“…we are witnessing the fusing of many sciences, all concerned with one or another aspect of behaviour, into one coherent science…”

Niko Tinbergen, 1963. On Aims and Methods of Ethology. *Zeitschrift fur Tierpsychologie*. 20, 410-433 (This journal was renamed *Ethology* in 1986).

University of Alberta

The Neural Encoding of Heterospecific Vocalizations in the Avian Pallium: An Ethological Approach

by

Marc Thomas Avey

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Psychology

Fall 2011
Edmonton, Alberta

Dedication

For all the songbirds who made this thesis and all my research possible.

Abstract

Songbirds (order Passeriformes, suborder Oscines) have captured the attention of scientists and non-scientists alike with their vocal signals. The black-capped chickadee (genus *Poecile*) uses its namesake call, *chick-a-dee*, to convey a variety of information. In Chapter 2 and 3, I examine the relationship between season and diurnal cycle and the production of three vocalizations of black-capped chickadees. In the natural habitat *chick-a-dee* call production was highest in autumn and winter generally at the meridian. *Fee-bee* song production increased once in the winter and once in the spring, and occurred almost exclusively at dawn. *Gargle* production did not differ significantly by season but most occurred during the meridian (Chapter 2). In the laboratory, the patterns of production were in general agreement with the patterns in the natural habitat (Chapter 3). In Chapter 4, I examined what role the phylogenetic relatedness of a heterospecific individual had on neural activity, measured via an immediate early gene, in the auditory brain areas. Using natural calls I found that there was no difference in the amount of neural response from closely individuals but there was less response from a distantly related individual. To further examine this I used calls that were more similar in their bioacoustic structures. With these calls I found no difference in the amount of neural activity regardless of phylogenetic proximity. In Chapter 5, I used mobbing calls to explore whether 'degree of threat' is encoded in the auditory processing brain areas. Degree of threat was indicated by the amount of neural activity with high threat mobbing calls and high threat predator calls generating the most activity followed by low threat mobbing calls and low threat

predator calls. Thus the ‘degree of threat’ was related to the amount of neural activity and within a threat level there was difference in the amount of activity regardless of the source of the threat. Finally, hand-reared birds had greater neural activity in response to mobbing calls which they had experience with than predator calls which they had no experience with. This result suggests that threat is a learned response and that the neural response is affected.

Acknowledgements

It is difficult to overstate my gratitude to my supervisor, Dr. Christopher B. Sturdy without whom my progress and completion of thesis would not have been possible. I would like to thank my examination committee, Dr. Clayton Dickson, Dr. Jonathan Prather, Dr. Dr. Cynthia Paszkowski, and Dr. Dallas Treit, as well as Dr. Christopher Westbury who was part of my candidacy examination committee. I would also like to thank my teaching mentor Dr. Connie Varnhagen for inspiring to engage education with the same zeal as my research.

I would like to thank my lab mates who shared the toil and joy of working in the laboratory and field with me: Dr. Laurie Bloomfield, Lauren Guillette, Marisa Hoeschele, Dr. Darren Proppe, and Michele Moscicki. The students I supervised who hopefully learned as much about science as I did about teaching: Craig Anderson, Jillian Avis, Rabil Chaudry, Cierra Darragh, Alanna Friesen, Homan Lee, Erin Irwin, Richard Kanyo, Kris Kowal, Fletcher Quince, and Alejandra Rodriguez, and Jessica Vlasveld.

I would also like to thank my collaborators: Dr. Todd Freeberg, Dr. Julie Elie, Thibault Grava, Dr. Ken Otter, and Jessica Owens. I would also like to thank Dr. Toni Bayans for her support in pursuing my interest in animal welfare during my graduate studies.

I would like to thank Pay Avey for graciously taking me into her home as I transitioned between positions and making life so much easier as I finished this thesis. I would like to thank all of my grandparents for their love and support over the years. I would like to thank both my brother Jonathan E. Avey and Aaron R.

Massecar (Bear) for keeping me human and grounded. I wish to thank my parents, Karin E. Avey, and Thomas V. Avey for patiently waiting for me to finish this journey, supporting me, and tolerating me while I grew up. Lastly, I would like to thank my wife, Laura H. Coleman for loving me. Thank-you.

Table of Contents

List of Tables

List of Figures

Chapter 1 General Introduction

Introduction

The study of animal behaviour developed rapidly as a scientific discipline in the 20th century with the birth of ethology by Konrad Lorenz (Lorenz, 1981), Niko Tinbergen (Tinbergen, 1951), and Karl von Frisch (von Frisch, 1953) joined by the creation of behaviourism and reflexology by B.F. Skinner (1938) and Ivan Pavlov (1927). The early ethologists were generally focused on inherited behaviours, whereas behaviourists generally focused on learning (Pfuger and Menzel, 1999). Developments in neuroscience in the early 20th century began linking the physiology of the nervous system to behaviours as led by researchers such as Ramon y Cajal, Charles Sherrington, Edgar Adrian, Alan Hodgkin, Andrew Huxley, Erich von Holst and Theodore Bullock. Modern neuroethology emerged from these traditions (Bullock, 1999; Pfuger and Menzel, 1999; Spiro and White 1998), but unlike Skinner's behaviourism, its focus has been to unravel the black box of the nervous system and its relationship to natural behaviour (Pfuger and Menzel, 1999). To understand the link between behaviour and brain, neuroethologists often take a top-down approach; that is, they study a natural behaviour in detail and then attempt to discover its neurobiological mechanisms (Spiro and White, 1998). The history of the study of birdsong reflects this top-down approach.

Song Learning

The study of birdsong, and in particular song learning, became the subject of focus for William Thorpe (Thorpe, 1951, 1954, 1958), and his student Peter

Marler (Marler and Tamura, 1964, Marler, 1970, Marler, et al. 1972). Basic principles of song learning were established through numerous studies, but in particular research on white-crowned sparrows (*Zonotrichia leucophrys*) in the laboratory (Marler and Tamura, 1962, 1964). Song learning is now well known to be a combination of nature and nurture, and it was Marler's (1970) work that established this and many of its basic attributes of song learning such as: Sensitive periods for song learning, the structure of the innate song as distinct from local dialects, and the rejection of non-relevant sounds during vocal development. Once song learning came to be understood as a well-defined behaviour that encompasses both imitative learning and innate preferences it was not long until the neurobiological mechanisms became the focus of study.

If a white-crowned sparrow is deafened, before or after exposure to a model song, the song does not develop normally (Konishi, 1965b). These results indicated that the model song was a guide for vocal development and output was modified until it matched the model (Marler and Peters, 1982). Konishi (1965b) also noted that isolate-reared white-crowned sparrows had songs that included tonal whistles present in wild-reared sparrow songs but not in the song of early deafened sparrows. The isolate-reared white-crowned sparrows were able to use auditory feedback to shape their song development in the direction of wild-reared sparrows even though they had no model. This ability to shape vocal development by auditory feedback is absent in non-vocal learners such as domestic fowl (*Gallus domesticus*; Konishi, 1963) and ring-doves (*Streptopelia risoria*; Nottebohm and Nottebohm, 1971). Konishi's experiments (Konishi 1965a, Konishi 1965b) on auditory feedback suggested that song learning required a template that a bird could use as a guide while practicing its song(s) during the critical periods discovered by Marler (1970), and that songbirds were a special case of vocal learners in the avian taxa. We now know that songbirds, parrots, and hummingbirds are the only known vocal learners among birds and humans, bats, whales and dolphins among mammals (Jarivs, 2004)

In 1976 the brain nuclei responsible for vocal learning and vocal production, which directly or indirectly innervated the syrinx, were discovered through lesion studies (Nottebohm, et al. 1976). These nuclei are now known as HVC (formerly the Higher Vocal Centre but now used as a proper name; Reiner et al. 2004), which projects to both RA (Robust nucleus of arcopallium), and X (Area X). RA in turn projects to nXIIts (tracheosyringeal nucleus), which innervates the syrinx (See Fig. 1-1). Further research has established the importance of these brain nuclei in the production of song and in learning (for a review see Bolhuis et al. 2010). Although much of the preceding research focused on the behaviour and brain of song learning and song production, the perception of song (Konishi, 1965) was also an important part of those discoveries.

Thorpe, Marler, Nottebohm, and Konishi’s pioneering work, along with insights into neurogenesis (Nottebohm, 1985), have caused song learning and songbird vocalizations to become a staple area of study in neuroscience because of the tractable nature of studying vocal output, the well-defined neural circuit for learning, production, and perception of song, and the analogous nature between birdsong and imitative learning during human speech acquisition (Bolhuis et al. 2010; Doupe and Kuhl, 1999).

Auditory Perception

Konishi's (1965) work on auditory feedback highlighted the importance of hearing to the development of normal song. The neuroanatomical structures and pathways of the auditory system are now well understood (Jarvis, 2004; Mooney, 2009; Pinaud and Terleph 2008, Reiner et al. 2004; Terleph and Pinaud 2010). The relationship of these structures to other behaviours such as social context (Woolley and Doupe, 2008) and cognitive functions such as category perception (e.g. Prather et al. 2009) are beginning to be understood as well. In songbirds, auditory information ascends from hair cells to the cochlear ganglion to cochlea nuclei in the brainstem to the lemniscal nuclei. From the lemniscal nuclei projections to the midbrain (MLd: nucleus mesencephalicus lateralis, pars dorsalis) and then to the thalamus (Ov: nucleus ovoidalis) connect the auditory pathway to the cerebrum. The thalamic nuclei project to the primary auditory cell populations in the avian pallium (L2; part of field L). Neurons in field L, L1 and L3 are putatively similar to mammalian layers 2 and 3 of the auditory cortex (see Jarvis, 2004 for a review), and Jarvis proposes the caudomedial mesopallium (CMM) and caudomedial nidopallium (NCM), which Jarvis (2004) proposes form reciprocal intrapallial connections similar to mammalian layers 2 and 3. CMM may share connectivity with the caudal striatum (CSt) auditory nuclei (Jarvis, 2004).Understanding the functional role of CMM and NCM in processing auditory information has been aided through the use of labels for immediate early genes.

ZENK (zif-268, egr-1, NGFI-A, or Krox-24), an immediate early gene, is induced in neurons after extracellular stimulation and increases in cytosolic Ca^{2+} (Ghosh et al. 1994). Immediate early genes are activity dependent with low baseline expression in neurons but expression increases rapidly and transiently in response to neuron depolarization (Sheng and Greenberg, 1990). Low baseline expression levels of ZENK and its responsiveness to membrane depolarization make ZENK a sensitive marker for neuronal activation (Chauduri, 1997).

In 1992, Mello and colleagues found that CMM and NCM are selectively sensitive to complex vocal signals of conspecific songs. Zebra finches (*Taeniopygia guttata*) or canaries (*Serinus canaria*) exposed to conspecific vocalizations had greater ZENK activity in CMM and NCM than when played heterospecific songs and synthetic noises (Mello et al. 1992). The processing of conspecific song in these regions has been verified by tract tracing, electrophysiology, and lesion studies (Chew et al. 1995; MacDougall-Shackleton et al. 1998; Vates et al. 1996), and both CMM and NCM exhibit immediate early gene expression in relation to complex stimuli that implicates them in higher order auditory processing (Chew et al. 1995; Chew et al. 1996, Pinaud and Terleph, 2008).

ZENK encodes for a protein that binds to several genes (Christy et al. 1989), and RNA levels of ZENK increase during nerve growth factor-induced neuronal differentiation (e.g. Bartel et al. 1989) as well as induction of long-term potentiation (Wisden et al. 1990). Mello and colleagues (1992) speculate that induction of ZENK could be related to activation of cellular events that modulates long-lasting changes to particular sensory stimulation (Mello et al. 1992). ZENK does not express in throughout the entire auditory system however; in field L, which is part of the primary auditory centre in the songbird brain and projects to both CMM and NCM, there is not significant induction of ZENK to playback of songs (e.g. Mello et al. 1992). Field L is physiologically active to sound stimuli, including conspecific song why measured electrophysiologically and with other techniques (Margoliash, 1983; 1986; Muller and Leppelsack, 1985; Muller and Scheich, 1985; Williams and Nottebohm, 1985). Moreover, tone burst which do not elicit ZENK expression in NCM do elicit electrophysiological activity (Muller and Leppelsack, 1985; Muller and Scheich, 1985) which suggest that the ZENK response is dependent on neuronal activity but this activity alone is not sufficient for its induction (Mello et al. 1992).The preferential response of CMM and NCM to conspecific song raises questions about what aspects of the vocalizations, the development and natural history of the individual, and the species under study have on neural response.

Bird Calls, Not Songs

Marler (2004) notes that although information about birdsong and the underlying neural mechanisms has increased dramatically, bird calls remain the “neglected orphans of avian behavioural neurobiology”. Songs and calls can be readily distinguished from each other, despite some overlap, they generally have particular characteristics. Bird songs are generally more complex than calls, used only within a limited time frame (breeding), and used for the primary functions mate attraction and territory defence (Marler, 2004). Bird calls are generally simpler in structure than songs, produced in a more erratic and opportunistic manner than the stereotypy associated with song, and serve more functions in more contexts than songs (Marler, 2004). Bird calls have often been regarded as simple and innate vocalizations, although researchers have been documenting the complexity that these calls convey and the learning and plasticity are involved in their production (e.g. Mundinger, 1970, Zann, 1984). Bird calls have been found to perform complex functions in the communication system not only of conspecifics, but also heterospecifics.

The vocalizations and vocal interactions of fellow conspecifics can provide a listener with information including the fitness of that individual or threat represented by predators (Mennill et al., 2002, Templeton et al. 2005). Heterospecifics also respond to the information contained in songs and calls (e.g. Rabatsky, 1997). For instance, the mobbing call of black-capped chickadees is responded to by numerous heterospecifics (Hurd, 1996) and the subtle information about the degree of threat is even responded to by at least one heterospecific species (Templeton & Greene, 2007). Such complex information conveyed by call and perceived by both conspecific and heterospecific individuals strongly suggests that bird calls represent a wealth of natural behaviour for investigation. One area in particular is neurobiological mechanisms of auditory perception.

The Black-capped Chickadee

A neuroethological approach to understanding the central nervous system can be broadly defined as an attempt to discover the neural mechanisms underlying the biologically relevant stimuli that elicit natural behaviour. I propose to use a top-down (behaviour to brain) neuroethological approach to study the auditory perception of calls in a songbird. To do so, a model species which can be studied in the laboratory under semi-natural conditions is required. One such model species is the black-capped chickadee (*Poecile atricapillus*). The black-capped chickadee is one of the most common and widespread birds in North America (Foote et al. 2010).

The behavioural ecology of the black-capped chickadee has been studied extensively in the field (Otter, 2007; Smith, 1991), as has its cognition, behaviour, and neurobiology. (e.g., Sturdy et al. 2007; Avey et al. 2008; Hoshooley et al. 2007; Phillmore et al. 2003; Pravosudov and Smulders 2010, Hampton et al. 1995). The black-capped chickadee is a non-migratory species whose range extends from the Atlantic to Pacific coasts and from the northern two thirds of the United States to the far north of the boreal forest in Canada and Alaska. Its closest relative is the mountain chickadee, (*Poecile gambeli*) whose range is confined to the montane coniferous forests from southern Arizona to the Yukon (McCallum et al. 1999; Gill et al. 2005). In the autumn, black-capped chickadee form flocks of three to twelve individuals and social interactions are structured by a linear dominance hierarchy. In the spring, the flock separates and monogamous pairs settle on individual territories where the male uses his *fee-bee* song to defend his territory from rivals and attract his mate (Smith, 1991). Throughout the year, vocal behaviour plays a crucial role in the lives of chickadees.

Vocalizations

The vocal behaviour and communication system of black-capped chickadees is complex and sophisticated. Adults have 16 known vocalization types (Smith 1991) and these can be broadly separated into songs and calls. Songs are generally produced in the spring to attract mates and defend territories, whereas calls are generally produced year round and serve numerous functions. The *fee-bee* song of the black-capped chickadee is comprised of two clear tones approximately one second in total length (Ficken et al. 1978). *Fee-bee* songs are produced by males, and sometimes females, for territory defence and attracting mates in the spring (Mennill and Otter 2007), but they are also produced frequently in winter (Avey et al. 2008b). Although, the *fee-bee* song is a learned vocalization (Shackleton and Ratcliffe, 1993) chickadees produce more call types than song types providing a number of calls and associated behaviours for study (Ficken et al. 1978; Hailman and Ficken, 1996).

The *chick-a-dee* call is a complex and variable vocalization that is produced by both sexes and used year round (Avey et al. 2008). This call is produced by all members of genus Poecile that also produce species typical variants (Ficken, Hailman, & Hailman, 1994; Ficken, Mclaren, & Hailman, 1996; Ficken et al. 1978; Gaddis, 1985; Hailman and Ficken, 1996; Smith, 1972), and is at least in part learned by black-capped chickadees (Hughes, Nowicki, and Lohr, 1998). The call is composed of four possible note types (A, B, C, D) that can be arranged differently, with variable repeats and omissions (Ficken et al. 1978), allowing for an open-ended variety of *chick-a-dee* call types (Hailmen et al. 1987). The ordering of the notes (A-D) is fixed syntactically, but any of the notes can be repeated or omitted to produce a wide variety of types in order to convey information (Hailman et al. 1985).

The information encoded by the *chick-a-dee* call is as varied as the behavioural repertoire of chickadees. The *chick-a-dee* call can encode subtle information, such as flock identity (Mammen and Nowicki 1981; Nowicki, 1983), or highly complex information such as the degree of threat of predators when chickadees perform mobbing behaviours (Odum 1942; Templeton et al. 2005). The *chick-a-dee* call is also used to give the “all-clear” after a predator has left (Ficken and Witkin, 1977) and in foraging as a signal to flock mates that a new food source has been discovered (Ficken, 1981). The *chick-a-dee* call is used in many situations, and as more research is conducted, our understanding of the complexity and variety of its use will only expand.

Chickadees have numerous other calls such as the *gargle* call which also has learned components (Ficken et al. 1985), but it is the *chick-a-dee* call that is arguably the best studied. The perception of *chick-a-dee* calls has been studied in detail using operant conditioning paradigms (e.g. Bloomfield et al. 2003). Black-capped chickadees perceive their *chick-a-dee* calls and their note types as belonging to natural, open-ended categories (Bloomfield, 2003; Sturdy et al. 2000). The neural mechanisms underlying perception have received limited study in chickadees to date (Phillmore et al. 2003; Avey et al. 2008a) but differences have already been found in the neural response between the perception of *fee-bee* song and *chick-a-dee* calls that varies depending on whether the producers or perceiver is male or female (Avey et al. 2008a). Because the *chick-a-dee* call is used year round and across the entire day, unlike *fee-bee* song, understanding the variation in its seasonal and diurnal pattern of production is essential to understanding its perception.

Seasonal and Diurnal Patterns

Vocal behaviour in black-capped chickadees is closely related to the seasonal and diurnal cycle (Avey et al. 2008b). Black-capped chickadees undergo hormonal changes timed with the annual light cycle that accompany changes in seasons (MacDougall-Shackleton et al. 2003). Male songbirds in general, unlike mammals, have a much greater change seasonally in their testicular mass, several hundred-fold, compared to two to five-fold in mammals (Dawson et al. 2001). The larger change in annual testicular mass is caused by a greater regression outside of the breeding season and is important for the reduction in weight for animals that fly (Dawson et al. 2001). The physiological states that accompany these changes in birds are photosensitive, photostimulated, and photorefractory. These states are related to periods of reproduction and non-reproduction and the changes in physiological state such as regressed gonads during the photorefractory period. These states are caused by differences in levels of gonadotropin-releasing hormone (Dawson et a. 2001). During the short photoperiod of winter the gonads are regressed until the change in day length in spring when dramatic increases in testicular mass occur and birds become photostimulated. Continued long periods of light results in gonadal regression and evidence supports that it is the first long period of light that triggers this photorefractory state (Dawson and Goldsmith, 1983). When photostimulated, black-capped chickadees produce large numbers of *fee-bee* song during a brief period of time in the spring (Avey et al. 2008b; MacDougall-Shackleton et al. 2003). These photoperiod-triggered physiological changes are interrelated to seasonal variation in vocal production. All of these seasonal and diurnal changes in vocal production have important implications for the study of auditory perception.

Neuroethological studies of chickadees often involve long-term housing in laboratory facilities. Designing experiments to examine natural behaviour in the laboratory can be challenging when trying to control extraneous variables while maintaining the natural system of study. This difficulty may be compounded by housing conditions and their impact on the natural behaviour and state of the animals. For instance, black-capped chickadees normally form flocks in the fall and then pair and defend territories in the breeding season, but these behaviours are severely limited in laboratory housing conditions. One of the most fundamental questions to be asked by any researcher studying vocal production or perception in songbirds is “what impact does housing chickadees in a laboratory setting have on their natural cycle of vocal production”? Indeed, the seasonal and diurnal patterns of vocal production in the field and the laboratory form the basis for the study of auditory perception. In chickadees, certain changes in vocal production (e.g. *fee-bee* song) are so dramatic and circumscribed that the perception of these vocalizations outside of their seasonal norms may affect behavioural and neural responses.

Summary of Current Studies

First, I investigate the seasonal and diurnal pattern of vocal behaviour of the black-capped chickadee for three of its major vocalizations: the *fee-bee* song, the *chick-a-dee* call and the *gargle* call (Chapter 2). This study lays the foundation for subsequent research by sampling the natural pattern of vocal production as a basis for the seasonal timing of future auditory perception experiments. This study builds on previous research (e.g., Odum 1941) which were primarily focused on the *fee-bee* song and more limited sampling procedures. Next, I investigate whether wild-caught chickadees housed in the laboratory continue their natural pattern of vocal production for the same three vocalizations (Chapter 3). This study is the first formal investigation of effects of housing conditions on black-capped chickadees and provides crucial information for future ethological studies using wild-caught black-capped chickadees under laboratory conditions.

Subsequent chapters (4 and 5) are neuroethological investigations of auditory perception in black-capped chickadees. First, I examine the response of neurons in CMM and NCM using the immediate early gene ZENK to determine if phylogenetic distance, and not bioacoustic similarity of heterospecific vocalizations, is what causes IEG to in these regions (Chapter 4). This experiment aims to address three important issues: I) to tease apart how phylogenetic relatedness and acoustic similarity make unique contributions to the auditory response ZENK in CMM and NCM; II) to determine whether the auditory system has evolved to process certain heterospecific vocalizations based on phylogenetic relatedness; III) to address whether heterospecific vocalizations are an appropriate control condition, as they have been used in previous experiments to examine the auditory response of ZENK in CMM and NCM.

Finally, I further investigate the role and function of heterospecific calls and how they impact the auditory response of ZENK in CMM and NCM (Chapter 5). This experiment examines how complex information, the degree of threat of predators, as encoded by the mobbing calls of black-capped chickadees is expressed. This experiment aims to address four questions: I) Is degree of threat from predators represented by the pattern of neural activity in CMM and NCM? II) Does the response of functionally relevant information, (i.e., degree of threat), generate the same pattern of neural activity in a conspecific and heterospecific individual? III) Can the signal of the functionally relevant information be not only conspecific or heterospecific, but also a heterospecific cue that did not evolve to convey the functionally relevant information (i.e., predator calls that serve as a cue to prey that the predator is near)? IV) Is the neural response learned or innate?

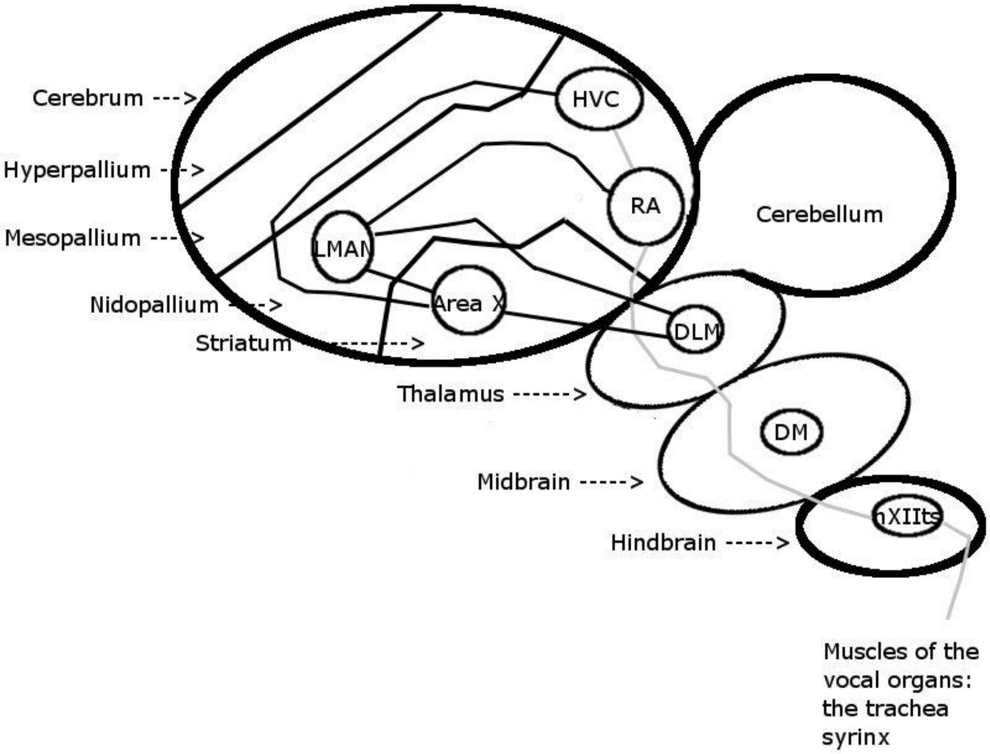

Cerebrum --->
Hyperpallium --->
Mesopallium --->
Nidopallium --->
Striatum ---------->
Thalamus ------>
Midbrain ----->
Hindbrain ----->
HVC
RA
LMAN
Area X
DLM
DM
nXIIts
Cerebellum
Muscles of the vocal organs: the trachea syrinx

Figure 1-1.

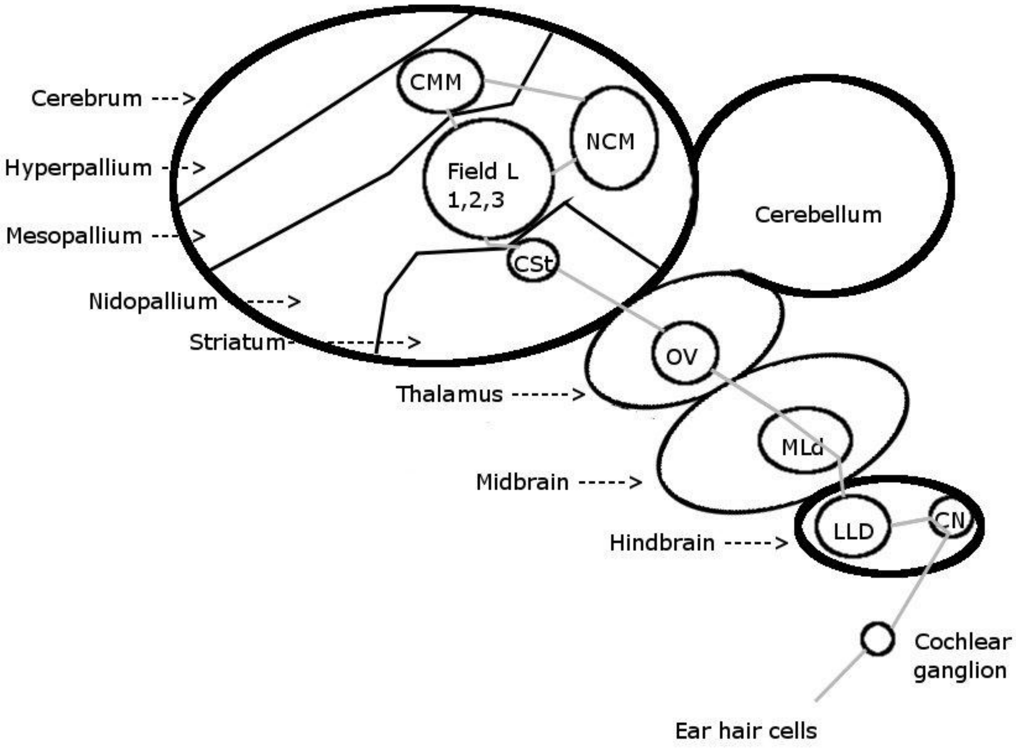

Cerebrum --->
Hyperpallium --->
Mesopallium --->
Nidopallium ----->
Striatum----------->
Thalamus ------>
Midbrain ----->
Hindbrain ----->
CMM
NCM
Field L
1,2,3
CSt
Cerebellum
OV
MLd
LLD
CN
Cochlear
ganglion
Ear hair cells

Figure 1-2.

References

Avey, M.T., Kanyo, R.A., Irwin, E.L., Sturdy, C.B. 2008a. Differential effects of vocalization type, singer and listener on ZENK immediate early gene response in black-capped chickadees (*Poecile atricapillus*). Behav Brain Res. 188, 201-208.

Avey M.T., Quince A.F., Sturdy C.B. 2008b. Seasonal and diurnal patterns of black-capped chickadee (Poecile atricapillus) vocal production. Behav Processes. 77,149-55.

Bartel, D.P., Sheng, M., Lau, L.F, Greenberg, M.E. 1989. Genes Dev, 3, 304-313

Bloomfield, L.L., Sturdy, C.B., Phillmore, L.S., Weisman, R.G. 2003. Open-ended categorization of chick-a-dee calls by black-capped chickadees (*Poecile atricapilla*). J Comp Psychol. 117, 290-301.

Bolhuis, J.J., Okanoya K., Scharff, C. 2010. Twitter evolution: converging mechanisms in birdsong and human speech. Nat Rev Neurosci. 11, 747-59.

Bullock, T.H. 1999. Neuoethology has pregnant agendas. J Comp Physiol A. 185: 291-295.

Chaudhuri, A. 1997. Neural activity mapping with inducible transcription factors. Neuroreport. 8, 3-7.

Chew, S.J., Mello, C., Nottebohm, F., Jarvis, E., Vicario, D.S. 1995. Decrements in auditory responses to a repeated conspecific song are long-lasting and require two periods of protein synthesis in the songbird forebrain. Proc Natl Acad Sci U S A. 92, 3406-3410.

Chew, S.J., Vicario, D.S., Nottebohm, F. 1996. A large-capacity memory system that recognizes the calls and songs of individual birds. Proc Natl Acad Sci U S A. 93, 1950-1955.

Christy, B., Lau, L., Nathans, D. 1989. DNA binding site of the growth factor-inducible protein Zif268. Proc Natl Acad Sci. USA 86, 8737-8741.

Dawson, A., King, V.M., Bentley, G.E., Ball, G.F. 2001. Photoperiodic control of seasonality in birds. J Biol Rhythms. 16, 365-380.

Dawson, A., Goldsmith, A.R. 1983. Plasma prolactin and gonadotrophins during gonadal development and the onset of photorefractoriness in male and female starlings (*Sturnus vulgaris*) on artificial photoperiods. J Endocrinol. 97, 253-260.

Doupe, A.J., Kuhl, P.K. 1999. Birdsong and human speech: common themes and mechanisms. Annu Rev Neurosci. 22, 567-631.

Ficken, M.S. 1981a. Food finding in Black-capped Chickadees: altruistic communication? Wilson Bulletin. 93. 393-394.

Ficken, M.S., Ficken, R.W., Apel, K.M. 1985. Dialects in a call associated with pair interactions in the Black-capped Chickadee. Auk. 102, 145-151.

Ficken, M.S., Ficken, R.W., Witkin, S.R. 1978. Vocal repertoire of the black-capped chickadee. Auk. 95, 34-48.

Ficken, M.S., Hailman, E.D., Hailman, J.P. 1994. The chick-a-dee call system of the Mexian chickadee. Condor. 96, 70-82.

Ficken, M.S., Mclaren, M.A., Hailman, J.P. 1996. Boreal Chickadee (Poecile hudsonicus), The Birds of North America Online (A. Poole, Ed.). Ithaca:

Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/254 doi:10.2173/bna.254

Ficken, M.S., Witkin, S.R. 1977. Responses of Black-capped Chickadee flocks to predators. Auk. 94, 156-157.

Foote, J.R., Mennill, D.J., Ratcliffe, L.M., Smith, S.M. 2010. Black-capped Chickadee (*Poecile atricapillus*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/039 doi:10.2173/bna.39

Gaddis, P.K. 1985. Structure and variability in the vocal repertoire of the mountain chickadee. Wilson Bulletin. 97, 30-45.

Ghosh, A., Ginty, D.D., Bading, H., Greenberg, M.E. 1994. Calcium regulation of gene expression in neuronal cells. J Neurobiol. 25, 294-303.

Hailman, J.P., Ficken, M.S. 1996. Comparative analysis of vocal repertoires, with reference to chickadees. Pages 136-159 *in* Ecology and evolution of acoustic communication in birds (Kroodsma, D.E., Miller, E.H. eds.). New York: Cornell University Press.

Hailman, J.P., Ficken, M.S., Ficken, R.W. 1987. Constraints on the structure of combinatorial "chick-a-dee" calls. Ethology. 75, 62-80.

Hailman, J.P., Ficken, M.S., Ficken, R.W. 1985. The “chick-a-dee” calls of Parus atricapillus: a recombinant system of animal communication compared with written English. Semiotica. 56, 191-224.

Hailman, J.P., Ficken, M.S., Ficken, R.W. 1987. Constraints on the structure of combinatorial "chick-a-dee" calls. Ethology. 75, 62-80.

Hampton, R.R., Sherry D.F., Shettleworth, S.J., Khurgel, M., Ivy, G. 1995 Hippocampal volume and food-storing behavior are related in parids. Brain Behav Evol. 45. 54-61.

Hoshooley, J.S., Phillmore, L.S., Sherry, D.F., Macdougall-Shackleton, S.A. 2007. Annual cycle of the black-capped chickadee: seasonality of food-storing and the hippocampus. Brain Behev Evol. 69, 161-168.

Hughes, M., Nowicki, S., Lohr, B. 1998. Call learning in Black-capped Chickadees (*Parus atricapillus*): The role of experience in the development of 'chick-a-dee' calls. Ethology. 104, 232-249.

Hurd, C.R. 1996. Interspecific attraction to the mobbing calls of black capped chickadees (Parus atricapillus). Behav Ecol Sociobiol. 38, 287-292.

Jarvis, E. D. 2004. Learned birdsong and the neurobiology of human language. Ann N Y Acad Sci. 1016, 749-777.

Konishi, M. 1963. The role of auditory feeback in the vocal behavior of the domestic fowl. Z Tierpschol. 20, 349-367.

Konishi, M. 1965a. Effects of deafening on song development in American robins and black-headed grosbeaks. Z Tierpschol. 22, 584-599.

Konishi, M. 1965b. The role of auditory feedback in the control of vocalization in the white-crowned sparrow. Z Tierpschol. 22, 770-783.

Lorenz, K. 1981. The foundations of ethology. Springer-Verlag. New York.

MacDougall-Shackleton, S.A., Hernandez, A.M., Valyear, K.F. Clark, A.P. 2003.

Photostimulation induces rapid growth of song-control brain regions in male and female chickadees (*Poecile atricapilla*). Neurosci Lett. 340, 165-168.

MacDougall-Shackleton S.A., Hulse S.H., Ball G.F. 1998. Neural bases of song preferences in female zebra finches (*Taeniopygia guttata*). Neuroreport. 9, 3047-3052.

