the chamber of the non-preferred male (treatment) or to observe males
without a model female (control). We replicated this experiment to demonstrate reliability and to
increase the sample size to around the same numbers as used for the MQB experiments (replicate
1 - treatment: n = 9, control: n = 9; replicate 2 - treatment: n = 9, control: n = 9).

(e) Statistical Analysis

For both experiments, the Mann-Whitney U was used to test whether test females in the
two conditions differed in the strength of their change in preference between pre- and post-model
preference trials whiles tests of differences in the absolute change in preference were analyzed using a binomial sign test for each group independently. To test if birds assigned to each condition differed in their preference for their preferred male on the pre-model preference trial, we used a Mann-Whitney U to compare the proportion of time spent in proximity to the preferred male between the conditions. Wilcoxon signed ranks were used to test whether females changed the proportion of time spent with the initially non-preferred male between the pre-model and post-model trials for birds within each condition. For the MQB experiment only, to test whether the effect of past experience with WF females altered female preference patterns, we examined the interaction between preference changes and aviary of origin (experience with or without WF females). For strength of preference-change, we analyzed data using a two-way ANOVA, and for the absolute preference-change data, we used a binary logistic regression with an exact inference method based on a maximum likelihood model. For the ANOVA, we angularly transformed proportion data, but still had a non-normal distribution. Since ANOVA is robust to violations of normality assumptions [260] and we used concurrence between the strength of change and absolute change, parametric and non-parametric tests respectively, the combination of these analyses allows us to determine if aviary of origin had any effect. All statistical tests were conducted using SPSS 22, except the binary logistic regression that was conducted using SAS 9.2. Raw data is provided in the Supplementary Material.

3. RESULTS

(a) Experiment 1: Mate Quality Bias

One treatment female from the second MQB replicate was eliminated because of side preference. Previous exposure to WF females in the aviary of origin had no effect on the strength
of preference change \((F(1,25) = 47, p = 0.72)\) or absolute preference change \((p > 0.99)\). As aviary had no influence on preference, we combined data across the aviaries. Separate analyses for each data set support the same conclusions as the combined data.

WF/PM condition test females compared to CF/PM condition test females had a greater strength of preference change from the initially non-preferred male between pre- and post-model trials \((U = 53.00, p = 0.02)\). Furthermore, choice reversal occurred in 7 of the 14 WF/PM females \((p = 1.00)\), but only 1 of the 15 CF/PM females \((p = 0.001)\). CF/PM and WF/PM birds did not initially differ in the proportion of time spent in proximity to the preferred male (pre-model trial: \(U = 96, p = 0.69\); Table 2.1). Between groups, after viewing males interact with models, WF/PM birds spent more time with their non-preferred male than did CF/PM birds \((U = 47, p = 0.01)\). Between the pre- and post-model trials and within groups, WF/PM females spent a significantly higher proportion of their time in proximity to their initially non-preferred male \((Z = -2.75, p = 0.006; \text{Figure 2.2})\) whereas CF/PM females did not \((Z = -1.78, p = 0.08)\).
Table 2.1. Descriptive statistics for the amount of time spent by the observer female in different parts of the testing chamber. Figure shows all replicates but the statistics in the manuscript are based on combined data for MQB. Values are time spent (s; mean±SE) in proximity to the male during each 20-min (1200-sec) preference trial, percentage of total time in shown in parentheses (%TT), and percentage of total viewing time (with either male) is shown in square brackets (%VT). Percentages have been rounded to the nearest whole number. Values are calculated from

<table>
<thead>
<tr>
<th>Time spent with male</th>
<th>Preferred</th>
<th>Non-preferred</th>
<th>Neither</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SE (%TT)[%VT]</td>
<td>Mean±SE (%TT)[%VT]</td>
<td>Mean±SE (%TT)</td>
</tr>
<tr>
<td>MCC Replicate 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>1106±59 [92%]</td>
<td>94±57 [8%]</td>
<td>0±0</td>
</tr>
<tr>
<td>Post</td>
<td>483±179 [40%] a</td>
<td>717±179 [60%] a</td>
<td>0±0</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>1199±0.2 [99.9%]</td>
<td>0.22±0.22 [0.01%]</td>
<td>0±0</td>
</tr>
<tr>
<td>Post</td>
<td>883±147 [74%] a</td>
<td>317±147 [26%] a</td>
<td>0±0</td>
</tr>
<tr>
<td>MCC Replicate 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>996±76 (83%) [85%]</td>
<td>170±67 (14%) [15%]</td>
<td>34±12 (3%)</td>
</tr>
<tr>
<td>Post</td>
<td>908±71 (76%) [79%]</td>
<td>242±65 (20%) [21%]</td>
<td>50±13 (4%)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>974±94 (81%) [87%]</td>
<td>144±75 (12%) [13%]</td>
<td>81±40 (7%)</td>
</tr>
<tr>
<td>Post</td>
<td>490±154 (41%) [48%] a</td>
<td>526±162 (44%) [52%] a</td>
<td>184±94 (15%)</td>
</tr>
<tr>
<td>MQB Replicate 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WF/PM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>919±133 (77%) [96%]</td>
<td>43±28 (4%) [5%]</td>
<td>238±47 (20%)</td>
</tr>
<tr>
<td>Post</td>
<td>578±237 (48%) [51%] a,b</td>
<td>560±223 (47%) [49%] a,b</td>
<td>62±11 (5%)</td>
</tr>
<tr>
<td>CF/PM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>1066±92 (89%) [99%]</td>
<td>7±5 (0.6%) [1%]</td>
<td>127±26 (11%)</td>
</tr>
<tr>
<td>Post</td>
<td>1133±57 (94%) [96%]</td>
<td>46±43 (3.8%) [4%]</td>
<td>21±60 (2%)</td>
</tr>
<tr>
<td>MQB Replicate 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WF/PM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>929±146 (78%) [88%]</td>
<td>123±57 (10%) [12%]</td>
<td>146±51 (12%)</td>
</tr>
<tr>
<td>Post</td>
<td>423±91 (35%) [41%] a,b</td>
<td>559±111 (51%) [59%] a,b</td>
<td>165±36 (14%)</td>
</tr>
<tr>
<td>CF/PM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>815±101 (69%) [80%]</td>
<td>198±55 (17%) [20%]</td>
<td>165±44 (14%)</td>
</tr>
<tr>
<td>Post</td>
<td>625±89 (53%) [61%]</td>
<td>396±101 (34%) [39%]</td>
<td>116±32 (13%)</td>
</tr>
</tbody>
</table>
the recorded values. a Significant change (p<0.05) from initial. b Significantly different than the control.

Figure 2.2. The increase in preference for the initially non-preferred male (mean ±SE) is statistically significant in the treatment group only, supporting the MQB hypothesis. Between trials, the WF/PM group viewed their non-preferred male with a wild type model female while their preferred male was paired with a white-feathered model female, and the CF/PM group viewed their non-preferred male with a white-feathered model and their preferred male with a wild type model. Time increase was calculated as (% Post-Observation time with non-preferred - % Initial time with non-preferred). Statistics were conducted on rank values.
(b) Experiment 2: Mate Choice Copying

Two treatment females and one control female were eliminated from the second MCC replicate due to side preference. Whereas MQB treatment altered female preference, for both of the MCC replicates there was no difference between treatment birds and control birds in the measure of preference change between pre- and post-model trials (replicate 1: $U = 24, p = 0.14$; replicate 2: $U = 15.00, p = 0.13$). Similarly, neither treatment nor control groups had a significant number of birds reverse their choice in either MCC replicate with treatment birds in replicate 2 showing a significant lack of reversal (replicate 1- treatment: 5 out of 9, $p = 1.00$, control: 3 out of 9, $p = 0.51$; replicate 2 – treatment: 0 out of 7, $p = 0.02$, control: 4 out of 8, $p = 1.00$; Table 1). Control and treatment birds did not differ in the proportion of time spent in proximity to the non-preferred male during the pre-model trial (replicate 1: $U = 25.00, p = 0.09$; replicate 2: $U = 27.00, p = 0.91$; Table 1), nor the post-model trial (replicate 1: $U = 21.00, p = 0.08$; replicate 2: $U = 19.50, p = 0.33$). The trending $p = 0.08$ seen in replicate 1 is due to birds, that were later randomly assigned to the control group compared to the treatment group, happening to spend a non-significant but greater proportion of their time with their non-preferred male in both the pre- and post-model trails. In fact, mean ranks of control and treatment birds are nearly identical in the pre-model trial and the post-model trial further verifying that treatment had no effect. Thus, two replicates of MCC showed no treatment effect.

4. DISCUSSION

As predicted, I was unable to find support for MCC but did find evidence for MQB in the socially monogamous zebra finch. Previous studies of MCC in the zebra finch have yielded equivocal results (Table 2.2). In my MCC study, females exhibited considerably greater interest in the non-preferred male after seeing him in association with a female, but this change in choice
behavior was not statistically significant when compared to the shift in behavior of the control birds, which never saw their non-preferred male with a female. My results are consistent with the findings of Doucet et al. [235], but not with those of the other authors in Table 2.2. Rosa et al. [232] suggested that the lengthy observation period allowed by Swaddle et al. [173] compared to the relatively brief observations permitted by Doucet et al. [235] might explain the differences in their results. However this explanation is unlikely given Rosa et al. [232] and others subsequently found significant evidence of MCC with only a two-hour observation period. Instead, a more likely explanation for the significant findings of some studies is that they either did not employ a control group for comparison, and/or they analyzed a more conducive subset of their subjects after pruning the data set of those that either did not show strong initial preferences for a male, appeared to have a side-bias in the testing arena, or were largely inactive. Individual variation in female use of public information during mate choice [233] parallels individual female zebra finch behavior in other sampling scenarios such as during foraging [232], and may represent a tactic that females in poor condition use to reduce fitness costs. Thus non-significant results in studies of MCC could be attributable to variation in female condition. Experimentally inducing variation in female condition is a technique worthy of investigation in future studies of MCC in this species.
<table>
<thead>
<tr>
<th>Source</th>
<th>Experimental Change (%)</th>
<th>No. of Individuals that Switched Preference/ Total</th>
<th>Control Change (%)</th>
<th>No. of Individuals that Switched Preference/ Total</th>
<th>Observation Period</th>
<th>Male Trait Being Tested</th>
<th>Controlled for Male Behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>51</td>
<td>5/9</td>
<td>26</td>
<td>2/9</td>
<td>2h</td>
<td>Natural Variation</td>
<td>No</td>
</tr>
<tr>
<td>B</td>
<td>31(^a)</td>
<td>13/15</td>
<td>n/a</td>
<td>n/a</td>
<td>2h</td>
<td>Natural Variation</td>
<td>No</td>
</tr>
<tr>
<td>C*</td>
<td>57(^a)</td>
<td>11/15</td>
<td>n/a</td>
<td>n/a</td>
<td>40m (1(^{st}) d); 1h (2(^{nd}) d)</td>
<td>Leg Band Colour</td>
<td>No</td>
</tr>
<tr>
<td>D**</td>
<td>22(^a,b)</td>
<td>?/24</td>
<td>20</td>
<td>?/18</td>
<td>2h</td>
<td>Artificial Crest</td>
<td>No</td>
</tr>
<tr>
<td>E</td>
<td>30</td>
<td>?/20</td>
<td>35</td>
<td>?/20</td>
<td>30m</td>
<td>Natural Variation</td>
<td>No</td>
</tr>
<tr>
<td>F**</td>
<td>11(^a)</td>
<td>?/15</td>
<td>n/a</td>
<td>n/a</td>
<td>1h/d x 10d</td>
<td>Symmetrical/asymmetrical bands</td>
<td>No</td>
</tr>
<tr>
<td>G (Ex 1)</td>
<td>No initial test given</td>
<td>?/20</td>
<td>n/a</td>
<td>n/a</td>
<td>?h/d x 14d</td>
<td>Natural Variation</td>
<td>No</td>
</tr>
<tr>
<td>G (Ex 2)</td>
<td>Not enough info. in paper provided to determine(^a)</td>
<td>?/22</td>
<td>n/a</td>
<td>n/a</td>
<td>?h/d x 14d</td>
<td>Leg Band Colour</td>
<td>No</td>
</tr>
</tbody>
</table>

**Table 2.2.** A comparison of the relative strength of mate choice copying effects in zebra finches from published studies. Experimental and Control Change is the change in the mean percent of time spent with the non-preferred male (Post-observation trial – Initial trial). A. this study; B. [232]; C. [233]; D. [234]; E. [235]; F. [14]; G. [173]. *Includes only females with a strong preference during the initial trial. **Median values. Data extracted from authors’ Fig. 2.

