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Effects of Altered Prenatal Sensory Stimulation on Postnatal Contingency Learning in Bobwhite Quail Neonates (*Colinus Virginianus*)

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

EFFECTS OF ALTERED PRENATAL SENSORY STIMULATION ON POSTNATAL
CONTINGENCY LEARNING IN BOBWHITE QUAIL NEONATES (*COLINUS*
VIRGINIANUS)

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

PSYCHOLOGY

by

Namitha Raju

2014

To: Interim Dean Michael R. Heithaus
College of Arts and Sciences

This dissertation, written by Namitha Raju, and entitled Effects of Altered Prenatal Sensory Stimulation on Postnatal Contingency Learning in Bobwhite Quail Neonates (*Colinus virginianus*), having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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The dissertation of Namitha Raju is approved.

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Florida International University, 2014

DEDICATION

I dedicate this dissertation to my husband whose encouragement and love has made this achievement possible.

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I am deeply grateful to my major professor, Dr. Robert Lickliter for his patience, guidance, and encouragement throughout this process. I am also very thankful to my committee member, Dr. Anthony Dick, for his invaluable help and advice on this dissertation. Lastly, I would like to extend my special thanks to Michael A. Logan who has been a tremendous help with data collection for this project.

ABSTRACT OF THE DISSERTATION
EFFECTS OF ALTERED PRENATAL SENSORY STIMULATION ON POSTNATAL
CONTINGENCY LEARNING IN BOBWHITE QUAIL NEONATES (*COLINUS
VIRGINIANUS*)

by

Namitha Raju

Florida International University, 2014

Miami, Florida

Professor Robert Lickliter, Major Professor

Preterm infants are exposed to high levels of modified early sensory experience in the Neonatal Intensive Care Unit (NICU). Reports that preterm infants show deficits in contingency detection and learning when compared to full-term infants (Gekoski, Fagen, & Pearlman, 1984; Haley, Weinberg, & Grunau, 2006) suggest that their exposure to atypical amounts or types of sensory stimulation might contribute to deficits in these critical skills. Experimental modifications of sensory experience are severely limited with human fetuses and preterm infants, and previous studies with precocial bird embryos that develop in ovo have proven useful to assess the effects of modified perinatal sensory experience on subsequent perceptual and cognitive development. In the current study, I assessed whether increasing amounts of prenatal auditory or visual stimulation can interfere with quail neonates' contingency detection and contingency learning in the days following hatching.

Results revealed that augmented prenatal visual stimulation prior to hatching does not disrupt the ability of bobwhite chicks to recognize and prefer information learned in a

contingent fashion, whereas augmented prenatal auditory stimulation disrupted the ability of chicks to benefit from contingently presented information. These results suggest that specific types of augmented prenatal stimulation that embryos receive during late prenatal period can impair the ability to learn and remember contingently presented information. These results provide testable developmental hypotheses, with the goal of improving the developmental care and management of preterm neonates in the NICU setting.

Keywords: contingency learning, preterm birth, prenatal sensory stimulation

TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION	1
II. LITERATURE REVIEW	3
Sensory Stimulation in the Uterine Environment	3
Premature Birth and Changes in Sensory Organization	4
Contingency Learning in Preterms	5
Historical and Theoretical Views.....	7
Development of Sensory Systems	8
Importance of Using Animal Models	10
Effects of Sensory Stimulation on Arousal.....	11
Effects of Sensory Stimulation on Perceptual Learning	12
The Effects of Altering the Amount of Premature Stimulation.....	12
The Effects of Altering the Type of Premature Stimulation.....	15
The Effects of Altering the Timing of Premature Stimulation	16
III. SCOPE AND DESIGN OF THE STUDY.....	20
IV. PRELIMINARY EXPERIMENTS.....	23
Touch-Screen Paradigm 1	24
Touch-Screen Paradigm 2.....	25
Touch-Screen Paradigm 3.....	26
Vocalization Contingency Paradigm with bobwhite maternal call.....	27
Vocalization Contingency Paradigm with Japanese quail maternal call ...	27
V. HYPOTHESES AND PREDICTIONS	29
VI. GENERAL METHODS.....	32
Subjects	32
Apparatus	32
Contingency Training	33
Testing.....	35
Data Analyses	36
VII. EXPERIMENT 1: EQUATING VISUAL AND AUDITORY STIMULI	39
Method	39
Results and Discussion	40
VIII. EXPERIMENT 2: SETTING UP CONTROLS	44
Method	44
Results and Discussion	45