Mammen, D.L. Nowicki, S. 1981. Individual differences and within-flock convergence in chickadee calls. Behav Ecol Sociobiol. 9,179-186.

Margoliash, D. 1983. Acoustic parameters underlying the responses of song-specific neurons in the white-crowned sparrow. J. Neurosci. 3, 1039-1057.

Margoliash, D. 1986. Preference for autogenous song by auditory neurons in a song system nucleus of the white-crowned sparrow. J Neurosci. 6, 1643-1661.

Marler, P. 1970. A comparative approach to vocal learning – song development in white-crowned sparrows. J Comp Physiol Psychol. 71, 1-25.

Marler, P. 2004. Bird calls: their potential for behavioral neurobiology. Ann N Y Acad Sci. 1016, 31-44.

Marler, P., Mundinge, P., Waser, M. S., Lutjen, A. 1972. Effects of acoustical stimulation and deprivation on song development in red-winged blackbirds (Agelaius-phoeniceus). Anim Behav. 20, 586-606.

Marler, P., Peters, S. 1982. Long-term storage of bird songs prior to production. Anim Behav. 30, 479-482.

Marler, P., Tamura, M. 1962. Song 'dialects' in three populations of white-crowned sparrows, Condor. 64, 368-377.

Marler, P., Tamura, M. 1964. Culturally transmitted patterns of vocal behavior in sparrows. Science. 146, 1483-1486.

McCallum, D., Archibald, R.G., Donald L.D. 1999. Mountain Chickadee (*Poecile gambeli*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/453 doi:10.2173/bna.453

Mello, C., Vicario, D., Clayton, D.F. 1992. Song presentation induces gene expression in the songbird forebrain. Proc Natl Acad Sci U S A. 89, 6818-6822.

Mello C.V., Velho, T.A., Pinaud, R. 2004. Song-induced gene expression: a window on song auditory processing and perception. Ann N Y Acad Sci. 1016, 263-281.

Mennill, D.J., Otter, K.A. 2007. Status signaling and communication networks in chickadees: complex communication with a simple song. Pages 215-233 *in* Ecology and behavior of chickadees and titmice: an integrated approach. (Otter, K.A., Ed.) Oxford University Press, Oxford, UK.

Mooney, R. 2009. Neurobiology of song learning. Curr Opin Neurobiol. 19, 654-660.

Muller, C.M., Leppelsack, H. Feature extraction and tonotopic organization in the avian auditory forebrain. 1985. Exp. Brain Res. 59, 587-599.

Muller, S.C., Scheich, H.P. Social stress increases [14C]2-deoxyglucose incorporation in three rostral forebrain areas of the young chick. 1985. J Comp Physiol. 156, 1-12.

Mundinger, P.C. 1970. Vocal imitation and individual recognition of finch calls. Science. 168, 480-482.

Nottebohm, F., Stokes, T.M., Leonard, C.M. 1976. Central control of song in the canary, *Serinus canaries*. J Comp Neurol. 165, 457-486

Nottebohm, F. 1985. Hope for a New Neurology. Ann N Y Acad Sci. 457, 1-238.

Nottebohm, F., Nottebohm, M.E. 1971. Vocalizations and bredding behaviour of surgically deafened ring doves (Streptopelia risoria). Anim Behav. 19, 313-327.

Nowicki, S. 1983. Flock-specific recognition of chickadee calls. Behav Ecol Sociobiol. 12, 317-320.

Odum, E.P. 1941. Annual cycle of the black-capped chickadee—1. AUK 58, 314-333.

Otter, K.A. 2007. Ecology and Behavior of Chickadees and Titmice. An Integrated Approach. Oxford University Press, New York.

Pavlov, I. 1927. Conditioned Reflexes. Dover, New York.

Phillmore, L.S., Bloomfield, L.L., Weisman, R.G. 2003. Effects of songs and calls on ZENK expression in the auditory telencephalon of field- and isolate-reared black capped chickadees. Behav Brain Res. 147, 125-134.

Pfuger, H.J., Menzel, R. 1999.Neuroethology, its roots and future. J Comp Physiol A. 185, 389-392.

Pinaud , R., Terleph, T.A. 2008. A songbird forebrain area potentially involved in auditory discrimination and memory formation. J Biosci. 33, 145-155.

Prather, J.F., Nowicki, S., Anderson R. C., Peters, S., Mooney, R. Neural 2009. Correlates of categorical perception in learned vocal communication. Nat Neurosci. 12, 221-228.

Pravosudov, V.V., Smulders T.V. 2010. Integrating ecology, psychology and neurobiology within a food-hoarding paradigm. Philos Trans R Soc Lond B Biol Sci. 365, 859-867.

Reiner, A., Perkel, D.J., Mello, C.V., Jarvis, E.D. 2004. Songbirds and the revised avian brain nomenclature. Ann N Y Acad Sci. 1016, 77-108.

Ribeiro, S., Cecchi, G.A., Magnasco, M.O., Mello, C.V. 1998. Toward a song code: evidence for a syllabic representation in the canary brain. Neuron, 21. 359–371.

Shackleton, S.A. Ratcliffe L. 1993. Development of song in hand-reared Black-capped Chickadees. Wilson Bulletin. 105, 637-644.

Sheng, M., Greenberg M.E. 1990. The regulation and function of c-fos and other immediate early genes in the nervous system. Neuron. 4, 477–485.

Skinner, B.F. 1938. The Behavior of Organisms. Appleton-Century-Crofts, New York.

Smith, S.M., 1991. The Black-capped Chickadee: Behavioral Ecology and Natural History. Cornell University Press, Ithaca.

Smith, S.T. 1972. Communication and other social behavior in *Parus carolinensis*. Publ. Nuttall Ornithol. Club No. 11.

Smulders, T.V., DeVoogd, T.J. 2000. Expression of immediate early genes in the hippocampal formation of the black-capped chickadee (*Poecile atricapillus*) during a food-hoarding task. Behav Brain Res. 114, 39–49.

Spiro J.E., White, S.A. 1998. Neuroethology: A meeting of brain and behaviour. Neuron. 21, 981-989.

Sturdy, C.B., Bloomfield, L.L., Farrell, T.M., Avey, M.T., Weisman, R.G. 2007. Category perception as a natural cognitive activity in songbirds. Comparative Cognition & Behavior Reviews. 2, 93-110.

Sturdy, C.B., Phillmore, L.S., Weisman, R.G. 2000. Call-note discriminations in black-capped chickadees (*Poecile atricapillus*). J Comp Psychol. 114 , 357-364.

Templeton, C.N., Greene, E. 2007. Nuthatches eavesdrop on variations in heterospecific chickadee mobbing alarm calls. Proc Natl Acad Sci U S A. 104, 5479-5482 .

Templeton, C.N., Greene, E., Davis, K. 2005. Allometry of alarm calls: black-capped chickadees encode information about predator size. Science. 308, 1934-1937.

Terleph, T.A., Pinaud , R. 2010. Neural coding of temporal information and its topography in the auditory cortex. J Biosci. 35, 499-500.

Thorpe, W.H. 1951. The learning abilities of birds. IBIS. 93, 252-296.

Thorpe, W.H. 1954. The process of song-learning in the chaffinch as studied by means of the sound spectrograph. Nature. 173, 465-469.

Thorpe, W.H. 1958. Further studies on the process of song learning in the chaffinch (*Fringilla coelebs gengleri*). Nature. 182, 554-557.

Tinbergen, N. 1951. The Study of Instinct. Oxford University Press, New York.

Vates, G.E., Broome, B. M., Mello, C.V., Nottebohm, F. 1996. Auditory pathways of caudal telencephalon and their relation to the song system of adult male zebra finches (*Taenopygia guttata*). J Comp Neurol. 366, 613-642.

von Frisch, K. 1953. The Dancing Bees - An Account of the Life and Senses of the Honey Bee. Harvest Books, New York.

Wisden, W., Errington, M.L., Williams, S., Dunnett, S.B., Waters, C., Hitchcock, D., Evan, G., Bliss, T.V.P., Hunt, S.P. 1990. Differential expression of immediate early genes in the hippocampus and spinal cord. 4, 603-614.

Williams, H., Nottebohm, F. 1985. Auditory responses in avian vocal motor neurons: a motor theory for song perception in birds. Science 229, 279-282.

Woolley, S.C., Doupe, A. J. 2008. Social context-induced song variation affects female behavior and gene expression. PLoS Biology. 6, e62.

Zann, R. 1984. Structural variation in the zebra finch distance call. Z Tierpschol. 66, 328-245.

Chapter 2 Seasonal and Diurnal Patterns of Black-capped Chickadee (*Poecile Atricapillus*) Vocal Production[1]

---

[1] *A version of this chapter has been published. Avey M.T., Quince, A.F., and Sturdy, C.B. (2008). Seasonal and diurnal patterns of black-capped chickadee (Poecile atricapillus) vocal production. Behavioural Processes. 77.149-155.*

Introduction

Seasonal variation in the production of vocalizations of chickadees *has* been studied previously (e.g., classic studies by Odum 1941a; Odum 1942). In spite of this, the seasonal pattern of vocal production, external variables (weather; e.g., Johnston 1942), and mechanisms that control chickadee vocal behaviour are still not clearly understood (Philmore et al. 2006; Smulders et al. 2006). In more than 60 years since Odum’s pioneering works chickadee researchers still describe chickadee vocal behaviour as “[sic] showing a peak in *fee-bee* song production in the spring and other vocalizations, such as *chick-a-dee* calls, predominating for the rest of the year”. My goal here is to move beyond these sweeping generalizations and instead provide a solid, quantifiable measure of what chickadees sing and when they sing it.

Previously, the seasonal pattern for *fee-bee* song production in black-capped chickadees is reported to occur mostly in spring (Odum 1942; Saunders 1947), spring and early summer (Dixon and Stefanski 1970; Ficken et al. 1978; Phillmore et al. 2006). However, in each of the above cases, the sampling patterns have been, at best, incomplete, selectively sampling at times when song production is assumed to be high, such as in the spring, and not sampling at times before or after. In one of the most systematic and quantitative effort to date to capture the pattern of *fee-bee* vocal production was conducted by Saunders (1947). In it he concludes that “I have found it impossible to determine definite dates when regular singing begins, because the singing always seems to be irregular” (pg. 99).

To summarize, in none of the studies previously mentioned was sampling done over more than 4 of 12 months. In few if any of these studies was the actual number of songs tallied. And in none of the studies was sampling done across the day to look at the pattern of vocalizing as a function of time of day! My first main aim in the current study then was to fill in these gaps in our knowledge of *fee-bee* song production in the black-capped chickadee by (1) sampling patterns of singing in a highly systematic manner across the entire years and (2) by sampling at three distinct times during the day, dawn, midday and dusk.

In common with the seasonal analysis of *fee-bee* song production, even fewer studies have focused on the seasonal pattern of *chick-a-dee* and *gargle* call production (but see Ficken et al. 1978;Odum 1942 and Smith 1991 for rare exceptions). The *chick-a-dee* call is produced by both sexes (Ficken et al. 1978) and primarily while in flocks (in the autumn and winter; Smith 1991) and is thought to coordinate flock movements and signal predators and food, amongst other functions (Freeberg and Lucas 2002; Mammen and Nowicki 1981; Templeton et al. 2005). Males produce the *gargle* call for aggression amongst black-capped chickadees (Ficken et al. 1978) and primarily during the summer and autumn season in the afternoon (Avey et al., this study). Although the vocal repertoire of the back-capped chickadee is extensive and its vocal behaviour has been studied extensively, no study has systematically quantified the usage of these three vocalization types across the year and at different times of day. The importance of understanding the seasonal nature of behaviour was recently highlighted by Pravosudov (2006). In this review, Pravosudov highlighted the potential pitfalls and blind alleys that can result from hypotheses and predictions made based on limited field observations using seed storage and retrieval in chickadees as his example. The crux of his argument is that by basing experimental designs on an incomplete sample of seasonal field data, scientists can be led astray. One example given in his review was that because most scientists took one paper as gospel regarding when birds store seeds, they were unaware that there were other periods just before and after this time when seed storing activity was high.

I envision analogous pitfalls and similar implications for studies of seasonal variations in vocal behaviour in chickadees as they are currently investigated and known (Christie et al. 2004; Gammon 2004; Gammon & Baker 2004; Hansen et al. 2005; MacDougall-Shackleton 2003; Odum 1941a; Otter et al. 1997; Otter & Ratcliffe 1993; Phillmore et al. 2006; Smulders et al. 2006) where precious time and resources may be squandered if a complete understanding is not achieved *a priori*. In line with my ideas, Amrhein et al. (2004) emphasized the importance of understanding the interaction between season, diurnal cycle, and social environment when studying the dawn chorus. I agree and here extend this rationale to understanding the seasonal and diurnal patterning of vocal production in black-capped chickadees. Indeed, neuroethology and related disciplines (e.g., neuroecology; Sherry 2006) will require a comprehensive understanding of the factors affecting natural vocal behaviour across seasons to describe adequately the evolution of cognition and brain structures without misinterpreting the link between what is observed in the laboratory versus what is observed in the field.

The purpose of this study was to determine quantitatively the amount and proportions of each vocalization type (*fee-bee*, *chick-a-dee*, and *gargle*) produced across one year to understand the seasonal and diurnal pattern of vocal production in black-capped chickadees. By tallying the number all three vocalization types produced across seasons, I could determine what effects photoperiod/season and diurnal cycle had on vocal behaviour. I predicted that *fee-bee* song production would vary both with the photoperiod/season, with most singing in spring, and with the diurnal cycle, with most singing at dawn (Otter and Ratcliffe 1993). I further predicted that the *chick-a-dee* call would also be linked to photoperiod/season, with most calling occurring in autumn and winter (Smith, 1991), and with diurnal cycle, with most calling at meridian coinciding with the periods of higher activities with which the chick-a-dee call is associated (Ficken et al., 1978; Templeton et al., 2006). Finally, I had no *a priori* prediction for *gargle* call production as previous authors had simply noted it was produced across all seasons (Smith 1991).

Methods

Study Site and Sampling

The study was conducted in the River Valley along the North Saskatchewan River in Edmonton Alberta, roughly between the western side of William Hawrelak Park (53°31.698’N, 113°31.220’W) and the eastern side of the University of Alberta Research Park (53°31.437’N, 113°32.731’W). The River Valley is ~7,400 ha and is the largest urban parkland in North America, supporting numerous avian species and even large ungulates such as moose (*Alces alces*). A stretch of forested trail along the river, approximately 4.2 km long, was used to conduct each sampling walk that lasted approximately one hour in duration, with the starting point randomly selected for each walk between Hawrelak Park and the University of Alberta Research Park. Sampling walks began on 4 October 2004 and finished on 23 September 2005. Three sampling walks were conducted biweekly for 12 months on randomly selected days of the week. The diurnal cycle for each sampling walk were either sunrise, meridian, or sunset (diurnal period), randomly assigned to each of the three days of the sampling weeks. Sampling walks began approximately 30 min before sunrise, meridian, or sunset (pre), and concluded approximately 30 min after sunrise, meridian, or sunset (post). All walks were conducted by one of two observers who tallied the data in real time.

Species and Criteria

Black-capped chickadee *fee-bees*, *chick-a-dees* and *gargles* were tallied during the walks. Both observers were trained for one month both in the lab and in the field until both observers could identify, and discriminate the three vocalization types. Both observers conducted practice walks in the field at dawn, meridian, and sunset until agreement on the type and numbers of vocalizations was ~100%. The *fee-bee* song consists of two clear whistled notes with the second lower in pitch. Criterion for the *fee-bee* song was that it contained the typical two notes ‘fee’ and ‘bee’ (Ficken, et al. 1978; Smith 1991). The *chick-a-dee* call is made up of one to four types, A, B, C (“chick-a-”), and D (“dee”), that can be repeated a variable number of times (Ficken, et al. 1978).The criterion for the *chick-a-dee* was that it had to contain at least one ‘dee’ note. The *gargle* call consists from two to thirteen syllables which can be used to comprise at least fifteen *gargle* types (Ficken & Weise 1984). The criteria for the *gargle* call was that it had to contain at least two syllables in succession that were identifiable as *gargle* syllables for the local population.

Statistical Analyses

A multivariate analysis of variance (MANOVA) comparing season × diurnal cycle × pre/post on the total number of vocalizations for each type (*fee-bee, chick-a-dee, gargle*) using SPSS version 11.5 (SPSS Inc.). Season was determined by the solstices and equinoxes. Winter was defined as the period from the winter solstice (21 December 2004) to the spring equinox (20 March 2005) and spring was defined as the period from the spring equinox to the summer solstice (21 June 2005). Summer was defined as the period from the summer solstice to the autumn equinox (22 September 2005) and autumn was defined as the period from the autumn equinox to the winter solstice (21 December 2004). Diurnal cycle was defined as dawn, meridian, and sunset for each one hour sample. The pre/post factor was determined by dividing each one hour sampling period into two equal portions with data scored during the 30 minutes pre-sunrise and 30 minutes post-sunrise analyzed separately (the same was done with meridian and sunset).

Results

For the season × diurnal cycle × pre/post MANOVA there was a significant main effect of season with *fee-bee* song, F(3, 162) = 8.453, $p < 0.001$, and with *chick-a-dee* calls, F(3, 162) = 8.574, $p < 0.001$, but not with *gargle* calls, F(3, 162) =1.533, p = 0.209 (see Table 2-1 for means). There was also a significant main effect of diurnal cycle for *fee-bee* song, F(2, 162) = 14.677, $p < 0.001$, for *chick-a-dee* calls, F(2, 162) = 3.668, p = 0.028, and for *gargle* calls F(3, 162) = 7.885, p = 0.001 (see Table 2-1 for means). There was no significant main effect of pre/post for *fee-bee* song, F(1, 162) = 0.24, p = 0.625, *chick-a-dee* calls, F(1, 162) = 0.103, p = 0.749, or *gargle* calls, F(1, 162) = 1.090, p = 0.298.

Fee-bee Song

Post hoc analyses (Tukey's HSD) were conducted on season for *fee-bee* song (Fig. 1). There were significant differences in the production of *fee-bee* songs between spring and summer ($p < 0.001$) and between spring and autumn ($p < 0.001$). Most *fee-bee* singing occurred during the spring although winter *fee-bee* singing was intermediary between spring and the other seasons (Fig. 1A). Post hoc analyses (Tukey's HSD) were conducted on the diurnal cycle for *fee-bee* song (Fig. 1B). There were significant differences in the production of *fee-bee* songs between dawn and meridian ($p < 0.001$) and between dawn and sunset ($p < 0.001$), with most *fee-bee* singing occurring at dawn. Furthermore, there was a significant interaction between season and diurnal cycle for *fee-bee* song, F(6, 162) = 7.5, $p < 0.001$. Most *fee-bee* singing occurred at dawn during the spring and winter, and least *fee-bee* singing occurred during all other seasons and diurnal periods.

Chick-a-dee Calls

Post hoc analyses (Tukey's HSD) were conducted on season for *chick-a-dee* calls (Fig. 1A). There were significant differences in the production of *chick-a-dee* calls between spring and winter (p = 0.004), spring and autumn (p < 0.001), and summer and autumn (p = 0.004). Most *chick-a-dee* calling occurred during the autumn and winter; however, calling during the winter was intermediary between autumn and summer while the least *chick-a-dee* calling occurred during the spring. Post hoc analyses (Tukey's HSD) were conducted on the diurnal cycle for *chick-a-dee* calls (Fig. 1B). There was a significant difference in the production of *chick-a-dee* calls between the meridian and sunset (p = 0.012). There were no significant interactions between season and diurnal cycle for *chick-a-dee* calls, F(6, 162) = 1.613, p = 0.148. There was a significant interaction between diurnal cycle and pre/post for *chick-a-dee* calls, F(2, 162) = 10.372, p < 0.001, with most *chick-a-dee* calling occurring post-sunrise and pre-sunset with little difference between pre and post meridian (Fig. 2)

***Gargle* Calls**

Post hoc analyses (Tukey's HSD) were conducted on the diurnal cycle for *gargle* calls (Fig. 1A). There were significant differences in the production of *gargle* calls between dawn and meridian (p = 0.004) and between meridian and sunset (p = 0.001), with most *gargle* calling occurring during the meridian. There were no significant interactions between season and diurnal cycle for *gargle* calls, $F(2, 162) = 0.952$, $p < 0.460$.

Discussion

Here I report the results of a year-long observational study of black-capped chickadee vocal behaviour. I quantified vocal production across the season and across different times of day. In so doing I built on existing landmark studies on chickadee communication and confirmed some observations, questioned others and extended our knowledge about what chickadees produce and when they produce it beyond what was previously known.

In general, I found significant differences in the amount of vocal production for the three vocalization types studied, namely *fee-bee* song, *chick-a-dee* calls and *gargle* calls, both across seasons as well as within the diurnal cycle. Some general trends, some of which match to varying degrees what was previously reported about chickadee communication, included observing the most *fee-bee* singing in the spring and at dawn, the most *chick-a-dee* calling in autumn at the meridian, and consistent levels of *gargle* calling observed across all four seasons but concentrated mainly at the meridian. However, there were several interesting and largely unexpected departures (e.g. winter *fee-bee* song) from these general trends that bear further examination, both here (see below) and in future research. Below I discuss each of the three vocal classes studied in turn, and the influence that season and diurnal cycle appeared to have on these vocalizations.

Fee-bee Song **Production Across Season and Diurnal Cycle**

The rate of *fee-bee* song production varied significantly with the seasonal cycle. In spring, *fee-bee* song production occurred almost entirely around the dawn (30 minutes pre and post sunrise) sampling period, consistent with research supporting dawn as the peak time for song production (Ratcliffe & Otter 1996). There was some *fee-bee* song production at the meridian whereas at sunset there was almost no *fee-bee* song production. Immediately following the spring equinox, when daylight hours exceed night hours, *fee-bee* song production increased dramatically. The sharp increase in vocal production at this time is in line with the evidence that photostimulated birds sing more than those that are either photorefractory or photosensitive (MacDougall-Shackleton et al. 2003). *Fee-bee* song production peaked before May and decreased to very low levels before the summer solstice. This decrease likely reflects the fact that by this point in the season most territories are well established and breeding is underway, thus eliminating the typical context of *fee-bee* singing (i.e., mate attraction and territorial advertisement).

In addition to the peak of *fee-bee* song production that occurs in the spring, there is one other aspect of seasonal variation in *fee-bee* production that bears discussion. That is, the highly circumscribed nature of the increase in *fee-bee* song production during this period of peak production. One explanation for this dynamic rise and fall in song production is likely related to the asymmetrical nature of the breeding behaviour of temperate songbirds. Resources are relatively plentiful in spring and summer; however temperate songbirds must divide their activities during this time between breeding in the spring and molting in the summer (Dawson et al. 2001). The rapid increase and subsequent decrease in the production of *fee-bee* song in spring may be part of the larger need to initiate and complete breeding in time to molt during the summer months and further, to allow fledglings enough time to develop before the autumn and winter.

The summer season, beginning at the summer solstice, represents the peak in the ratio of daylight to nighttime hours, but also marks the beginning of a steady decrease in daylight hours. The summer solstice itself does not serve as a trigger for the reduction of *fee-bee* song, as the decrease in production starts well in advance of the solstice. Based on previous research in black-capped chickadees, as well as in other songbirds, exposure to long days should instate photorefractoriness; however, the exact timing of this phase is unknown (MacDougall-Shackleton et al. 2003). Summer solstice and the concomitant decrease in daylight hours may also serve as a trigger for molting.

The autumn season, beginning at the autumn equinox, represents a shift in the daytime to nighttime ratio, with the amount of daytime becoming less than the amount of nighttime. This change in the ratio of day to night has no discernable affect on *fee-bee* production, although the shift to short days eventually moves the black-capped chickadees into a state of photosensitivity (MacDougall-Shackleton et al. 2003; Phillmore et al. 2006).

The winter season, beginning at the winter solstice when daylight hours begin to increase and when black-capped chickadees would most certainly be photosensitive (MacDougall-Shackleton et al. 2003), had a strong affect on *fee-bee* song production. The increase in *fee-bee* song production observed during this season was similar to that observed in the spring, but smaller, a large increase in production occurred mid-season. However, the end of the transient increase in song production does not see the song rates return to the low baseline level of song production seen in the summer and autumn. *Fee-bee* song production in winter occurred mostly at dawn although some song production occurred at the meridian. In spite of the fact that black-capped chickadees are in flocks during the winter, *fee-bee* song output during the winter may be due to initial competition for mates and territories. The dawn chorus at this time of year would certainly not be influenced by female nesting activity (Mace 1987, Gammon 2004); however, there are many alternative theories regarding dawn chorus, any of which either alone or in combination may be influencing song production. Singing behaviour occurs with the shift to increasing daylight that is coupled with a change in hormonal state to photosensitivity; the question as to how *fee-bee* song production functions in the winter months is unknown.

In summary, *fee-bee* song production and the interaction of season and diurnal cycle were clearly related. Confirmation of the dawn chorus at spring was expected; however, the similar dawn chorus in winter was an unexpected departure from previous literature. The limited use of song at the meridian and essentially non-use at sunset also expand our understanding of the daily cycle of black-capped chickadee vocal behaviour. Further research into understanding the function of *fee-bee* song production in the winter via natural observation will be necessary. Moreover, the dynamics of within day (dawn) and within season production of *fee-bee* song warrant further study.

***Chick-a-dee* Call Production Across Season and Diurnal Cycle**

The rate of *chick-a-dee* call production also varied with the seasonal cycle, although relative to the *fee-bee* song production, seasonal variation of *chick-a-dee* calling was not as dramatic or as season-specific. Furthermore, the diurnal cycle of *chick-a-dee* call production was also more variable. The most *chick-a-dee* calling occurred during the autumn and winter; however call production during the winter was not significantly different from autumn or summer while it was significantly higher than *chick-a-dee* calling in the spring. On average across seasons, the most *chick-a-dee* calling occurred around the meridian.

Examination of variations in *chick-a-dee* call production across the diurnal cycle and season, however, showed a complex relationship. In agreement with previous findings (e.g., Smith 1991) most calling occurred during the autumn and winter and around the meridian. The trend for *chick-a-dee* calling in the spring was an overall decrease in call production compared to winter, with most calling during this period occurring at the meridian. This trend persisted until the summer solstice when call production once again increased. Decreases in *chick-a-dee* call production during the spring likely occurred, at least in part, because as *fee-bee* song production increases, the ability to produce other vocalizations decreases.

The general decrease in *chick-a-dee* call production towards late spring may be a result of mated pairs minimizing vocal production and activity not related to raising their clutches. Calling did not increase again until after the summer solstice. There was little difference between pre and post meridian in *chick-a-dee* calling, but more calling occurred post-dawn and pre-sunset which is likely a function of general activity being restricted to light at the beginning and end of the day.

In summary, I confirmed that *chick-a-dee* call production occurs primarily in autumn and winter and during the meridian, but the nuances found in calling behaviour (e.g., spring decreases in calling coupled with increases in *fee-bee* song production) demonstrate that the vocal behaviour is much more complex than previous literature had assumed. Although season and diurnal cycle are good predictors of *chick-a-dee* calling behaviour, the relationship is complicated and likely significantly affected by other factors (e.g., flocking behaviours). Further research on the natural behaviours and more in-depth observations within days as well as around critical photoperiodic events will be required to determine how these factors interact and affect vocal production of the *chick-a-dee* call. The variation seen between seasons and within days demonstrates that further quantification of this behaviour will be required.

***Gargle* Call Production Across Season and Diurnal Cycle**

Gargle call production did not vary significantly across season or to the same degree as *fee-bee* song and *chick-a-dee* call production. In fact, *gargles* were produced least amongst all three vocal types. Similar to the diurnal pattern observed for *chick-a-dee* call production, *gargle* call production was focused around the meridian. This may be related to a period of high activity for chickadees, leading to increased and aggressive behavioural interactions. Although no significant seasonal differences were observed, some potential trends across season were apparent. Before the spring equinox there was an increase in *gargle* call production that may be related to aggression amongst flock members as dominance status and mates are challenged before the breeding season. Similar to *fee-bee* song and *chick-a-dee* call production, *gargle* call production decreased steadily across the spring and did not increase again until after the summer solstice. Following the autumn equinox there was another increase in *gargle* production that may be related to increased interactions amongst chickadees as flocks are formed and dominance relationships are re-established in autumn. The trend for *gargle* production at dawn and sunset was that most occurred during the autumn and winter, times when resources are low and competition for microhabitat is high.

In summary, I expanded our understanding of how *gargle* call production is shaped by season, diurnal cycle and its relative production to *fee-bee* song and *chick-a-dee* calls. *Gargle* call production was the most variable and in turn the most difficult to predict from the seasonal and diurnal cycle. Increased sampling within days and around relevant photoperiod events as well as increased field observations of natural behaviour, will be required to further explain use and factors affecting production of this complex call.

Summary, Shortcomings, Future Directions

Our results indicate that the relationship between vocal behaviour and photoperiod is not a straightforward one. Each vocal class investigated was produced differentially with respect to seasonal photoperiod and within diurnal cycle. This suggests that the mechanism by which individual vocalizations are modulated may be impacted differently at hormonal and neural levels of control. These results also indicate the importance of examining multiple vocalizations within the same study because decreases in one vocalization may be related to increases in another (e.g., song circuit).

One potential shortcoming in the current research is the effect or effects that changes in bird density across the season in the sampling area may have had on results. Could density have affected results? Possibly, but likely not significantly. Consider the following illustration. In spring, when vocal output is highest (see Figure 3) with *fee-bee* song production, there are likely about 50-60 birds in the sampling area with 42 in pairs and some unmated floaters (based on territory sizes compiled in Smith 1991 and the study area size). In contrast, in the fall I had approximately 3-4 times the number of birds in the study area and yet the amount of *chick-a-dee* call production is less than half of that observed for *fee-bee* song production observed in the spring when there are many fewer birds present. Therefore, although bird density is variable across the year, this is not enough on its own to explain the variations in vocal production observed. I suggest that other more salient factors are driving differences in the distribution of vocal production such as season and time of day. This is not to say that understanding the effects that fluctuating population density may have on vocal production is not worthy of further study, but rather that it is beyond the scope of the current investigations and in the present research does not appear to play a major role in the results.

Future research in the field should be directed at expanding the types of calls studied for black-capped chickadees (e.g., *tseets*) as well as for other species both within the genus (e.g., mountain chickadee, *Poecile gambeli*) and outside the genus (e.g., nuthatches, titmice). Indeed, studying the interactions of season and diurnal cycle on the vocalizations would provide insight into the form and function of vocal production as this is highly evolved in Passeriformes with differing ecologies. Future research could also focus on the interaction that climate and weather, food availability, and latitude have on these patterns. Researchers that study either laboratory bred or wild caught birds in laboratory settings would also be well served to determine if housing conditions produce normal patterns of vocalizations across seasons. Given the wide range that black-capped chickadees inhabit, studies examining populations at different latitudes may help determine the effect(s) that day length has on behaviour. Future research in endocrinology and behavioural neuroscience on black-capped chickadees can now use these results as a roadmap for interactions between the environment and complex vocal production. Indeed, in my efforts to understand the function and biological mechanisms for cognition in animal species, I must continue to investigate natural behaviour as the foundation for understanding cognition.

Table 2-1. Interaction of Season and Diurnal Cycle: Means and Standard Deviations

Vocalization	Season	Diurnal Cycle	Mean	Std. Dev.
fee-bee songs	Winter	Dawn	37.25	41.33
		Meridian	16.00	21.74
		Sunset	0.00	0.00
	Spring	Dawn	108.50	121.08
		Meridian	4.86	5.49
		Sunset	0.00	0.00
	Summer	Dawn	2.64	8.52
		Meridian	0.38	1.03
		Sunset	0.13	0.50
	Autumn	Dawn	2.43	8.80
		Meridian	0.00	0.00
		Sunset	0.17	0.39
chick-a-dee calls	Winter	Dawn	14.17	22.03
		Meridian	19.67	14.04
		Sunset	24.92	42.92
	Spring	Dawn	3.71	6.11
		Meridian	14.43	13.87
		Sunset	1.43	4.24
	Summer	Dawn	13.71	18.86
		Meridian	16.75	10.96
		Sunset	1.31	3.70
	Autumn	Dawn	25.07	17.66
		Meridian	27.58	17.00
		Sunset	16.42	13.77
gargle calls	Winter	Dawn	4.67	5.33
		Meridian	10.75	12.56
		Sunset	2.75	4.09
	Spring	Dawn	2.36	4.41
		Meridian	5.50	9.97
		Sunset	0.07	0.27
	Summer	Dawn	0.57	1.65
		Meridian	8.31	14.13
		Sunset	1.38	4.18
	Autumn	Dawn	3.36	4.99
		Meridian	4.33	5.30
		Sunset	4.08	6.49

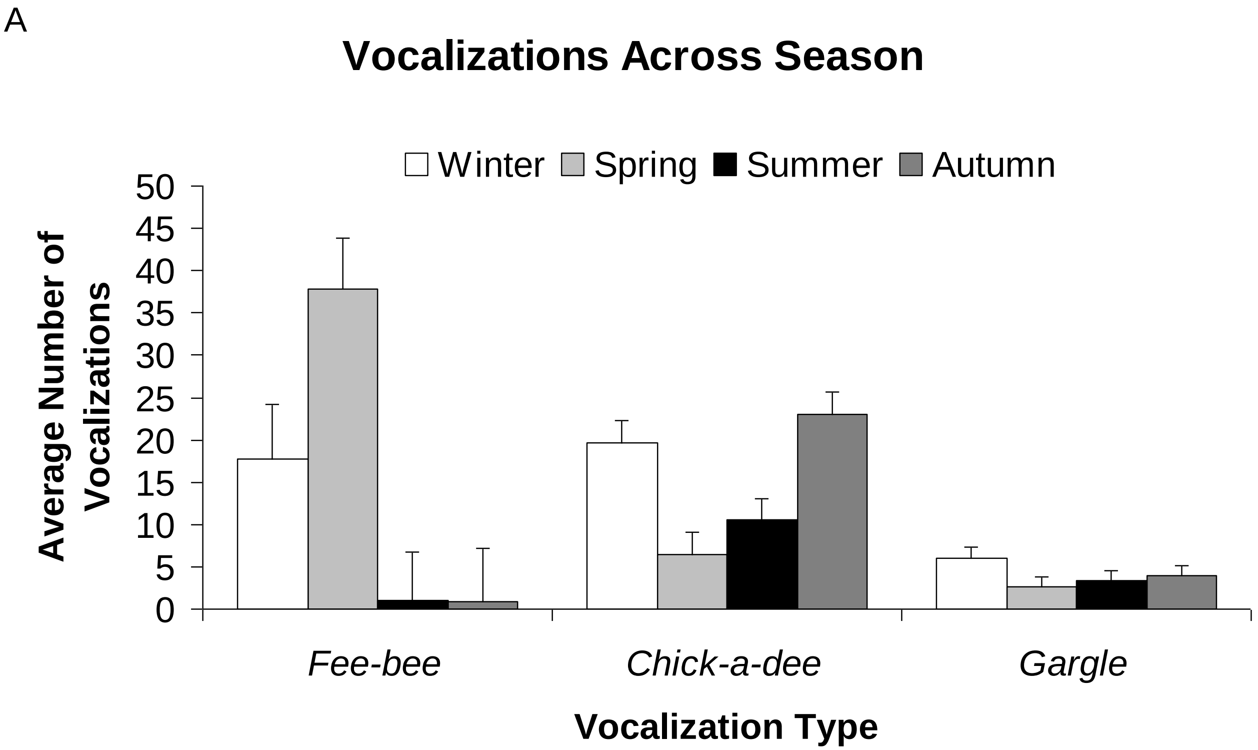

A
Vocalizations Across Season
☐ Winter
▨ Spring
■ Summer
▩ Autumn
Average Number of Vocalizations
50
45
40
35
30
25
20
15
10
5
0
Fee-bee
Chick-a-dee
Gargle
Vocalization Type

Figure 2-1A.