\(^a\) Significant change (p<0.05) from initial. 
\(^b\) Significantly different from the control.
Consistent with my predictions, I found strong evidence for mate quality bias. The low quality, WF model altered preference for the previously preferred male whereas pairing with the CF model did not alter preference and this effect was significant regardless of females’ past aviary experience with WF birds. Quality-disassortative pairings of model females with males (i.e. WF with preferred male, and CF with non-preferred male), changed the post-observational attractiveness of males. As far as I am aware, this study is the first to report such a MQB effect in a monogamous bird species.

Overall the results of my experiments are mostly consistent with those found in humans [120, 251-253] and particular fish species [71, 248, 249]. For example, Waynforth [120] found no evidence that the presence of a female caused an increase in attractiveness of human males, but significant observer preferences did appear when the attractiveness of the accompanying female was taken into account. In humans, attractive females increased a previously unattractive male’s attractiveness while unattractive females decreased a previously attractive male’s attractiveness. In zebra finches, if the WF is considered by the observer females to be an unattractive (i.e. low quality) individual, then his association with a WF female should decrease the preferred male’s attractiveness, as I have shown. This interpretation is reinforced by my pairing the non-preferred male with a CF female and showing that partner switching did not occur.

The fact that not every WF/PM female in the MQB experiment altered her preference could have 2 explanations: a) some males may have been so attractive relative to the non-preferred male that pairing them with WF females was insufficient to change the observer’s
preference and b) observer females may vary in their assessment criteria based on their own individual quality. Further research that controls for male quality and observer quality is needed to sort out the reasons why only some females engaged in quality bias.

While it appears that the quality of a model female (or at least her plumage colouration) can alter an observer’s preference for a male associated with the model, the effect of this phenomenon on the strength of sexual selection in wild zebra finches is unclear. Zebra finches are socially monogamous with extremely low rates of extra-pair copulations (EPC) [259]. However when they do engage in EPCs, females seek males more attractive than their mate [261]. This creates a potential conundrum for attractive males, who presumably will already be paired with high quality females. Theoretically due to the MQB effect, an attractive male may be unable to obtain additional EPCs if he is first observed engaging in an extra-pair association with a low quality female. Thus despite the inexpensive material (i.e. ejaculate) cost of an EPC with a low quality female, conveying that negative public information may impair a male’s future reproductive success. Because EPC is uncommon in this species, a more likely effect of MQB occurs in future monogamous pairings. Zebra finches have been shown to switch partners from between breeding seasons, and may have more than one breeding partner sequentially within a single season [259]. Thus MQB may play a role in the sequential pairing opportunities of males.

In conclusion, my results agree with the findings of previous studies that found that MCC is not characteristic of zebra finch females. Uniquely, I conclude that MQB is a biologically relevant strategy employed by a monogamous taxon other than humans, and may influence mating strategies and the fitness benefits of sexual selection in the zebra finch. Further mate
choice copying studies, in both captive and wild populations, which consider natural variation in body condition and other measures of mate quality relevant to the natural history of the zebra finch, are warranted.
CHAPTER 3: OBSERVATIONAL CONDITIONING IN THE ZEBRA FINCH

FOREWORD

In Chapter 2, I showed that the zebra finch demonstrates non-independent mate choice in the form of mate quality bias (MQB). Although the exact learning mechanism in MQB is unclear, it is posited that during MQB, the females either learn an associate to a particular male or an association to a particular trait. Association-to-male and association-to-trait are similar; however in the first case, the observer female prefers a male seen with or near a female to one seen alone [262]. In the second case, the female prefers any male displaying the same traits as the male seen with the other female [263]. The commonality between the alternative hypotheses is that either local or stimulus enhancement is responsible for the acquisition of mate choice information. Local or stimulus enhancement is believed to be a less cognitively demanding form of observational learning [46, 47, 64]. To determine whether zebra finches were capable of more cognitively demanding type of observational learning, I examined observational conditioning of a CS-US (tone-shock) association here in Chapter 3. The results of this study will be submitted to Proceedings of the Royal Society of London B.

ABSTRACT

Learning that a stimulus is troublesome can be acquired either directly through experience or vicariously through observing conspecifics reacting with a threat response to an aversive stimulus. Using a standard fear conditioning chamber adapted for a passerine bird, I tested zebra finches’ acquisition of direct and observational fear conditioning to an acoustic
stimulus. I found that zebra finches can learn to associate a tone with an impending shock. Observation of a conspecific reacting fearful to the tone was not sufficient for observers to show a conditioned response to the tone in a probe trial immediately following observation; however, observers compared to non-observers did show savings in the acquisition of fear conditioning after the observation period. To my knowledge, this is the first study to use a standard fear conditioning chamber to demonstrate fear conditioning in a passerine bird and the only study to show a role of observational learning in classical conditioning of fear in birds.

1. BACKGROUND

Fear conditioning (FC) is a form of classical (Pavlovian) conditioning during which an animal learns to associate an initially neutral stimulus (the conditioned stimulus; CS) with an aversive stimulus (the unconditioned stimulus; US) that generates species-typical fear responses (unconditioned responses; URs). Following learning of the CS-US association, subjects will respond to the CS alone (the conditioned response; CR) [17]. In FC, CRs are fear-related threat responses similar to the defensive URs elicited by the US [264]. For example, in rodents foot shock as a US elicits jumping or other escape responses whereas the threat response of freezing is the CR [264-267]. FC has become the standard mechanism for studying conditioned fear acquisition and emotional learning because it is rapidly acquired, leads to clear, long-lasting associative learning of threat responses in both vertebrate and invertebrate species, and because the neuronal circuits underlying FC are known to be highly conserved in mammals and other vertebrates [13, 17, 264, 268-277].

The social transmission of fear signals have been documented, implying animals are able to send and correctly perceive conspecific signals of aversive or dangerous stimuli [103, 111-113, 159, 278-281]. Fear behaviors in a conspecific aid in alerting the observer to dangerous
stimuli and assign a threat value to the stimulus. In observational FC, the conspecific’s fear response may serve as an US which elicits fear responses in the observer because the fear response becomes paired with a particular novel stimulus, the CS [82, 111, 279-283]. The ability to detect and respond to indicators of threat and pain from observing a conspecific rather than having to experience the threat oneself is thought to bestow selective advantages to individuals that possess this ability [112].

The emergence of fear response to a novel stimulus when it is paired with a conspecific’s defensive behaviors has been demonstrated in several species including birds [157-159, 278], fish [68], mice [279], cats [103], cows [113] and primates [75, 81, 82, 111, 112, 280-282]. However, this acquisition might not reflect observational conditioning and may instead be explained as response facilitation or social contagion. To be considered observational conditioning, a Pavlovian association must be established by the demonstrator and this learned association, indicated by the display of a CR in response to a CS, must have the possibility of being demonstrated when the subject is not in the direct presence of the demonstrator. For example, biting flies with their biting parts removed are not aversive to mice; however, after viewing a demonstrator reacting to being bitten by flies, observer mice will recognize and attempt to avoid biting flies, even when the flies have their biting parts removed [279]. Similarly, rhesus monkeys will engage in threat responses to a plastic snake after watching a conspecific reacting fearfully to snakes [81, 112]. In these examples, the observer mice and monkey responses occur even after the demonstrator has been removed. In contrast, contagion and social facilitation do not require the learning of an association by the observer. Contagion is when 2+ individuals engage simultaneously in a behavior that is usually species-typical and the behavior of one individual appears to act as a releaser for the behavior in another individual [46, 284].
social facilitation, the presence of the conspecific is thought to increase the general arousal level of an individual [46]. This increase in arousal level may then alter the observing individual’s activity level or behaviors [46, 285].

Previous studies of observational fear learning in avian species have used paradigms where the demonstrator and observer are, at least initially, simultaneously responding to the CS, which may confound the interpretation of what stimulus is being learned by the observer. For example, threat responses towards humans wearing specific mask while trapping American crows can be transmitted to naïve conspecifics and juveniles. After observing experienced crows mobbing and vocally scolding researchers wearing the trapping-masks, but not scolding humans wearing neutral masks, the observer birds will begin mobbing and scolding only those humans wearing trapping-masks [157, 158]. Observers will begin mobbing and scolding trapping-mask individuals independently after several facilitated mobbings with the experienced birds; however, they never learn to discriminate as well as the experienced crows [157, 158]. Furthermore, fear responses may occur even when the stimulus producing defensive behaviors in the demonstrator is not seen by the observer [159]. Female zebra finches will respond with threat responses when observing their male partner perform learned threat responses to the presentation of a starling (e.g. flying at the starling, excited behaviors like tail and wing flicks, and excited calls), even though the females only see and hear the male responses rather than seeing the starling. Thus, studies where observers must perform responses in the absence of demonstrators are needed to distinguish observational conditioning from contagion or facilitation.

Thus, I sought to examine whether zebra finch can acquire fear conditioning under direct and observational training. Notably, previous studies of fear conditioning have been performed under conditions that did not allow free flight and in species for which the conditioned response
to shock is freezing or tonic immobility, pigeons and chicks respectively [273-275, 286-288].
Additionally, FC in pigeons has been performed using an invasive technique of inserting
electrodes into the pubis and fitting the birds with a harness that secures the electrical inputs
[286-289]. Such an invasive technique may cause stress responses that could alter physiological
and neurological processes in the bird and therefore skew interpretations of the data or detract
from the generalizability of the study

A necessary prerequisite to creating a laboratory model of fear conditioning for freely
moving birds that respond to threat with flight, such as zebra finches and other passerines, is the
development of a non-invasive test of fear conditioning. Thus, I adapted a standard mouse fear
condition chamber and adjusted typical shock protocols to test whether zebra finches could be
classically conditioned to associate a tone and shock and whether this same tone could more
readily elicit a fear response in a zebra finch that previously observed a conspecific undergoing
FC training compared to zebra finches that observed conspecifics responding to the tone only.
Presumably, the lack of an appropriate apparatus may explain why my experiment is the first, to
my knowledge, to address direct learning of fear conditioning in the classic FC chamber in birds
that respond to threat with flight, and is the only study, of which I am aware, to examine
observational fear conditioning in birds.