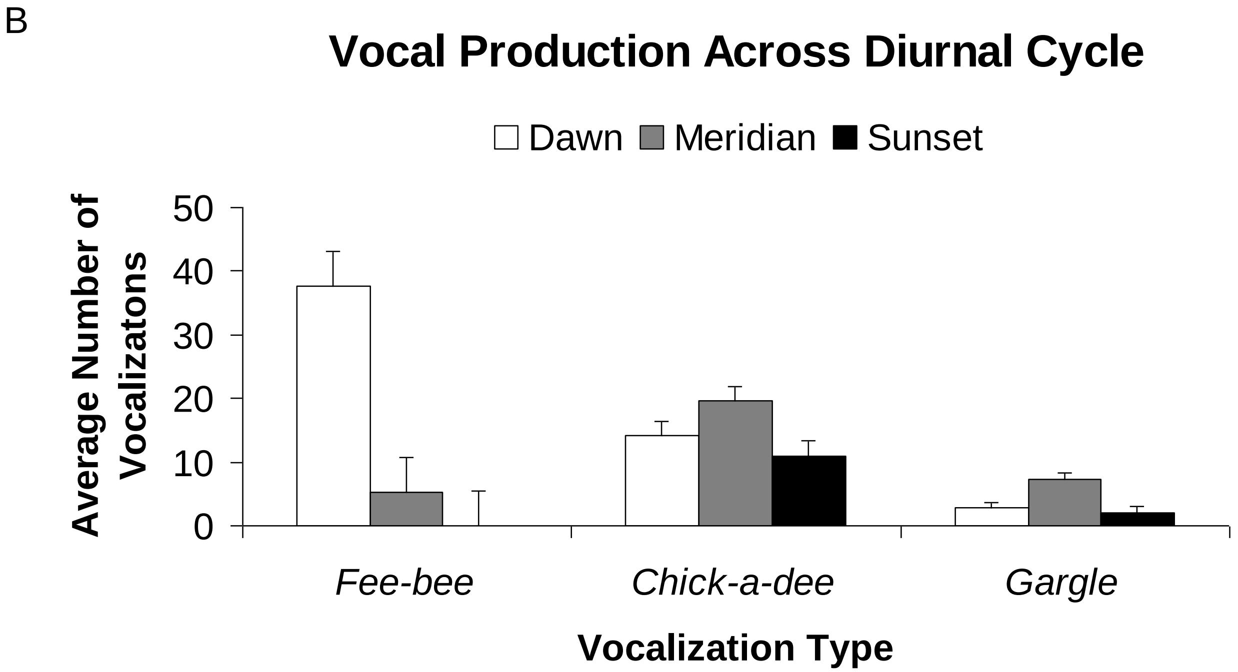

B
Vocal Production Across Diurnal Cycle
Dawn
Meridian
Sunset
Average Number of Vocalizatons
50
40
30
20
10
0
Fee-bee
Chick-a-dee
Gargle
Vocalization Type

Figure 2-1B.

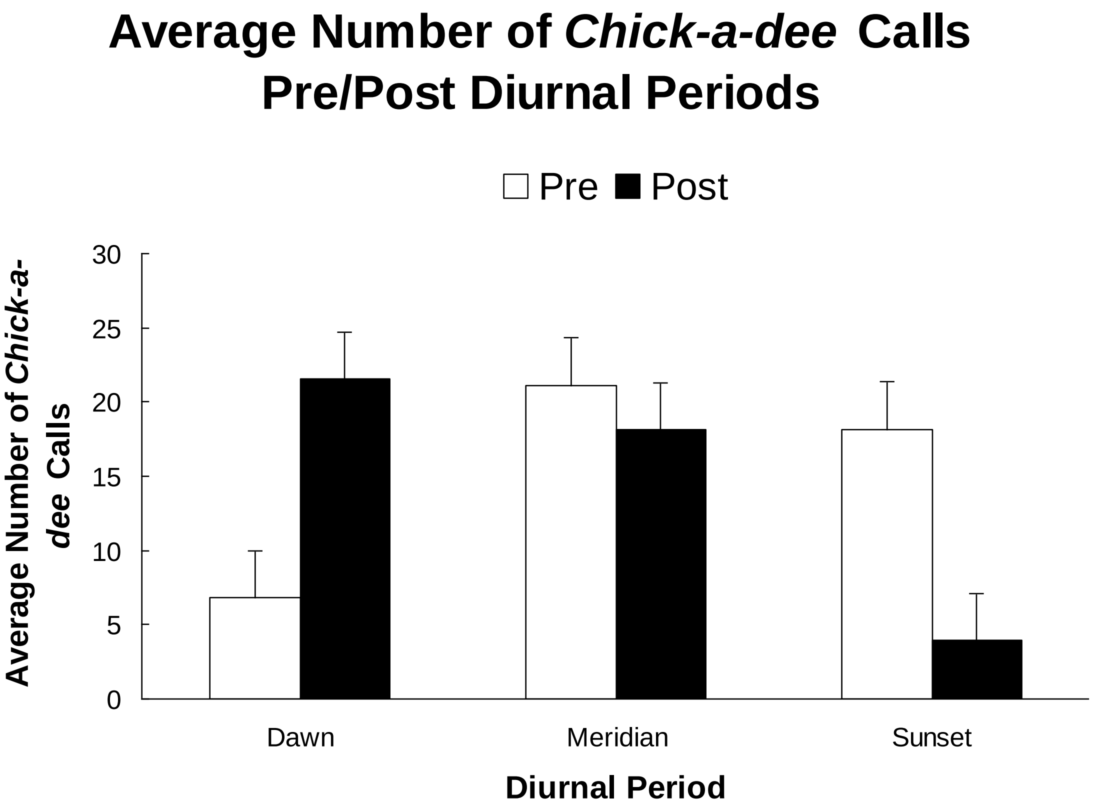

Average Number of *Chick-a-dee* Calls
Pre/Post Diurnal Periods
☐ Pre ■ Post
Average Number of *Chick-a-dee* Calls
30
25
20
15
10
5
0
Dawn
Meridian
Sunset
Diurnal Period

Figure 2-2.

References

Amrhein,V., Kunc, H.P., Naguib, M. 2004. Seasonal patterns of singing activity vary with time of day in the nightingale (*Luscinia megarhynchos*).Auk. 121, 110-117.

Christie, P.J., Mennill, D.J., Ratcliffe, L.M. 2004. Chickadee song structure is individually distinctive over long broadcast distances. Behaviour 141, 101-124.

Dawson, A., King, V.M., Bentley, G.E., Ball, G.F. 2001. Photoperiodic control of seasonality in birds. J. Biol. Rhythm. 16, 365-380.

Dixon, K.L., Stefanski, R.A. 1970. An appraisal of song of black-capped chickadee. Wilson Bull. 82, 53-62.

Ficken, M.S.,Weise, C.M. 1984. A complex call of the black-capped chickadee (*Parus atricapillus*). 1. Microgeographic variation. Auk. 101, 349-360.

Ficken, M.S., Ficken, R.W. Witkin, S.R., 1978. Vocal repertoire of the blackcapped chickadee. Auk. 95, 34-48.

Freeberg, T.M., Lucas, J.R. 2002. Receivers respond differently to chick-a-dee calls varying in note composition in Carolina chickadees, Poecile carolinensis. Anim. Behav. 63, 837-845.

Gammon, D.E. 2004. Black-capped Chickadee dawn chorus and subsequent sexual activity. Wilson Bull. 116, 252-256.

Gammon, D.E., Baker, M.C. 2004. Song repertoire evolution and acoustic divergence in a population of black-capped chickadees, *Poecile atricapillus*. Anim. Behav. 68, 903-913.

Hansen, I.J.K., Otter, K.A. van Oort, H., Holschuh, C.I., 2005. Communication breakdown? Habitat influences on black-capped chickadee dawn choruses. Acta Ethol. 8, 111-120.

MacDougall-Shackleton, S.A. Hernandez, A.M., Valyear, K.F., Clark, A.P., 2003. Photostimulation induces rapid growth of song-control brain regions in male and female chickadees (*Poecile atricapilla*). Neurosci. Lett. 340, 165-168.

Mace, R. 1987. The dawn chorus in the great Tit *Parus Major* is directly related to female fertility. Nature 330, 745-746.

Mammen, D.L., Nowicki, S. 1981. Individual-differences and within-flock convergence in chickadee calls. Behav. Ecol. Sociobiol. 9, 179-186.

Odum, E.P. 1941. Annual cycle of the black-capped chickadee—1. Auk. 58, 314-333.

Odum, E.P. 1942. Annual cycle of the black-capped chickadee—3. Auk. 59, 499-530.

Otter, K., Ratcliffe, L. 1993. Changes in singing behavior of male black-capped chickadees (*Parus atricapillus*) following mate removal. Behav. Ecol. Sociobiol. 33, 409-414.

Otter, K., Chruszcz, B., Ratcliffe, L. 1997. Honest advertisement and song output during the dawn chorus of black-capped chickadees. Behav. Ecol. 8, 167173.

Phillmore, L.S., Hoshooley, J.S., Sherry, D.F. MacDougall-Shackleton, S.A., 2006. Annual cycle of the black-capped chickadee: seasonality of singing rates and vocal-control brain regions. J. Neurobiol. 66, 1002-1010.

Pravosudov, V.V. 2006. On seasonality in food-storing behaviour in parids: do we know the whole story? Anim. Behav. 71, 1455-1460.

Ratcliffe, L.M., Otter, K. 1996. Sex differences in song recognition. In: Kroodsma, D.E., Miller, E.H. (Eds.), Ecology and Evolution of Acoustic Communication in Birds. Cornell University Press, Ithaca, pp. 339-355.

Saunders, A.A. 1947. The seasons of bird song. The beginning of song in spring. Auk. 64, 97–107.

Sherry, D.F. 2006. Neuroecology. Annu. Rev. Psychol. 57, 167-197.

Smith, S.M. 1991. The Black-capped Chickadee: Behavioral Ecology and Natural History. Cornell University Press, Ithaca.

Smulders, T.V., Lisi, M.D., Tricomi, E., Otter, K.A., Chruszcz, B., Ratcliffe, L.M.,DeVoogd,T.J. 2006. Failure to detect seasonal changes in the song system nuclei of the black-capped chickadee (*Poecile atricapillus*). J. Neurobiol. 66, 991-1001.

Templeton, C.N., Greene, E., Davis, K. 2005. Allometry of alarm calls: black-capped chickadees encode information about predator size. Science 308, 1934-1937.

Chapter 3 Seasonal variation of vocal behaviour in a temperate songbird: Assessing the effects of laboratory housing on wild-caught, seasonally-breeding birds[2]

---

[2] *A version of this chapter has been published. Avey M.T., Rodriguez, A., and Sturdy, C.B. (2011). Seasonal variation of vocal behaviour in a temperate songbird: Assessing the effects of laboratory housing on wild-caught, seasonally-breeding birds. Behavioural Processes.*

Introduction

Songbirds have increasingly been used in laboratory settings for fundamental scientific research, although the impact of captivity on the behaviour of songbirds housed in laboratories has not been studied extensively (Collins et al., 2008). The diversity among songbird species and their behaviour should make identifying and describing the effects of housing conditions on songbird behaviour a priority. Collins et al. (2008) estimate that 50% of research being conducted on passerines uses the zebra finch (*Taeniopygia guttata*), a species which has starkly different natural cycles and behaviours than a temperate bird such as the black-capped chickadee (*Poecile atricapillus*). Although the behavioural patterns of black-capped chickadees have been studied extensively in natural settings (Smith, 1991), little research has focused on what impact housing conditions have on their behaviour patterns.

The importance of studying the seasonal variation in behaviour for black-capped chickadees was demonstrated by Pravosudov (2006) who challenged the assumption of a single seasonal peak in food-caching activity. Changes in seasonal variation of food-caching behaviour may be compounded by laboratory conditions that provide an unnatural environment and may, for instance, negatively affect memory performance (Pravosudov, 2006). Likewise, changes in the diurnal patterns of behaviour found in the wild (Avey et al., 2008B) may have impacts on the behaviour in the laboratory. Songbirds’ vocal behaviour diurnally, from dawn to sunset, is an important parameter for population estimation and modeling vocal patterns (Thogmartin, 2010). The diurnal pattern is modulated by the light cycle in natural settings and the effect of this on the behaviour of captive chickadees has not been examined to date.

Typically in black-capped chickadees, and most songbirds, the song is the focal vocal behaviour and calls are often the ‘neglected orphans’ of study (Marler, 2004). Calls serve numerous functions in the life cycle of songbirds and also vary across season and throughout the day making their study as important as song. The black-capped chickadee is a vocal learner that learns its *fee-bee* song (Fig. 3-1a) and components of its *chick-a-dee* (Fig. 3-1b) and *gargle* calls (Fig. 3-1c; Shackleton and Ratcliffe, 1993; Hughes et al., 1998; Clemmons and Howitz, 1990). Both male and female black-capped chickadees produce all three of these vocalizations, although their function and frequency of production has been observed to vary (Avey et al., 2008B, Odum, 1942; Ficken et al., 1978). The *fee-bee* song consists of two clear, tonal, whistled notes with the second note sung lower in pitch than the first note. The *chick-a-dee* call consists of one to four types, A, B, C (“*chick-a*”), and D (“*dee*”), that can be repeated a variable number of times (Ficken et al. 1978). The *gargle* call consists of two to 13 syllables which can be used to produce at least 15 *gargle* types (Ficken & Weise, 1984).

Males predominately use the *fee-bee* song during the spring for courtship and territory defense (Odum 1942; Smith 1991; Otter and Ratcliffe, 1993), although females also produce the occasional *fee-bee* song (Dwight, 1897; Odum, 1942). There is a gradual increase in *fee-bee* song production during the winter before a dramatic increase that occurs at the spring equinox (Avey et al., 2008B). The *chick-a-dee* call is produced by both sexes and frequency of use varies across season (Avey et al., 2008B, Ficken et al., 1978). Chickadees form flocks in autumn (Odum, 1941A; Odum, 1941B; Odum ,1942) of about three to seven birds (Dhondt and Lowe, 1995) and there is an increase in the amount of *chick-a-dee* calling during this time and in the winter while chickadees are still in flocks (Avey et al., 2008B). These flocks remain together until spring, when male-female pairs select and defend territories and reproduce (Odum, 1941A; Odum, 1941B; Odum, 1942).The *chick-a-dee* call serves many functions such as flock cohesion (Nowicki, 1983) and mobbing of predators (Templeton et al. 2005). The *gargle* call is primarily produced by males and is used for aggression against other black-capped chickadees and is often accompanied by physically aggressive interactions (Dixon and Stefanski, 1970; Ficken et al., 1978). The *gargle* call is produced throughout the year (Avey et al., 2008B) and an individual chickadee typically uses between three to 18 distinct gargle vocalizations (Baker et al., 2000).

Differences between results in field and laboratory experiments can be the result of housing conditions. Indeed, even results between laboratory studies can be caused by different housing conditions (Calisi and Bentley, 2009). Chickadees and other songbirds are highly dependent on photoperiod to trigger behavioural and physiological changes (e.g. De Groof et al., 2009; Foerster et al., 2002; Hahn and MacDougall-Shackleton, 2008). For instance, black-capped chickadees begin to increase gradually the amount of *fee-bee* vocalizations as winter (photosensitive period) progresses until the spring equinox, when *fee-bee* song increases dramatically for a short time and then is essentially undetected until the following winter (Avey et al., 2008B). Male birds entering the photosensitive periods can have annual testicular mass change that is several hundred-fold compared to up to five-fold in mammals (Dawson, 2000). Despite these dramatic behavioural and physiological changes in songbirds caused by changes in photoperiod, little is known about how captivity may distort the natural behaviour of songbirds during their seasonal cycles.

The aim of the current study is to determine whether the pattern of vocal behaviour of wild-caught birds housed in the laboratory matches or is analogous to the pattern of vocal behaviour of wild birds in their natural habitat by assessing vocal behaviour across seasons in the laboratory. It was conducted concurrently with Avey et al. (2008B), a study that sampled the vocal behaviour across seasons in the field. To determine the impact of captivity on behaviour I investigated whether wild-caught birds housed in a laboratory continue patterns of natural vocal behaviour.

I assessed three different vocalizations which are known to vary both seasonally and diurnally (*fee-bee* songs, *chick-a-dee* and *gargle* calls) in black-capped chickadees, for one year. By examining how captivity potentially distorts the natural cycle of vocal behaviour I address two questions: Does captivity change the seasonal pattern of the vocal behaviour of wild birds? Does captivity change the diel pattern of vocal behaviour of wild birds?

Methods

I consulted the ARRIVE guidelines (Kilkenny et al., 2010) for the methodological information reported below. Ireport the methods used in Avey et al. 2008B in brief following the laboratory methods.

Experimental Subjects

Adult black-capped chickadees were captured from several regions within Edmonton, Alberta, Canada (53°34' N 113°31' W) and Kananaskis Country, Alberta, Canada (51°02'N, 115°03'W). Black-capped chickadees were housed individually in cages (30 cm wide × 40 cm high × 40 cm deep) in a colony room (365 cm wide × 287 cm high × 365 cm deep) with the natural seasonal light cycle for Edmonton, Alberta, Canada (53°34' N 113°31' W). Each cage contained three perches, two food cups, a water bottle, cuttle bone, a nest box and a ground shelter. Mazuri small bird maintenance food (Purina Mills LLC, St. Louis, MO), and water was provided *ad libitum* and colony room temperatures were maintained at approximately 20° C. Dietary supplements were provided, including eggs with spinach two times per week, meal worms three times per week, and three to five shelled sunflower seeds each week day. All birds were treated in accordance with Canadian Council on Animal Care Guidelines and Policies with approval from the Animal Care and Use Committee for Biosciences for the University of Alberta.

Vocal Sampling, Recording Equipment and Procedure

Because the experiment was designed to test the actual housing conditions all recording took place in the animal housing room. The number of chickadees and sex ratio within the housing room were not manipulated as they are not standardized under typical housing conditions as described above. There were 38 chickadees on average in the housing room over the course of the year with a range of 21-58. There were no modifications to individual housing or the daily routine of animal service staff except that during recording sessions no staff entered the room. Recording of vocalizations began on 4 October 2004 and finished on 23 September 2005. Laboratory vocal behaviour was collected concurrently with the collection of field data on seasonality of vocal output in black-capped chickadees conducted in Edmonton, Alberta, Canada (53°34' N 113°31' W; Avey et al., 2008B). Three recording sessions per week were conducted every other week for 12 months on randomly selected weekdays. The diurnal pattern for each recording session corresponded to either sunrise, meridian, or sunset and was randomly assigned to one of each of the three recording days. Because the light cycle in the housing was binary (either on or off) I conducted a series of test recordings before and after the lights came on and found no vocal behaviour occurred before the room lights were switched on. Thus, I began recording the sunrise session when the lights came on and finished recording the sunset session when the lights turned off, with each session lasting 1 hr. The meridian sample began 30 min prior to the onset of the meridian and concluded 30 min post meridian.

At the start of each recording session, a researcher entered the housing room to initiate the recording session, using a Sony SME Modified TCM-5000EV Bird Version tape recorder (frequency response: 90 – 12 000 Hz; Sony, Tokyo, Japan) connected to an AKG C 1000 S microphone (AKG Acoustics, Vienna, Austria) parabola (Dan Gibson Parabolic Microphone) with Maxell High Bias XLII audio cassettes (Hitachi Maxell, Tokyo, Japan) and then immediately left the colony room. The same researcher then re-entered the room after 30 min of recording to switch the tape side and record for an additional 30 min. The parabola was used to minimize the effects of reverberation in the recordings and was randomly aimed at either the east or west wall during each session.

Recordings were scored for the total number of vocalizations per session and each session was scored in 12 five min blocks. The first five mins of each 30 min block of recording (i.e., the first 5 min on each side of the tape) were excluded from analysis to eliminate possible increases or decreases in vocal behaviour related to the presence of the researcher. I further divided the total number of vocalizations by the number of individuals present in the colony room during recordings to adjust for number of individuals present during any given recording session. *Fee-bees*, *chick-a-dees*, *dees* (calls containing D notes only), and *gargles* were tallied. Criterion for the *fee-bee* song was that it contained both the '*fee*' and '*bee*' notes (Ficken et al., 1978; Smith, 1991). The criterion for the *chick-a-dee* was that it had to contain at least one "*chick-a-*" (A, B, or C note type) and at least one '*dee*' note. The '*dee'* call consisted of any number D notes that were not preceded an A, B, or C note (Ficken et al., 1978) The criterion for the *gargle* was that it had to contain at least two syllables in succession that were identifiable as *gargle* syllables for the local population.

Statistical Analysis

Using SPSS version 15.0 (SPSS Inc.) I conducted a multivariate analysis of variance (MANOVA) with Season × Diurnal Pattern as fixed factors and the adjusted number (see methods) of vocalizations for each type (*fee-bee, chick-a-dee, dee*, *gargle*) as dependent variables with an alpha level of 0.05. Season was determined by the solstices and equinoxes. Winter was defined as the period from the winter solstice (21 December 2004) to the spring equinox (20 March 2005). Spring was defined as the period from the spring equinox to the summer solstice (21 June 2005). Summer was defined as the period from the summer solstice to the autumn equinox (22 September 2005). Autumn was defined as the period from the autumn equinox to the winter solstice (21 December 2004). Diurnal pattern was defined as dawn, meridian, and sunset for each 1 hr sample. I also conducted Tukey HSD posthoc tests on diurnal pattern and season. To compare the field data (Avey et al., 2008B) to the current results I MANOVA with Season × Diurnal Pattern × Sampling Location (field or laboratory) as fixed factors. I transformed the number of each vocalization (*fee-bee, chick-a-dee, dee, gargle*) into z-scores and entered them as dependent variables with an alpha level of 0.05.

Avey et al., 2008B Methods in Brief

The study was conducted in the River Valley along the North Saskatchewan River in Edmonton Alberta, roughly between the western side of

William Hawrelak Park (53°31.698′N, 113°31.220′W) and the eastern side of the University of Alberta Research Park (53°31.437′N, 113°32.731′W). A stretch of forested trail along the river approximately 4.2 km long was used to conduct each sampling walk that lasted approximately 1h in duration, with the starting point randomly selected for each walk between Hawrelak Park and the University of Alberta Research Park. Sampling walks began on 4 October 2004 and finished on 23 September 2005. Three sampling walks were conducted biweekly for 12 months on randomly selected days of the week. The diurnal cycle for each sampling walk were either sunrise, meridian, or sunset (diurnal period), randomly assigned to each of the 3 days of the sampling weeks. Sampling walks began approximately 30 min before sunrise, meridian, or sunset (pre), and concluded approximately 30 min after sunrise, meridian, or sunset (post). Black-capped chickadee *fee-bees*, *chick-a-dees* and *gargles* were tallied during the walks.

Results

MANOVA Results for Songs and Calls in the Laboratory: Season × Diurnal Pattern

Songs and calls differed in how they changed across the treatment condition for Season in captivity (F(4,168) = 5.578, P < 0.001). There was also a significant difference for songs and calls for the treatment condition Diurnal Pattern in captivity (F(8,110) = 4.775, P < 0.001). Interactions between Season × Diurnal Pattern, were significant among treatment conditions (F(24,228) = 2.631, P < 0.001). The MANOVA revealed a significant main effect of Season for *fee-bee* songs (F(3,57) = 7.958, P < 0.001), *dee* calls (F(3,57) = 2.796, P = 0.048), and *gargle* calls, F(3,57) =16.073, P < 001, but not for *chick-a-dee* calls, F(3,57) = 0.484, P = 0.695. There was also a significant main effect of Diurnal Pattern for *fee-bee* songs (F(2,58) = 6.422, P = 0.003), *gargle* calls (F(2,57) = 14.481, P < 0.001), but not for *chick-a-dee* calls, F(2,57) = 2.037, P = 0.140) or *dee* calls, F(2,57) = 0.368, P = 0.694.

The diurnal change in songs and calls was also affected by season, as shown by significant interactions between Season and Diurnal Pattern for *fee-bee* songs (F(6,57) = 2.715, P = 0.022), *chick-a-dee* calls, (F(6,57) = 2.663, P = 0.024), and *gargle* calls, (F(6,57) = 6.114, P < 0.001), but not for *dee* calls, (F(6,57) = 1.392, P = 0.234; see Table 1. for means).

Post hoc Analyses for Songs and Calls in the Laboratory: Season × Diurnal Pattern

Post hoc analyses were conducted for Season × Diurnal Pattern for all vocalizations (Figs. 3-2 o 3-55) for captive birds. There were significant differences in the total production of *fee-bee* songs between spring and summer (P = 0.007), between spring and autumn (P < 0.007), and between spring and winter (P = 0.024), with more songs occurring in spring. There was a significant difference between dawn and sunset for *fee-bee* songs (P = 0.008) with very little song production recorded at sunset in any season. For *chick-a-dee* calls these analyses revealed no significant differences as a function of season or diurnal pattern. For *dee* calls there was a significant difference between winter and summer (P = 0.039), with more *dee* calls occurring in summer. There was no significant diurnal effect or interaction for dee calls. For g*argle* calls there were significant differences between summer and winter (P < 0.001), summer and spring (P = 0.003), summer and autumn (P < 0.001) with more *gargle* calls occurring in summer. There were significant differences in the production of *gargle* calls between dawn and sunset (P < 0.001) and between meridian and sunset (P < 0.001), with dawn and meridian having higher levels of *gargle* call production than sunset.

MANOVA Results for Songs and Calls Between the Field and Laboratory: Season × Diurnal Pattern × Sampling Location

Results for both Season and Diurnal Pattern did not differ from the above analysis or from Avey et al. 2008B, here I report only the comparison between field and laboratory conditions. The treatment condition Sampling Location (field versus laboratory) was non-significant for *fee-bee* song (F(1, 138) = 0.588, P = .445; Fig. 3-6), *chick-a-dee* calls (F(1, 138) = 0.003, P = 0.959; Fig. 3-7), and for *gargle* calls (F(1, 138; Fig. 3-8) = 0.154, P = 0.696).

Discussion

In the laboratory, the pattern of *fee-bee* song production peaked in the spring with most singing at dawn although there was also a large amount of singing at the meridian. There was no difference across season or diurnal pattern for *chick-a-dee* calls but for *dee* calls there was a large increase in the summer.

Gargle calls increased in the summer with most calling occurring at dawn and the meridian.

Patterns of vocalizations demonstrate general agreement between field and laboratory. Only *chick-a-dee* calls varied significantly between field and laboratory in diurnal patterning in the winter and the seasonal cycle at dawn and sunset. In the laboratory, *fee-bee* song production increased rapidly in the spring at dawn, indicating that the birds were photostimulated as the light cycle changed to long days. In captive birds, *chick-a-dee* calls did not vary with season or time of day and the most *dee* calls occurred during the summer and the least in winter; however, I was unable to compare field and laboratory patterns for this call (Avey et al., 2008B). In the laboratory, most *gargle* calls occurred during the summer during the dawn and meridian.

A comparison between the absolute numbers of vocalizations in the field and laboratory is difficult because we did not standardize the number of individuals sampled in the field or in the housing conditions which would have been difficult in the field and not a true test of typical housing arrangement in the laboratory (Avey et al., 2008B). However, the proportion of *fee-bee* songs relative to other vocalizations is dramatically lower in the laboratory than in the field (Table 2.). One hypothesis to explain this result is that individual housing, as opposed to group housing in flight aviaries, may modify the other natural behaviours associated with *fee-bee* song, resulting in a decrease in overall production of this vocalization.

The proportion of *chick-a-dee* calls is also lower in the laboratory than in the field (including '*dee*' calls; Table 2.). The reasons for this result are unclear; this may be a result of housing conditions eliminating other natural behaviours such as foraging where *chick-a-dee* calling is often used. Direct comparison between the pattern of *dee* calls in the laboratory and field are not possible because *dee* calls were not isolated in the scoring of vocal behaviour in the field (Avey et al., 2008B). The pattern for *dee* calls in the laboratory indicates that there is a large 'spike' in the summer, although not significantly higher than from spring and autumn. Large increases in summer and a marked decrease in winter suggest that changes in vocal behaviour are linked to changes in the light cycle in the colony room. Interpreting the pattern for *dee* calls highlights the importance of understanding the functional difference even within categories of calls (e.g., different *chick-a-dee* calls can have different meanings; Templeton et al., 2005) to gain a more complete understanding of vocalization behaviour.

Gargle calls were produced proportionally more frequently in the laboratory than the field although patterns did not differ (Table 2.). The large proportion of *gargle* calls in the laboratory may be a result of housing conditions where birds are either housed individually and therefore cannot physically interact and establish dominance through physical contests (e.g., displacement), or housed on higher and lower shelves where they are unable to see one another, thus increasing the amount of vocalizations required to maintain contact. *Gargle* calls communicate interspecific aggression and increases in the laboratory warrant further investigation regarding features in the laboratory cause departure from natural behaviour.

Our results demonstrate that the vocal behaviour in wild-caught black-capped chickadees housed in captivity closely matches that of bird remaining in native population in the field across the entire year. Black-capped chickadees are well studied in the field, particularly with respect to their behavioural ecology (Smith, 1991). However, determining which environmental conditions are relevant for housing black-capped chickadees in the laboratory remains difficult. In the wild, the spring increase in *fee-bee* song production includes other vocalizations not recorded here (e.g., faint '*fee*') and other natural behaviours such courtship and mating as well as territory defense (Smith, 1991). Intraspecific interactions as well as other conditions such as nutrition may change everything, from the behaviour itself to the interpretation of the molecular mechanisms controlling behaviour (e.g. Avey et al., 2008A; 2011).

As Calisi and Bentley (2009) note, the differences between field and laboratory settings should not be viewed as negatives but either as variables to control or information to incorporate into the interpretation of study results. Just as there can be differences between housing conditions in different laboratories or even the same laboratory over time, field studies also use populations of animals in different habitats which can affect the behaviour of free-ranging animals. Whether working in the field or in the laboratory, housing conditions or natural settings are both part of any research design and will impact results and their interpretation.

Conclusion

Our results provide strong evidence that under a standard housing regime vocal behaviour of black-capped chickadees in the laboratory was similar to that of birds in the natural source population across season and time of day. *Fee-bee* song, which is a good indicator of normal physiological state and cycle, followed the same pattern as in the field, suggesting that laboratory housing environment was well designed. Future research should focus on investigating the effects of housing on other natural behaviours such as flocking, courtship and dominance in chickadees as well as other temperate songbirds. Such comparisons are important for research on behaviour that may be altered by housing conditions. One final consideration is how changes in the behaviour of wild birds in captivity affects the welfare (e.g. stress levels) of those animals. Because behaviour can be directly observed and measured it can function as a non-invasive indicator of welfare (Nogueira et al., 2010; Albentosa and Cooper 2004). Thus, future studies should investigate if vocal behaviour itself can be an indicator of welfare (e.g. Zimmerman et al., 2003) in songbirds.

Table 3-1. Mean Number of Songs and Calls per Capita for Season and Diurnal Period in the Laboratory – Means and Standard Error of the Mean (SEM)

Vocalization	Diurnal pattern	Spring	SEM	Summer	SEM	Autumn	SEM	Winter	SEM
Fee-bee	Dawn	3.51	0.65	0.61	0.58	0.33	0.58	1.32	0.58
	Meridian	3.28	0.65	0.25	0.49	0.46	0.49	0.27	0.53
	Sunset	0.07	0.49	0.13	0.53	0.23	0.49	0.05	0.53
Chick- a- dee	Dawn	2.39	0.57	3.48	0.51	2.14	0.51	1.90	0.51
	Meridian	1.67	0.57	1.21	0.43	1.71	0.43	2.76	0.46
	Sunset	1.50	0.43	1.18	0.46	2.62	0.43	2.22	0.46
dee	Dawn	0.29	0.25	0.82	0.23	0.46	0.23	0.22	0.23
	Meridian	0.30	0.25	0.99	0.19	0.35	0.19	0.31	0.21
	Sunset	0.35	0.19	0.26	0.21	0.69	0.19	0.16	0.21
Gargle	Dawn	16.16	3.21	21.47	2.87	3.80	2.87	4.47	2.87
	Meridian	10.23	3.21	26.39	2.43	3.27	2.43	3.72	2.62
	Sunset	3.00	2.43	1.26	2.62	3.98	2.43	1.84	2.62

Table 3-2. Total Mean Vocalizations as a Percentage Field and Laboratory

Vocalization	Percentage (Field)	Percentage (Laboratory)
Fee-bee	45.84	7.50
Chick-a-dee (including *dees*)	42.69	21.40
Gargles	11.47	71.10

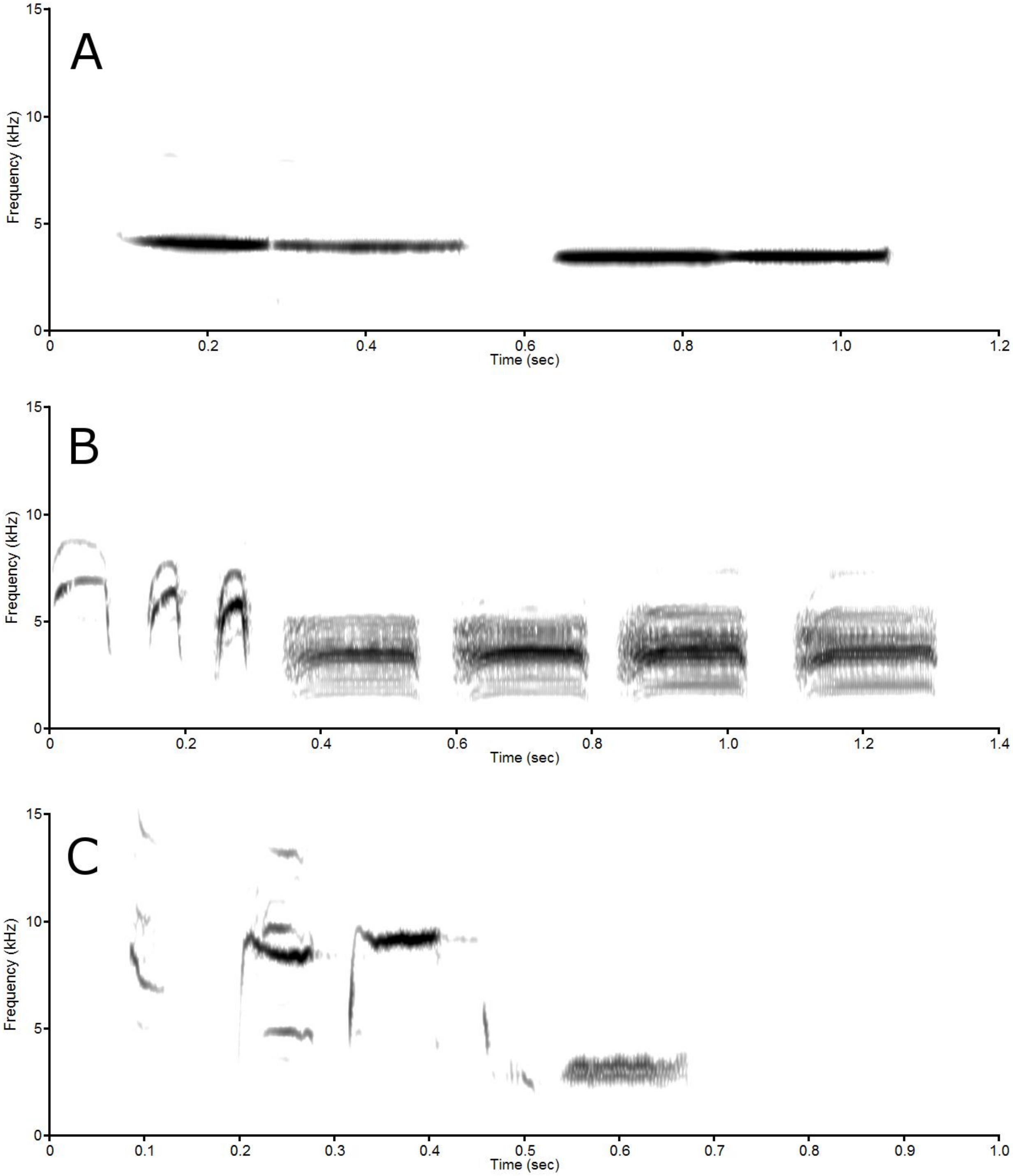

A
B
C
Frequency (kHz)
0
5
10
15
Time (sec)
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0.1
0.3
0.5
0.7
0.9

Figure 3-1.

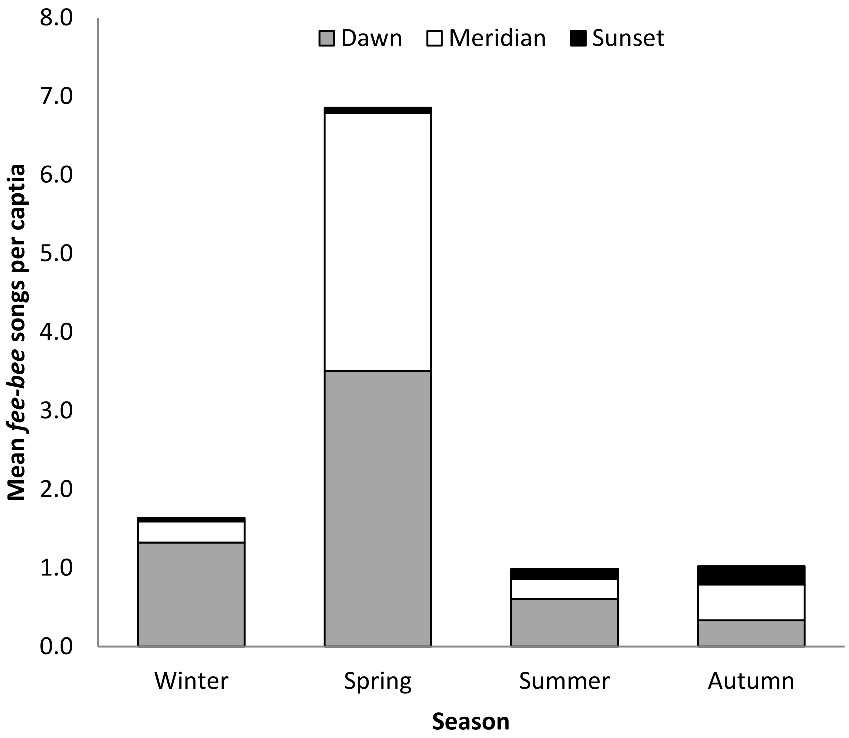

8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Mean *fee-bee* songs per captia
▨ Dawn
☐ Meridian
■ Sunset
Winter
Spring
Summer
Autumn
Season

Figure 3-2.

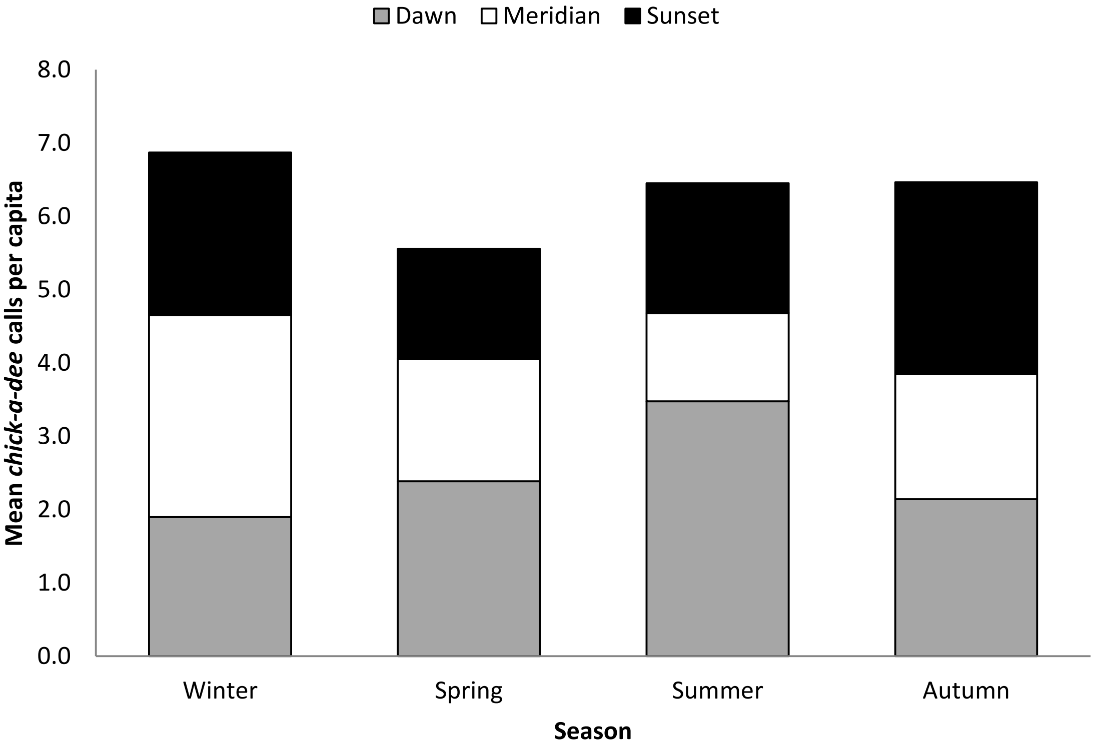

Dawn
Meridian
Sunset
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Mean *chick-a-dee* calls per capita
Winter
Spring
Summer
Autumn
Season

Figure 3-3.

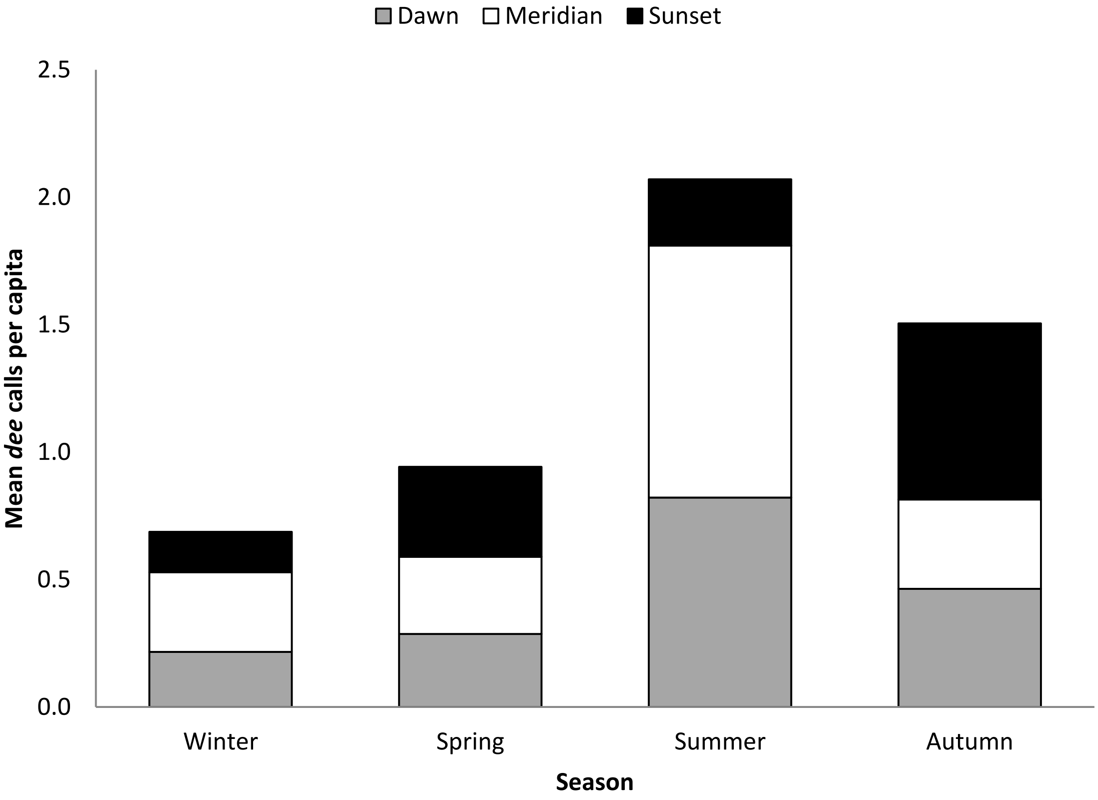
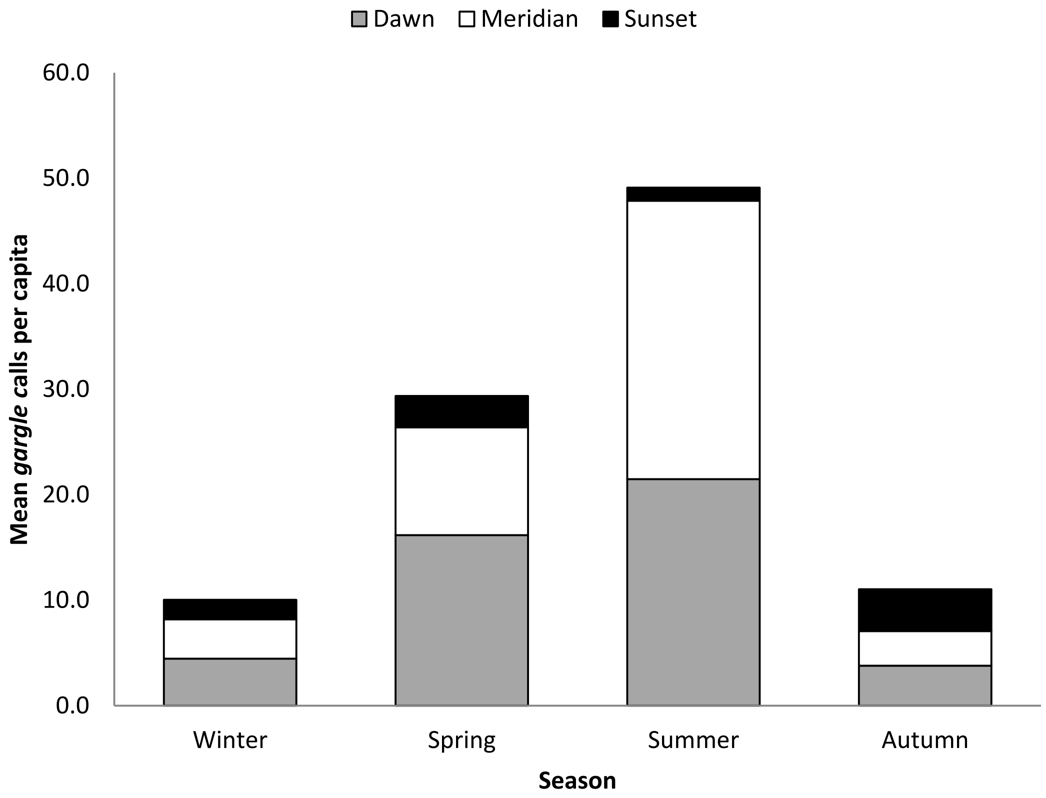

Dawn
Meridian
Sunset
2.5
2.0
1.5
1.0
0.5
0.0
Mean *dee* calls per capita
Winter
Spring
Summer
Autumn
Season

Figure 3-4.



▨ Dawn
☐ Meridian
■ Sunset
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Mean *gargle* calls per capita
Winter
Spring
Summer
Autumn
Season

Fig 3-5.

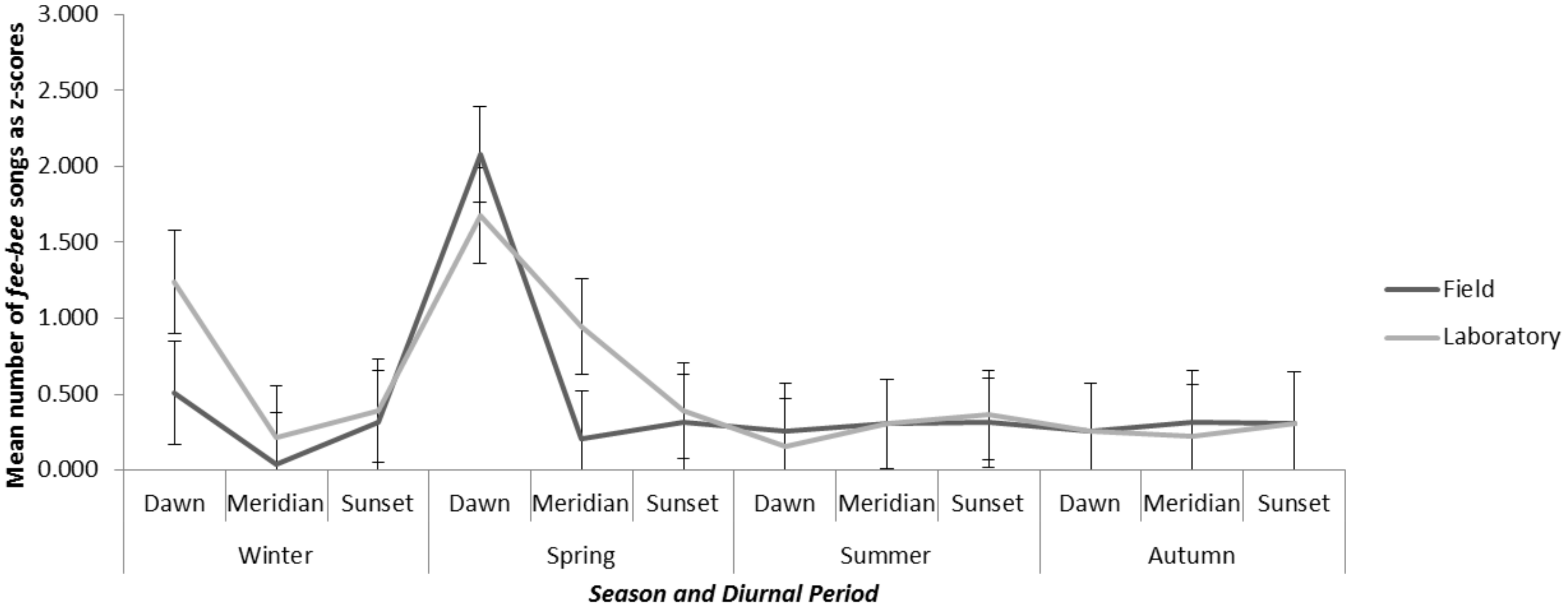

3.000
2.500
2.000
1.500
1.000
0.500
0.000
Mean number of *fee-bee* songs as z-scores
Dawn
Meridian
Sunset
Winter
Spring
Summer
Autumn
Season and Diurnal Period
Field
Laboratory

Figure 3-6.

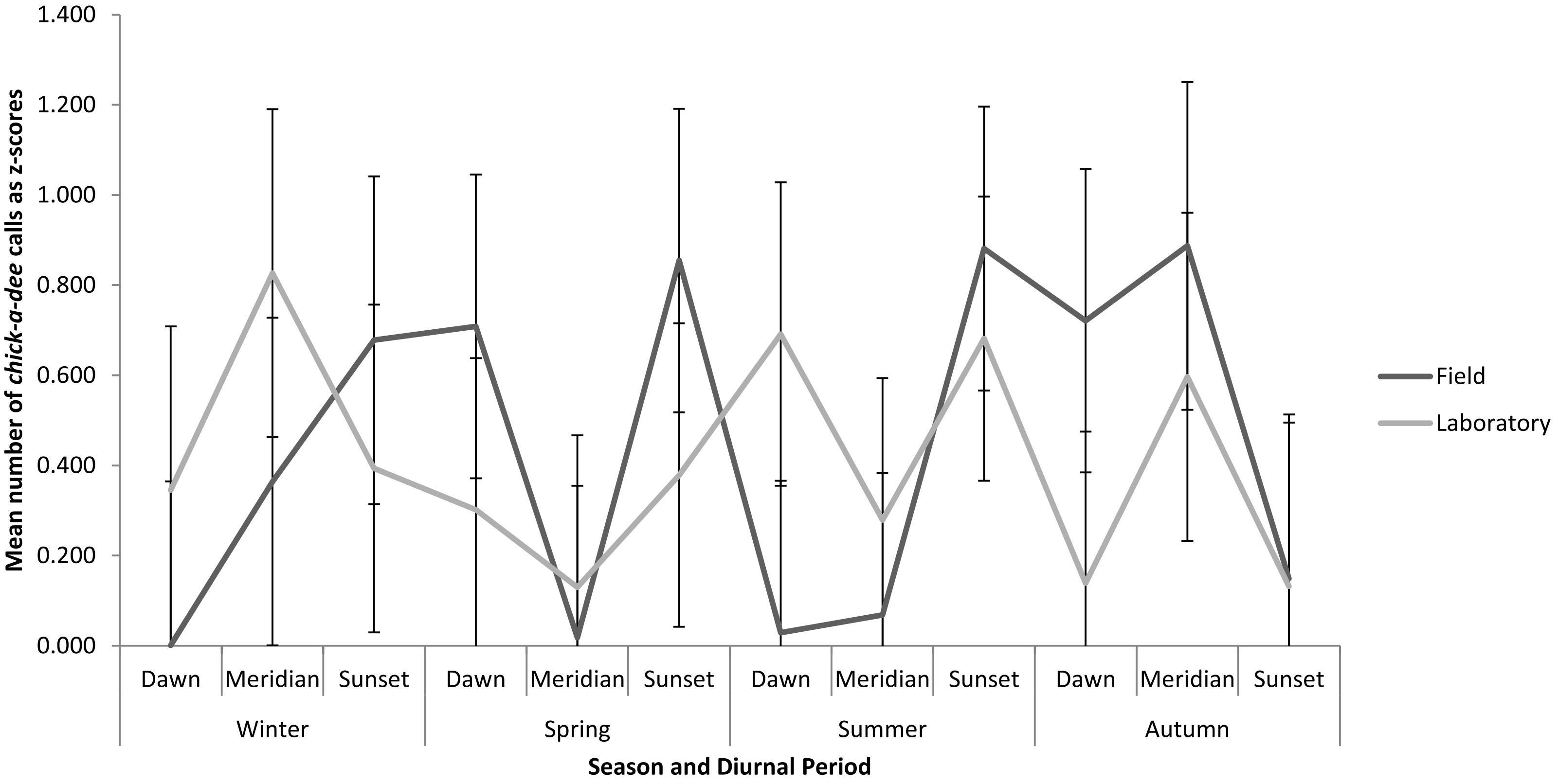

1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
Mean number of *chick-a-dee* calls as z-scores
Dawn
Meridian
Sunset
Dawn
Meridian
Sunset
Dawn
Meridian
Sunset
Dawn
Meridian
Sunset
Winter
Spring
Summer
Autumn
Season and Diurnal Period
Field
Laboratory

Figure 3-7.

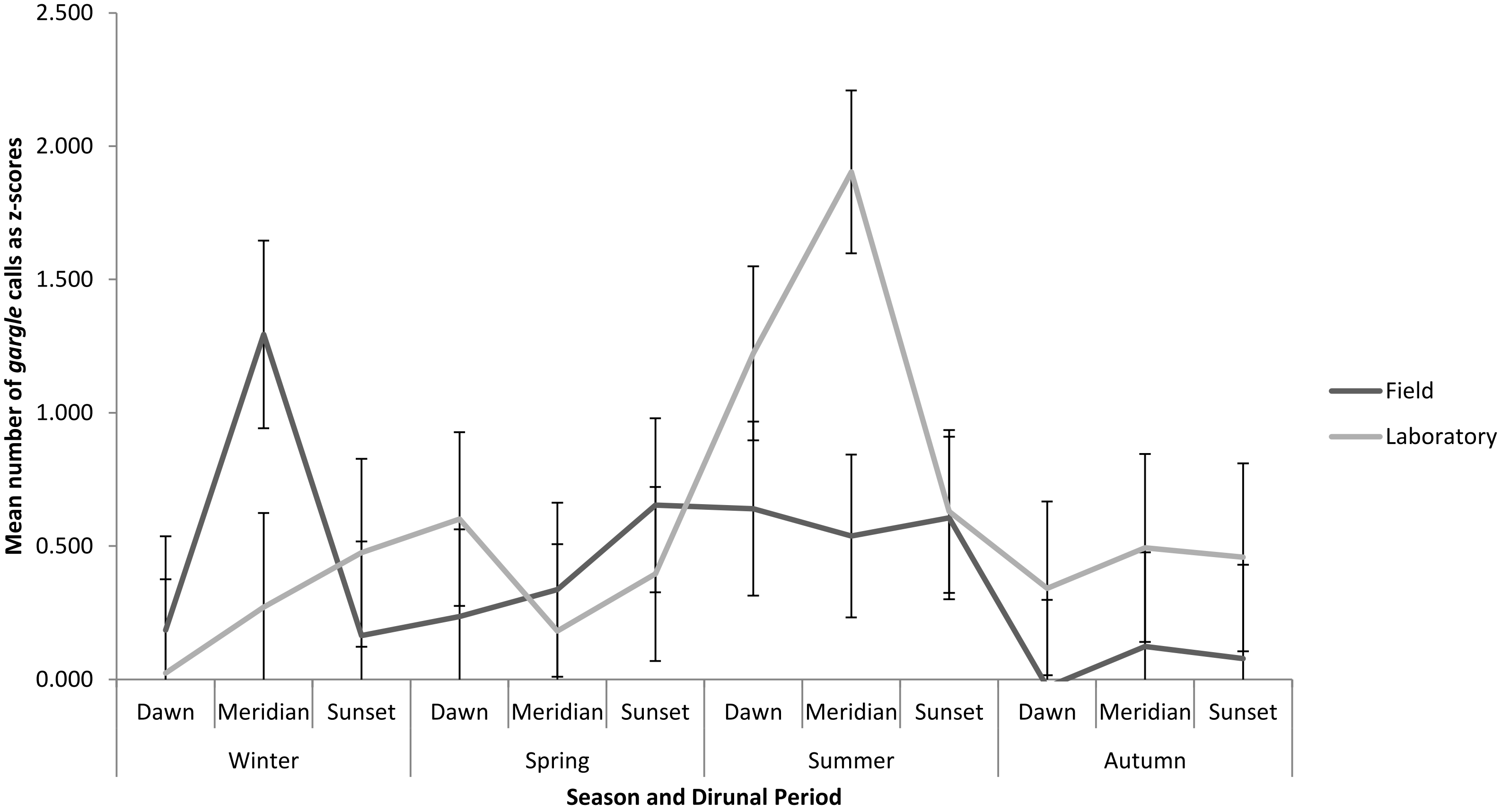

2.500
2.000
1.500
1.000
0.500
0.000
Mean number of *gargle* calls as z-scores
Dawn
Meridian
Sunset
Dawn
Meridian
Sunset
Dawn
Meridian
Sunset
Dawn
Meridian
Sunset
Winter
Spring
Summer
Autumn
Season and Dirunal Period
Field
Laboratory

Figure 3-8.

References

Albentosa, M.J., Cooper, J.J., 2004. Effects of cage height and stocking density on the frequency of comfort behaviours performed by laying hens housed in furnished cages. Anim. Welf. 13, 419-424.

Avey, M.T., Hoeschele, M., Moscicki, M.K., Bloomfield, L.L., Sturdy, C.B., 2011., Neural Correlates of Threat Perception: Neural Equivalence of Conspecific and Heterospecific Mobbing Calls Is Learned. PLoS ONE 6(8): e23844. doi:10.1371/journal.pone.0023844

Avey, M.T., Kanyo, R.A., Irwin, E.L., Sturdy, C.B., 2008A., Differential effects of vocalization type, singer and listener on ZENK immediate early gene response in black-capped chickadees (*Poecile atricapillus*). Behav. Brain Res. 188, 201-208.

Avey, M.T., Quince, A.F., Sturdy, C.B., 2008B., Seasonal and diurnal patterns of black-capped chickadee (*Poecile atricapillus*) vocal production. Behav. Processes. 77, 149-155.

Baker, M.C., Howard, T.M., and Sweet, P.W. 2000., Microgeographic variation and sharing of the gargle vocalization and its component syllables in black-capped chickadee (Aves, Paridae, *Poecile atricapillus*) populations. Ethology. 106, 819-838.

Calisi, R.M., Bentley, G.E., 2009. Lab and field experiments: Are they the same animal? Horm. Behav. 56, 1-10.

Clemmons, J., Howitz. J.L., 1990. Development of early vocalizations and the chick-a-dee call in the Black-capped Chickadee, *Parus atricapillus*. Ethology. 86, 203-223.

Collins, S.A., Archer, J.A., Barnard, C.A., 2008., Welfare and mate choice in zebra finches: effect of handling regime and presence of cover. Anim.Welfare. 17, 11-17.

Dawson, A., King, V.M., Bentley, G.E., Ball, G.F., 2001. Photoperiodic control of seasonality in birds. J. Biol. Rhythms. 16, 365-380.

De Groof, G., Verhoye, M., Poirier, C., Leemans, A., Eens, M., Darras, V., Van der Linden, A., 2009. Structural changes between seasons in the songbird auditory forebrain. J. Neurosci. 29, 13557-13565.

Dixon, K.L., and Stefanski, R.A., 1970. An appraisal of the song of the black-capped chickadee. Wilson Bull. 82, 53-62.

Dhondt, A.A., Lowe, J.D., 1995. Variation in black-capped chickadee group size. Birdscope, 9, 1.

Dwight, J., Jr., 1897. The whistled call of *Parus atricapillus* common to both sexes. Auk, 14, 99.

Ficken, M.S., Ficken, R.W., Witkin, S.R., 1978. Vocal repertoire of the black-capped chickadee. Auk. 95, 34-48.

Ficken, M.S., Weise, C.M., 1984. A complex call of the black-capped chickadee (*parus atricapillus*) .1. Microgreographic variation. Auk. 101. 349-360.

Foerster, K., Poesel, A., Kunc, H., Kempenaers, B., 2002. The natural plasma testosterone profile of male blue tits during the breeding season and its relation to song output. J. Avian Biol. 33, 269-275.

Hahn, T.P., MacDougall-Shackleton, S.A., 2008. Adaptive specialization, conditional plasticity and phylogenetic history in the reproductive cue response systems of birds. *Phil. Trans. R. Soc. B. 363, 267-286.*

Hughes, M., Nowicki, S., Lohr. B., 1998. Call learning in Black-capped Chickadees (*Parus atricapillus*): The role of experience in the development of 'chick-a-dee' calls. Ethology.104, 232-249.

Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M., Altman, D.G., 2010. Improving Bioscience Research Reproting: The ARRIVE Guidleines for Reporting Animal Research. PLoS Biol. 8(6), e1000412. doi:10.1371/journal.pbio.1000412

Marler, P., 2004. Bird calls: their potential for behavioral neurobiology. Ann. N. Y. Acad. Sci. 1016, 31-44.

Nogueira, S.S. da C., Silva, M.G., Dias, C.T. dos S., Pompeia, S., Cetra, M., Nogueria-Filho, S.L.G., 2010. Social behaviour of collared peccaries (*Pecari tajacus*) under three space allowances. Anim. Welf. 19, 243-248.

Nowicki, S., 1983. Flock-specific recognition of chickadee calls. Behav. Ecol. Sociobiol. 12, 317-320.Odum, E.P., 1941A. Annual cycle of the Black-capped Chickadee-1. Auk. 58, 314-333.

Odum, E.P., 1941B. Annual cycle of the Black-capped Chickadee-2. Auk. 58, 518-534.

Odum, E.P., 1942. Annual cycle of the Black-capped Chickadee-3. Auk. 59, 499-531.

Otter, K., Ratcliffe, L., 1993. Changes in singing behavior of male black-capped chickadees (*parus atricapillus*) following mate removal. Behav. Ecol. Sociobiol. 33, 409-414.

Pravosudov, V.V., 2006. On seasonality in food-storing behaviour in parids: do we know the whole story? Animal Behav. 71, 1455-1460.

Shackleton, S.A., Ratcliffe, L., 1993. Development of song in hand-reared black-capped chickadees. Wilson Bull. 105, 637-644.

Smith, S.M., 1991. The Black-capped Chickadee: Behavioral Ecology and Natural History. Cornell University Press, New York.

Templeton, C.N., Greene, E., Davis, K., 2005. Allometry of alarm calls: Black-capped chickadees encode information about predator size. Science. 308, 1934-1937.

Thogmartin, W.E., 2010. Sensitivity analysis of North American bird population estimates. Ecological Modelling. 221, 173-177.

Zimmerman, P.H., Lundberg, A., Keeling, A., Koene, P., 2003. The effect of an audience of the gakel-call and other frustration behaviours in the laying hen (*Gallus gallus domesticus*). Anim. Welf. 12, 315-326.

Chapter 4 Conspecific or Heterospecific Vocalizations? ZENK Activation in the Nidopallium of Black-capped Chickadees[3]

---

[3] *A version of this chapter has been submitted for publication and is in revision . Avey M.T., et al. Conspecific or Heterospecific Vocalizations? ZENK Activiation in the Nidopallium of Black-capped Chickadees.Jounral of Comparative Neurology.*

Introduction

A diverse range of species has been studied for the ability to respond to heterospecific as well as conspecific signals (e.g., Galapagos marine iguana, *Amblyrhynchus cristatus*, Vitousek et al. 2010; zebrafish, *Danio rerio,* Saverino and Gerlai, 2008; grey mouse lemur, *Microcebus murinus*, Braune et al. 2008; tungara frog, *Physalaemus pustulosus*, Hoke et al. 2004; honeybee, *Apis cerana* and *Apis mellifera,* Tan et al. 2008; and numerous songbird species such as zebra finch, *Taeniopygia guttata*, Mello et al. 1992; canary*, Serinus canaria*, Lynch & Ball, 2008; and black-capped chickadee, *Poecile atricapillus* (Avey et al. 2008A; 2011). Many species respond to heterospecific acoustic signals, such as alarm calls, and are able to respond in a signal-appropriate manner that suggests that they can decode signals, such as the size and type of predator (Rainey et al. 2004; Templeton et al. 2005; Templeton and Greene, 2007). It has been demonstrated in songbirds that there is a perceptual preference or bias for conspecific heterospecific signals (Dooling et al. 1992; Marler and Peters, 1989) although the bias may be altered by experience during development or even adulthood (Dooling et al. 1992; Marler and Peters, 1989).

Songbirds are vocal learners, and this trait is shared by humans, cetaceans, bats, elephants, hummingbirds, and parrots (Jarvis, 2004; Poole et al. 2005). Songbirds, hummingbirds, and parrots all share analogous forebrain regions that are involved in the production and perception of vocalizations (Gahr, 2000; Jarvis and Mello, 2000). Neural activity in the auditory forebrain of songbirds is associated with the behavioural relevance of the signal (e.g., Lynch and Ball, 2008). Immediate early gene (IEG) expression in these brain regions has been used extensively to study neural activity of the vocal perception systems of songbirds (Mello, 2002).

In songbirds, the caudomedial mesopallium (CMM) and caudomedial nidopallium (NCM) both display increased neuronal activation in response to conspecific vocalizations (e.g., Mello and Clayton, 1995, Pinaud and Terleph 2008). IEG expression measured using markers for ZENK (zif-268, egr-1, NGFI-A, Krox-24; e.g., Mello, 2002) is generally higher following playback of conspecific vocalizations relative to heterospecific vocalizations while both of these stimulus classes generally elicit greater ZENK expression compared to artificial stimuli such as tones (Mello and Clayton, 1994, 1995; Chew et al. 1995, 1996) and silence (e.g. Hernandez and MacDougall-Shackleton, 2004; Mello et al. 1992; Stripling et al. 2001; Velho et al. 2005). While conspecific vocalizations generate the most IEG expression in previous studies, heterospecific stimuli also generate significant amounts of IEG expression. However, past research has neglected to focus on IEG expression caused by heterospecific acoustic signals.

Research using heterospecific stimuli has often not directly focused on the ecological relevance for the listener of the heterospecific stimulus at hand. Indeed, heterospecific stimuli are usually selected from species that do not overlap in natural environments with the species being tested (Table 1; but see Lynch and Ball, 2008). Hence, the choice of which heterospecific stimuli are used is often not ethologically relevant to the species studied, but may be chosen for other reasons such as acoustic similarity (e.g. Bailey and Wade, 2003; Terpstra, 2005).

The constraints by and focus of previous research methodology makes interpretation and comparisons of the effects of heterospecific stimuli difficult. It is possible that the neural mechanisms for processing auditory information may have adapted to process not only conspecific signals, but also heterospecific signals. Methodologies employed in previous research do not allow this question to be directly addressed.

Here I examine ZENK expression in the black-capped chickadee, a temperate North American songbird species that has a large repertoire of vocalizations (Ficken et al. 1978; Smith, 1991) that vary seasonally (Avey et al. 2008B). Studies of CMM and NCM in black-capped chickadees have revealed robust ZENK expression in response to their tonal conspecific *fee-bee* song and acoustically complex *chick-a-dee* call (Phillmore et al. 2003; Avey et al. 2008A). The genus *Poecile* is a closely related group of species (Gill et al. 2005), all of which use a learned complex call (the namesake *chick-a-dee* call). Chickadee species are split by relatedness into two subgroups, the black-headed and brown-headed subgroups, with the former using a whistled song in the spring and the latter using a combination of their complex calls in lieu of a song. The black-capped chickadee is most closely related to the mountain chickadee *(Poecile gambeli)* and both are members of the black-headed subgroup. These two species are more distantly related to the boreal (*Poecile hudsonicus*) and chestnut-backed (*Poecile rufescens*) chickadees which are members of the brown headed subgroup (Gill et al. 2005).

I conducted two experiments to test whether phylogenetic proximity of closely related heterospecific species is related to the amount of ZENK expression observed in CMM and NCM following playback of heterospecific vocalizations. If the neural mechanism for encoding heterospecific vocalizations vary based on the degree of relatedness, I expect that black-capped chickadees exposed to the calls of their own species will show a similar amount of ZENK expression to birds exposed to their (closely-related) sister species (mountain chickadee) and this ZENK expression will be greater than that shown by black-capped chickadees exposed to the calls of the more distantly related species such as the boreal and chestnut-backed chickadee.