2. METHODS

(a) Animals and Treatment

Adult male (n=16) and female (n=16) zebra finches (6-18 months of age) bred in an
aviary at University of Mississippi were used in this experiment. The birds were housed in cages
of 8 same-sex individuals on a 14:10 light:dark photoperiod at an ambient temperature of 21-
24°C. Seed (Kaytee; Chilton, WI, USA) and water were provided ad libitum.
(b) Apparatus

I modified a standard mouse FC chamber (30.5 cm x 24.1 cm x 21.0 cm: Med Associates, Inc., St. Albans, VT, USA) for a flying biped, the zebra finch. To prevent the birds from perching on a single one of the parallel conductive metal rods (diameter: 3.2 mm), and thus averting electric shock, the floor was flattened by placing a 6.35 mm thick polyvinyl chloride (PVC) sheet cut to fit the floor dimensions and routed to accommodate approximately half the grid bar’s diameter (Fig. 3.1). The electrical resistance of the avian foot is over 10 MΩ [289] compared to 100-150 kΩ in the mouse [290]. To increase conductance, I applied electrode gel (Signagel®, Parker Laboratories, Inc., Fairfield, NJ, USA) to the bars before inserting the PVC flooring and to birds feet prior to placing each bird in the chamber. These alterations resulted in a consistent defensive response to the US, a 3.5 mA foot shock.

The CS was a 90-dB, 3000 Hz pure tone emitted from a speaker mounted to the side of the chamber. This frequency and amplitude are within the zebra finch audibility range (500-6000 Hz) but outside the fundamental frequencies of male song syllables (400-2000 Hz) [291]. Thus, past experience was unlikely to affect response to this distinct tone. The duration and temporal patterning of the CS and US were controlled using Video Freeze® software (MED Associates, Inc., St. Albans, VT, USA). Behavior was recorded using a video camera (DMK 31AF03.AS; The Imaging Source; Bremen, Germany) mounted in front of the chamber and the footage was relayed to an image analyzer (Ethovision; Noldus Information Technology, Wageningen, The Netherlands). As explained below, Ethovision software was used to acquire measures of flight duration and the latency to first flight response after the tone onset.
Figure 3.1. Picture of fear conditioning chamber with modifications for the zebra finch (insert shows close-up of how the PVC sheeting ensures contact with multiple bars).
For observational learning, observer females were placed in a small, clear plastic viewing chamber (11.5 x 15 x 18 cm) with a slotted lid to aid in transmission of the acoustic tone. The females’ observation chamber was mounted adjacent to the camera allowing the female to view the entire FC chamber without blocking the camera (Fig. 3.2).

Figure 3.2. Diagram of the observational chamber’s position in relation to the camera and the fear conditioning chamber.

(c) Unconditioned Responses and Escape Strategies in Zebra Finches

Prior to initiating my experiments, I used 5 male zebra finches and the “direct conditioning” protocol described below to determine the URs and CRs displayed by zebra finches in this fear chamber. Associative learning in the standard fear chamber has been characterized by the freezing or tonic immobility that occurs at the onset of the CS in rodents [264-267], pigeons [286-288], and chicks [273-275]. For the free flying zebra finch, I developed
novel dependent measures of associative learning. Shock elicited flight (UR) and the pairing of shock and tone (US-CS) came to elicit two main response strategies (CRs). Either, the bird would take flight at the beginning of the tone and attempt to remain in flight for the 5s duration of the tone, or the bird would wait until just before or right at the onset of the shock to take flight and attempt to remain aloft until the tone ceased. To capture this variation in CRs, I measured two dependent variables. The total time the bird remained in flight (> 2cm off the cage floor) during a trial, “flight response duration”, and the time between the onset of the tone and the start of flight, “flight response latency”. I similarly designed my “probes”, which confirm associative learning, to account for alternative CRs. The “CS-only probe trial” was a single 5s CS-only presentation. If a bird’s strategy involved taking flight at the beginning of or within the first 2s of the tone onset, conditioning would be confirmed by flight response latencies that were below 2s and lower than unconditioned birds. In the “CS-US probe trial”, 2s after the onset of a 5s CS, a 1 s shock was delivered. If a bird’s strategy involved waiting for the beginning of the shock to take flight, conditioning would be confirmed by flight response latencies lower than unconditioned birds and close to, but below, 2s to avoid the shock.

(d) Direct Conditioning

Before training began each day, birds were placed in the fear chamber and given 120s to habituate. Experimental males (n = 8) received 1 block of 5 paired CS-US trials per day for 11 days (a total of 55 trials). The CS (tone) duration was 5 s. The onset of the shock occurred 2s after the initiation of the tone (the interstimulus interval) and was sustained for 3s with the tone and shock co-terminating. Control males (n = 8) were treated similarly but were exposed to the CS only. The intertrial interval during training varied randomly between 60-120s with intermediate intervals of 80 and 100s. This conditioning protocol is effective in chickens [273-
Following conditioning trials, all males were run in the 2 different types of probe trials to determine if learning had occurred. Probes were separated by 30s.

(e) Observational Conditioning

Before testing, male direct learners (i.e. demonstrators) and female observers were randomly paired and pairs were assigned at random to either the threat response observer group (n = 8) or no threat response naïve group (n = 8). Females were placed in the observation viewing chamber at the same time the male was placed in the fear chamber, and were given 120s to habituate. The females then watched their assigned male as he underwent 11 days of either CS-US paired trials (observers) or CS alone trials (naïves) as described in the Direct Conditioning section.

I looked at the effect of observational learning in two ways: by conducting the two probe trial types and by using measuring learning savings. Probes were conducted within 2hrs of viewing the last male trial. The following day all females began direct conditioning trials identical to those of the experimental males (5trials/day) except for 5 days only. The following day, the 6th day, all females were placed in the chamber and the two probe trials were repeated. Given that the direct learning of males ran concurrently with observational learning by females, I did not design an unpaired shock-tone group. What observer females would learn from watching males react to the shock alone is unclear and could obviously influence female behavior in the probes and direct learning trials in unpredictable ways.

(f) Analysis

Qualitative observations on the type of escape strategy being employed by conditioned subjects are reported. To analyze quantitative data, flight response duration and flight response latency for the 5 daily trials were averaged for each bird. If the bird did not respond with flight >
2cm off the floor following the tone, the response latency was recorded as 5s. To analyze learning in CS-US paired males, I used a one-way repeated measure ANOVA across days followed by a test for a linear contrast when there were significant day effects. Differences between CS-US males and CS only males, which rarely flew during training or on the CS-only probe trial, were so extreme that qualitative rather statistical analyses were used to describe group differences. Differences between the control and experimental males on CS-US probe trial were analyzed using a t-test.

Differences between observer and naïve females on probes prior to direct training were analyzed using t-tests along with performing one-sample t-test to compare responses in CS-US probe in relation to shock onset times. Learning savings was examined using a two-way repeated-measures ANOVA (days x treatment) with Greenhouse-Geisser corrections as appropriate. If both groups combined showed day effect, then each groups’ day effects were analyzed using a one-way repeated measures ANOVAs followed by linear contrasts. Performance on post-direct training probes was analyzed as for other probes. All statistical tests were conducted using SPSS 22 (IBM Corp., Armonk, NY, USA) and differences were considered significant at an α level of 0.05. I report means and standard errors as mean ± SE.

3. RESULTS

(a) Direct Learning Results

Fear conditioning was evident in experimental males. Across days, flight duration increased \((F(10, 70) = 8.40, p < 0.001, \text{ linear contrast: } F(1, 7) = 18.80, p < 0.01)\) and latency to respond decreased \((F(10, 70) = 3.08, p < 0.01, \text{ linear contrast: } F(1, 7) = 9.301, p < 0.02; \text{ Fig.3. 3})\). As would be expected, control males showed no evidence of conditioning. Flight duration did not vary across conditioning blocks and latency to respond did not vary across days in a linear
manner ($F(10, 70) = 3.13, p = 0.002$, linear contrast: $F(1, 7) = 0.02, p = 0.92$; Fig. 3.3). The CS alone did not produce threat responses; only one control male responded to the CS on the first day of testing and this male responded on just one trial. Collectively, the control males only took flight on 23 out of the 440 total trials (0.05%). No single male took flight more than 6 times, flights were of short ($1.63s \pm 0.31$), and flight did not immediately follow the onset of the tone ($3.11s \pm 0.32$).

On the post-direct training CS-only probe, only experimental males responded to the tone with a flight response (duration: $1.28s \pm 0.47$; latency $2.02s \pm 0.68$; Fig. 3.4) demonstrating that only the CS-US paired group was conditioned to respond to the CS. On the CS-US probe, the experimental males remained in flight longer than the controls suggesting prior knowledge about the typical length of the shock ($t(14) = 2.37, p < 0.03$), but the latency to flight response was not different between the groups ($t(7.23) = 1.53, p = 0.17$; Fig. 3.5) verifying that the US was capable of eliciting a rapid defensive response without previous association.
Figure 3.3. Mean (±SE) flight duration and latency to response across trial blocks by group. The treatment group received tone-shock pairings while the control group received CS only trials.
Figure 3.4. Mean (±SE) flight duration and latency to response by group in males post-conditioning on the CS-only probe (probe 1).
Figure 3.5. Mean (±SE) flight duration and latency to response by group in males post-conditioning on the CS-US probe (probe 2). The dashed line indicates the delivery time of the 1 sec shock. * Significantly different than the control.
(b) Observational Learning Results

None of the females showed a flight response during the pre-direct training, CS-only probe. On the CS-US probe, all birds except two naïve birds responded to the shock. While observers did not differ from naïves in flight duration \( (t(14) = 0.79, p > 0.46) \) or latency \( (t(9.62) = 1.62, p = 0.13; \) Fig. 3.6); however, the observers but not the naïves performed a flight response before the onset of the shock suggesting prior learning by observers \( (t(7) = 1.83, p = 0.055) \), but not naïves \( (t(7) = 0.961, p = 0.18) \).

During direct training, observer females responded to the tone significantly faster \( (F(1, 14) = 9.33, p < 0.01) \) and had flight duration times that were marginally longer than the naïve females \( (F(1, 14) = 3.90, p < 0.07; \) Fig. 3.7). There was a significant day effect for females for both flight duration \( (F(4, 56) = 8.15, p < 0.0001) \) and flight latency \( (F(4, 56) = 13.91, p < 0.0001) \); with each group demonstrating a reduction in latency to response (observers – \( F(4, 28) = 8.14, p < 0.001 \), linear contrast: \( F(1, 7) = 10.91, p = 0.013 \); naïves – \( F(4, 28) = 5.96, p = 0.001 \), linear contrast: \( F(1, 7) = 22.24, p = 0.002 \) and an increase in flight response duration (observers – \( F(4, 28) = 2.98, p = 0.036 \), linear contrast: \( F(1, 7) = 5.52, p = 0.05 \); naïves – \( F(4, 28) = 6.08, p = 0.001 \), linear contrast: \( F(1, 7) = 19.75, p = 0.003 \); Fig. 3.7).