Experiment 1

In experiment 1, I examine the degree to which phylogenetic proximity of heterospecific vocalizations elicit ZENK expression through playback of the familiar *chick-a-dee* call of black-capped, mountain, and boreal chickadees. In addition, I used gray-crowned rosy-finch (*Leucosticte tephrocotis*) calls as a distant phylogenetic comparison which I expect would generate less ZENK expression than any of the three chickadee species’ calls.

Methods

Subjects

Ten adult male black-capped chickadees were captured from several regions in and around Edmonton, Alberta, Canada (53°32′N, 113°29′W) and Kananaskis Country, Alberta, Canada (51°02′N, 115°03′W). Sex was initially determined by DNA analysis (Griffiths, 2000) and subsequently confirmed by post-mortem examination of reproductive organs. Prior to experimental sessions, chickadees were housed in individual cages in a colony room with a light cycle that approximated the natural weekly light cycle for Edmonton. Food and water was provided *ad libitum* and colony room temperatures were maintained at about 20°C. All studies were conducted in accordance with the Canadian Council on Animal Care Guidelines and policies with approval from the Animal Care and Use Committee for Biosciences for the University of Alberta (Protocol number 682/12/11), and the University of Calgary Life and Environmental Sciences Animal Care Committee (BI11R-10). Chickadees were captured under an Environment Canada Scientific permit (Permit number 09-MB-SC027), Alberta Sustainable Resource Development (Fish and Wildlife Division) Collection and Research permits (Permit numbers 47908 and 47910), and a City of Edmonton Parks Permit.

Stimuli

Birds from which vocalizations were recorded and used as playback stimuli were not produced by subjects in the experiment nor birds housed with individuals used in the experiment. Black-capped, mountain and boreal chickadees were recorded individually between 0900 and 1700 by placing their home cage in a large sound-attenuating chamber (inner dimensions 58 × 168 × 83cm; Industrial Acoustics Corporation, Bronx, New York, USA). Birds were recorded using an AKG C 1000S (AKG Acoustics, Vienna, Austria) condenser microphone (frequency response: 50–20,000 Hz), and a solid-state recorder (Marantz PMD670, D&M Professional, Itasca, IL, USA). Gray-crowned rosy-finch calls were selected from *Stokes Field Guide to Bird Songs: Western Region* (Time Warner AudioBooks, New York, New York, USA) and *Alberta Birding by Ear* (Barbara Beck, Edmonton, Alberta, Canada). All vocalizations were bandpass filtered between 1,000 Hz and 10,000 Hz using Goldwave (Goldwave, St. John's, Newfoundland & Labrador, Canada) to remove background noise and equalized using SIGNAL version 4.0 sound analysis software (Engineering Design 2003, Berkeley, CA, USA) with 32 bit depth at 44,100 Hz sample rate.

I constructed five stimulus sets consisting of: (1) black-capped chickadee calls, (2) mountain chickadee calls, (3) boreal chickadee calls, (4) gray-crowned rosy-finch calls, and (5) silence (Fig. 4-1). Each avian stimulus set consisted of three vocalizations from each of three different individuals' none of the individuals were the same amongsets. These three vocalizations were repeated twice (i.e., a-b-c-a-b-c) over a period of 20 s and then were followed by 40 s of silence. Each 60 s sequence of playback and silence was repeated 30 times for each stimulus set for a total of 30 minutes of stimulus-silence playback. Stimulus sets were constructed as in Avey et al. (2007) to maintain a natural presentation rate; there were no significant differences among call lengths (analysis of variance, ANOVA (F(3,8)=2.39, p = 0.144)).

Playback Equipment

Stimuli were played back through a speaker (Realistic Minimus-7 Cat. no. 40-2034; input 8 OHMS, 40W max, Radio Shack Fort Worth, TX, USA) and amplifier (Cambridge Audio A300, London, UK) with a compact disc player (Sony D-SJ301 S2 Sports CD Walkman, Sony, Toronto, ON, Canada). The amplitude was measured at the level of the perches from the centre position of the cage. Playback amplitude was set to approximately 74 db with a sound-level meter (A weighting, slow response; Radio Shack 33-2055).

Playback Procedure

The experiment was conducted before winter solstice in December when *chick-a-dee* calling is naturally high and *fee-bee* song production is low (Avey et al. 2007b). There were two black-capped chickadees in each of five groups. The playback was conducted in a sound attenuating chamber (inner dimensions 58 × 168 × 83cm; Industrial Acoustics Corporation, Bronx, New York, USA). Individual birds were housed overnight in this chamber in a modified home cage which contained three perches at the level of the speaker and four water bottles and two food cups located at either end of the cage (two water bottles and one food cup at each end of the cage). The light cycle used in the chamber was the same as that used in the colony room. Pre-playback baseline (30 min of silence) and playback sessions (30 min) were recorded (audio and video) using bullet cameras (Swann Bullet-cam, SW-P-BCC, Swann, Santa Fe Springs, CA, USA) starting at approximately 10:00 hrs each day. Following the 30 min playback period, the chamber lights were extinguished for 1 h.

Histology

Immediately following the playback procedure just described, the bird was given an overdose (0.03 ml) of 100 mg/ml ketamine and 20 mg/ml xylazine intramuscularly (1:1) and then transcardially perfused with heparanized 0.1M phosphate buffered saline (PBS) followed by 4% paraformaldehyde. Following perfusion, the brain was removed and placed in 4% paraformaldehyde for 24hrand then placed in a 30% sucrose PBS solution for approximately 24hruntil saturated. The brains were then frozen in dry ice and stored at −80˚C until immunocytochemistry (ICC) for ZENK protein was performed. For each bird, forty-eight 40μm sagittal sections were collected from each hemisphere. Sectioning started from the midline and proceeded laterally. Sections were taken using a cryostat and placed into 0.1M PBS. I processed brains in batches randomized across treatment groups. Sections were washed for 5 min in 0.1M PBS, incubated in 0.5% H_2O_2 for 15 min, and washed for 5 min again in 0.1M PBS. Next, sections were incubated in 10% normal goat serum for 20hrat room temperature, followed by incubation in the primary antibody (egr-1, catalogue # sc-189, Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a concentration of 1:5,000 in 0.1M PBS containing Triton X-100 (PBS/T) for 24 h. Sections were then washed in PBS/T and incubated in biotinylated goat-antirabbit antibody (Vector Labs, Burlington, ON, Canada) for 1 hr (1:200 dilution in PBS/T). Next, sections were washed for 5 min in PBS/T, incubated in avidin–biotin horseradish peroxidase (ABC Vectastain Elite Kit; Vector Labs, Burlington, ON, Canada) for 1 hrand washed for 5 min in 0.1M PBS. Finally, the sections were visualized using 3,3´-diaminobenzidine tetrachloride (Sigma FastDAB, D4418, Sigma–Aldrich, Santa Fe Springs, CA, USA), mounted on gelatin-coated microscope slides, dehydrated in a graded series of ethanol, cleared with citrisolv (Fisher Scientific, Ottawa, ON, Canada) and protected with cover slips affixed with Permount (Sigma–Aldrich).

Analysis

ZENK immunoreactivity (ZENK-ir) was quantified for three auditory brain regions: the caudomedial mesopallium (CMM) and the ventral and dorsal parts of the caudal medial nidopallium (NCMv, NCMd; Figs. 4-2 and 4-3). Images were captured from locations used previously (Avey et al. 2005, 2008A). The lateral ventricle defined the dorsal, ventral, and caudal borders of NCM, and Field L defined the rostral border. ZENK-ir in NCM was assessed at a dorsal and ventral location. ZENK-ir in CMM was quantified in the same sections used for NCM and was assessed in the most caudal area bounded by the lateral ventricle and the caudal-ventral boundary of the mesopallial lamina (LaM). For each black-capped chickadee, eight sections per hemisphere were measured for ZENK-immunoreactivity. Quantification began with the first section in which mesopallium was contiguous with the rostral portion of the nidopallium to ensure that the orientation of the neostriatum was correct. Using the method described above, for each bird 16 images (0.40 × 0.30mm) of each brain region, eight per hemisphere, were captured using a Leica microscope (DM 5500B, Leica, Richmond Hill, ON, Canada) with a 20× objective and a Retiga EX*i* camera (Qimaging, Surrey, British Columbia, Canada) using Openlab 5.1 (Perkin Elmer Inc., Waltham, Massachusetts, USA). I cropped each image to a size of 0.20 × 0.15mm and then counted the number of immunoreactive cells following a semi-automated protocol using ImageJ (NIH, v.1.36b, NIH, Bethesda, Maryland, USA). For CMM, an image was captured from the most caudal part of the region. For NCM, a dorsal image was captured from the most dorso-caudal part of NCM and a ventral image was captured from the centre of the ventro-rostral region in an area of relatively high immunoreactivity. This sampling method, from which I counted the number of immunoreactive cells, captured images from the areas with the highest density of immunopositive cells within these auditory regions. This method has reliably found differences in previous studies (Avey et al. 2008A, 2005; Gentner et al. 2001).

Statistical Analysis

I conducted the statistical analysis following Avey et al. (2005). In brief, I conducted a repeated measures Analysis of Variance to examine the effects of Playback Condition (black-capped, mountain or, boreal chickadee, gray-crowned rosy-finch, silence) as a between-subject factor and Brain Region (CMM, NCMv or NCMd), Hemisphere (left and right) and Medial-lateral Position (section numbers 1-8) as within-subject factors. Subsequent to this analysis, I conducted three separate one-way ANOVAs examining Brain area × Playback Condition for each of the three brain areas of interest. For these analyses, I used the average number of ZENK-ir cells across the eight sampled sections for each hemisphere as the dependent measure and conducted Tukey’s post hoc analysis on playback condition.

Results

Overall Pattern of ZENK Epression

There was ZENK expression in all four experimental conditions as well as in the silence condition (Fig. 4-3). Figure 4-3 shows images of ZENK expression in CMM, NCMd, and NCMv in sagittal sections captured at 200× magnification. ZENK expression was very limited or non-existent in Field L but there was a robust response in both CMM and NCM. The results indicated that the amount of ZENK expression varied among the stimulus groups and brain areas (Fig. 4-4). The repeated measures ANOVA revealed significant main effects of Brain Region (F(2, 10)=31.14, p<0.01; CMM, M=147.92, SD=4.64; NCMd, M=133.80, SD=6.54; NCMv, M=91.08, SD=2.99), and Hemisphere (F(1, 5)=24.80, p<0.01; Left, M=118.70, SD=2.29; Right, M=129.84, SD=2.93). The main effect for Medial-lateral Position was not significant (F(7, 35)=3.53, p=0.06). The interaction between Brain Region and Playback Condition was significant (p<0.05; Fig. 4-4) with greater amounts of ZENK expression in CMM and NCMd relative to NCMv for all conditions except silence. The Hemisphere × Playback Condition interaction was also significant (p<0.01; Fig. 4-5) with greater ZENK expression in the right hemisphere to playback of mountain and boreal chickadees, closely related species, than in the left hemisphere.

Caudomedial Mesopallium

A one-way ANOVA revealed a significant main effect of playback condition in CMM ($F(4, 5)=103.71$, $p<0.01$; Fig. 4-6A). Post hoc comparisons (Tukey HSD) indicated no significant differences in mean ZENK expression to the playback of black-capped and mountain ($p=0.31$), black-capped and boreal, ($p=0.89$), and mountain and boreal ($p=0.13$) chickadee calls. In addition, there was no significant difference in expression to the playback of gray-crowned rosy-finch calls and silence ($p=0.84$) although the gray-crowned rosy-finch calls ($M=41.38$, $SD=0.35$) resulted in more ZENK expression than the silence condition ($M=26.34$, $SD=3.76$). However, the amount of expression to each chickadee playback condition differed significantly from the amount of expression to gray-crowned rosy-finch calls and the silence condition (all ps<0.01), with a greater amount of ZENK expression to the chickadee playback conditions (Fig. 4-6A).

Caudal Medial Nidopallium (Dorsal)

A one-way ANOVA revealed a significant main effect of playback condition in NCMd ($F(4, 5)=47.72$, $p<0.01$; Fig. 4-6B). Post hoc comparisons (Tukey HSD) indicated no significant differences in mean ZENK expression to the playback of black-capped and mountain ($p=0.72$), black-capped and boreal ($p=0.99$), and mountain and boreal ($p=0.91$) chickadee calls. However, the amount of expression to each chickadee playback condition differed significantly from the amount of expression to gray-crowned rosy-finch calls and the silence condition (all ps<0.01), with greater ZENK expression to the chickadee playback conditions (Fig. 4-6B).

Caudal Medial Nidopallium (Ventral)

The one-way ANOVA revealed a significant main effect of playback condition in NCMv (F(4, 5)=87.98, p<0.01; Fig. 4-6C). Post hoc comparisons (Tukey HSD) indicated no significant differences in mean ZENK expression to the playback of black-capped and mountain (p=1.00), black-capped and boreal (p=0.80), and mountain and boreal (p=0.85) chickadee calls. However, the amount of expression to each chickadee playback condition differed significantly from the amount of expression to gray-crowned rosy-finch calls and the silence condition (all ps<0.01), with greater ZENK expression to the chickadee playback conditions (Fig. 4-6C).

Experiment 2

In experiment 2, I examine the degree to which bioacoustic complexity interacts with phylogenetic markers in the ‘*dee*’ portion of the chick-a-dee call along the phylogenetic continuum. I compare species that produce ‘*dee*’-like notes with similar bioacoustic properties (e.g., broad band, harmonic-like frequency stacks) that also have a similar duration as black-capped chickadee D notes. Chestnut-backed chickadee (Hoeschele et al. 2009), tufted titmouse (*Baeolophus* *bicolor;* Owens and Freeberg, 2007), and female and male zebra finch (*Taeniopygia guttata;* Zann, 1983) calls all share similar bioacoustic properties (e.g. loudest frequency of ‘*dee*’-like notes) but move from phylogenetically closely related to distantly related (Gill et al. 2005).

Methods

Subjects

Thirty adult male black-capped chickadees were captured from several regions in and around Edmonton, Alberta, Canada (53°32′N, 113°29′W) and Kananaskis Country, Alberta, Canada (51°02′N, 115°03′W). All other aspects were the same as Experiment 1.

Stimuli

As in Experiment 1, birds used for recording of stimuli were neither used in the experiment nor housed with birds used in the experiment. Black-capped chickadees were recorded at Elk Island National Park (53°36′N, 112°51′W) using a Marantz PMD670 solid state recorder and a Sennheiser ME67 directional microphone (Saul Mineroff Electronics, Elmont, New York, USA). Chestnut-backed chickadee calls were recorded on Vancouver Island, Canada using a MiniDisc recorder (model MZ-N1, Sony Corp., Tokyo, Japan) connected to a Sennheiser omnidirectional microphone (model ME62, Sennheiser Corp., Wedemark, Germany) or from the Maccaulay Library of Natural Sounds at the Cornell Laboratory of Ornithology, which consisted of recordings from many different individuals with different recording equipment. The tufted titmouse calls were recorded at field sites and in an aviary at the University of Tennessee, Knoxville using a Fosted recorder (Fosted FR-2 Field Memory Digital Recorder) and Sennheiser directional microphone (Me-66). The zebra finch calls were recorded at the University of California, Berkeley in a soundproof room in cages (40 × 25 × 35cm) using a Marantz recorder (Marantz PMD 670) and Sennheiser omni-directional microphone (Sennheiser MD42). All vocalizations were bandpass filtered between 500 Hz and 14,000 Hz in Goldwave (Goldwave, St. John's, Newfoundland & Labrador, Canada) to remove background noise and equalized using SIGNAL version 5.0 sound analysis software (Engineering Design 2003, Berkeley, CA, USA). All stimuli were 32 bit and with a 44,100 Hz sample rate.

I constructed five stimulus sets for each five separate playback conditions: black-capped chickadee '*dee*' note calls, reversed black-capped chickadee '*dee*' note calls, female zebra finch calls, chestnut-back chickadee '*dee*' note calls, tufted titmouse '*dee*' note calls, and male zebra finch calls (Fig. 4-7 a-f). Each stimulus set consisted of two '*dee*' note-type calls from two separate individuals. These two vocalizations repeated 30 times with 50 secs of silence between each iteration of both calls (i.e., a-b-silence-a-b-silence etc), for a total of 30 mins of stimulus-silence playback. Stimulus sets were constructed with one call from each species such that each two 'dee' note type call for each species was matched in duration for the black-capped chickadee, reversed black-capped chickadee, chestnut-backed chickadee, and tufted titmouse. The male zebra finch calls were within ~1 ms of duration to the matching stimulus sets of the black-capped chickadee, reversed black-capped chickadee, chestnut-backed chickadee, and tufted titmouse sets. The female zebra finch calls, naturally longer in duration than the '*dee*' notes of chickadees, were ~250ms longer in total duration than the other stimulus sets (see Table 2. for durations).

Playback Equipment and Procedure

Playback equipment and procedure was the same as in Experiment 1 except the following: Playbacks were recorded using an AKG C 1000S condenser microphone (frequency response: 50–20,000 Hz; AKG Acoustics, Vienna, Austria), and a solid-state recorder (Marantz PMD670, D&M Professional, Itasca, IL, USA). I randomly selected one of six playback conditions to present to individual adult black-capped in sound attenuating chambers: There were five adult black-capped chickadees in each playback condition. Stimuli were played back through a Fostex FE108Σ speaker (Fostex Corp., Japan; frequency range 80–18,000 Hz) and amplifier (Cambridge Audio A300; London, UK) with an MP3 player (Creative ZEN; Singapore). The amplitude was measured at the level of the perches from the centre position of the cage and playback amplitude was set to approximately 74 dB with a sound-level meter (Radio Shack 33-2055; Radio Shack, Fort Worth, TX, USA). The playback was conducted in one of six sound attenuating chambers (inner dimensions 58 × 168 × 83cm; Industrial Acoustics Corporation, Bronx, New York, USA).

Histology

Histological methods were identical to Experiment 1.

Analysis

The analysis was conducted the same as in Experiment 1 except that images were captured using a Leica microscope (DM 5500B; Wetzlar, Germany) with a 40× objective to eliminate the need to crop them. Images were captured from the same brain locations as experiment 1.

Statistical Analysis

I conducted a repeated-measures ANOVA to examine the effects of Playback Condition (black-capped, reversed black-capped, chestnut-backed, tufted titmouse, female or male zebra finch) as a between-subject factor and Brain Region (CMM, NCMv or NCMd), Hemisphere (left or right) and Medial-lateral Position (section numbers 1-8) as within-subject factors, and conducted Tukey’s post hoc analyses on Playback Condition.

Results

Overall Pattern of ZENK Expression

There was ZENK expression in all experimental conditions (Fig. 4-8). Figure 4-8 show images of ZENK expression in CMM, NCMd, and NCMv in sagittal sections captured at 400× magnification. ZENK expression was limited or non-existent in Field L, but there was a robust response in both CMM and NCM.

The results indicated that the amount of ZENK expression varied among the Brain Regions (Fig. 4-9a) and Playback Condition (Fig.4-9b). The repeated measures ANOVA revealed significant main effects for ZENK expression of Brain Region (F(2, 46)=8.51, p<0.01; CMM, M=125.50, SE=3.27; NCMd, M=116.79, SE=2.94; NCMv, M=112.11, SE=2.09), with the most ZENK expression in CMM. There was also a significant main effect for ZENK expression for Medial-lateral Position (F(7, 161)=10628.89, p<0.01; Fig. 4-9c) with progressively more expression in sections from medial to lateral. There was no significant main effect Hemisphere (F(1, 23)=0.03, p=9.54; Left, M=118.25, SE=3.09; Right, M=118.08, SE=2.73). There were no significant interactions between the within subjects factors or the between subject factor. The between subjects factor, playback condition, was a significant main effect (F(5, 23)=6.62, p<0.01; Fig. 4-9b) and a post hoc comparison was performed.

Post Hoc Comparisons

Post hoc comparisons (Tukey HSD) for the between subject factor, playback condition, indicated no significant differences in mean ZENK expression between any of the playback conditions except for reverse black-capped chickadee '*dee*' notes (Fig. 4-9b) which differed significantly less ZENK expression compared to all other playback conditions (black-capped chickadee, p=0.03; chestnut-backed chickadee, p<0.01; tufted titmouse, p=0.02; female zebra finch, p=0.04; male zebra finch, p=0.01). These differences were consistent for all brain regions.

Discussion

In Experiment 1, heterospecific chickadee species' vocalizations generated the same amount of ZENK expression as conspecific black-capped chickadee vocalizations but more than the more distantly related heterospecific vocalization of the gray-crowned rosy finch. In Experiment 2 there was no significant difference in the amount of expression of ZENK whether the vocalizations were conspecific or heterospecific. The results from Experiment 1 suggest that phylogenetic proximity to other species may, at least in part, predict IEG expression in CMM and NCM in response to vocalizations. The results from Experiment 2 suggest that, as bioacoustic properties of signals converge, the amount ZENK expression becomes indistinguishable.

In Experiment 1, I used the acoustically complex *chick-a-dee* call because it is used by all three chickadee species (black-capped, mountain and boreal). While the bioacoustic properties of the note that comprise the each species *chick-a-dee* call vary, the calls are very similar in overall structure (Ficken et al. 1978). I have shown in previous work that the *chick-a-dee* call induces ZENK expression (Avey et al. 2008A) and the pattern of ZENK expression in black-capped chickadees in the present study is also consistent with previous research in other songbirds (e.g., zebra finches and canaries; Mello et al. 1992).

The current results demonstrate the novel finding that the calls of very closely related heterospecific individuals generate the same amount of ZENK expression as the calls of conspecific individuals. In Experiment 1, vocalizations from the most distantly-related heterospecific, the gray-crowned rosy-finch, generated significantly less ZENK expression than did the closely related chickadee playbacks. This result suggested that ZENK expression varied along a phylogenetic continuum for distantly related species (chickadees versus the finch), however, there are greater bioacoustic differences between the chickadee calls of the three species of chickadees, and the gray-crowned rosy finch calls (Fig. 4-1), which offers an alternate explanation of the result. Interestingly, ZENK expression in the right hemisphere of groups that heard playback of mountain or boreal chickadees was significantly higher than in the left hemisphere but this effect was not seen for the gray-crowned rosy-finch vocalizations.

In Experiment 2, I used the acoustically complex *'dee'* notes from two species' *chick-a-dee* calls and the equivalent calls from tufted titmouse and the more distantly related zebra finch because the '*dee*' note has been shown to contain acoustic features used by black-capped chickadees for species identification in behavioural paradigms (Bloomfield et al. 2008A; Bloomfield and Sturdy 2008; Bloomfield et al. 2008B; Guillette et al. 2010) and it is relatively simple in structure for matching between species. Relative to the stimulus sets in Experiment 1, the '*dee*' note calls were much more similar bioacoustically (e.g., max frequency) and were selected to have equivalent durations (except female zebra finch calls which were longer). These results suggest that, as bioacoustic similarity is increased, the amount of ZENK expression no longer reliably indicates differences in response to between conspecific and heterospecific vocalizations in CMM and NCM. Similar to Experiment 1, the most ZENK expression occurred in CMM although in Experiment 2 there was no difference between the dorsal and ventral portions of NCM. Furthermore, in Experiment 2 there were no significant differences in the hemispheric response to the stimulus although there was a significant increase in ZENK expression from medial to lateral in both hemispheres. These differences may reflect neural processing differences driven by the complexity of the signals between experiments. Surprisingly, the reversed black-capped '*dee*' notes resulted in significantly less ZENK expression than the other playback condition although the absolute amount of expression to these reversed '*dee*' notes was still high. Which particular acoustic features of the reversed black-capped '*dee*' notes caused this difference is unclear. The reversed '*dee*' note calls appear to driving greater levels of ZENK response than the reversed whole calls suggesting that the reversed whole calls are not being attended to as natural stimuli but the reversed '*dee*' not calls may be. Future behavioural testing via field playback and operant discrimination paradigms can explore this finding.

Response to Heterospecific Stimuli and the Caudomedial Mesopallium

Hernandez and MacDougall-Shackleton (2004) found that in adult female house finches (*Carpodacus mexicanus*), ZENK expression was greatest to conspecific vocalizations and intermediary to heterospecific vocalizations compared to controls. Similarly, Arc expression in CMM is greatest in response to conspecific vocalizations but intermediary to heterospecific vocalizations and low to controls in zebra finches (Velho et al. 2005). In CMM, prior to lesions, female zebra finches prefer conspecific over heterospecific songs, but lesions to CMM disrupt this preference (MacDougall-Shackleton et al. 1998). In female canaries (*Serinus canaria),* treatments with N-(2-chloroethyl)-N-2-bromobenzyl-amine hydrochloride (DSP-4), a noradrenergic neurotoxin, reduced the global amount of ZENK expression and eliminated differences between conspecific and heterospecific stimuli in CMM while saline treatments still showed increased ZENK expression to conspecific stimuli relative to heterospecific stimuli (Lynch and Ball, 2008). Bailey and Wade (2003) are the only researchers to have found no difference between expression of ZENK (and c-FOS) for conspecific and heterospecific stimuli using juvenile female zebra finches; however, a difference in expression was detectable in juvenile male zebra finches. These differences disappear by day 45 and expression in response to conspecific vocalizations increases relative to heterospecific vocalizations (Bailey and Wade, 2003).

The available evidence suggests that CMM plays a role in the processing of heterospecific signals, including the ability to discriminate between relevant conspecific and heterospecific vocalizations. These results suggest that IEG expression in CMM can be as robust a response to heterospecific as conspecific stimuli in adults of a songbird species. Previous studies (Table 4-1) did not select heterospecific stimuli from closely related species or bioacoustically similar vocalizations (see Table 1.) as did the current study, which may explain the consistent difference between conspecific and heterospecific ZENK expression in the former studies in other studies. The results from Experiment 2 strongly suggest that as the bioacoustic information becomes similar between conspecifics and heterospecific vocalizations, differences in the amount of ZENK expression disappear altogether, which may make the use heterospecific stimuli as a control condition unsuitable.

Our inability to detect a significant difference between the ZENK expression for birds hearing the three chickadee species in Experiment 1 may have been caused by a ceiling effect for the number of cells responding within these brain regions. The results from Experiment 2 suggest that ZENK expression alone as a neural activity marker, quantified and compared via the number of ZENK-positive cells, cannot explain the neural mechanism for species discrimination between conspecificand heterospecific stimuli or heterospecific stimuli that differ in the degree of their relatedness. Neural encoding for species may occur outside these brain regions, or different neurons may encode for different relevant stimuli. For instance, ZENK expression for conspecific stimuli may be induced in different neurons than for heterospecific stimuli, thus there may be distinct neurons for different stimuli (i.e., selectivity), although the overall number of neurons may be similar. Future studies using *in vivo* electrophysiological paradigms should investigate if cells in these regions are responsive specifically to heterospecific stimuli.

Response to Heterospecific Stimuli and the Caudal Medial Nidopallium

Research in songbirds has found that NCM also plays a significant role in the processing of heterospecific signals. Bailey and Wade (2003) found no difference in ZENK expression in NCM between conspecific and heterospecific stimuli in juvenile female zebra finches but found differences in juvenile males. Like CMM, differences in the pattern of ZENK and c-FOS expression in males and females disappear by day 45, leading to greater expression to conspecific than heterospecific stimuli (Bailey and Wade, 2005).

Patterns of ZENK expression in NCM in other songbirds is similar to that seen in CMM, with conspecific stimuli generating more expression than heterospecific stimuli. In male zebra finches (Mello et al. 1992; Stripling et al. 2001), as well as in female house finches (Hernandez and MacDougall-Shackleton, 2004), conspecific song presentation generates more ZENK expression than heterospecific song and heterospecific song generates more ZENK expression than artificial stimuli or silence in NCM. In zebra finches, Arc expression in NCM is greatest in response to conspecific vocalizations but intermediate to heterospecific vocalizations and low to controls (Velho et al. 2005).

In NCM, neurons habituate to the presentation of the same stimulus in both zebra finches and canaries (Chew et al. 1995; Terleph et al. 2006). Neurons that habituate and stop firing in response to song stimulation are selective for conspecific song, with habituation lasting longer for conspecific song stimuli than for heterospecific song stimuli (Chew et al. 1996). NCM neurons also respond with more rapid neuronal firing rates (physiological activity) to conspecific songs than to heterospecific songs, and to heterospecific songs than to artificial stimuli (Chew et al. 1996; Stripling et al. 2001; but see Stripling et al. 1997 for a study that did not find this effect). Treatments with DSP-4 reduced the overall amount of ZENK expression as well as the difference between conspecific and heterospecific expression in dorsal NCM and reduced the amount of ZENK expression in ventral NCM (Lynch and Ball, 2008). These studies suggest that, similar to CMM, NCM, and specifically the activity of IEGs, play a role in the processing of heterospecific signals. The dorsal portion of NCM responds more robustly than the ventral portion to both conspecific and heterospecific stimuli suggesting hierarchical processing in this auditory nucleus.

In conclusion, I show, in certain cases, that the IEG expression of ZENK is not always greater to conspecific than to heterospecific signals. The degree of relatedness of the heterospecific species and similarity of their vocalizations predicts ZENK expression in black-capped chickadee CMM and the dorsal and ventral portion of NCM when the full calls are used. The degree of phylogenetic relatedness does not predict differences in ZENK expression in CMM or NCM when only a portion of the entire natural signal is used that is bioacoustically very similar between black-capped chickadees and heterospecific species. The findings suggest that the amount of neuronal signal processing from closely related heterospecific species is not different from the processing of conspecific signals, but more distantly related species are processed by fewer neurons at least when full calls are used. When heterospecific signals offer less information and that information becomes very similar to conspecific signals the number of active neurons becomes similar. Whether the neuronal populations that process heterospecific vocalizations in black-capped chickadees are identical to those that process conspecific vocalizations remains unknown. Future research needs to determine whether these neuronal populations are the same and what basic constituents of heterospecific signals control this response.

Table 4-1. Behavioural Neuroscience Research Articles Investigating Vocal Perception by Avian Species that use Vocal Stimuli from Heterospecific Species

Article	Species Studied	Vocal Learner	Stimulus	Technique†
Brenowitz, 1991	Canary	Yes	CON, HET (White-crowned Sparrow)	Lesion
Mello et al. 1992	Zebra Finch & Canary	Yes	CON, HET (Zebra Finch & Canary), TON, SIL	ZENK
Chew et al. 1995	Zebra Finch	Yes	CON, HET (Canary, Human Words), TON, WN	EP
Chew et al. 1996	Zebra Finch	Yes	BOS, CON, NOV, HET (Canary, Bengalese Finch, Silver Bill, Human Speech) TON, WN	EP
Stripling et al. 1997	Zebra Finch	Yes	BOS, CON, HET (White-crowned Sparrow), TON, WN, SIL	EP
MacDougall-Shackleton et al. 1998	Zebra Finch	Yes	CON, Artificial CON, HET (European nightingale)	Lesion
Scharff et al. 1998	Zebra Finch	Yes	CON, HET (Canary)	Lesion
Bentley et al. 2000	Canary & Song Sparrow	Yes	CON, HET (Zebra Finch), SIL	Behaviour
Rosen and Mooney, 2000	Zebra Finch	Yes	BOS, Reverse BOS, Reverse Syllable BOS, CON, HET (Bengalese Finch), WN	EP
Mooney et al. 2001	Swamp Sparrow	Yes	BOS, NOV, HET (Song Sparrows)	EP
Stripling et al. 2001	Zebra Finch	Yes	CON,Reverse CON, NOV, HET (White-crowned Sparrow), TON, WN	EP
Bailey et al. 2002	Zebra Finch	Yes	CON, HET (*), TON, SIL	ZENK
Long et al. 2002	Chicken & Quail	No	CON, HET (Chicken or Quail)	ZENK
Bailey and Wade, 2003	Zebra Finch	Yes	CON, HET (**), TON, SIL	FOS
Hernandez and MacDougall-Shackleton, 2004	House Finch	Yes	Local NOV, Foreign NOV, HET (White-crowned Sparrow)	ZENK
Bailey and Wade, 2005	Zebra Finch	Yes	Female directed NOV, HET (**), SIL	ZENK
Terpstra et al. 2005	Ringdove	No	NOV, HET (Zebra Finch)	ZENK
Velho et al. 2005	Zebra Finch	Yes	NOV, HET (Canary), TON, WN, SIL	ZENK
Bailey and Wade., 2006	Zebra Finch	Yes	CON, HET (**), TON, SIL	FOS, ZENK
Huchzermeyer et al. 2006	Zebra Finch	Yes	CON, HET (Bengalese Finch)	ZENK
Lynch and Ball, 2008	Canary	Yes	NOV, HET (Cassin's Finch)	ZENK
Gee et al. 2009	California & Gambel's Quail	No	NOV, HET (California or Gambel's Quail), TON	ZENK

†Lesions; immediate early gene labeling studies for ZENK, C-FOS, EP, electrophysiological studies; Behaviour, behavioural studies.
Songbird Species: Canary, House Finch, Song Sparrow, Swamp Sparrow, Zebra Finch.
Non-Songbird Species: Chicken, California Quail, Gambel's Quail, Quail, Ringdove.
Stimulus: Bird's Own Song (BOS), Conspecific (CON), Novel Conspecific (NOV), Heterospecific (HET (species)), Tone (TON), Silence (SIL), White Noise (WN).* American Robin, Summer Tanager, Bell's vireo, White Breasted Nuthatch, Marsh Wren, Connecticut Warbler, Cassin's Finch, Baird's Sparrow, Scott's Oriole, Western Meadowlark.
** American Robin, Baird's Sparrow, Bell's Vireo, Cassin's Finch, Connecticut Warbler, Marsh Wren, Scott's Oriole, Summer Tanager, Western Meadowlark and White-Breasted Nuthatch

Table 4-2. Duration of ‘dee’-like Notes per Iteration for Each Stimulus Set

Stimulus Set	Black-capped Chickadee	Reversed Black-capped Chickadee	Chestnut-backed Chickadee	Tufted Titmouse	Female Zebra Finch	Male Zebra Finch
1	643 ms	643 ms	643 ms	643 ms	850 ms	642 ms
2	645 ms	645 ms	645 ms	645 ms	848 ms	646 ms
3	626 ms	626 ms	626 ms	626 ms	847 ms	626 ms
4	680 ms	680 ms	680 ms	679 ms	846 ms	681 ms
5	664 ms	664 ms	664 ms	664 ms	855 ms	663 ms

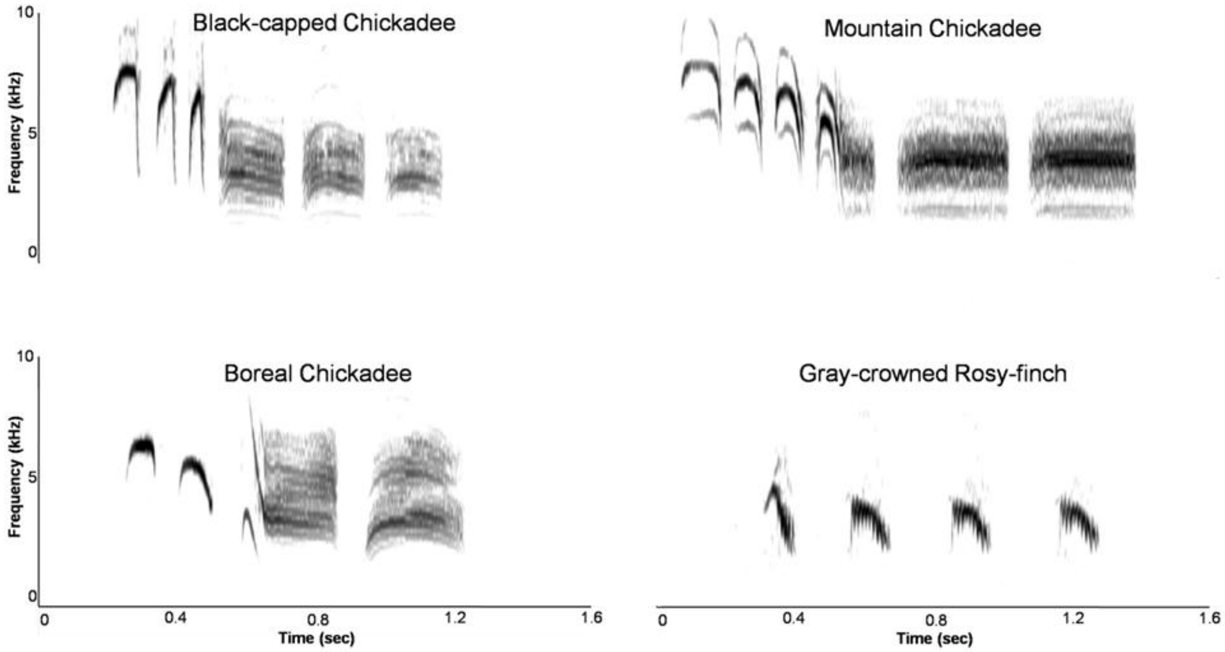

Black-capped Chickadee
Mountain Chickadee
Boreal Chickadee
Gray-crowned Rosy-finch
Frequency (kHz)
10
5
0
Time (sec)
0
0.4
0.8
1.2
1.6

Figure 4-1.