On post-direct training CS-only probe, there was no significant difference between the groups in flight response duration or latency to response between the groups (Fig. 3.8). On the subsequent CS-US probe, observer females had significantly faster flight responses to the tone compared to naïve females \( (t(14) = 2.35, p < 0.03) \), but there was no difference in flight response duration (Fig. 3.9).
Figure 3.6. Mean (±SE) flight duration and latency to response by group in females preconditioning on the CS-US probe (probe 2). The dashed line indicates the delivery time of the 1 sec shock.
Figure 3.7. Mean (±SE) flight duration and latency to response across trial blocks by group. Observers viewed the males during fear conditioning (tone-shock pairings) while naïves viewed males that received CS-only trials.
Figure 3.8. Mean (±SE) flight duration and latency to response by group in females post-conditioning on the CS-only probe (probe 1).
Figure 3.9. Mean (±SE) flight duration and latency to response by group in females post-conditioning on the short-shock probe (probe 2). The dashed line indicates the delivery time of the 1 sec shock. * Significantly different than the naïves.
(c) Qualitative Analysis of Escape Strategies

Observed threat responses in males and females (male = 8; female = 16) during fear conditioning are described here. By day 2 of direct training, approximately 70% of birds would take flight at the beginning of the tone (CS) and attempt to remain in flight for the whole 5s duration of the tone. Approximately 13% of birds waited for 1.5s before taking flight. The remaining 17% of birds would wait until the onset of the shock before taking flight. Near equal portions of males and females used each strategy.

4. DISCUSSION

My results show that zebra finches readily acquire an association between a tone and a shock via direct fear conditioning training. Additionally, prior observation of a conspecific undergoing conditioning confers an advantage to the observer which results in a savings in learning. This study demonstrates the presence of direct learning of emotional CS-US association in the zebra finch. Previous studies in chickens have yielded equivocal results [273-275]; however, ours is the first, to my knowledge, to examine this type of learning in a species with a flight response and the first to demonstrate observational conditioning in the zebra finch. My study is unique in examining this effect as a majority of the past studies conducted on avian species focus on mobbing behavior in which the conspecific acting as a learning model (or demonstrator) and the observer are engaging in the fear behavior concurrently and therefore may reflect contagion or social facilitation [157-159, 278]. In my study the observer must learn and store the association and use it later in the absence of the demonstrator ensuring this paradigm is examining observational conditioning.

Overall, the results of direct conditioning in the zebra finch are consistent with those seen in cued FC in rodents. Similar to time freezing in rats and mice, flight response duration
increased as learning occurred, and this response began to occur before the US presentation [292]. Likewise, females showed increases in flight response duration across days, but the two groups only differed in flight response latency with the observer females having a significantly shorter latency than the naïve females. The lack of difference in flight duration may reflect the physical limitation of the birds and therefore, latency may be the best measurement for analysis. However, I suggest that the measurement of flight response duration may not be as useful as an indicator of learning as the measurement of latency to response. Flight response duration is contingent on the bird’s physical ability to maintain hovering flight within the confines of the box. Most of my subjects were simply not capable of sustained flight for the whole 3 s of the US presentation. In contrast, latency to response is not limited by the bird’s physical ability and a latency and a latency faster than the interstimulus interval clearly indicates the bird has learned to anticipate the foot shock after hearing the tone begin. Additionally, the zebra finch has been shown to not engage in early flushing (i.e. to fly from cover) in response to a fearful stimulus (i.e. a human) [293]. These factors support why, unlike rodent studies where percentage of time engaged in defensive freezing is the most meaningful (and reported) measure, response latency might be more important in flighted responses.

Lastly, this study describes how to adapt a fear chamber for birds and a protocol for training that produces strong and consistent results. I showed that fear conditioning is possible in the finch and ways to quantify a flighted threat response. The procedures described here may prove useful for several different kinds of neurobiological and comparative studies. Already, these techniques have been used in studies of cerebellar function (Chapter 4) and in a study examining the effect of adrenergic receptor antagonists on fear memories (Webb, Hribar & Day, 2015; data yet unpublished). The development of similar paradigms for mammals and birds is
essential for optimal cross-taxa comparison. The methodology described here may allow for a comparison of the neural mechanisms and brain regions involved in emotional associative learning and observational conditioning between mammals and birds, and allow a better understanding of how the brain evolved to support this essential type of learning.
CHAPTER 4: CEREBELLAR INVOLVEMENT IN CLASSICAL
FEAR CONDITIONING OF ZEBRA FINCHES

FOREWARD

My first fear conditioning study involved an examination of the observational learning abilities of the zebra finch in a fear conditioning task (observational conditioning; Chapter 3). I predicted that the zebra finch would be capable of acquiring the tone-shock association from viewing conspecific undergoing direct conditioning. Although acquisition was not found on the pre-training probe trials, a significant savings in learning was shown. In this chapter, I investigated whether lesions to deep cerebellar nuclei, the lateral nuclei, would impair fear conditioning acquisition. To examine the effect of cerebellar damage on observational learning, one must first show that cerebellar damage blocks direct learning of the task. If cerebellar deactivation blocks instead of impairs learning acquisition, then any learning prior to lesioning cannot not be confound with any learning that occurs post-lesion. I predicted that cerebellar lesioning would create significant deficits in fear conditioning thereby allowing me to examine the role of the cerebellum in observational conditioning acquisition.

ABSTRACT

Fear conditioning has yielded considerable data on the brain structures and systems involved in emotional associative learning. The limbic system has traditionally been implicated as the main brain regions involved in fear conditioning; however, mounting evidence indicates the cerebellum may play a pivotal role in the acquisition of emotional associations. Using a standard fear conditioning chamber adapted for a passerine bird, I tested whether bilateral
ablation of the lateral deep cerebellar nuclei would affect the acquisition of fear conditioning to an acoustic stimulus in the zebra finch. I found that cerebellar lesions did not cause any deficits in fear conditioning acquisition. Lesioned birds performed similarly to the sham control birds across training days in both flight response duration and latency to flight response. To my knowledge, this is the first study to examine the role of the cerebellum in avian fear conditioning.

1. BACKGROUND

In classical fear conditioning (FC), a neutral stimulus such as a tone, light or context (conditioned stimulus; CS) is repeatedly paired with an aversive stimulus like a foot shock (unconditioned stimulus; US) until the US reliably elicits a specific behavioral response (conditioned response; CR) [17, 294]. FC has yielded considerable data on the brain structures and systems involved in emotional associative learning. The limbic system, especially the amygdala and hippocampus, have traditionally been implicated as the main brain regions involved cued (or delayed) FC and contextual FC, respectively [17, 294-296]. However, mounting evidence indicates another brain region, the cerebellum, may serve a pivotal role in FC [297-301].

The cerebellum (CB) has also been shown to be essential for the consolidation of fear responses to acoustic stimuli and context in rats [298, 299]. Two CB regions, the vermis (VE) and the interpositius nucleus (CBi), are both necessary for consolidation; however, their role in memory formation differs. The CBi is involved in the memorization of the freezing response to an acoustic CS whereas the VE is involved in the memory formation of the freezing response to both the acoustic CS and to context. Additionally, these regions’ role in the consolidation of fear responses may extend as long as those of the limbic system [298, 299]. When reversible inactivation of either the CBi or the VE by tetrodotoxin (TTX) was administered in the rat at
increasing post-acquisition delays (i.e. at different points during consolidation), data indicated that functional integrity of both CB regions for at least 8 days post-acquisition is required for consolidation of the cued and contextual fear memory traces[298]. This is similar in function integrity duration found in the basolateral amygdala, dorsal hippocampus and the perirhinal cortex [17, 294, 295, 302, 303], and may suggest that the CB is involved in memory trace storage like the amygdala and perirhinal cortex [303, 304].

While the mammalian cerebellum is slightly more elaborate than the avian cerebellum, the underlying microcircuitry and anatomy are highly conserved [29]. In comparison to the 4 deep cerebellar nuclei found in mammals, the avian cerebellum contains 3 nuclei: the lateral, the medial, and the vestibular nuclei [207]. Due to similarities in projections to midbrain and forebrain structures, it is thought that the lateral cerebellar nuclei (CBl) are homologous to the mammalian dentate while the medial nuclei are the presumed homolog and fused combination of the mammalian fastigal and interpositus nuclei [305-309]. The vestibular nuclei are the most conserved nuclei across taxa [206]. While studies using neuroanatomical tracing methods have found similarities in projection pathways between mammals and birds, how conserved the nuclei and their projection pathways are in their contribution to cognitive processes remains unclear.

Although the pigeon was among the first species used to explore cerebellar function in the early 1800s [31, 32], avian studies of cerebellar function, particularly in cognitive processes, have since been significantly overshadowed by mammalian studies. Only in the past half-decade have investigations into cerebellar cognitive functions in birds been conducted [209-211, 310, 311]. These studies have shown that cerebellar lesions in birds create deficits in motor, match-to-sample, spatial and vocal learning [209-211, 310, 311]. These studies provided the evidence of a
role for the avian cerebellum in cognition; however, the complete cognitive functions of the cerebellum have yet to be determined.

In the present study, my aim was to investigate the role of the avian cerebellum in aversive associative learning, more specifically whether bilateral ablation of the CBl would prevent the acquisition of FC. This was a necessary immediate step toward examining the role of the CB in observational conditioning because in order to be able to determine if conditioning was influenced by prior observation, it is necessary to block learning during actual FC trial performances. This is particularly important in this task because FC is acquired rapidly through direct conditioning (Chapter 3). If acquisition is possible despite CBl lesioning, it would be difficult to ascertain whether the CB has a significant role in observational conditioning (see Table 4.1). Additionally, I had to ensure that the birds maintained intact flight response abilities.
<table>
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<td>No Deficits in Training/ Learning</td>
<td>CB required for procedural acquisition via observation</td>
<td>Acquisition occurred during the observation period before lesioning</td>
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<tr>
<td>Lesion – Observation – Training</td>
<td>Deficits in Training/ Learning</td>
<td>CB required for procedural acquisition via observation</td>
<td>Lesioning before the observation period prevented acquisition during observation; if no deficit was seen, it would difficult to determine if learning occurred during observation or training</td>
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<tr>
<td>Lesion – Training</td>
<td>Deficits in Training/ Learning</td>
<td>CB required for procedural acquisition</td>
<td>Lesioning before training prevented acquisition during training; this is a necessary condition needed for comparison with the first group to ensure acquisition occurred only during observation and not training</td>
</tr>
<tr>
<td>Training – Lesion</td>
<td>No Deficits in Training/ Learning</td>
<td>CB required for procedural acquisition; CB is not needed for motor behavior</td>
<td>Acquisition occurred during training before lesioning and lesioning does not impair the motor response</td>
</tr>
<tr>
<td>Control</td>
<td>No Deficits in Training/ Learning</td>
<td>CB required for procedural acquisition</td>
<td>Acquisition occurred during training</td>
</tr>
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</table>

**Table 4.1.** A comparison of cerebellar lesion timing in relation to observation and training on training deficits. It is important to note that only by comparing the “lesion-training” group with the “observation-lesion-training” group can the role of the CB in observational learning of a task be ascertained.

### 2. METHODS

(a) **Animals and Treatment**

Adult male zebra finches (N = 16) of similar age were obtained from a professional vendor and kept in the university aviary on a 13:11 light:dark photoperiod at an ambient temperature of 21-24°C for 3 weeks prior to surgery. Seed (Kaytee; Chilton, WI, USA) and water were provided *ad libitum*. The birds were randomly assigned to 2 treatment groups: a sham control group (n = 8) or a CB lesioned group (n = 8). These groups allowed for the examination
of effects of lateral CB nuclei lesioning on FC learning. All procedures were approved by the University of Mississippi IACUC (protocol #15-008).