Figure 4-2.

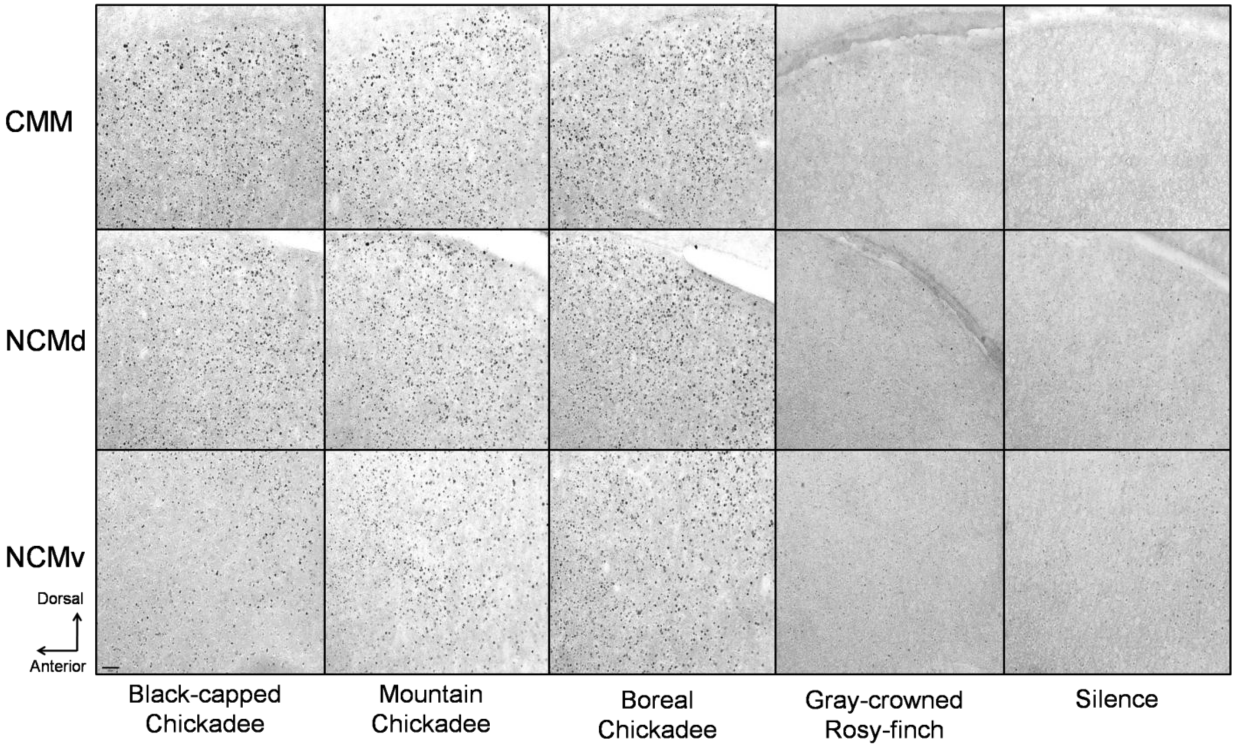

CMM
NCMd
NCMv
Dorsal
Anterior
Black-capped Chickadee
Mountain Chickadee
Boreal Chickadee
Gray-crowned Rosy-finch
Silence

Figure 4-3.

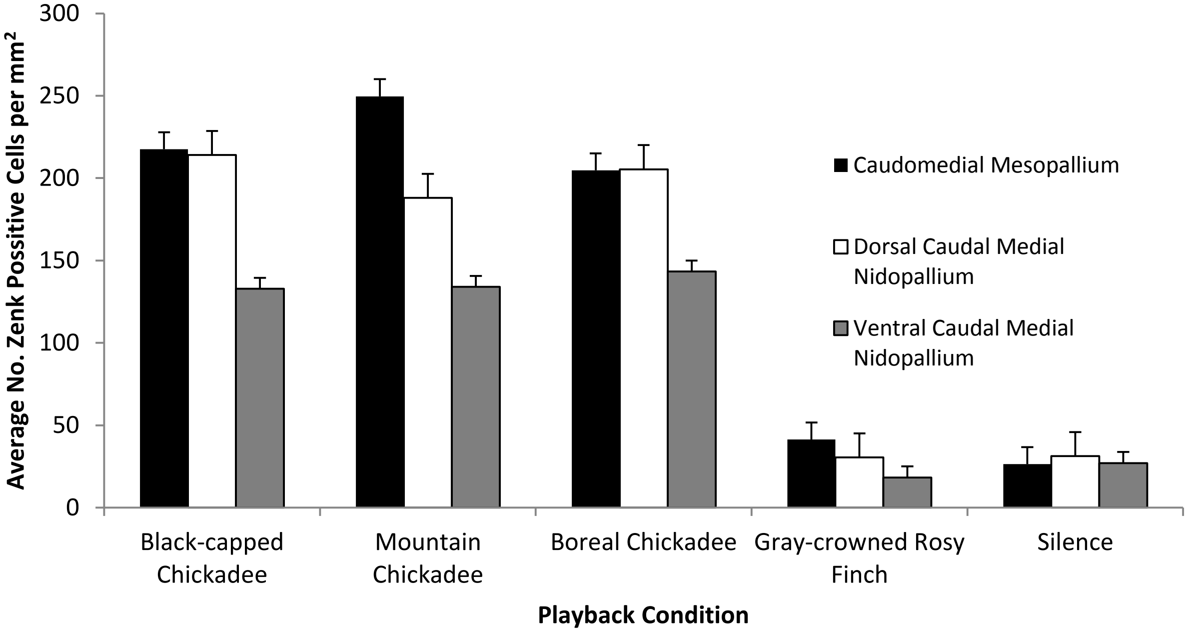

300
250
200
150
100
50
0
Average No. Zenk Possitive Cells per mm²
■ Caudomedial Mesopallium
□ Dorsal Caudal Medial Nidopallium
▨ Ventral Caudal Medial Nidopallium
Black-capped Chickadee
Mountain Chickadee
Boreal Chickadee
Gray-crowned Rosy Finch
Silence
Playback Condition

Figure 4-4.

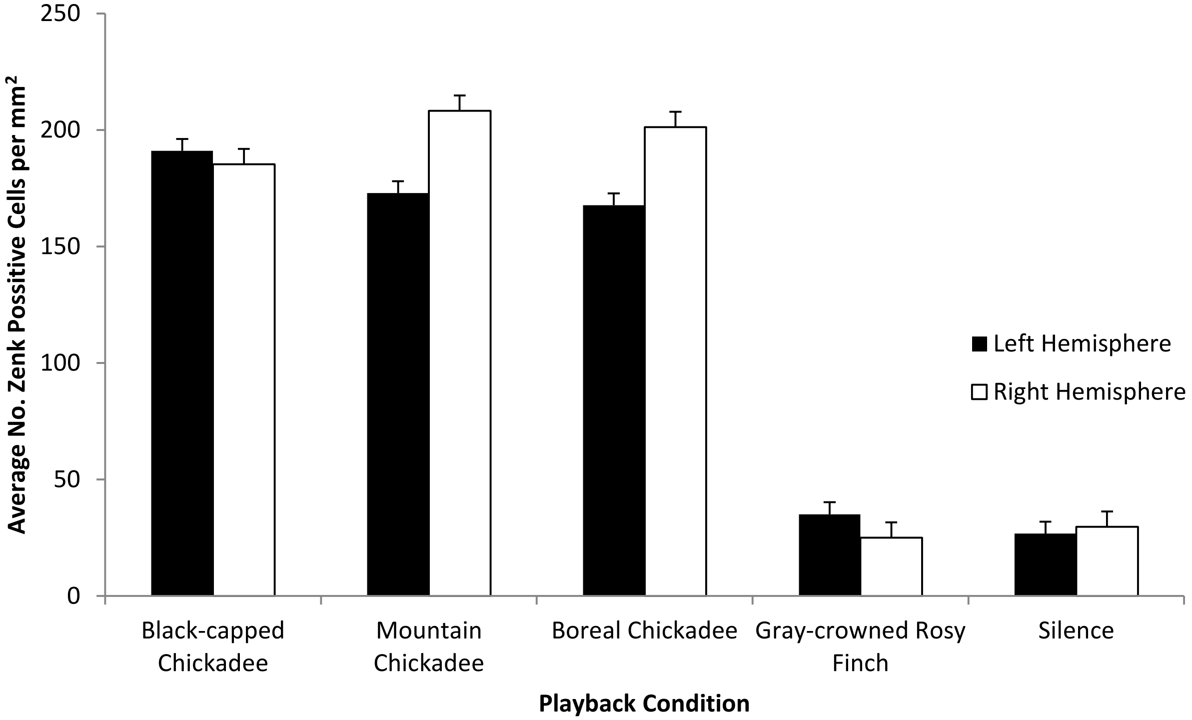

250
200
150
100
50
0
Average No. Zenk Possitive Cells per mm²
■ Left Hemisphere
□ Right Hemisphere
Black-capped Chickadee
Mountain Chickadee
Boreal Chickadee
Gray-crowned Rosy Finch
Silence
Playback Condition

Figure 4-5.

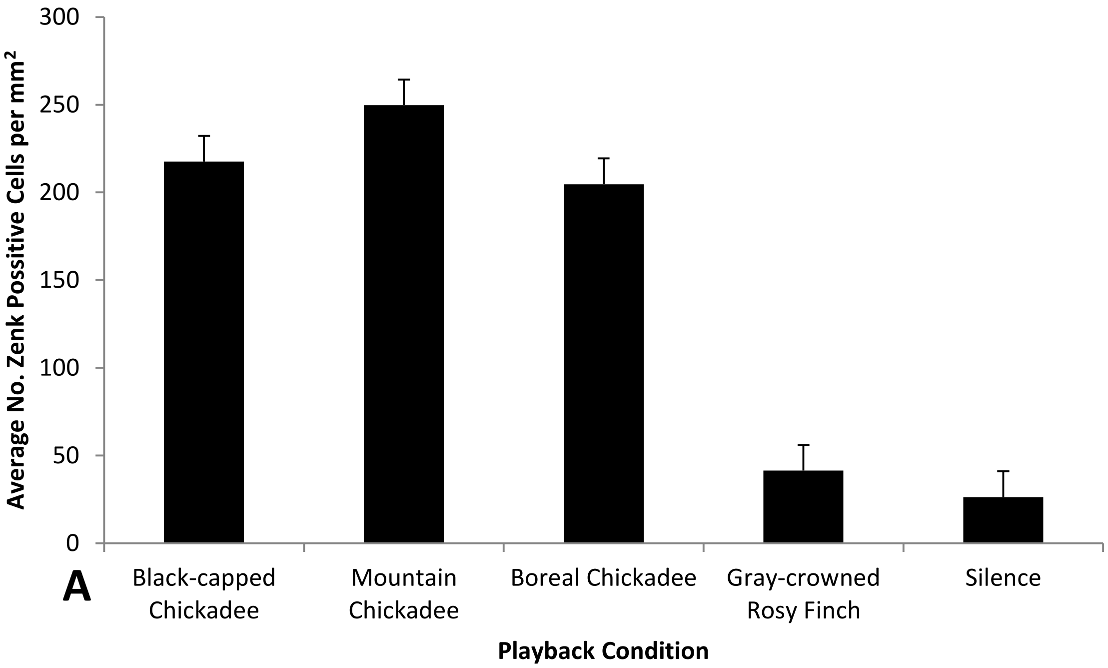
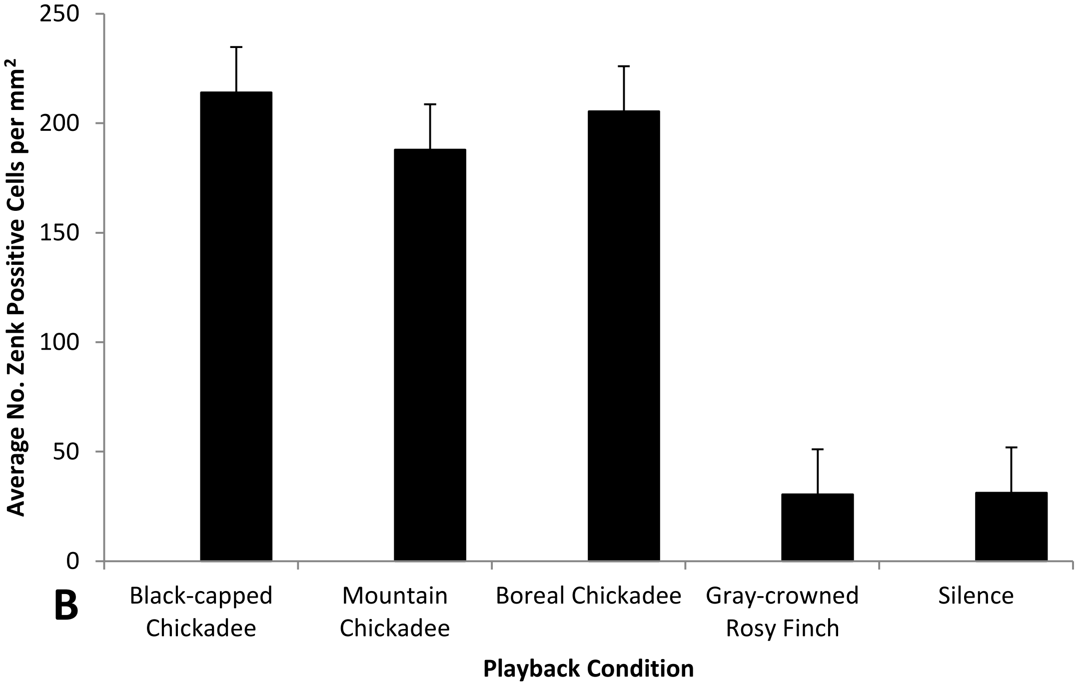
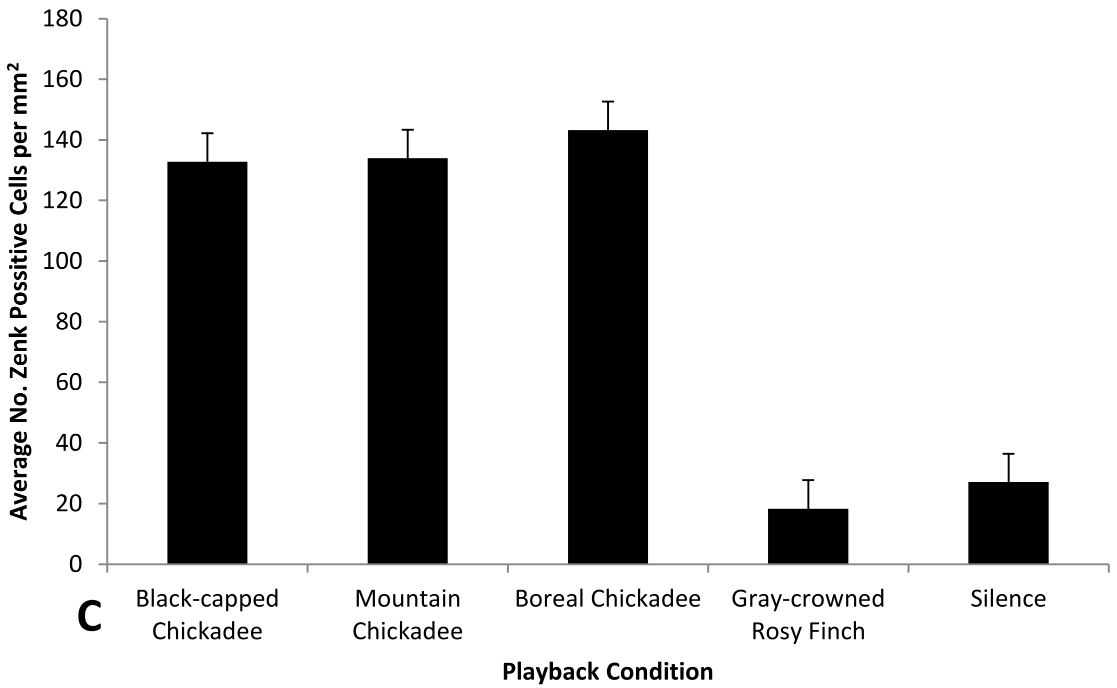

300
250
200
150
100
50
0
Average No. Zenk Possitive Cells per mm²
A
Black-capped Chickadee
Mountain Chickadee
Boreal Chickadee
Gray-crowned Rosy Finch
Silence
Playback Condition

Figure 4-6A.



Average No. Zenk Possitive Cells per mm²
250
200
150
100
50
0
B
Black-capped Chickadee
Mountain Chickadee
Boreal Chickadee
Gray-crowned Rosy Finch
Silence
Playback Condition

Figure 4-6B.



180
160
140
120
100
80
60
40
20
0
Average No. Zenk Possitive Cells per mm^2
C
Black-capped Chickadee
Mountain Chickadee
Boreal Chickadee
Gray-crowned Rosy Finch
Silence
Playback Condition

Figure 4-6C.

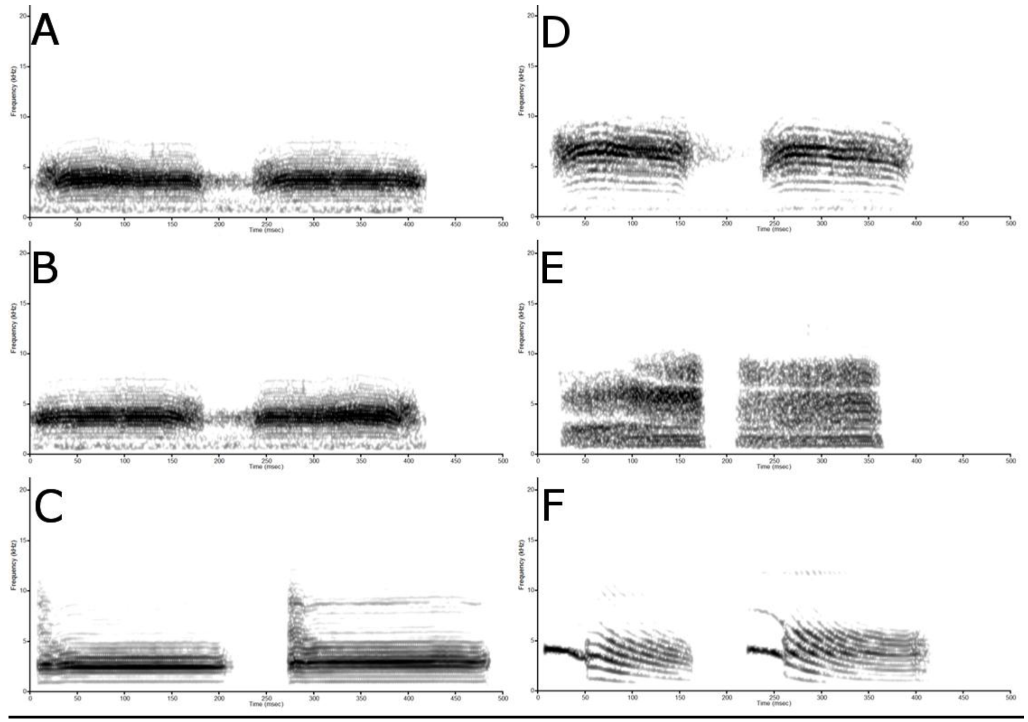

A
B
C
D
E
F
Frequency (kHz)
0
5
10
15
20
Time (msec)
0
50
100
150
200
250
300
350
400
450
500

Figure 4-7A-F.

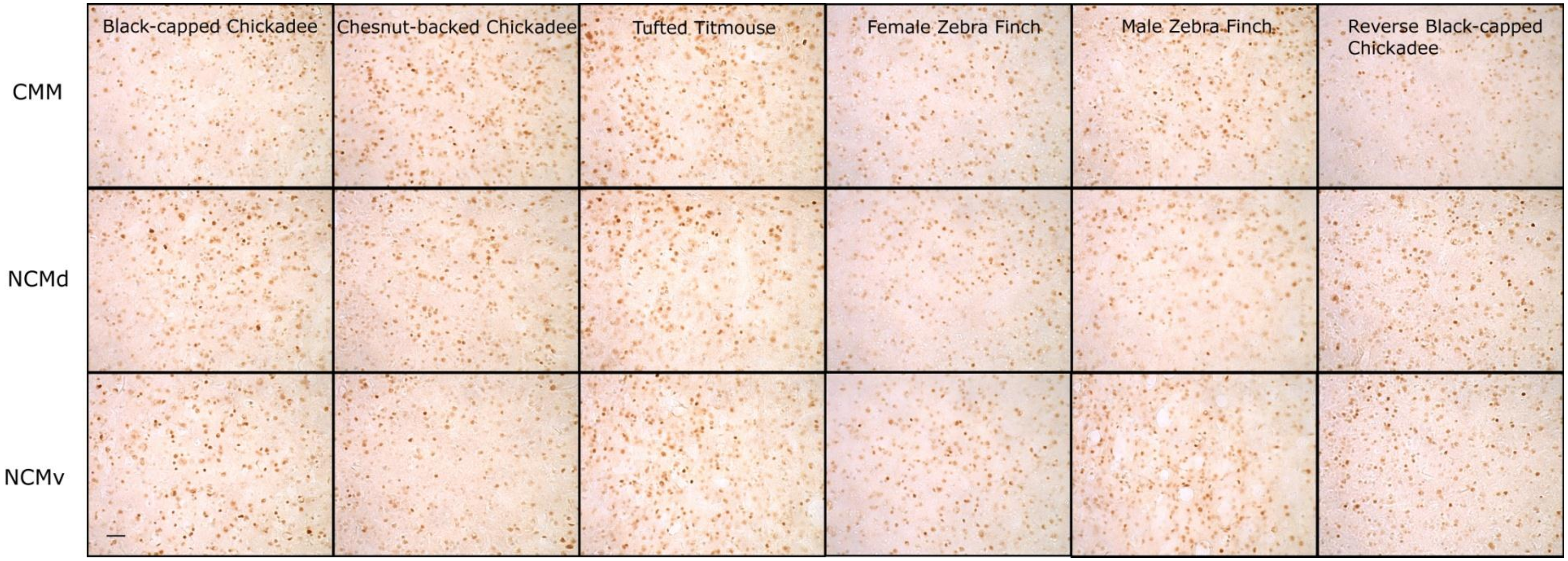

Black-capped Chickadee
Chesnut-backed Chickadee
Tufted Titmouse
Female Zebra Finch
Male Zebra Finch
Reverse Black-capped Chickadee
CMM
NCMd
NCMv

Figure 4-8.

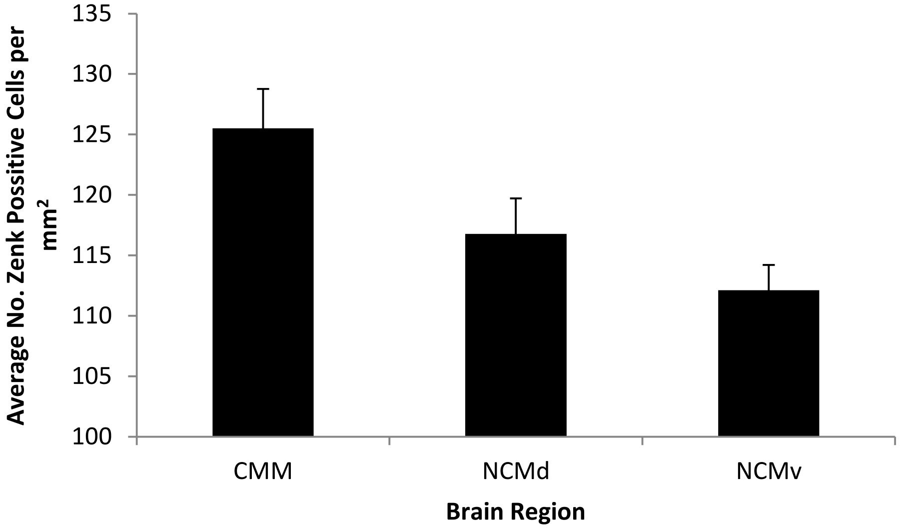

135
130
125
120
115
110
105
100
Average No. Zenk Possitive Cells per mm²
CMM
NCMd
NCMv
Brain Region

Figure 4-9A.

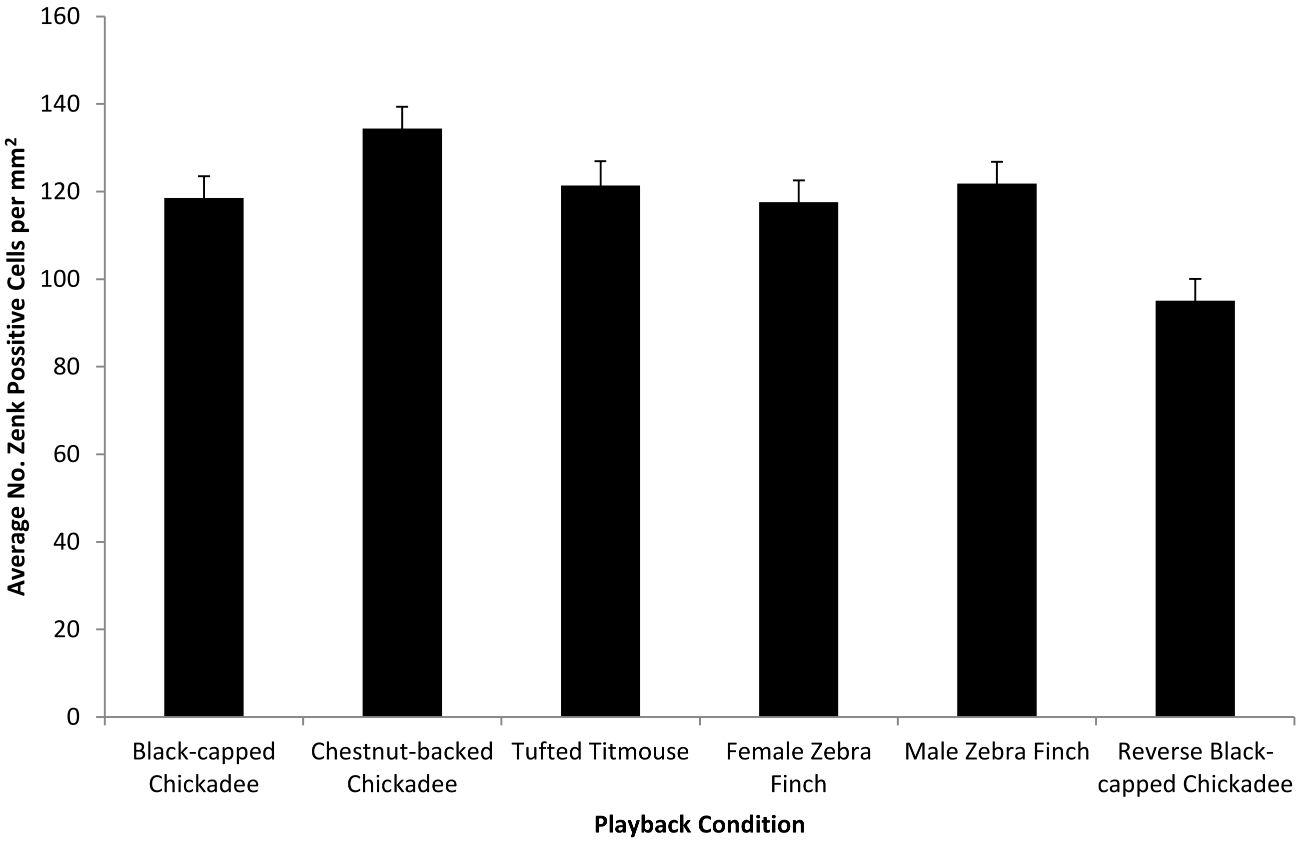

160
140
120
100
80
60
40
20
0
Average No. Zenk Possitive Cells per mm²
Black-capped Chickadee
Chestnut-backed Chickadee
Tufted Titmouse
Female Zebra Finch
Male Zebra Finch
Reverse Black-capped Chickadee
Playback Condition

Figure 4-9B.

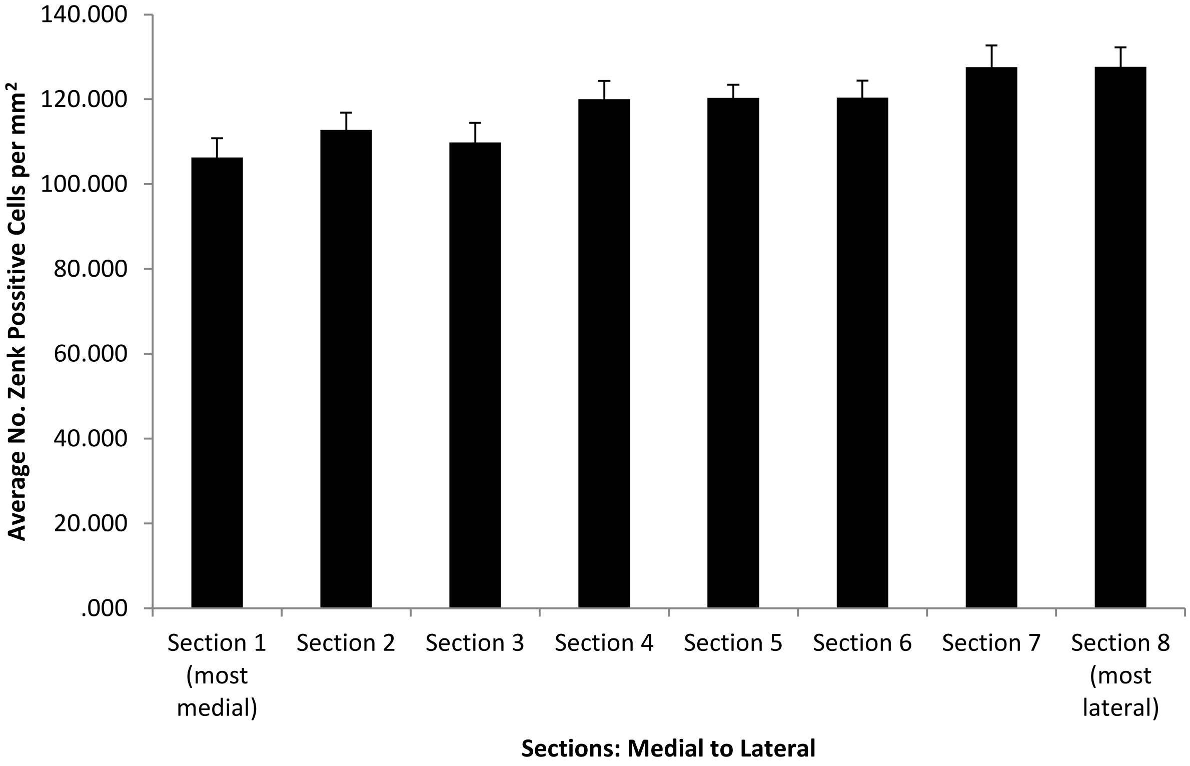

140.000
120.000
100.000
80.000
60.000
40.000
20.000
.000
Average No. Zenk Possitive Cells per mm²
Section 1 (most medial)
Section 2
Section 3
Section 4
Section 5
Section 6
Section 7
Section 8 (most lateral)
Sections: Medial to Lateral

Figure 4-9C.

References

Avey, M.T., Hoeschele, M., Moscicki, M.K., Bloomfield, L.L., Sturdy, C.B., 2011. Neural Correlates of Threat Perception: Neural Equivalence of Conspecific and Heterospecific Mobbing Calls Is Learned. PLoS ONE 6(8): e23844. doi:10.1371/journal.pone.0023844

Avey, M.T., Kanyo, R.A., Irwin, E.L., Sturdy, C.B. 2008A. Differential effects of vocalization type, singer and listener on ZENK immediate early gene response in black-capped chickadees (*Poecile atricapillus*). Behav Brain Res. 188, 201-208.

Avey, M.T., Phillmore, L.S., MacDougall-Shackleton, S.A. 2005. Immediate early gene expression following exposure to acoustic and visual components of courtship in zebra finches. Behav Brain Res. 165, 247-253.

Avey, M.T., Quince, A.F., Sturdy, C.B. 2008B. Seasonal and diurnal patterns of black-capped chickadee (*Poecile atricapillus*) vocal production. Behav Processes. 77, 149-155.

Bailey, D.J., Rosebush, J.C., Wade, J. 2002. The hippocampus and caudomedial neostriatum show selective responsiveness to conspecific song in the female zebra finch. J Neurobiol. 52, 43-51.

Bailey, D.J., Wade, J. 2003. Differential expression of the immediate early genes FOS and ZENK following auditory stimulation in the juvenile male and female zebra finch. Brain Res Mol Brain Res. 116, 147-154.

Bailey, D.J., Wade, J. 2005. FOS and ZENK responses in 45-day-old zebra finches vary with auditory stimulus and brain region, but not sex. Behav Brain Res.162, 108-115.

Bailey, D.J., Wade, J. 2006. Sexual dimorphism in song-induced ZENK expression in the medial striatum of juvenile zebra finchesnch. Neurosci Lett. 401, 86-91.

Bentley, G.E., Wingfield, J.C., Morton, M.L., Ball, G.F. 2000. Stimulatory effects on the reproductive axis in female songbirds by conspecific and heterospecific male song. Horm Behav. 37, 179-89.

Bloomfield, L.L., Farrell, T.M., Sturdy, C.B. 2008A. All "chick-a-dee" calls are not created equally. Part II: Mechanisms for discriminations by sympatric and allopatric chickadees. Behav Processes. 77, 87-99.

Bloomfield, L.L., Sturdy, C.B. 2008. All "chick-a-dee" calls are not created equally. Part I: Open-ended categorization of chick-a-dee calls by sympatric and allopatric chickadees. Behav Processes. 77, 73-86.