(b) Surgical Procedures

Surgeries were performed 24hr prior to the first fear conditioning trial. Birds were given a brief dose of isoflurane gas then injected with 30 µl of equithesin to initiate light anesthesia and allow for positioning in a stereotaxic device (Kopf with small bird beak holder; David Kopf Instruments, Tujunga, CA). While in the device, a deep plane of anesthesia was achieved and maintained with a mix of isoflurane and oxygen delivered through a tube inserted into the bird’s mouth [210, 211]. A craniotomy was performed over the cerebellum. The midpoint of the caudal border of the central sinus was used as the zero for the coordinate system. This zero point corresponds to brain atlases created for the zebra finch [312]. We made bilateral mechanical lesions to the cerebellum with a 26 gage needle inserted into the brain, held in place for 1 min and then slowly withdrawn [209]. Lesion coordinates were L.M. ±1.1, R.C. -2.7, D.V. -4.5. Sham controls underwent the same treatment as the CB lesioned group except the needle was not inserted into the brain.

(c) Apparatus

We used the same apparatus as in my previous studies of direct and observational learning of fear conditioning as this apparatus was shown to be effective in zebra finch fear conditioning in a previous study (see Chapter 3).

(d) Conditioning Training and Probe Trial

Protocols were the same as for direct conditioning in Chapter 3 with the exception that the birds received 1 block of 5 CS-US pairings per day for 5 days (a total of 25 trials). This protocol was shown to be effective in chickens [273-275] and zebra finches (see Chapter 3). In
the zebra finch, it was effective for both females who had viewed a male demonstrator receiving CS-US pairings and in females who observed a male receiving CS only trial (5/d x 10d). On the 6th day, all birds were placed in the chamber and presented with the 2 different types of probe trials, the CS-US probe trial and CS-only probe trial.

All measurements of conditioning were the same as for Chapter 3, in brief, birds’ responses to the tone were video recorded by a video camera mounted in front of the chamber and the footage was relayed to an image analyzer (Ethovision; Noldus Information Technology, Wageningen, The Netherlands). Flight response duration and flight response latency following tone onset were calculated by the software and used for analysis.

(e) Histology

Two days after the probe trials, the CB lesioned males were sacrificed by isoflurane overdose and brains were fast frozen on dry ice and stored at -80°C. The CB was sliced coronally on a cryostat (40 μm) and mounted directly onto slides. Slides were Nissl-stained with cresyl violet to allow for lesion confirmation.

(f) Analysis

Three subjects (1 CB lesioned bird and 2 control birds) were unable to take or maintain flight thereby prohibiting flight response analysis by the software. For these birds, the trial videos were scored by experimenters viewing video footage to detect the onset of a behavioral response (i.e. repeated hopping or attempted flight) and the duration of this response. Videos were scored by two independent individuals and the correlation between scoring was significant ($r (81) = 0.99, p < 0.01$). Latency to response was averaged between scorers and included in the group analysis but these 3 birds’ scores were eliminated from the flight duration analysis.
Response duration and latency to respond to the tone were averaged across the five trials for each bird each day. Differences in learning curves between lesion and control groups were analyzed using two-way repeated-measures ANOVAs (trial blocks x treatment) followed by a test for a linear contrast when there were significant day effects, and differences in probe trials were analyzed using a paired t-test. Greenhouse-Geisser correction was used when appropriate. All statistical tests were conducted using SPSS 22 and differences were considered significant at an $\alpha$ level of 0.05.

3. RESULTS

(a) Lesion confirmation

The lesions hit the CBl at least unilaterally on 5 of the 8 subjects. White matter superficial to or surrounding CBl was hit on all other lesions thus connectivity with CBl was damaged in all subjects (Fig. 4.1).

Figure 4.1. Photomicrograph of nissl-stained brain tissue showing the lesions to the CBl. Black arrows point to the position of the lesion.
(b) Behavioral Results

No significant differences were observed in flight response duration or latency to response between the sham control and CB-lesioned birds (duration: $F(1, 11) = 1.03, p = 0.33$; latency: $F(1, 11) = 0.0001, p = 0.98$). Both groups increased (duration: $F(4,44) = 3.43, p = 0.02$; linear contrast: $F(1, 11) = 13.69, p = 0.004$) and decreased latency ( $F(2, 32) = 3.39, p = 0.01$; linear contrast: $F(1, 11) = 8.57, p = 0.01$) indicating learning (Fig. 4.2). Follow-up pairwise comparisons with Bonferroni corrections for multiple comparisons showed that flight response duration was significantly higher on days 4 and 5 than on day 1 ($p = 0.009; p = 0.006$), and latency to response was significantly lower on days 4 and 5 than day 1 ($p = 0.001; p = 0.005$). This implies that about 15 paired stimuli were sufficient for the majority of birds to acquire the fear response. There was no difference in flight response duration or latency to respond between the treatment groups on probe trial1 (Fig. 4.3); however, the latency to response of the CB lesioned birds was faster on probe 2 than the sham control birds ($t(14) = 2.12, p = 0.05$; Fig. 4.4) while duration did not differ between the groups.
Figure 4.2. Mean (±SE) flight duration and latency to response across trial blocks by group.
Figure 4.3. Mean (±SE) flight duration and latency to response by group in males post-conditioning on the CS-only probe (probe 1).
Figure 4.4. Mean (±SE) flight duration and latency to response by group in males post-conditioning on the CS-US probe (probe 2). The dashed line indicates the delivery time of the 1 sec shock. * Significantly different than the control.
4. DISCUSSION

Birds were able to learn the CS-US association after CBl lesion at levels similar to sham control birds. This indicates that functional integrity of the avian CBl is not necessary for the acquisition of fear-related behaviors. However, lesioned birds displayed a faster latency to response on the CS-US probe (probe 2) which may indicate that lesioning has an effect on extinction rates, with CBl lesions inhibiting extinction at the same rate as non-lesioned birds. My results suggest that at least the avian CBl does not have a role in fear memory consolidation or in the performance of the avian flight response.

The placement of the lesion may be responsible for the lack of effect seen in this experiment. I chose to lesion the CBl because of its connections with higher cognitive and motor brain regions [305-309]. However, in studies on the rat, lesions to the interpositus nuclei were shown to create deficits in FC memory consolidation (although dentate lesioning was not conducted) [298, 299]. Perhaps lesions to the medial nuclei of the CB would impair avian FC. Future studies examining the role of the medial nuclei should be conducted using the protocols outlined in this study. This would potentially allow for investigation into the role of the avian CB in observational conditioning; however studies would first need to be conducted to ensure the medial nuclei are not a memory trace storage site. If the medial nuclei are involved in the storage of the memory similar to that proposed for the interpositus nuclei of the rat [298, 299], lesioning after observational conditioning may erase the previously acquired memory making the link between the CB and observational conditioning impossible to determine.

Some other possible explanations for the failure of my lesions to produce any deficits in conditioning may be contributed to issues in the protocol. Mammalian studies have shown that
the CB is only involved in classical conditioning under certain constraints [17, 313-317]. One constraint is the interstimulus interval (i.e. the time between the CS and the US; ISI). Studies in rabbits and rats have shown that CB involvement occurs only in protocols with relatively short ISIs of under 5s [17, 313-315]. A second constraint is the complexity of the behavioral response. Results in mammalian studies suggest that the CB is involved only in associative learning tasks where simple responses (e.g. reflex reactions) are conditioned [316]. Lastly, the CB has been shown to be differentially involved in aversive and appetitive conditioning. In rabbits and rats, lesions to the CB interrupted aversive conditioning (e.g. eyeblink conditioning or tone-shock pairing), but did not disrupt appetitive conditioning (e.g. jaw movement conditioning with juice or tone-food pairing) [315, 317]. While these constraints were considered in the development of my protocol and steps were taken to forstall any issues based on these constraints, it is possible that the factors that influence CB involvement in classical conditioning vary by taxa (e.g. mammals versus birds). Future studies examining possible taxonomic differences in these constraints may reveal how evolutionary conserved these pathways are in cognition.
CHAPTER 5: EXAMINING DIRECT AND OBSERVATIONAL LEARNING OF A NOVEL SPATIAL MAZE IN THE ZEBRA FINCH

FOREWORD

In Chapters 2 and 3, I showed that the zebra finches are capable of observational conditioning and learning via stimulus enhancement. In this Chapter, I examined whether zebra finches were capable of mindful imitation in a spatial maze task. Mindful imitation is one of the most cognitively demanding forms of observational learning, demanding the individual recognize and encode the demonstrator’s behavior and intentions so they can reproduce the behavior and achieve the same goal as the demonstrator in the future [48]. To date, mindful imitation of spatial information has only been demonstrated in one non-human animal, the rat [18-20, 37, 212]. I predicted that zebra finch performance of a spatial maze task would be enhanced by prior observation of a conspecific learning and successfully navigating the maze. The results of this study will be submitted to the Journal of Experimental Psychology.

ABSTRACT

While several maze types and tasks have been developed to examine spatial learning and memory in non-food-caching birds, one fundamental downfall emerges – a majority of these mazes and tasks employ modifications such as feeders that may confound interpretations of the data. Here we describe the development of a Morris water maze (MWM)-analogue for the zebra finch (Taeniopygia guttata) which like the MWM contains no proximal, spatially-contiguous cues. The birds, which were released from different starting locations within the maze, had to
locate the maze exit to escape a hot floor using only extra-maze cues positioned around the arena. In a series of three experiments, we examined direct and observational learning of the maze analogue. Data showed that the zebra finches were adept at learning the task under direct training protocols, but prior observation of a conspecific learning and successfully navigating the maze did not have an effect on post-observational maze performance.

1. BACKGROUND

Most animals live in environments in which resources are not uniformly distributed. Therefore, the ability to acquire and retain pertinent information the environment may strongly affect their fitness and influence the evolution of learning. One type of learning heavily affected would be spatial learning. While there is significant debate over the definitions of spatial learning, for the purposes of this study, I will define spatial learning is the ability to use distal cues, as opposed to local cues, to successfully navigate to a target or goal. I refer to distal cues as stimuli not spatially contiguous with the target and local cues as those which are spatially contiguous with the goal [318].

The first experiments of spatial learning where conducted using rodents since spatial navigation tasks were naturalistic and easy for them to acquire [319-321]. This research led to numerous paradigms based on the premise of having an animal either learns to locate a particular goal or locate a target area to avoid an aversive stimulus. Various mazes have been developed to test spatial abilities, including the starburst maze, the spiral Battig maze, radial mazes, open-area mazes, runway mazes, and water mazes. While each of these mazes have made unique contributions to spatial learning research, the two main mazes used to assess spatial learning and memory are the radial arm mazes and the Morris water maze (MWM).
These two main mazes developed initially for mammals have been adapted for birds [322, 323]; however, design flaws make it difficult to determine which strategies are being employed by the subjects. Two so-called radial maze analogues, based on the Olton-type radial maze have been developed [322-324]. Adaptations include in having to learn the location of baited versus non-baited feeders but the feeders are presented in an open space instead of having the bird walk down narrow arms (making this task arguably more of an open field task than a radial maze) [322] and upscaling the original maze design so birds may walk or fly through the radial tunnels to reach feeders at the end of the arms [323]. These radial arm-like mazes have a significant downfall in testing spatial cognition. Due to the regular geometry of the apparatus, the task can be solved successfully by repeating a definite egocentrically oriented response. Another type of open field maze was developed for testing spatial cognition where one baited and three empty feeders are placed in an aviary. The birds are then released from different starting points and have to use maze cues to find the feeder containing the food reward [324]. The authors claim this maze is similar to the MWM, but unlike the MWM, this maze is square, has only four possible goal positions (the MWM has numerous) and has a visible target location. The issue with the aforementioned tasks adapted for avian use is they contain goals that are clearly identifiable or an arena that is non-homogeneous and therefore it is difficult, if not impossible, to determine if the subjects are using local cues, egocentric or taxis strategies, or spatial memory.