Bloomfield, L.L., Farrell, T.M., Sturdy, C.B. 2008B. Categorization and discrimination of “chick-a-dee” calls by wild-caught and hand-reared chickadees. Behav Processes. 77, 166-176.

Braune, P., Schmidt, S., Zimmermann, E. 2008. Acoustic divergence in the communication of cryptic species of nocturnal primates (*Microcebus ssp.*). BMC Biol. 6, 19.

Brenowitz, E. 1991. Altered perception of species-specific song by female birds after lesions of a forebrain nucleus. Science. 251, 303-305.

Chew, S.J., Mello, C., Nottebohm, F., Jarvis, E., Vicario, D.S. 1995. Decrements in auditory responses to a repeated conspecific song are long-lasting and require two periods of protein synthesis in the songbird forebrain. Proc Natl Acad Sci U S A. 92, 3406-3410.

Chew, S.J., Vicario, D.S., Nottebohm, F. 1996. A large-capacity memory system that recognizes the calls and songs of individual birds. Proc Natl Acad Sci U S A. 93, 1950-1955.

Dooling, R., Brown, S., Klump, G., Okanoya, K. 1992. Auditory perception of conspecific and heterospecific vocalizations in birds: Evidence for special processes. J Comp Psychol. 106, 20-28.

Ficken, M.S., Ficken, R.W., Witkin, S.R. 1978. Vocal repertoire of the black-capped chickadee. Auk. 95, 34-48.

Gahr, M. 2000. Neural song control system of hummingbirds: comparison to swifts, vocal learning (Songbirds) and nonlearning (Suboscines) passerines, and vocal learning (Budgerigars) and nonlearning (dove, owl, gull, quail, chicken) nonpasserines. J Comp Neurol. 426, 182-196.

Gee, J.M., Tomaszycki, L., Adkins-regan, E. 2009. Sex-dependent species discrimination in auditory forebrain of naturally hybridizing birds. Brain Behav Evol. 74, 258-267.

Gill, F., Slikas, B., Sheldon, F. 2005. Phylogeny of titmice (Paridae): II. Species relationships based on sequences of the mitochondrial cytochrome-b gene. Auk. 122, 121-143.

Griffiths, R. 2000. Sex identification using DNA markers. In: Baker, A.J. (Eds.), Molecular Methods in Ecology (pp. 295–321). Malden, MA. Blackwell Science.

Guillette, L.M., Farrell, T.M., Hoeschele, M., Sturdy, C.B. 2010. Acoustic mechanisms of a species-based discrimination of the chick-a-dee call in sympatric black-capped (*Poecile atricapillus*) and mountain chickadees (*P. gambeli*). Frontiers in Comparative Psychology. 1:229. doi: 10.3389/fpsyg.2010.00229.

Hernandez, A.M., MacDougall-Shackleton, S.A. 2004. Effects of early song experience on song preferences and song control and auditory brain regions in female house finches (*Carpodacus mexicanus*). J Neurobiol. 59, 247-258.

Hoeschele, M., Gammon, D.E., Moscicki, M.K., Sturdy, C.B. 2009. Note types and coding in Parid vocalizations: the chick-a-dee call of the chestnut-backed chickadee (*Poecile rufuscens*). J Acoust Soc Am. 126, 2088-2099.

Hoke, K.L., Burmeister, S.S., Fernald, R.D., Rand, A.S., Ryan, M.J., Wilczynski, W., et al. 2004. Functional mapping of the auditory midbrain during mate call reception. J Neurosci. 24, 11264-11272.

Huchzermeyer, C., Husemann, P., Lieshoff, C., Bischof, H.J. 2006. ZENK expression in a restricted forebrain area correlates negatively with preference for an imprinted stimulus. Behav Brain Res, 71, 154-161.

Jarvis, E. 2004. Learned birdsong and the neurobiology of human language. Ann N Y Acad Sci. 1016, 749-777

Jarvis, E.D., Mello, C.V. 2000. Molecular mapping of brain areas involved in parrot vocal communication. J Comp Neurol. 419, 1-31.

Long, K.D., Kennedy, G., Salbaum, J.M., Balaban, E. 2002. Auditory stimulus-induced changes in immediate-early gene expression related to an inborn perceptual predisposition.J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 88, 25-38.

Lynch, K.S., Ball, G.F. 2008. Noradrenergic deficits alter processing of communication signals in female songbirds. Brain Behav Evol. 72, 207-214.

MacDougall-Shackleton, S.A., Hulse, S.H., Ball, G.F. 1998. Neural bases of song preferences in female zebra finches (*Taeniopygia guttata*). Neuroreport. 9, 3047-3052.

Marler, P., Perters, S. 1989. Species Differences in Auditory Responsiveness in Early Vocal Learning. Pages 243-273 *in* The comparative psychology of audition: Perceiving complex sounds (Dooling, R.J., Hulse, S.H. eds.). Hillsdale, NJ: Erlbaum.

Mello, C.V. 2002. Mapping vocal communication pathways in birds with inducible gene expression. J Comp Physiol A Neuroethol Sens Neural Behav Physiol, 188. 943-959.

Mello, C.V., Clayton, D.F. 1995. Differential induction of the ZENK gene in the avian forebrain and song control circuit after metrazole-induced depolarization. J Neurobiol. 26, 145-161.

Mello, C., Clayton, D. 1994. Song-induced ZENK gene expression in auditory pathways of songbird brain and its relation to the song control system. J Neurosci. 14, 6652-6666.

Mello, C., Vicario, D., Clayton, D.F. 1992. Song presentation induces gene expression in the songbird forebrain. Proc Natl Acad Sci U S A. 89, 6818-6822.

Mooney R., Hoese, W., Nowicki, S. 2001. Auditory representation of the vocal repertoire in a songbird with multiple song types. Proc Natl Acad Sci U S A. 98, 12778-12783.

Owens JL, & Freeberg TM. 2007. Variation in chick-a-dee calls of tufted titmice, Baeolophus bicolor: note type and individual distinctiveness. J Acoust Soc Am. 122, 1216-1226.

Phillmore, L.S., Bloomfield, L.L., Weisman, R.G. 2003. Effects of songs and calls on ZENK expression in the auditory telencephalon of field- and isolate-reared black capped chickadees. Behav Brain Res. 147, 125-134.

Pinaud R., Terleph, T.A. 2008. A songbird forebrain area potentially involved in auditory discrimination and memory formation. J Biosci. 33, 145-155.

Poole, J.H., Tyack, P.L., Stoeger-Horwath, A.S., Watwood, S. 2005. Animal behaviour: elephants are capable of vocal learning. Nature. 434, 455-456.

Rainey, H.J, Zuberbühler, K., Slater, P.J. 2004. Hornbills can distinguish between primate alarm calls. Proc Biol Sci. 271, 755-759.

Rosen M.J., Mooney, R. 2000. Intrinsic and extrinsic contributions to auditory selectivity in a song nucleus critical for vocal plasticity. J Neurosci. 20, 5437-5448.

Scharff, C., Nottebohm, F., Cynx, J. 1998. Conspecific and heterospecific song discrimination in male zebra finches with lesions in the anterior forebrain pathway. J Neurobiol. 36, 81-90.

Smith, S.M. 1991. The Black-capped Chickadee: Behavioral Ecology and Natural History. Ithaca: Cornell University Press.

Stripling, R., Kruse, A.A., Clayton, D.F. 2001. Development of song responses in the zebra finch caudomedial neostriatum: role of genomic and electrophysiological activities. J Neurobiol. 48, 163-180.

Stripling, R., Volman, S.F., Clayton, D.F. 1997. Response modulation in the zebra finch neostriatum: relationship to nuclear gene regulation. J Neurosci. 17, 3883-3893.

Tan, K., Yang, M.X., Radloff, S.E., Hepburn, H.R., Zhang, Z.Y., Luo, L.J., et al. 2008. Dancing to different tunes: heterospecific deciphering of the honeybee waggle dance. Naturwissenschaften. 95, 1165-1168.

Tchernichovski, O., Schwabl, H., Nottebohm, F. 1998. Context determines the sex appeal of male zebra finch song. Anim Behav. 55, 1003-1010.

Templeton, C., Greene, E. 2007. Nuthatches eavesdrop on variations in heterospecific chickadee mobbing alarm calls. Proc Natl Acad Sci U S A. 104, 5479-5482 .

Terleph, T.A., Mello, C.V., Vicario, D.S. 2006. Auditory topography and temporal response dynamics of canary caudal telencephalon. J Neurobiol. 66, 281-292.

Terpstra, N.J., Bolhuis, J.J., Den Boer-Visser, A.M., Ten Cate, C. 2005. Neuronal activation related to auditory perception in the brain of a non-songbird, the ring dove. J Comp Neurol. 488, 342-351.

Velho, T.A., Pinaud, R., Rodrigues, P.V., Mello, C.V. 2005. Co-induction of activity-dependent genes in songbirds. Eur J Neurosci. 22, 1667-1678.

Vitousek, M.N., Adelman, J.S., Gregory, N.C., Clair, J.J. 2007. Heterospecific alarm call recognition in a non-vocal reptile. Biol Lett. 3, 632-634.

Zann, R.A. The Zebra Finch: A Synthesis of Field and Laboratory Studies. Oxford University Press, Oxford (1996).

Chapter 5 Neural correlates of threat perception: Neural equivalence of conspecific and heterospecific mobbing calls is learned[4]

---

[4] *A version of this chapter has been submitted for publication. Avey M.T., Hoeschele M., Moscicki M.K., Bloomfield L.L. and Sturdy C.B., (2011). Neural Correlates of Threat Perception: Neural Equivalence of Conspecific and Heterospecific Mobbing Calls Is Learned. PLoS ONE 6(8): e23844. doi:10.1371/journal.pone.0023844*

Introduction

Bird calls, unlike songs, are a relatively understudied communication system in behavioural neurobiology (Marler, 2004). Calls serve numerous functions including signaling potential threats which are a primary concern for many species. Threat signals often involve complex behaviour that requires learning both the nature and degree of the potential threat (Evans et al. 1993). Such complex acoustic signaling systems are used to convey information about potential threats to conspecifics or heterospecific individuals (Evans et al. 1993; Ficken and Witkin, 1977; Griffin, 2004; Templeton et al. 2005; Zuberbuhler et al. 1999). Black-capped chickadees use a sophisticated vocal signaling system to indicate the type and degree of potential threat (Templeton et al. 2005). Black-capped chickadees use a high frequency, low amplitude *high zee* call to indicate the presence of an aerial predator, and a loud, complex *chick-a-dee* mobbing call to recruit conspecific individuals and other avian species to mob a perched predator (Baker and Becker, 2002; Ficken and Witkin, 1977; Hurd, 1996; Templeton and Greene 2007; Templeton et al. 2005). Templeton et al. (2005) demonstrated that the structure of the black-capped chickadee *chick-a-dee* mobbing call encodes the degree of threat of potential predators (Templeton et al. 2005). Generally, mobbing calls produced in response to smaller, higher-threat predators contain more “D” notes compared to those produced in response to larger, lower-threat predators. However, where and how the degree of threat is encoded in the brain is unknown.

Auditory processing nuclei in songbirds, such as the caudomedial mesopallium (CMM) and caudomedial nidopallium (NCM), putatively perform functions similar to those of the mammalian auditory cortex (Jarvis et al. 2005; Mello et al. 2004; Pinaud and Terleph, 2008). These regions may activate in response to degree of threat because they activate in response to complex auditory information [Mello et al. 1992; Jarvis et al. 2005; Mello et al. 2004; Pinaud and Terleph, 2008; Woolley and Doupe, 2008). Use of the immediate early gene ZENK (zif-268, egr-1, NGFI-A, or Krox-24) as a regional activity marker has established CMM and NCM as crucial in processing complex auditory information such as conspecific vocalizations (Mello et al. 1992). In general, conspecific vocalizations induce more ZENK positive cells in CMM and NCM compared to heterospecific vocalizations and tones that induce fewer ZENK positive cells (Mello et al. 2004). However, the conspecific signals used as playback stimuli are, necessarily, songs that are biologically relevant to the species' natural history. In contrast, heterospecific signals are often songs of other species that are not biologically relevant signals to the species being studied. In some situations, however, heterospecific vocalizations may be more salient than conspecific vocalizations and this may be reflected in the corresponding neural activity.

I investigated whether the degree of threat perceived by black-capped and mountain chickadees is correlated with ZENK activity in CMM and NCM. To achieve this, I played back one of six stimulus types: four threat stimuli and two control stimuli (Fig. 5-1 a-f), to either wild-caught adult black-capped or mountain chickadees. Thus, I extended the concept of degree of threat not only to differences in the mobbing calls of black-capped chickadees heard by a conspecific bird (Templeton et al. 2005), and mobbing calls of black-capped chickadees heard by a heterospecific bird (mountain chickadees; Templeton and Greene, 2007), but also to the heterospecific calls of predators that induced these mobbing calls. I used two degrees of threat: high threat (Fig. 5-1 a, d) and low threat (Fig. 5-1 b, e). Each degree of threat was conveyed by two distinct signals that shared the same referent, either chickadee mobbing calls to a predator or the corresponding predator calls (northern saw-whet and great horned owls). The calls of a red-breasted nuthatch (Fig 5-1. f), a heterospecific that flocks with both black-capped and mountain chickadees, was used as a control for threat. Reversed mobbing calls (Fig. 5-1 c) were used as a control to match for spectral and temporal complexity in the *chick-a-dee* mobbing call. To my knowledge, whether two signals from different classes of producers can both convey such complex information as degree of threat, and whether these two signals would produce similar amounts of ZENK expression in the brain, have not been tested. This design allowed me to determine whether the degree of threat is encoded in a neural response in CMM and NCM and whether the ZENK expression levels differ depending on the species identity of the caller (conspecific versus heterospecific individuals).

Materials and Methods

Subjects

For this experiment I used 18 wild-adult caught black-capped chickadees (12 male, 6 female), 18 wild-adult caught mountain chickadees (12 male, 6 female), and 16 adult hand-reared black-capped chickadees (7 male, 9 female). Adult black-capped and mountain chickadees were captured from several regions within Edmonton, Alberta, Canada (53°32′N, 113°29′W) and Kananaskis Country, Alberta, Canada (51°02′N, 115°03′W). Hand-reared black-capped chickadees were collected from four different broods (approximately 5–14 days post-hatch) within several regions of Edmonton, Alberta, Canada in June 2004 and June 2005 (Bloomfield et al. 2008). Adult black-capped and mountain chickadees were housed individually in cages in conspecific colony rooms immediately after being brought into the lab. Hand-reared black-capped chickadees were transferred into individual cages in either black-capped or mountain chickadee rearing colony rooms at approximately 35 days of age. Food and water were provided *ad libitum* and colony room temperatures were maintained at about 20°C with the natural seasonal light cycle for Edmonton. All studies were conducted in accordance with the Canadian Council on Animal Care Guidelines and policies with approval from the Animal Care and Use Committee for Biosciences for the University of Alberta (Protocol number 682/12/11), the University of Calgary Life and Environmental Sciences Animal Care Committee (BI11R-10). Chickadees were captured under an Environment Canada Scientific permit (Permit number 09-MB-SC027), an Alberta Sustainable Resource Development (Fish and Wildlife Division)

Collection and Research permits (Permit numbers 47908 and 47910, and a City of Edmonton Parks Permit.

Playback Stimuli

To obtain black-capped chickadee mobbing calls, male black-capped chickadees that were not used in the experiment were placed in a small sound-attenuating chamber (inner dimensions 58 × 168 × 83cm; Industrial Acoustics Corporation, Bronx, New York, USA) and left undisturbed for 24 hrs. The following day, between 0900 and 2000 chickadees were presented with a stuffed mount of either a perched northern saw-whet owl (*aegolius acadicus;* length = 175 mm, wing length = 91mm), great horned owl (*Bubo virginianus*; length = 645 mm, wing length = 349 mm), or a red-breasted nuthatch (length = 130 mm, wing length = 67 mm) in a randomized order with each mount presented twice for three mins and 1 h between each presentation. All recordings were made only while the mount was visible to the black-capped chickadee and were conducted between April 1st 2008 and June 19th 2008. Birds were recorded using an AKG C 1000S condenser microphone (frequency response: 50–20,000 Hz; AKG Acoustics, Vienna, Austria), and a solid-state recorder (Marantz PMD670, D&M Professional, Itasca, IL, USA). Vocalizations from black-capped chickadees that called in response to all three stuffed mounts were used to create the mobbing stimuli. Individual northern saw-whet owl, great horned owl, and red-breasted nuthatch calls were selected from Voices of North American Owls (Cornell Laboratory of Ornithology, Ithaca, New York, USA), Stokes Field Guide to Bird Songs: Western Region (Time Warner AudioBooks, New York, New York, USA), National Geographic Guide to Bird Sounds (Cornell Laboratory of Ornithology, Ithaca, New York, USA), Bird Songs of Canada's West Coast (Neville Recording, Salt Spring Island, British Columbia, Canada), and Alberta Birding by Ear (Barbara Beck, Edmonton, Alberta, Canada). All vocalizations were lowpass filtered at 10,000 Hz in Goldwave (Goldwave, St. John’s, Newfoundland & Labrador, Canada) to remove background noise and normalized using SIGNAL version 5.0 sound analysis software (Engineering Design, Berkeley, California, USA).

I generated two stimulus sets for each vocalization type (e.g. two sets of northern-saw whet owl calls). Each stimulus set consisted of three vocalizations from three different individuals (i.e. set one, individual northern-saw whet owl a-b-c; set two individual northern-saw whet owl d-e-f) within a 15 s window followed by 45 s of silence. This one min of playback was repeated 30 times resulting in a period of 30 mins with stimulus playback for each stimulus category. Stimulus sets were constructed as in previous studies (Avey et al. 2008a) to produce a stimulus presentation that was as natural as possible for the species selected. Thus, the duration of the stimuli varied but this variation did not correlate with expected results of the playback design (i.e., high threat calls were not longer). Within the 15 s window that playback calls occurred, the duration of the stimuli were: black-capped chickadee mobbing calls made to a northern saw-whet owl ~7100 ms; the calls of a northern saw-whet owl ~3100 ms; black-capped chickadee mobbing calls made to a great horned owl ~3400 ms; the calls of a great-horned owl ~8400 ms; the calls of a red-breasted nuthatch ~7100 ms;

and reversed playback of the black-capped chickadee mobbing call to a northern saw-whet owl ~7100 ms (the identical calls used above were reversed).

Playback

Individual birds were housed overnight in a chamber in a modified home cage which contained three perches at the level of the speaker and four water bottles and two food cups located evenly at either end of the cage. Playbacks were recorded using an AKG C 1000S condenser microphone (frequency response: 50–20,000 Hz; AKG Acoustics, Vienna, Austria), and a solid-state recorder (Marantz PMD670, D&M Professional, Itasca, IL, USA). I randomly selected one of six playback conditions to present to individual adult black-capped and mountain chickadees in sound attenuating chambers: 1) black-capped chickadee mobbing calls made to a northern saw-whet owl (high threat); 2) calls of a northern saw-whet owl (high threat); 3) black-capped chickadee mobbing calls made to a great horned owl (low threat); 4) calls of a great-horned owl (low threat); 5) calls of a red-breasted nuthatch (threat control); 6) reversed playback of the black-capped chickadee mobbing call to a northern saw-whet owl (methodological control). There were three adult black-capped and three adult mountain chickadees in each playback condition. Sample size was selected based on power to detect the interaction effect between black-capped and mountain chickadee and playback condition using R 2.12.2 (Good, 2001). There were four adult hand-reared black-capped chickadees in each of groups 1, 2, 5, and 6.

Stimuli were played back through a speaker (Realistic Minimus-7 Cat. no. 40-2034; input 8 OHMS, 40W max; Radio Shack, Fort Worth, TX, USA) and amplifier (Cambridge Audio A300; London, UK) with an mp3 player (Creative ZEN; Singapore). The amplitude was measured at the level of the perches from the centre position of the cage and playback amplitude was set to approximately 74 db with a sound level meter (Radio Shack 33-2055; Radio Shack, Fort Worth, TX, USA). I conducted the experiment before fall equinox in late August and early September when both *chick-a-dee* calling and *fee-bee* song production is low (Avey et al. 2008b). The playback was conducted in one of six sound attenuating chambers (inner dimensions 58 × 168 × 83cm; Industrial Acoustics Corporation, Bronx, New York, USA). Recording began at 1000 every day with 30 min of recording before playback with the lights illuminated, after which playback commenced and continued for 30 min. Following the playback period the lights were extinguished for 1 h. By playing back the calls in a sound chamber to one individual at a time I were able to control for other vocalizations and behaviours that would normally confound the auditory responses in natural settings.

Histology

Following the playback method just described, each bird was given an overdose of 0.03 ml of 100 mg/ml ketamine and 20 mg/ml xylazine intramuscularly (1:1) and then transcardially perfused with heparanized 0.1 M phosphate buffered saline (PBS) followed by 4% paraformaldehyde. Following perfusion, the brain was removed and placed in 4% paraformaldehyde for 24 h and then placed in 30% sucrose in PBS for approximately 24 h until saturated. The brains were then frozen in dry ice and stored at -80°C until immunocytochemistry (ICC) for ZENK protein was performed. For each bird, a cryostat was used to collect forty-eight 40 µm sagittal sections from each hemisphere starting from the midline and proceeding laterally. Sections were then placed into PBS. I processed brains in batches randomized across treatment groups. Sections were washed in 0.1 M PBS, incubated in 0.5% H_2O_2 for 15 min, and washed again in 0.1 M PBS. Next, sections were incubated in 10% normal goat serum for 20 h, followed by incubation in the primary antibody (egr-1, Santa Cruz Biotechnology, catalogue # sc-189; Santa Cruz, CA, USA) at a concentration of 1:5,000 in PBS containing Triton X-100 (PBS/T) for 24 h. Sections were then washed in PBS/T and incubated in biotinylated goat-anti-rabbit antibody for 1 h (1:200 dilution in PBS/T). Next, sections were washed in PBS/T, incubated in avidin–biotin horseradish peroxidase (ABC Vectastain Elite Kit; Vector Labs; Burlington, ON, Canada) for 1 h and washed in 0.1 M PBS. Finally, the sections were visualized using 3,3′-diaminobenzidine tetrachloride (Sigma FastDAB, D4418; Oakville, ON, Canada), mounted on gelatin-coated microscope slides, dehydrated in ethanol and protected with cover slips affixed with Permount (Sigma-Aldrich; Oakville, ON, Canada).

Analysis

ZENK immunoreactivity (ZENK-ir) was quantified for three auditory brain regions: the caudomedial mesopallium (CMM) and the ventral and dorsal parts of the caudal medial nidopallium (NCMv, NCMd; Fig. 5-3.). The lateral ventricle defined the dorsal, ventral, and caudal borders of NCM, and field L defined the rostral border. ZENK-ir in CMM was quantified in the same sections used for

NCM and was assessed in the most caudal area bounded by the lateral ventricle and the caudal-ventral boundary of the mesopallial lamina (LaM). For each chickadee, eight sections per hemisphere were measured for ZENK-ir. Quantification began with the first section in which mesopallium was contiguous with the rostral portion of the nidopallium to ensure that the orientation of the neostriatum was correct. This section, and the next seven sections moving laterally, were then mounted in the correct orientation. For each bird, 16 images (0.20 mm×0.15 mm) of each brain region, eight per hemisphere, were captured using a Leica microscope (DM 5500B; Wetzlar, Germany) with a 40× objective and a Retiga EX*i* camera (Qimaging, Surrey, British Columbia, Canada) using Openlab 5.1 (Perkin Elmer Inc., Waltham, Massachusetts, USA). Images were captured from locations used in previous studies (Phillmore et al. 2003). For CMM, an image was captured from the most caudal part of the region. For NCM, a dorsal image was captured from the most dorso-caudal part of NCM and a ventral image was captured from the centre of the ventro-rostral region in an area of relatively high immunoreactivity. This sampling method, from which I counted the number of immunoreactive cells following a semi-automated protocol using ImageJ (NIH, v.1.36b; 2), captured images from the areas with the highest density of immunopositive cells within these auditory regions. This method has reliably found differences in previous studies (Avey et al. 2005; Gentner et a. 2001; Phillmore et al. 2003.

Results

Wild-adult Chickadees

I quantified the number of ZENK positive cells in CMM and the dorsal (NCMd) and ventral (NCMv) portions of NCM in both hemispheres (Fig. 5-2 and 5-3). I conducted a repeated measures analysis of variance (RMANOVA) with Brain Area × Hemisphere as within subject factors and Listener Species × Playback Condition (Fig. 5-1) as between subject factors. The amount of ZENK expression varied significantly among Brain Areas (RMANOVA: $F(2, 48) = 7.59$, $P < 0.01$; CMM, M=103.23, SD=3.14; NCMd, M=105.68, SD=2.89; NCMv, M=92.5, SD=2.62). Pairwise comparisons (Bonferroni corrected) revealed that CMM and NCMd both had significantly more ZENK expression than NCMv ($P = 0.03$; $P < 0.01$). There was no significant difference between Hemispheres (RMANOVA: $F(1, 24) = 0.54$, $P = 0.47$).

There was no significant difference in the amount of ZENK expression between black-capped and mountain chickadee Listener Species (RMANOVA: $F(1, 24) = 0.72$, $P = 0.40$), indicating that conspecific and heterospecific mobbing calls induced similar ZENK expression in these closely related species. ZENK expression differed significantly among Playback Conditions for both black-capped and mountain chickadees (RMANOVA: $F(5, 24) = 89.57$, $P < 0.01$), and there was no significant interaction between Listener Species and Playback Condition (RMANOVA: $\mathrm{F}(5,24) = 0.85$, $P = 0.53$). Below I analyze the differences between playback conditions by pooling the black-capped and mountain chickadees groups.

Post-hoc comparisons (Tukey HSD) for Playback Condition indicated that playback of black-capped chickadee mobbing calls produced in response to the high threat northern saw-whet owl generated significantly more ZENK expression than black-capped chickadee mobbing calls produced in response to the low threat great-horned owl ($P<0.01$; Fig. 5-4). Thus, mobbing calls associated with higher threat generated more ZENK expression than mobbing calls associated with lower threat. Similarly, playback of the high threat northern saw-whet owl calls generated significantly more ZENK expression than the low threat great-horned owl calls ($P<0.01$; Fig. 5-4). Thus, the degree of threat, whether signaled by chickadee mobbing calls or predator calls, results in higher levels of ZENK expression for high threat signals, independent of whether the signal was produced by a chickadee or predator.

Post-hoc comparisons (Tukey HSD) for Playback Condition indicated that playback of black-capped chickadee mobbing calls produced in response to the high threat northern saw-whet owl did not differ significantly in ZENK expression from playback of northern saw-whet owl calls ($P=0.99$; Fig. 5-4). Similarly, ZENK expression following playback of black-capped chickadee mobbing calls produced in response to the low threat great horned owl calls did not differ significantly from ZENK expression following playback of great-horned owl calls ($P=0.35$). Although each threat level was associated with two distinct signals, one a chickadee mobbing call and one an owl call, there was no significant difference in the amount of ZENK expression induced within a threat level. This result suggests that degree of threat is driving the ZENK expression in CMM and NCM, and not species-specificity. All of the high and low threat playback conditions (mobbing calls and owl calls) differed significantly from the corresponding control condition. The threat control, red-breasted nuthatch calls, generated significantly more expression than the methodological control, reversed chickadee mobbing call (*P*<0.01; Fig. 5-4).

Hand-reared Chickadees

ZENK expression levels in black-capped chickadee auditory perception nuclei vary between high and low threat signals but not between different types of signals conveying the same degree of threat. The next step was to determine whether experience was necessary for the perception of degree of threat in these brain nuclei. To address this question, I hand-reared black-capped chickadees in colony rooms alongside adult chickadees. Hand-reared birds had no experience with either owl species or red-breasted nuthatches. I played back stimuli from one of four conditions to adult hand-reared black-capped chickadees: black-capped chickadee mobbing calls produced in response to a northern saw-whet owl (high threat conspecific vocalization), reversed black-capped chickadee mobbing calls to a northern saw-whet owl (control), northern saw-whet owl calls (high threat heterospecific predator), and red-breasted nuthatch calls (control; Fig. 5-1 a, c, d, f). Comparing ZENK expression following playback of black-capped chickadee mobbing calls produced in response to a northern saw-whet owl with the ZENK expression following playback of northern saw-whet owl calls allowed us to determine whether experience with predators modulates the number of ZENK positive cells in CMM and NCM.

I conducted a RMANOVA with Brain Area × Hemisphere as within subject factors and Playback Condition as the between subjects factor. In common with the results from wild-caught adult chickadees, results for hand-reared chickadees indicated that the amount of ZENK expression varied significantly among the brain areas. The RMANOVA revealed a significant main effect for Brain Area ($F(2, 24) = 9.94$, $P<0.01$; CMM, M=69.55, SD=3.44; NCMd, M=66.96, SD=4.92; NCMv, M=54.33, SD=2.82), with more ZENK expression in CMM and NCMd. There was no significant difference between Hemispheres (RMANOVA: $F(7, 84) = 0.19$, $P=0.48$).

The amount of ZENK expression also varied significantly between Playback Conditions (RMANOVA: $F(3, 12) = 14.80$, $P<0.01$). Post-hoc comparisons (Tukey HSD) indicated that playback of black-capped chickadee mobbing calls produced in response to the high threat northern saw-whet owl generated significantly more ZENK expression than playback of either northern saw-whet owl calls or red-breasted nuthatch calls (both $P<0.01$; Fig. 5-5). ZENK expression elicited by playback of northern saw-whet owl calls did not differ significantly from that elicited by playback of red-breasted nuthatch calls ($P=0.44$; Fig. 5-5). Unlike in wild-caught adult chickadees, ZENK expression levels in CMM and NCM in hand-reared black-capped chickadees, differ between the two high-threat signals (mobbing calls and predator calls), suggesting that perception of threat level is learned.

Discussion

In summary, I found that an increased number of ZENK positive cells correspond to increased degree of threat regardless of the producer species or the spectral and temporal features of the signal. In contrast to wild-caught adult chickadees, hand-reared chickadees responded to conspecific mobbing calls with an increased number of ZENK positive cells, but the number of ZENK positive cells did not vary with exposure to heterospecific predator calls and heterospecific non-predator calls. The activation patterns of ZENK positive cells in wild-caught adults and hand-reared black-capped chickadees support the idea that degree of threat is learned and that learning creates changes in the neural activation within CMM and NCM.

The black-capped chickadee mobbing call is a multi-note, broad band vocalization with complex harmonics (Charrier et al. 2005; Ficken and Witkin, 1977; Templeton et al. 2005), which is a striking contrast to the simple, tonal vocalization of the northern saw-whet owl call (Holschuh and Otter, 2005). Although the structure and duration of the signals differ, the mobbing calls and the corresponding owl calls still generated the same amount of activation in CMM and NCM of black-capped chickadees. Despite that the owl calls are not used to signal threat to heterospecific species, the wild-caught adult chickadees intercepting the owl calls perceive them as conveying the same degree of threat as the corresponding mobbing calls. The patterns of activation strongly support the idea that CMM and NCM are responding to the degree of threat and not simply the producer or spectral and temporal properties of the signal

Previous research initially reported differences in the amount of ZENK expression in CMM and NCM in response to conspecific and heterospecific vocalizations (Mello et al. 1992). Subsequently, researchers have used heterospecific signals as a control in ZENK expression studies (Bailey and Wade, 2006), but here I show that heterospecific signals can generate as much, if not more, ZENK expression depending on the information contained within, or the relevance of, that signal. I build upon previous studies by demonstrating that CMM and NCM do not simply respond in a graded fashion to conspecific and heterospecific signals, but that biological relevance of the stimuli can supersede the distinction between conspecific versus heterospecific signals. I confirmed this idea with two closely-related species of chickadee, one that heard conspecific vocalizations and one that heard heterospecific mobbing calls as well as both species hearing of heterospecific predator calls.

Chickadees have a sophisticated alarm call system for signaling threat. This study demonstrates that the information conveyed in the signal, the degree of threat, produces a differential response in the auditory perception nuclei I investigated. By studying this system, I was able to show that a conceptual category, such as threat, can be conveyed with very distinct stimulus types that differ in the species of the producer and the signal structure itself. In addition, by studying both wild and hand-reared chickadees, I showed that the degree of threat predators pose is learned, and this learning can be detected in the neural activity patterns of the auditory nuclei.

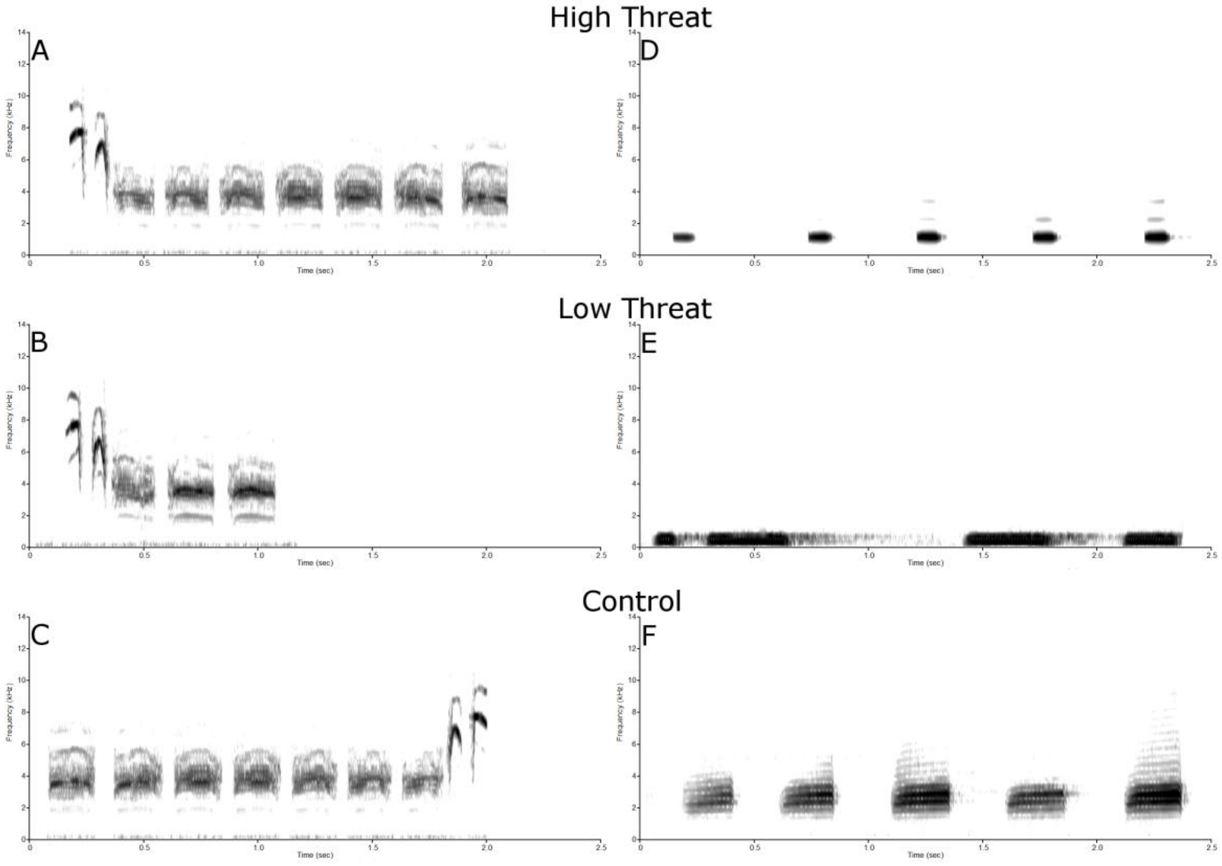

High Threat
A
D
Low Threat
B
E
Control
C
F
Frequency (kHz)
0
2
4
6
8
10
12
14
Time (sec)
0
0.5
1.0
1.5
2.0
2.5

Figure 5-1.