In order to test avian spatial learning and memory in the zebra finch, my lab developed a task analogous to the MWM, called the Day Escape Maze (DEM). The DEM consists of a clear cylinder with a hole cut into the side and a removable lid. Since the escape hole is not visible to the finch, the arena is homogeneous, and they location of the escape hole can be changed to several positions within the room, the DEM is closely in line with the MWM. The efficacy of the
MWM is due in large part to the water acting as a negative reinforcer and therefore motivating the subject to seek out and learn an escape strategy [225, 325]. In pilot studies, low levels of water resulted in the finches bathing and higher levels of water resulted in floating and cold stress as is sometimes seen in mice [326]. Replacing the water with ice also failed to be effective in motivating the finches. Finally, heat was tested as a motivator since it has been shown to be effective in both MWM and Barnes maze analogs in insects [226, 327-329]. Heat was successful in motivating the finches to locate the escape hole in the maze.

There is evidence that spatial tasks can be learned not only by actual execution of the task but also via observation of a demonstrator as they execute the task. This occurs via mindful imitation where the observer must recognize and encode the demonstrator’s behavior and intentions for the behavior (i.e. the goal) so they may reproduce the demonstrator’s behavior and achieve the same goal [47, 48]. For example, rats suspended in an observer chamber over a MWM and allowed to watch 200 trials performed by a companion rat significantly outperformed their naïve counterparts in learning of the task (as indicated by significantly faster escape latencies and significantly less distance traveled within the maze) [20]. While mindful imitation has been demonstrated in birds [189], their ability to acquire a spatial information via observation has not been tested.

In the present study, I aimed to investigate whether the zebra finch could learn and successfully navigate a maze that lacked proximal cues or cues that were spatially-contiguous with the goal. For this study, I used a novel MWM-analog. In addition, I examined whether prior observation of conspecifics learning and successfully completing the task would have a subsequent effect on maze performance, indicating the task could be acquired through observational learning.
2. METHODS

(a) Animals and Treatment

We used experimentally naïve males and females (6 – 12 months) age-matched within each experiment. Birds were housed in cages of 6-10 same-sex individuals on a 14:10 light:dark photoperiod at an ambient temperature of 21-24°C. Seed and water were provided ad libitum. All housing protocols and procedures performed in these experiments approved by the University of Mississippi IACUC (protocol #10-025).

(b) Apparatus

The DEM consisted of a clear cylinder (30cm in diameter and 15.2cm tall), made from extruded Plexiglas with a 5.4cm diameter escape hole cut 7 cm above the hotplate and a clear Plexiglas lid (Fig. 5.1). The floor of the maze was a ceramic tile heated uniformly by an electric hot plate maintained at ~50°C. The escape maze was elevated to raise it from the floor and bring it closer to the camera. The maze was placed within a flight cage (148.6 x 71.1 x 188.2cm) lined with black cloth so no external light, objects or the experimenters could be seen by the birds while in the maze. Four cues were attached to the black lining cloth ~10cm from the maze bottom at artificial compass points designated as north, south, east and west. These artificial compass points were used to divide the maze arena into 4 quadrants (northeast, northwest, southeast, and southwest). The escape hole was oriented to be in the northeast quadrant in all experiments. Two perches were attached to the flight cage 25cm from the cage top and on opposite ends equidistant from the wall. These perches were provided to allow the zebra finch to rest comfortably after escaping the maze. A camera and the observation deck (used to house observers while viewing conspecifics in the maze) were secured to the top of the aviary with the camera directly over the maze.
Figure 5.1. Cross-sectional diagram of the spatial maze and its position in the aviary.
(c) General Methods

During direct training, all birds completed blocks of four trials per day (the number of blocks varied by experiment). In all experiments, birds were released facing the outer wall at artificial cardinal points labeled north, south, east, and west centered on the 4 maze quadrants. Each release point was used in random order across each block of four trials, but was the same order for each bird on that day. Birds were allowed a maximum of 120s to locate the escape hole and 60s of rest upon escape. If the bird was unable to locate the escape hole within 120s, latency was recorded as 120s, and the bird was gently guided toward the escape. The bird was then returned to a holding cage where they were individually housed between trials. All subjects in the group completed trial 1 before the second set began, and this cycle continued for the entire four-trial block. The intertrial interval for each bird was approximately 10-15 min. The paths taken by the birds within the maze were video recorded by a camera mounted on the ceiling and relayed to an image analyzer (Ethovision; Noldus Information Technology, Wageningen, The Netherlands). Three dependent measures were recorded: escape latency (s), distance traveled (cm), and velocity (m/s).

Following direct training, a 120s probe trial (transfer test) was conducted to confirm learning. Probe trial procedures varied slightly by experiment and are described for each experiment. For analysis, only the first 30s of the probe trial was used since it was observed that past 30s the birds began frantically and aimlessly searching for a new escape.

Observers were suspended in small cages above the spatial maze either individually (Exp. 1) or in a group (Exp. 2 and 3). In all the experiments, all sides of the observation cage were opaque except the floor which was a metallic grid. This ensured that the female would not
be distracted by each other or by other stimuli in the aviary. Through the grid, the females watched as male demonstrators underwent direct learning of the maze. Video of the females’ behavior was recorded in Exp. 1 and examined to ensure the females were attending to the males in the maze. All females were found to attend to the male by directing their gaze to the maze below them. The observation period and method of suspension varied slightly by experiment and is fully describe within each experiment.

(d) Analysis

For direct and observational learning, average latency to escape, distance traveled, and velocity were averaged across trials for each bird each day. One-way repeated measures ANOVAs or two-way repeated measures ANOVAs (trials blocks x treatment or gender) were used as appropriate. Post-hoc analyses were conducted using sequential Bonferroni correction.

For the probe trials, the amount of time spent and distance traveled in the cued quadrant (i.e. the quadrant that previously contained the escape hole) versus the average of the three uncued quadrants was transformed for analysis and distance data was corrected for velocity. T-tests or one-way ANOVAs were used as appropriate. In the case of non-normally distributed data, Wilcoxon sign tests or Mann Whitney U tests were used.

All statistical tests were conducted using SPSS 22 for Windows, employing two-tailed tests of probability and an alpha level of 0.05.

3. EXPERIMENT 1

It was my aim to determine if the zebra finch was capable of learning the novel complex spatial task and if spatial learning could occur through observation. I tested this using the DEM, a task based on the classic MWM. In my maze, the birds were required to escape from a hot-
plate heated surface out of an escape hole using cues on walls. Observer birds watched as
demonstrators learned the task. In rats, observation prior to being placed in the MWM
significantly improves their performance [18-20, 35, 37], and thus, I expected the same positive
effect on performance in my observers.

(a) Direct and Observational Training

Eight male demonstrators and 16 females were selected at random from the university
aviary and were housed in groups of 8 same-sex individuals by group. Birds were run in two
batches consisting of 4 male demonstrators, 4 observer females and 4 naïve females. Females
were randomly assigned to either the observer or naïve treatment group. Each observer female
was paired with a demonstrator male for the duration of the observation period. During the
observation period, females were individually suspended directly over the spatial maze while
their paired male ran his direct trials. Each observer female viewed 4 direct trials a day for 5 days
(i.e. 20 trials). Naïve females were suspended in for an equated time but in an empty black-
clothed aviary. Following the observation period, all females underwent direct training in the
spatial maze for 4 trials a day over 4 days (16 trials).

Immediately following their respective direct training, males and females received a
probe trial in which the normal maze wall with an escape hole was replaced with a solid
cylindrical maze wall and the cues on the aviary wall were rotated 180°. The bird was given 120
sec to search for the escape after which the trial was terminated. Observer females did not view
male probe trials.
(b) Results and Discussion

Latency ($F(1, 10) = 66.79, p < 0.001$; Linear Contrast: $F(1, 7) = 108.51, p < 0.001$) and distance decreased across blocks ($F(2, 12) = 15, p = 0.001$; Linear Contrast: $F(1, 7) = 25.76, p = 0.01$) supporting learning of the task (Fig. 5.2) Velocity did not change across blocks suggesting latency differences are due to more efficient rather than faster escape. The proportion of total distance ($t(7) = 3.04, p = 0.02$) and total time ($t(7) = 2.78, p = 0.03$) spent in the previously cued quadrant was greater than the average of the other three quadrants, indicating males learned the location of the escape hole in relation to the spatial cues provided Fig. 5.3).

For females, latency and distance traveled decreased across blocks while velocity did not (Fig. 5.2). This was true for both Observers and Naïves (Observer distance: $F(3, 21) = 12.12, p < 0.001$, Linear Contrast: $F(1, 7) = 38.94, p < 0.001$; latency: $F(3, 21) = 50.68, p < 0.001$, Linear Contrast: $F(1, 7) = 131.86, p < 0.001$; Naïves distance: $F(3, 18) = 16.02, p < 0.001$, Linear Contrast: $F(1, 6) = 42.21, p = 0.001$; latency: $F(3, 18) = 33.68, p < 0.001$, Linear Contrast: $F(1, 6) = 63.63, p < 0.001$). Observers and Naïve learned to escape the maze with similar distance traveled ($F(1, 13) = 1.07, p = 0.32$), and latencies to escape ($F(1, 13) = 0.04, p = 0.84$), and had similar patterns of improvement across blocks for distance ($F(2, 21) = 0.96, p = 0.38$), and latency ($F(2, 24) = 0.56, p = 0.56$). On the probe trial, both groups traveled significantly more in the previously correct quadrant than in the average of the other three quadrants (observers: $Z = -2.10, p = 0.04$; naïves: $Z = -2.10, p = 0.04$; Fig. 5.3) and there was no effect of treatment ($U = 15, p = 0.15$). For latency, Observers spent significantly more time in the previously correct than in the average of the other three quadrants ($Z = -2.10, p = 0.04$) but the Naïves did not ($Z = -1.35, p = 0.18$); and the difference between the groups was not significant ($U = 12.5, p = 0.07$). Probe data indicates all females traveled significant more in the previously
**Figure 5.2.** The performance of males, observer females and naïve females during training trials for Experiment 1: A. distance traveled in the maze, B. latency to escape, and C. average speed (velocity) the bird traveled within the maze. (Data points are averages for blocks of four training trials. See text for results of statistical analysis.)
Figure 5.3. The performance of males, observer females and naïve females during probe trial for Experiment 1: A. portion of distance traveled in quadrants, and B. proportion of time spent in quadrants. *Significantly different from the average of other three quadrants. (See text for results of statistical analysis.)
correct than in the average of the other three quadrants indicating that maze learning had occurred but there was no difference between the Observers and Naïves. However, this was not true for latency, where the Naïves in contrast to the observers did not spend more time in the previously correct quadrant. Video analysis of traveling pathways implies this is probably because the naïve birds flew at the wall more often and their momentum propelled them into the adjacent quadrants.

The males and the naïve females had similar experience (i.e. no prior exposure to the maze or the cues) before undergoing direct learning and therefore, their performance in the maze could be compared to determine if gender differences exist in maze learning. Since there was also no effect in treatment between the observers and the naïve females, all the female data was combined and compared to the male’s maze performance. There was no significant interaction of trial-block with gender but there was a significant effect of gender on distance traveled within the maze. Specifically, females traveled a significantly shorter distance to find the escape hole ($F(1, 21) = 8.53, p = 0.008; \text{Fig. 5.2}$). There was no effect of gender on either escape latency ($F(1, 21) = 1.19, p = 0.29$). Comparison of probe trials showed that while within their groups, both the males and females traveled more and spent more time in the cued quadrant versus the average of the other three quadrants (Fig. 5.3), there was no difference in these measurements between the groups suggesting that females may be more efficient at escaping, but their spatial learning of the maze did not differ.

The results indicate that zebra finches are capable of learning the novel Escape Maze through direct training, and that gender differences are only in the distance traveled to escape (females travel a shorter distance) and not in the time spent escaping. There was no effect of
prior observation on direct learning which implies observational learning did not provide a savings in learning under this protocol.

4. EXPERIMENT 2

In the first experiment, the observer females only watched 20 trials performed by their demonstrator male. While 20 trials is sufficient to learn the maze via direct training in the zebra finch, the results of Exp 1 indicated that it was not effective in learning the maze via observation. Similarly in rats, approximately 20 trials are required for learning to occur in the MWM [225]. Studies showing observational learning in the rat used 200 conspecific demonstrator trials [18-20, 35, 37]. Thus, my use of 20 trials of observation in Exp 1 may have been insufficient for observational learning. Therefore, in Exp. 2, I used the same basic methods as in Exp. 1, but allowed the observers to view 200 demonstrator trials.

(a) Direct and Observational Training

Five male demonstrators and 12 females were selected at random from the university aviary and were housed in groups of 5 or 6 same-sex individuals by group. Females were randomly assigned to either the observer or naïve treatment group. For this experiment, a 6-individual observation deck was constructed so all the observer females were able to view all 5 males as they underwent direct training. Males received two 4-trial blocks per day, one in the morning (~9am) and another in the late afternoon (~3pm) for 5 days (40 trials total). Since the females viewed all 5 males, they viewed 200 trials over the 5 days. Naïve females were suspended for the same amount of time in the observation deck and allowed to view the maze and cues (with no demonstrator present). After the observation period, all females underwent direct training in the spatial maze for 4 trials a day over 4 days (16 trials).
Following their respective direct training, males and females received a probe trial in which the escape hole was removed by use of a solid cylindrical maze wall; however, in this probe, the cues were not rotated. Observer females did not view male probe trials.

(b) Results and Discussion

For the males, latency ($F (1, 5) = 9.22, p = 0.024$; Linear Contrast: $F(1, 4) = 9.59, p = 0.036$) and distance ($F (2, 12) = 6.70, p = 0.026$; Linear Contrast: $F(1, 4) = 16.67, p = 0.015$) decreased across blocks indicating learning of the task (Fig 5.4). Velocity did not change across blocks. On the probe trial, the proportion of total distance ($t(4) = 4.84, p = 0.008$) and total time spent ($t(4) = 6.25, p = 0.003$) in the previously cued quadrant was greater than the average of the other three quadrants, indicating males learned the location of the escape hole in relation to the spatial cues provided Fig. 5.5).

For the females, latency and distance traveled decreased across blocks while velocity did not (Fig. 5.4). This was true for both Observers and Naïves (Observers distance: $F (3, 15) = 25.13, p < 0.001$, Linear Contrast: $F (1, 7) = 20.18, p = 0.006$; latency: $F (3, 21) = 100.20, p < 0.001$, Linear Contrast: $F (1, 7) = 108.33, p < 0.001$; Naïves distance: $F (3, 15) = 5.91, p = 0.007$, Linear Contrast: $F (1, 5) = 4.86, p = 0.08$; latency: $F (3, 15) = 13.83, p < 0.001$, Linear Contrast: $F (1, 6) = 15.97, p = 0.01$). Surprisingly, there was trending main effect of treatment with the Naïves having a faster escape latency than the Observers (treatment: $F (1, 10) = 4.44, p = 0.06$; treatment x trial block: $F (2, 15) = 6.99, p = 0.01$). However, Observers and Naïves had similar patterns of improvement across blocks for distance traveled (treatment: $F (1, 10) = 1.44, p = 0.26$; treatment x trial block: $F (2, 13) = 3.69, p = 0.07$) or traveling velocity (treatment: $F (1, 10) = 0.041, p = 0.84$; treatment x trial block: $F (2, 21) = 1.44, p = 0.34$).
**Figure 5.4.** The performance of males, observer females and naïve females during training trials for Experiment 2: A. distance traveled in the maze, B. latency to escape, and C. average speed (velocity) the bird traveled within the maze. (Data points are averages for blocks of four training trials. See text for results of statistical analysis.)
Figure 5.5. The performance of males, observer females and naïve females during probe trial for Experiment 2: A. portion of distance traveled in quadrants, and B. proportion of time spent in quadrants. *Significantly different from the average of other three quadrants. (See text for results of statistical analysis.)
On the probe trial, Observers traveled significantly more in the previously correct quadrant than in the average of the other three quadrants \((Z = -2.20, p = 0.03)\) but there was no difference between the quadrants for latency \((Z = -0.32, p = 0.75)\). The Naïves spent significantly more time in the previously correct quadrant than in the average of the other three quadrants \((Z = -2.20, p = 0.03)\) but the distance traveled between the quadrants was not significant but trending \((Z = -1.78, p = 0.075)\). There no significant effect of treatment on probe latency \((U = 17, p = 0.94)\) or distance traveled \((U = 9 p = 0.18)\). These results show that both groups traveled more in the previously correct quadrant than in the average of the other three quadrants indicating maze learning occurred. In contrast to Exp. 1, in this experiment, the Observers spent less time in the previously correct quadrant and video analysis showed that the observer females flew at the wall more often and their starting point for the flight was outside the previously correct quadrant. In addition, the momentum from their flight would propel them in to the adjacent quadrants.

Due to the difference in training protocols between the males and females (males receiving 8 trials/day and the females 4 trials/day), gender comparisons were not examined. Consistent with Experiment 1, the results of this experiment indicate that zebra finches are capable of learning the maze through direct learning, but not observational learning. Interestingly, escape latency between Observers and Naïves differed, with the Naïves escaping faster. This may indicate that prior exposure to the maze and cues allowed the Naïves to better memorize the cues and aviary dimension prior to testing which conferred an advantage during training.
5. EXPERIMENT 3

In the second experiment, the observer females again failed to acquire the escape procedure via observational learning. I posited that perhaps this was because the females only viewed 5 males as they performed 40 trials each. Since learning of the task only requires 16-20 trials, it may be that these males progressed too rapidly since they received 8 trials and therefore the females did not receive ample observation time to learn. Unexpectedly, the naïve females who viewed the maze and cues without the presence of a demonstrator showed a slight advantage in maze performance. This may be because they were able to memorize the cues and dimensions of the aviary without distraction from the male demonstrators. Therefore, in Exp. 3, I used eight males that only received 4 trials per day (thereby extending the males’ learning portion for the observers) and I blocked the view of the maze and cues from the naïve females.

(a) Direct and Observational Training

Ten male demonstrators and 12 females were selected at random from the university aviary and were housed in groups of 6 or 10 same-sex individuals by group. Females were randomly assigned to either the observer or naïve treatment group. As in Exp. 2, a 6-individual observation deck was used so all the observer females were able to view all 10 males as they underwent direct training. Males were divided into 2 groups (a morning and afternoon group) and each received one 4-trial block per day. Since the females viewed all 10 males, they viewed 200 trials over the 5 days. Naïve females were suspended for the same amount of time in the observation deck in a plain black-clothed aviary. Probe trials were conducted the same as Exp. 2.
(b) Results and Discussion

For the males, latency ($F(4, 36) = 31.77, p < 0.001$; Linear Contrast: $F(1, 9) = 46.29, p < 0.001$) and distance ($F(4, 36) = 13.79, p < 0.001$; Linear Contrast: $F(1, 9) = 22.54, p = 0.01$) decreased across blocks indicating learning of the task (Fig 5.6). Velocity did not change across blocks. On the probe trial, males traveled more ($t(9) = 6.47, p < 0.001$) in the previously correct quadrant than the average of the other three quadrants but did not spend more time in the previously cued quadrant ($t(9) = 1.52, p = 0.16$; Fig. 5.7).

On the pre-training probe, neither group of females traveled more or spent more time in the previously correct quadrant than the average of the other three quadrants nor was there any difference between the groups in these measurements. This indicated that observation alone was not enough for the females to learn the location of the escape hole. During the females’ training, there was a significant block effect across groups and each group demonstrated a reduction in latency and distance traveled but not velocity across trials (Observers distance: $F(3, 15) = 12.19, p < 0.001$, Linear Contrast: $F(1, 5) = 14.19, p = 0.013$; latency: $F(3, 15) = 8.37, p = 0.002$, Linear Contrast: $F(1, 5) = 8.09, p = 0.036$; Naïves distance: $F(3, 15) = 7.81, p = 0.002$, Linear Contrast: $F(1, 5) = 9.24, p = 0.029$; latency: $F(3, 15) = 9.39, p = 0.001$, Linear Contrast: $F(1, 5) = 9.16, p = 0.029$). There was no significant difference for the main effect of treatment (Observer versus Naïve) nor any trial-block x treatment effects for distance traveled in the maze (treatment: $F(1, 10) = 2.43, p = 0.15$; treatment x trial block: $F(3, 30) = 1.34, p = 0.28$), latency to escape (treatment: $F(1, 10) = 0.58, p = 0.47$; treatment x trial block: $F(3, 30) = 0.44, p = 0.73$) or speed traveling (treatment: $F(2, 10) = 1.07, p = 0.33$; treatment x trial block: $F(2, 20) = 2.52, p = 0.08$) within the maze.
Figure 5.6. The performance of males, observer females and naïve females during training trials for Experiment 3: A. distance traveled in the maze, B. latency to escape, and C. average speed (velocity) the bird traveled within the maze. (Data points are averages for blocks of four training trials. See text for results of statistical analysis.)
Figure 5.7. The performance of males, observer females and naïve females during probe trial for Experiment 3: A. portion of distance traveled in quadrants, and B. proportion of time spent in quadrants. *Significantly different from the average of other three quadrants. (See text for results of statistical analysis.)
On the post-training probe, both the observers and naïve females spent more time in the previously correct quadrant (Observers: $t(5) = 2.82, p = 0.04$; Naïves: $t(5) = 2.62, p = 0.047$) but there was no effect of treatment between the two groups ($F(1, 10) = 1.07, p = 0.32$). However, in distance traveled, neither group showed a significant preference for the cued quadrant although the observer group was trending (Observers: $t(5) = 2.42, p = 0.06$; Naïves: $t(5) = 1.31, p = 0.25$) and there was no difference between the groups ($F(1, 10)=3.00, p =0.12$). This implies that spatial learning was weak in both groups.