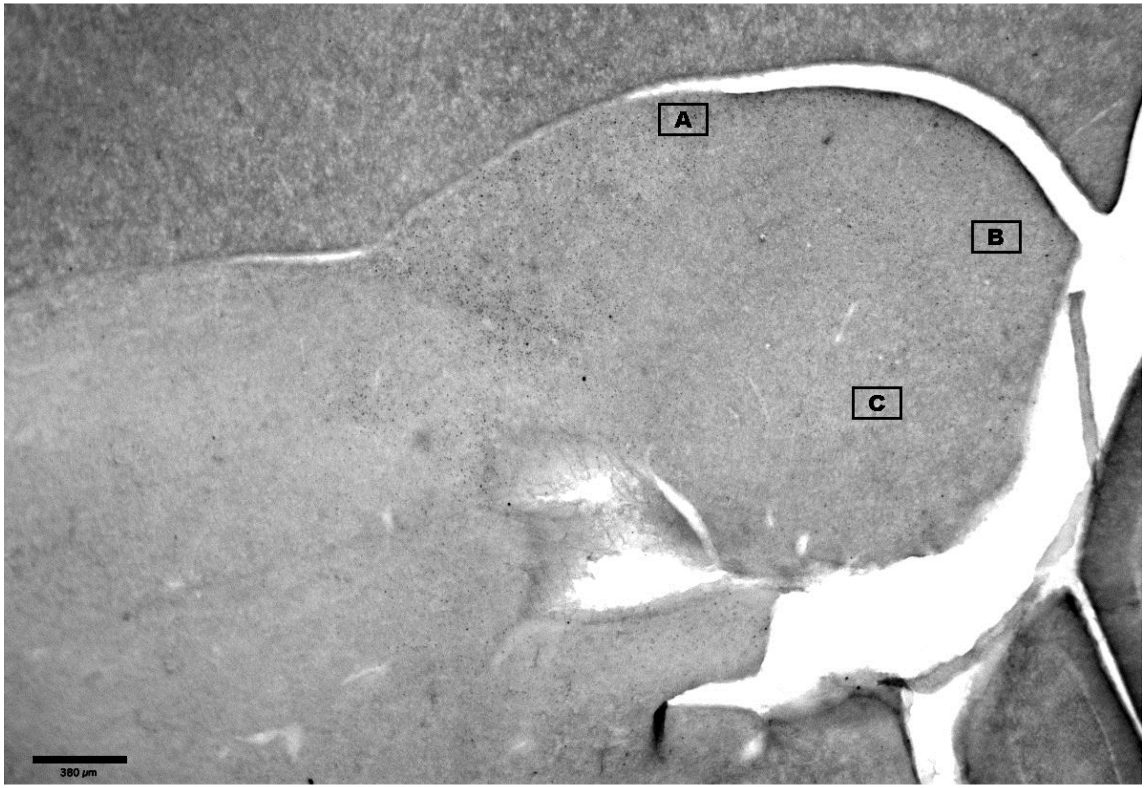


Figure 5-2

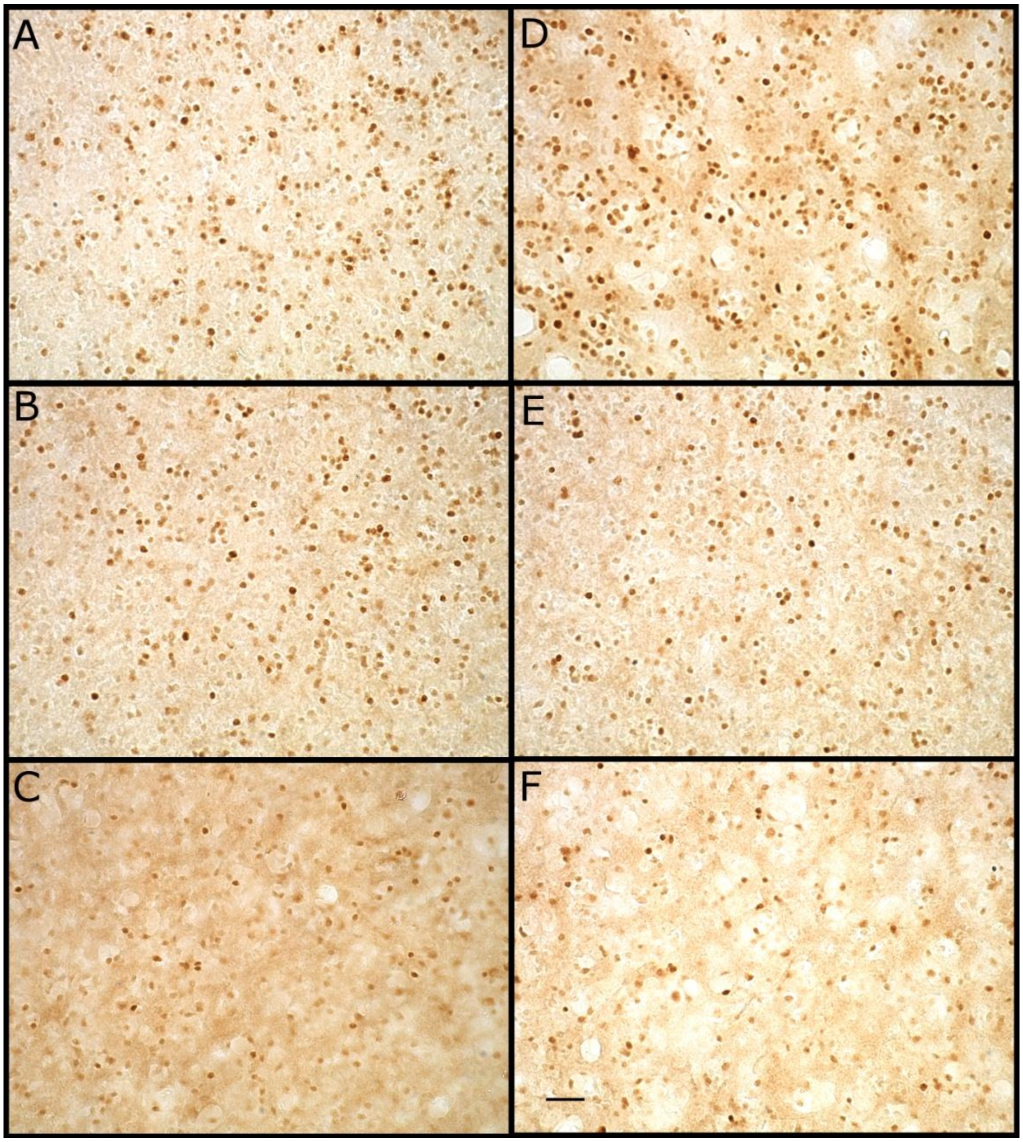
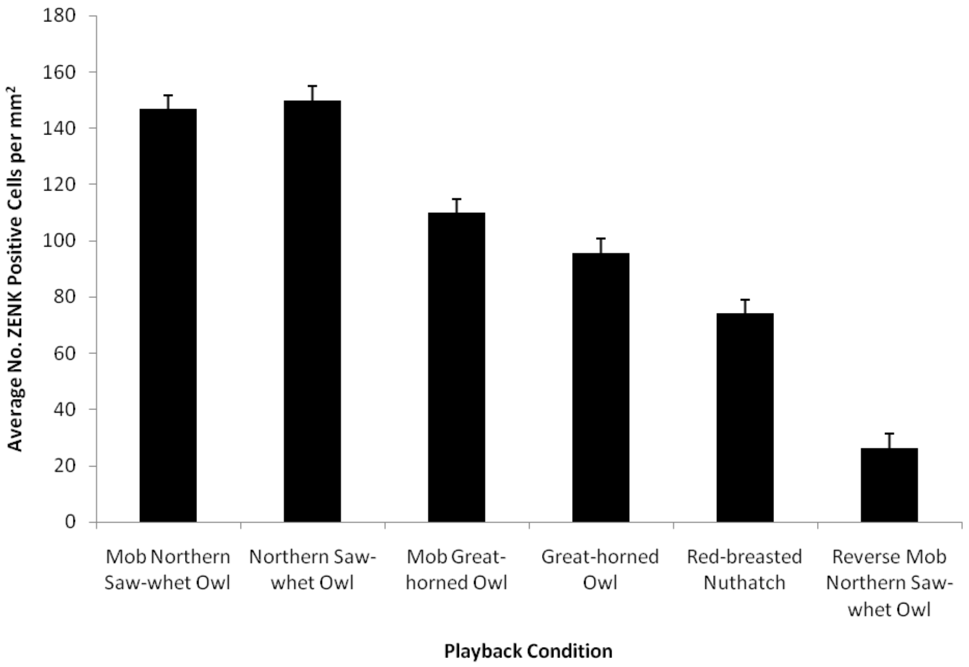


Figure 5-3.



Average No. ZENK Positive Cells per mm^2
180
160
140
120
100
80
60
40
20
0
Mob Northern Saw-whet Owl
Northern Saw-whet Owl
Mob Great-horned Owl
Great-horned Owl
Red-breasted Nuthatch
Reverse Mob Northern Saw-whet Owl
Playback Condition

Figure 5-4.

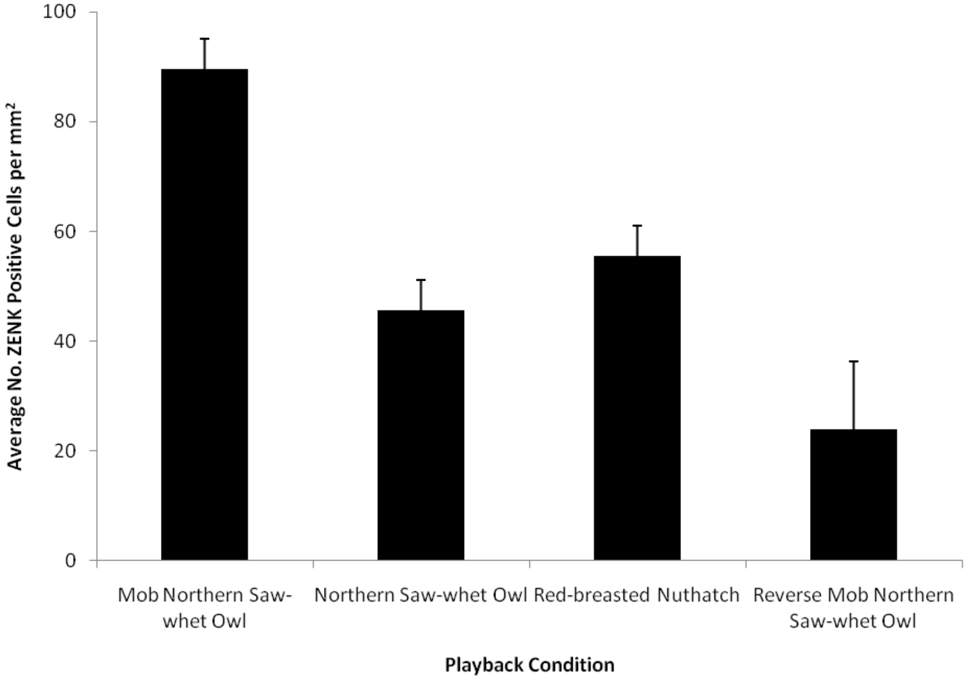

100
80
60
40
20
0
Average No. ZENK Positive Cells per mm²
Mob Northern Saw-whet Owl
Northern Saw-whet Owl
Red-breasted Nuthatch
Reverse Mob Northern Saw-whet Owl
Playback Condition

Figure 5-5.

References

Avey, M.T., Phillmore, L.S., MacDougall-Shackleton, S.A. 2005. Immediate early gene expression following exposure to acoustic and visual components of courtship in zebra finches. Behav Brain Res. 165, 247-253.

Avey, M. T., Quince, A. F., & Sturdy, C. B. 2008b. Seasonal and diurnal patterns of black-capped chickadee (*Poecile atricapillus*) vocal production. Behav Processes, 77, 149-155.

Avey, M.T., Kanyo R.A., Irwin E.L., Sturdy, C.B. 2008. Differential effects of vocalization type, singer and listener on ZENK immediate early gene response in black-capped chickadees (*Poecile atricapillus*). Behav Brain Res. 188, 201-208.

Baker, M. C., Becker, A.M. 2002. Mobbing calls of black-capped Chickadees: Effects of urgency on call production. Wilson Bull, 114, 510-516.

Bailey, D.J., Wade, J. 2006. Sexual dimorphism in song-induced ZENK expression in the medial striatum of juvenile zebra finches. Neurosci Lett. 401, 86-91.

Bloomfield, L.L., Farrell, T.M., Sturdy, C.B. 2008. Categorization and discrimination of “chick-a-dee” calls by wild-caught and hand-reared chickadees. Behav Processes. 77, 166-176.

Charrier, I., Bloomfield, L.L., Sturdy, C.B. 2004. Note types and coding in parid vocalizations. I: The chick-a-dee call of the black-capped chickadee (*Poecile atricapillus*). Can J Zool, 82. 769-779.

Evans, C.S., Evans, L., Marler, P. 1993. On the meaning of alarm calls: functional reference in an avian vocal system. Anim Behav. 46, 23-38.

Ficken, M.S., Witkin, S.R. 1977. Responses of black-capped chickadee flocks to predators. Auk. 94, 156-157.

Gentner, T.Q., Hulse, S.H., Duffy, D., Ball, G.F. 2001. Response biases in auditory forebrain regions of female songbirds following exposure to sexually relevant variation in male song. J Neurobiol. 46, 48-58.

Good, P. I. 2001. Resampling methods: A practical guide to data analysis. Boston: Birkhauser, 269 p.

Griffin, A.S. 2004. Social learning about predators: a review and prospectus. Learn Behav. 32, 131-140.

Holschuh, C.I., Otter, K.A. 2005. Using vocal individuality to monitor Queen Charlotte Saw-whet owls (*Aegolius acadicus brooksi*). J Raptor Res. 39, 134-141.

Hurd, C.R. 1996. Interspecific attraction to the mobbing calls of black capped chickadees (*Parus atricapillus*). Behav Ecol Sociobiol. 38, 287-292.

Jarvis, E.D., Güntürkün, O., Bruce, L., Csillag, A., Karten, H., et al. 2005. Avian brains and a new understanding of vertebrate brain evolution. Nature Rev Neurosci 6, 151-159.

Marler, P. 2004. Bird calls: Their potential for neurobiology. Ann N Y Acad Sci. 1016, 31-44.

Mello, C.V., Velho, T.A., Pinaud, R. 2004. Song-induced gene expression: a window on song auditory processing and perception. Ann N Y Acad Sci. 1016, 263-281.

Mello, C.V., Vicario, D., Clayton, D.F. 1992. Song presentation induces gene expression in the songbird forebrain. Proc Natl Acad Sci U S A. 89, 6818-6822.

Pinaud. R., Terleph, T.A. 2008. A songbird forebrain area potentially involved in auditory discrimination and memory formation. J Biosci. 33, 145-155.

Phillmore, L.S., Bloomfield, L.L, Weisman, R.G. 2003. Effects of songs and calls on ZENK expression in the auditory telencephalon of field- and isolate-reared black capped chickadees. Behav Brain Res. 147, 125-134.

Templeton, C. N., Greene, E. 2007. Nuthatches eavesdrop on variations in heterospecific chickadee mobbing alarm calls. Proc Natl Acad Sci U S A. 104, 5479-5482.

Templeton, C.N., Greene, E., Davis, K. 2005. Allometry of alarm calls: Black-capped chickadees encode information about predator size. Science. 308, 1934-1937.

Woolley, S.C., Doupe, A.J. 2008. Social context-induced song variation affects female behavior and gene expression. PLoS Biol. 6, 525-537.

Zuberbuhler, K., Jenny, D., Bshary, R. 1999. The predator deterrence function of primate alarm calls. Ethology. 105, 477-490.

Chapter 6 General Discussion

This thesis examined the vocal production and auditory perception of *chick-a-dee* calls in black-capped and mountain chickadees using an integrative approach. First, a study of the seasonal pattern of production of *chick-a-dee* calls in the field under natural conditions provided an ethological foundation for the timing of laboratory perceptual experiments. Second, the formal study of housing conditions and wild-caught chickadees' production of the *chick-a-dee* calls ensured that the artificial conditions in the laboratory did not radically change the patterns observed in the field. The subsequent perceptual experiments explored how neural activity, via the immediate early gene ZENK, is linked to *chick-a-dee* calls and heterospecific calls from closely related species to distantly related predators.

First, I determined what factors of heterospecific calls drive the ZENK response in CMM and NCM by exploring the relationship between phylogeny and bioacoustics and found that the relationship is not black and white. Second I explored the relationship between the functional relevance of signals and the ZENK response in CMM and NCM. Here I found that the ZENK response correlates with information conveyed by the calls, that is, the degree of threat from predators. Furthermore, I found that irrespective of whether the information is conveyed by a conspecific or a heterospecific call (either chickadee or predator), high threat vocalizations result in equivalent amount of ZENK expression. Finally, I exposed hand-reared black-capped chickadees to calls that were high threat but from two different sources, fellow black-capped chickadees and northern saw-whet owls. The hand-reared birds did not have equivalent

ZENK expression, suggesting that identifying the predator calls as threatening is a learned response and that ZENK expression is impacted by this learning. These studies build upon the research initially begun by Thorpe and Marler, studying the development of bird song, and Nottebohm, Konishi, Clayton, and Mello's research which set the foundation of neural circuits for the production and perception of song. Taken together, the results of these studies further our knowledge of the natural pattern of calling in a songbird species and how the neural response of ZENK is affected by heterospecific calls. Although calls remain an understudied area of songbird neurobiology, the results presented here open a window onto the processing of calls.

Seasonal Variation in Vocal Production

The results of the field study (Chapter 2) provide a better understanding of both the seasonal and diurnal pattern of vocal production for three major vocalization types in black-capped chickadees. Previous research did not examine all of these vocalizations together, nor did they investigate them in as much detail along with either the seasonal or diurnal dimension. I have expanded upon the previous research and recommendations of researchers such as Pravasudov (2006) to examine the behaviour across the entire season (and time of day). I also examined three major vocalizations in concert which allowed comparison within one study of the pattern of vocal production. I found differences in the amount of vocal production for *fee-bee* song, *chick-a-dee* calls and *gargle* calls, both across the different seasons as well as within the diurnal cycle.

Seasonal and Diurnal Patterns of *Fee-bee* Song in the Field and Laboratory

Most *fee-bee* singing occurs in the spring and at dawn as previously reported (Smith, 1991). Although there was some *fee-bee* song production at the meridian, at sunset there was almost no *fee-bee* song production. The dramatic increase in fee-bee song production immediately following the spring equinox is followed with a dramatic decrease in production before May and low levels well before summer. What individual behavioural changes are occurring in the population with this overall pattern of vocal production is an area for future investigation. Even in this case with the very well-studied *fee-bee* song in black-capped chickadees, questions about what behaviours account for the dramatic decrease (likely nesting) and continued low level use of *fee-bee* song in late spring (late breeders, extra pair copulations) have not been quantified in detail. The results for the study of *fee-bee* song raise more questions about the behaviours related to the increase in *fee-bee* song production during the winter.

The increase in *fee-bee* song production observed during winter was less than that observed in the spring, but similar in nature to that observed during the spring. However, the end of the transient increase in *fee-bee* song production did not see the song rates return to the low baseline level of song production of the summer and autumn. *Fee-bee* song production in winter occurred mostly at dawn, although some song production occurred at the meridian. In spite of the fact that black-capped chickadees are in flocks during the winter, *fee-bee* song output during the winter may be due to initial competition for mates and territories. The dawn chorus at this time of year is not influenced by female nesting activity

(Mace 1987; Gammon 2004); however, what behaviours are occurring and the function of this increase in vocal production warrant further analysis.

In the laboratory (Chapter 3), the pattern of *fee-bee* song production peaked in the spring, with most singing at the dawn similar to the field results, although there was also a large amount of singing at the meridian. The proportion of *fee-bee* songs relative to other vocalizations is dramatically lower in the laboratory than in the field. The housing conditions, such as individual cages, may have a direct impact on the amount of *fee-bee* song by impeding natural behaviours such as mating, and defending territories etc. The housing conditions may be directly reducing the amount of *fee-bee* song produced or indirectly by increasing the amount of other vocalizations being produced such as *chick-a-dee* or *gargle* calls.

The field and laboratory studies of *fee-bee* song suggest that season and diurnal cycle have strong impacts on the production of this vocalization. From these two studies it is clear that drive to produce this *fee-bee* song in the spring is powerful even when chickadees are unable to defend territories and mate. The winter role of *fee-bee* song production is unclear but the increase may be related to hormonal changes initiated at the winter solstice in males. Alternatively, changes in behaviour in females during the winter such as early mate selection may be driving the increases in *fee-bee* song production. Future field investigations should focus on determining what behaviours are associated with the increase in winter *fee-bee* song and if the is any benefit to fitness.. In the laboratory, there are many questions that should be asked about the effects of housing, including whether interfering with birds' ability to pair and defend territory in the spring impacts hormonal levels relative to wild birds.

Seasonal and Diurnal Patterns of *Chick-a-dee* Calling in the Field and Laboratory

The rate of *chick-a-dee* call production varied with the seasonal cycle in the field (Chapter 2), although relative to *fee-bee* song production, seasonal variation of *chick-a-dee* calling was not as dramatic or as season-specific. Most *chick-a-dee* calling occurred during the autumn and winter and on average across seasons, the most *chick-a-dee* calling occurred around the meridian.

Examination of variations in *chick-a-dee* call production across the diurnal cycle and season showed a complex relationship. Most calling occurred during the autumn and winter and around the meridian. The trend for *chick-a-dee* calling in the spring was an overall decrease in call production compared to winter, with most calling during this period occurring at the meridian. This trend persisted until the summer solstice when call production once again increased. Decreases in *chick-a-dee* call production during the spring likely occurred, at least in part, because as *fee-bee* song production increases, there is reduced opportunity to produce other vocalizations. The general decrease in *chick-a-dee* call production towards late spring may be a result of mated pairs minimizing vocal production and activity not related to raising their clutches. In summary, I confirmed that *chick-a-dee* call production occurs primarily in autumn and winter and during the meridian, but the nuances found in calling behaviour (e.g. spring decreases in calling coupled with increases in *fee-bee* song production) demonstrate that vocal behaviour is much more complex than previous literature had determined. Although season and diurnal cycle are good predictors of *chick-a-dee* calling behaviour, the relationship is complicated and likely significantly affected by other factors (e.g., flocking behaviours, climate). Further research on natural behaviours and more in-depth observations within days as well as around critical photoperiodic events will be required to determine how these factors interact and affect vocal production of the *chick-a-dee* call. The variation seen between seasons and within days demonstrates that even further observation and quantification of this behaviour will be required.

In the laboratory (Chapter 3), there was no difference across season or diurnal pattern for *chick-a-dee* calls, although there was a large increase in *dee* calls in the summer. The comparison between field and laboratory patterns of vocalizations demonstrates a general agreement. Only *chick-a-dee* calls varied significantly between field and laboratory in their pattern during the diurnal pattern in the winter and the seasonal cycle at dawn and sunset. *Chick-a-dee* calls did not vary across season or diurnal pattern and the most *dee* calls occurred during the summer and the least in the winter, although there is no direct comparison to field vocalization patterns for this call (Avey et al., 2008). The proportion of *chick-a-dee* calls is also lower in the laboratory than in the field (including '*dee*' calls). The reasons for this result are unclear; this may be a result of housing conditions eliminating other natural behaviours, such as foraging, where *chick-a-dee* calling is often used. I was not able to directly compare the pattern of *dee* calls in the laboratory and field because I was unable to reliably score *dee* calls separately in the field survey (Avey et al., 2008). The pattern for *dee* calls indicates that there is a large ‘spike’ in the summer, although not significantly different from spring and autumn. The large increase in the summer and marked decrease in the winter suggest that the changes in vocal behaviour are linked to changes in the light cycle in the colony room. Interpreting the *dee* calls highlights the importance of understanding the functional difference even within categories of calls (e.g., different *chick-a-dee* calls can have different meaning; Templeton et al., 2005) to gain a more complete understanding of the behaviour.

The field and laboratory studies of *chick-a-dee* calls suggests that season and diurnal cycle impact the production of this vocalization. From the two studies it is not possible to determine whether these changes are related to hormonal or physiological changes, as is known in the production of the *fee-bee* song. The changes I found are most likely driven by environmental and behaviour changes that occur. In the field, close studies of the immediate effect of weather, habitat, and behaviour interactions will yield insights into this variable call. In the laboratory, as in the field, the study of the artificial habitat and behaviours should be examined more closely. There are many questions that should be asked about the impact of laboratory housing. For instance, does housing chickadees in individual cages in one room across all seasons create one large flock with the associate dominance hierarchy? Does interfering with the ability to pair bond and defend territory in the spring impact hormonal levels?

Seasonal and Diurnal Patterns of Gargle Calling in the Field and Laboratory

In the field (Chapter 2), *gargle* call production did not vary significantly across season or to the same degree as *fee-bee* song and *chick-a-dee* call production. In fact, *gargles* were produced least amongst all three vocal types. Similar to the diurnal pattern observed for *chick-a-dee* call production, *gargle* call production was focused around the meridian. This may be related to a period of high activity for chickadees, leading to increased behavioural and aggressive interactions. I expanded our understanding of how *gargle* call production is shaped by season, diurnal cycle and its relative production to *fee-bee* song and *chick-a-dee* calls. *Gargle* call production was the most variable and in turn the most difficult to predict from the seasonal and diurnal cycle. Increased sampling within days and around relevant photoperiod events as well as increased field observations of the natural behaviour in future research will be required to further explain this use and factors affecting this complex call.

In the laboratory (Chapter 3), *gargle* calls increased in the summer, with most calling occurring at dawn and the meridian. Most *gargle* calls occurred during the summer during the dawn and meridian. *Gargle* calls were proportionally higher in the laboratory than the field although their patterns were not different. The large proportion of *gargle* calls in the laboratory may be a result of housing conditions, as discussed previously.

In two studies (Chapter 2 and 3) I described in detail the relationship between vocal production of and the seasonal and diurnal cycle for black-capped chickadees in both the field and laboratory. The results indicate that the relationship between vocal behaviour and photoperiod is not a straightforward one and the housing conditions can impact the natural vocal behaviour. However, there was general agreement between the field and laboratory in the pattern of vocal production for each vocal type studied. The description of vocal production of songbirds and in particular, the black-capped chickadee, has been done before, but attention to non-song vocalizations investigated here has been more limited. Even investigation into the vocal pattern of *fee-bee* song yielded new insights and this (Chapter 3) was the first study to formally investigate laboratory housing conditions on black-capped chickadees' vocal behaviour. Taken together these two studies provide a strong foundation for future research into the pattern of vocal production in the field and laboratory as well as an important foundation for the subsequently auditory perception experiments I performed in the laboratory.

The field and laboratory studies of *gargle* calls suggest that season and diurnal cycle have less impact on the production of this vocalization relative to *fee-bee* song and *chick-a-dee* calls. One striking contrast from these two studies is the increased proportion of *gargle* calls in the laboratory compared to the field. The gargle call is used for interspecific aggression and the laboratory housing conditions may be exacerbating conflict due to artificially large numbers of chickadees being housed in closed quarters where they can see one another but not directly interact. Postural signals may be an important component of *gargle* calls, however Susan Smith (2007) notes that one area of chickadee behavior that we know little about is visual displays. Differences in plumage can indicate, not only sex, but also dominance rank (Mennill et al. 2003; Doucet et al. 2004), but little is known of postural signals (Smith, 1991). The systemic study of visual displays in the laboratory combined with *gargle* calls may yield future insights. Finally, from the field and laboratory study, it is clear that changes in the light cycle across season and time of day are crucial for the natural behaviour of black-capped chickadees and any captive birds must be maintained on a natural cycle. Improvement in the housing would include dimming the lights on and off to reflect dawn and dusk. Housing the chickadee where it is possible for all birds to see one another may also decrease the amount of *gargle* calls.

Phylogeny, Heterospecifics, Bioacoustics, Function and ZENK Activity

In Chapters 4 and 5, I explored how ZENK expression in CMM and NCM is related to heterospecific stimuli. I attempted to control for relatedness and bioacoustic complexity (Chapter 4) and the function of calls and learning (Chapter 5). I played back complete calls of heterospecifics (Chapter 3 Experiment 1) and found that ZENK expression did not decrease for closely related heterospecifics but did for a more distant heterospecific call. This result can be explained either by the response of ZENK expression along a phylogenetic gradient or by the fact that the closely related species calls were bioacoustically more similar (both chickadee calls) to black-capped chickadee calls than the call of the gray-crowned rosy-finch. In the second experiment, I attempted to maintain ethological relevance of the signals while removing bioacoustic differences. I selected species' calls to play back to black-capped chickadees with which they had no experience, were arranged on a phylogenetic gradient, and all had '*dee*' like notes that shared important bioacoustic properties with the black-capped '*dee*' notes (e.g., broadband frequencies and similar durations). I found no differences along the phylogenetic gradient, even though the zebra finch is certainly more ecologically and experientially separated from chickadees than the the gray-crowned rosy finch of experiment 1. The results suggest that as bioacoustic similarity in the signal increases (towards the species' own signals) the amount of ZENK expression in CMM and NCM becomes more similar (higher) as well. I cannot conclude that the signals are indistinguishable to the chickadees nor that more and different neural activity may be revealed through different techniques. Indeed, because the reversed '*dee*' note condition yielded significantly less (although still relatively high amounts) of ZENK expression, some features of the '*dee*' note must be attended to.

Both experiments 1 and 2 (Chapter 4) support the idea that heterospecific signals cannot be used as an unqualified control for ZENK expression relative to conspecific signals. The features of calls or song needed for equivalent amount of ZENK induction remain unknown, but future research can examine what features and combinations of features are required to activate the ZENK response and how they related to phylogeny or the ecology of the species. These two experiments taken together attempt to reveal that the conspecific-heterospecific divide in ZENK expression is not as clear cut as previous research indicated. The relationship between bioacoustics and ZENK expression is not a simple reduction of relevant bioacoustic features to increased ZENK expression, but must include an analysis of the full signal, its relationship to the organism and even its context.

ZENK expression was not related to the bioacoustics of calls but to the function of calls when the function made the calls extremely salient or biologically relevant (Chapter 5). I found that ZENK expression mirrored the degree of threat of a call whether that call was produced by a conspecific chickadee, heterospecific chickadee, or predators' calls which vary greatly in their bioacoustic properties. Three conclusions can be drawn from this. First, that ZENK expression is affected by the function of the vocalization. Second, that ZENK expression is similar for signals that share the same function regardless of source. Third, bioacoustic properties of the signal alone do not drive the ZENK response. The hand-reared chickadees did not respond along the threat continuum as wild-adult chickadees, suggesting that the degree of threat or function of the signals for threat is learned. This supports evidence that the ZENK response is a learned response (Knapska and Kaczmarek, 2004). An alternative explanation of the mirroring of the ZENK expression with the degree of threat in the signals is that the ZENK response is also related stress (Knapska and Kaczmarek, 2004). If mobbing calls and owl calls induce more or less stress, the ZENK response may vary along this continuum. I was unable to eliminate the possibility that familiarity may have influenced the responding of the hand-reared birds' increased ZENK response to the conspecific mobbing calls relative to the two heterospecifics. This research (Chapter 5) investigated how the function of calls can mediate the ZENK response and whether learning is necessary for the response as well. This study advances our understanding of the complexity of the ZENK response to auditory stimulus and reinforces the importance of studying the functional aspect of the signals used and their ethological relevance to the organism.

Conclusion and Future Directions

The studies that comprised this thesis were designed to investigate the natural behaviour and neural mechanisms that underlie songbird auditory perception. I investigated the seasonal and diurnal patterns of the production of calls in both the field and laboratory, and through the use of an immediate early gene I investigated the neural activity associated with calls along phylogeny, bioacoustics, function, and learning. I used a comparative and ethological approach in an attempt to discover general principles that govern these processes as they evolved in the organisms under study. By studying both calls and song production as they vary across season and diurnal cycle, I resolved with greater clarity the nuances of these patterns. By studying in both the field population and under normal laboratory housing conditions I validated our laboratory studies' use of wild-caught birds for ethological experiments. I am now able to conclude that heterospecific signals can and do generate as much ZENK expression as conspecific signals and that the ZENK response is complex and varies with a host of factors including, but not limited to, phylogeny, bioacoustics, function, and learning.

Future research should focus on the use multiple labeling techniques to determine what cell types are responding in these brain regions as well as to determine if all vocalizations are encoded by the same cells or if there is specialization. The difficult nature of quantifying the bioacoustics differences between vocalizations could be aided by use of measures such as Wiener entropy to quantify the differences in the signals. Finally, more behavioural and ecological research needs to be done studying how black-capped chickadees respond to these signals in the natural environment, and in the laboratory using operant techniques to term the limits and range of responding to subtle differences in the structure of the signals.

References

Avey M.T., Quince A.F., Sturdy C.B. 2008b. Seasonal and diurnal patterns of black-capped chickadee (*Poecile atricapillus*) vocal production. Behav Processes. 77,149-155.

Doucet, S.M., Mennill, D.J., Montgomerie, R., Boag, P., Ratcliffe, L.M. 2004. Achromatic plumage reflectance predicts reproductive success in male black-capped chickadees. Behavl Ecol. 16, 119-133.

Gammon, D.E. 2004. Black-capped Chickadee dawn chorus and subsequent sexual activity. Wilson Bulletin. 116, 252-256.

Knapska, E., Kaczmarek, L. 2004. A gene for neuronal plasticity in the mammalian brain: Zif268/Egr-1/NGFI-A/Krox-24/TIS8/ZENK? ProgNeurobiol. 74, 183-211.

Mace, R. 1987. The Dawn Chorus in the Great Tit *Parus Major* is directly related to female fertility. Nature. 330, 745-746.

Mennill, D.J., Doucet, S.M., Montgomerie, R., Ratcliffe, L.M. 2003. Achromatic color variation in black-capped chickadees, *Poecile atricapilla*: black and white signals of sex and rank. Behav Ecol Sociobiol. 53, 350-357.

Pravosudov, V.V. 2006. On seasonality in food-storing behaviour in parids: do we know the whole story? Anim Behav. 71, 1455-1460.

Smith, S.M. 1991. The Black-capped Chickadee: Behavioral Ecology and Natural History. Ithaca: Cornell University Press.

Smith, S.M. 2007. Introduction to the North American Paridae. Pages 1-6 *in* Ecology and behavior of chickadees and titmice: an integrated approach. (Otter, K.A., Ed.) Oxford University Press, Oxford, UK.

Templeton, C.N., Greene, E., Davis, K. 2005. Allometry of alarm calls: black-capped chickadees encode information about predator size. Science. 308, 1934-1937.