Due to the difference in direct learning protocols between the males and females (females were given a pre-training probe), gender comparisons were not conducted. The results of this experiment confirm that direct training is effective for learning the spatial maze. Additionally, the results show that prior observation of conspecifics learning and correctly navigating the maze does not confer an advantage in maze learning or performance under this protocol.

6. DISCUSSION

I conducted an avian analog study of the MWM using a clear, cylindrical arena with an escape hole. It is important to note that my adaption of the MWM analog differed from the other avian spatial maze [322-324] as mine did not require pre-training the birds with food, lacked any proximal or spatial contiguous cues, and had a homogeneous arena. The results of my experiments indicated that zebra finches are capable of learning this complex spatial maze using only distal cues to guide them to the goal as is typical of mammals. Additionally, I found that there was no effect of gender on learning my spatial task, with males and females being capable of learning the task at the same speed.
I was not able to demonstrate that the zebra finch was capable of learning complex spatial skills by observing conspecifics performing the task. Observational learning of a spatial performance requires mindful imitation where the observer must learn and understand not only the motor behaviors of the demonstrator but also the goal the demonstrator is attempting to obtain. It is believed that during learning acquisition, the observer is extracting pertinent information from the demonstrator, encoding a mental representation of their behaviors and then storing this as a template to guide future behavior [47, 48, 330]. Repeated observations evoke the neural coding and the observed behavior is learned [331]. This hypothesis is supported by clinical and neuroimaging studies in humans [215, 332]. The fact that the zebra finches, unlike rats [18-20, 35, 37, 212] were incapable of learning spatial tasks through observation may imply they do not possess the cognitive abilities or neural networks required for encoding and replicating motor behaviors of a demonstrator, or more plausibly that the environmental demands on the species are different and therefore result in learning differences. It may be that observationally learned spatial information may not be as important for the survival of the zebra finch as it is for the rat, and necessitates the ability in the rat but not the finch.

Unlike several lab mammals, avian species do not readily acquire tasks that are not ecologically relevant. Perhaps using different types of mazes or target goals will allow for observational learning of a spatial task to occur. For example, more spatially contiguous cues or mazes in which the target is a food reward may be useful in examining observationally-acquired spatial information in the zebra finch as these situations more closely resemble natural foraging behaviors or group movements in the finch. One particular maze that may be useful, and has been shown effective in the zebra finch is the four-feeder open area task. In this task, birds must use arena geometry and cues to navigate to a baited feeder [324]. Since there are only four
possible target locations and the goal is spatially contiguous, it may be slightly less cognitively demanding on the observer. Additionally, some evidence exists showing the zebra finch will alter food preferences and increase feeding amounts following the observation of a conspecific’s foraging and eating behaviors [230, 231, 333]. This may increase the ecological relevance of the maze and the spatial information may be acquired by observers more readily.

Finally, this study describes how to construct a novel maze and a protocol for training. The procedures described here may prove useful for several different kinds of neurobiological studies. These techniques have already been exploited in studies of CB function, pharmacological studies on the effects of estrogens on spatial memory, and in a study examining the effect of adrenergic receptor antagonists on spatial memory. These studies have revealed that CB inactivation via mechanical lesions to the nuclei creates deficits in maze acquisition, and that pharmacological manipulation can alter learning and performance of the maze.

The benefits of the present maze and procedure are: 1) the speed of training, 2) no pre-training is required, 3) the apparatus fits within a compact space, 4) the experimental set up is easy to assemble and disassemble, and 5) the design is extremely cost-effective. The disadvantages are the inability to vary the motivation level or reinforcement magnitude so if a bird does not find the heat floored aversive, they may not attempt to escape the maze, and that the placement of the birds on a heated surface may cause stress responses which could interact with ablation of pharmacological manipulations. However, these disadvantages are shared by the MWM [225] which is still heavily used in studies on spatial learning. Most importantly, my procedure may allow for better ecologically correct comparisons on spatial learning between birds and mammals. This comparison is vital to our understanding of how the brain functions and has evolved to support spatial learning.
CHAPTER 6: GENERAL DISCUSSION

1. SUMMARY

In the preceding series of experiments, my aim was to establish which type of observational learning the zebra finch was capable of learning with the larger goal of examining the underlying brain regions that support avian observational learning. To achieve this aim, I conducted three experiments focusing on three types of observational learning (stimulus enhancement – Chapter 2; observational conditioning – Chapter 3; and, mindful imitation – Chapter 5) and one experiment looking at the role of the CB in fear conditioning acquisition. The first study (Chapter 2) investigated whether zebra finches are able to learn public information about male quality under two scenarios: mate choice copying (MCC) and mate quality bias (MQB). While I was unable to find support for MCC, I did find evidence of MQB in the socially monogamous zebra finch. This study suggests that MQB is a biologically relevant strategy employed by a monogamous species other than humans and may influence mate selection and therefore sexual selection in the zebra finch.

The second study (Chapter 3) examined the acquisition of fear conditioning (FC) in the zebra finch through both direct experience and observation. I found that zebra finches readily acquire an association between a tone and a shock via direct FC training. Additionally, prior observation of a conspecific undergoing conditioning confers an advantage to the observer which results in a savings in learning. These results suggested that zebra finches are capable of observational conditioning. In order to determine if observational conditioning could be used to
study the role of the CB in observational learning, I examine the CB role in the acquisition of FC (Chapter 4). This was necessary as FC is acquired rapidly and if CB inactivation did not block learning during FC trial performance, ascertaining the CB’s role in observational conditioning would be difficult. I found that lesions to the lateral cerebellar nuclei of the zebra finch did not produce differential deficits in fear conditioning acquisition.

My final study (Chapter 5) examined if the zebra finch could acquire spatial task information and learn to navigate an Escape Maze through observing conspecifics learning and ultimately successfully performing the task. While the zebra finches were capable of learning the task through performance, there was no evidence indicating that prior observation of the task conferred an advantage in subsequent maze performance. This implies that the zebra finch is not capable of mindful imitation in a spatial task.

Together the results from my three studies on observational learning show that zebra finches are capable of some but not all types of observational learning. These findings are interesting because of how they compare to learning in other taxa, particular mammalian species. In addition, they offer novel insights into avian observational learning and functional neuroanatomy. As such, they have important implications for observational learning evolution in vertebrates.

2. CONCLUSION

It is important that studies examining which types of observational learning are possible in a single species (like my series of investigations in the zebra finch) continue and are extended to species in all taxa. Developing learning inventories for each species, will allow researchers to compare and contrast the types of observational learning that occur by species, will aid in the
development of better hypotheses to explain the selection for observational learning based on the environment and behavioral constraints, and ultimately allow us to understand the evolution of learning constraints. This in turn may allow for researchers to better pinpoint brain regions to test for their involvement in observational learning through inductive reasoning. This approach was successfully used in discovering the brain pathways used for vocal learning, the rarest form of observational learning.

Vocal learning is found in three distantly related groups of birds: parrots, passerines, and hummingbirds. By looking for similarities in brain regions that could support this form of learning, researchers were able to identify the cortical pathways and nuclei necessary for avian vocal learning [176, 180]. This research was then applied to mammalian species that also possessed vocal learning abilities. It was hypothesized that analog brain structures and pathways in the same homologous region of the brain (i.e. the cortex) would be present in mammals with vocal learning, and these regions were discovered [101, 176, 180]. Investigations into the brain regions for vocal learning were based and significantly aided by the comparative behavioral evidence complied by researchers looking at learning capabilities and limitations. This same technique could be used for the different types of observational learning.

It is reasonable to posit that the more taxonomically distributed a particular type of learning is, the more likely the brain region responsible for the learning is highly conserved across species. Conversely, the rarer the behavior, like vocal learning, the more specialized and less conserved the brain regions may be. Perhaps observational learning inventories, like the one described in this dissertation, will help elucidate some of the mysteries surrounding the evolution of observational learning in animals.
3. FUTURE DIRECTION

Using this work as a foundation, several directions for further investigation may be taken. I will outline a few for each line of my research.

Mate Choice in the Zebra Finch

The effects of lesions on the acquisition of public information on potential mates and same-sex conspecifics have yet to be investigated. The CB receives input from almost every sensory system including vestibular and proprioceptive, visual, audition, somatosensation, and nociception [334] and has been shown to play a role in executive functions that require organization like planning and abstract reasoning in mammals [300]. As such, the CB may function in mate quality bias where the traits/qualities of multiple individuals, both male and female, must be compared. I believe this warrants further investigation. Additionally, studies into male choice and whether males use MQB are necessary to gain a complete understanding of the role of observational learning in zebra finch sexual selection.

Fear Conditioning and Cerebellar Involvement

Lesions to the CBl did not have a pronounced effect on fear conditioning acquisition. Therefore, the role of the CB in observational conditioning was unable to be tested. This lack of effect may be due to the position or size of the lesion, or that in contrast to mammals, the CB does not play a role in avian FC (although the latter is unlikely). To determine if the CB is involved in FC acquisition, investigations into immediate early gene expression in the CB during FC should be conducted. If the CB is found to function in FC, lesion or temporary deactivation studies may pinpoint the exact nuclei and pathways involved in FC.
Spatial Maze

Unlike the rodent and the human, the zebra finch was unable to acquire spatial information in our novel escape maze. This is likely because the task and/or the information being acquired is not biological relevant to the zebra finch. As previously mentioned in Chapter 5, studies into the observational acquisition of spatial information in the zebra finch should be extended to different spatial mazes that contain more spatially contiguous cues or mazes which use food rewards as goals. Additionally, given that it has now been shown that the zebra finch can detect fear or threat reactions in conspecifics, it is now possible to determine if the stress reactions from demonstrators learning the maze may influence observer behavior and tease this apart from the spatial component of the maze. Studies should be conducted where, following the observation period, Observer and Naïve females are given an initial probe with the hotplate disengaged (and therefore not aversive). If Observers learned the fear portion but not the spatial component (i.e. the goal location), then they should show an increased reaction or more movement compared to their Naïve counterparts.

4. CONCLUDING REMARKS

This dissertation provides several significant and novel results, and two novel procedures for testing cognition in birds. My MCC/MQB study (Chapter 2) is the first well-controlled study to show MQB in a non-human monogamous species. This result indicates that the zebra finch can identify morph traits associated with quality not just in potential mates, but also in same-sex conspecifics, and use this information to reduce errors in mate choice. It additionally lends support to the MQB hypothesis which predicts that species with monogamous mating systems will pay more attention to the quality of females interacting with a male than just the number of
females. My FC study (Chapter 3) was the first to demonstrate which responds to a threat stimulus with flight can be classically conditioned, and is the first well-controlled experiment to show observational conditioning in any avian species. This indicates that the zebra finch can learn to avoid the negative effects associated with threatening stimuli by watching conspecific reactions. Finally, I have provided the scientific community with two appropriate procedures and apparatuses to test FC and spatial memory in flighted birds.

Collectively, these studies show that zebra finches observationally learn certain information and what may determine which information is acquired may relate to the ecological relevance of the information. My results imply that observationally- acquiring information about mate selection and threatening stimuli, but not spatial information, confers a selective advantage for the finch. This in turn provides a more comprehensive understanding of zebra finch cognition and the driving factors behind the evolution of observational learning in this species.


210. Stinson G. 2010 Effects of estrogen on recovery of spatial function after cerebellar lesion. University, MS, University of Mississippi.

211. DiGuisto M. 2011 The role of the cerebellum in the zebra finch song circuit. University, MS, University of Mississippi.


VITA

Professional Preparation

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