IX. EXPERIMENT 3: ALTERING PRENATAL VISUAL STIMULATION	52
Method	52
Results and Discussion	53
X. EXPERIMENT 4: ALTERING PRENATAL AUDITORY STIMULATION	62
Method	62
Results and Discussion	63
XI. EXPERIMENT 4: ALTERING TIMING OF AUDITORY STIMULATION	72
Method	72
Results and Discussion	73
XII. GENERAL DISCUSSION.....	81
Limitations and Future Directions	86
Summary	88
REFERENCES	89
VITA.....	95

LIST OF TABLES

TABLE	PAGE
1. Mean and Standard Deviations of Behavioral Measures of Distance Traveled, Velocity, and Vocalizations	41
2. Mean Proportion of Total Duration (PTD) across the Trained and Untrained Control Conditions.....	47
3. Mean Proportion of Total Entries (PTE) across the Trained and Untrained Control Conditions.....	48
4. Mean Proportion of Total Latency (PTL) across the Trained and Untrained Control Conditions.....	49
5. Mean Proportion of Total Duration (PTD) scores across the Trained Controls and the Visual Conditions.....	55
6. Mean Proportion of Total Entries (PTE) across the Trained Control and Visual Conditions.....	56
7. Mean Proportion of Total Latency (PTL) across the Trained Control and Visual Conditions.....	57
8. Mean Proportion of Total Duration (PTD) scores across the Trained Controls and the Auditory Conditions.....	65
9. Mean Proportion of Total Entries (PTE) across the Trained Control and Auditory Conditions.....	66
10. Mean Proportion of Total Latency (PTL) across the Trained Control and Auditory Conditions.....	67
11. Mean Proportion of Total Duration (PTD) for the Trained Control, Short-Early, Short-Duration Auditory and Long-Duration Auditory Conditions	75
12. Mean Proportion of Total Entries (PTE) for the Trained Control, Short-Early, Short-Duration Auditory and Long-Duration Auditory Conditions	76
13. Proportion of Total Latency (PTL) scores for the Trained Control, Short-Early, Short-Duration Auditory and Long-Duration Auditory Conditions.....	77

LIST OF FIGURES

FIGURE	PAGE
1. A schematic diagram of the sequence in which sensory systems become functional in bobwhite quail embryos	9
2. Mean distance traveled for the Control, Visual, Auditory 1000 Hz, and the Auditory 250 Hz Conditions	41
3. Mean velocity for the Control, Visual, Auditory 1000 Hz, and the Auditory 250 Hz Conditions	42
4. Mean vocalizations for the Control, Visual, Auditory 1000 Hz, and the Auditory 250 Hz Conditions	42
5. Mean Vocalizations for Trained Controls During Training and Testing.....	46
6. Mean Proportion Scores of Duration for the Control Conditions.....	50
7. Mean Proportion Scores of Total Entries for the Control Conditions	51
8. Mean Vocalizations for Trained Controls, Short-Duration Visual, and Long-Duration Visual Conditions during Training and Testing.....	54
9. Mean Proportion Scores of Duration for the Trained Control and Visual Conditions ..	58
10. Mean Preference Scores of Total Entries for the Trained Control and Visual Conditions	59
11. Mean Vocalizations for Trained Controls, Short-Duration Auditory, and Long-Duration Auditory Conditions during Training and Testing.....	64
12. Mean Proportion Scores of Total Duration for the Trained Control and Auditory Conditions	69
13. Mean Proportion Scores of Total Entries for the Trained Control and Auditory Conditions	69
14. Mean Vocalizations in Trained Controls, Short-Duration Auditory, Short-Early Auditory and Long-Duration Auditory Conditions	74
15. Mean Proportion Scores of Total Duration for the Trained Control, Short-Duration Auditory, Short-Early Auditory and Long-Duration Auditory Conditions.....	79

16. Mean Proportion Scores of Total Entries for the Trained Control, Short-Duration
Auditory, Short-Early Auditory and Long-Duration Auditory Conditions.....79

CHAPTER I

INTRODUCTION

Sensory stimulation during prenatal as well as early postnatal periods plays a critical role in neurobehavioral, perceptual, and attentional development (Lickliter, 2000). Preterm human infants that are born weeks or even months before term experience a drastically different sensory environment during early development when compared to full-term infants. Infants born before 37 gestational weeks, as well as infants born with a birth-weight of less than 2500 grams, are designated as preterm. Preterm infants that are born close to 37 weeks in most cases do not need special care and can be taken home and cared for by parents. However the majority of preterm infants born earlier than 37 weeks require medical care only available in the Neonatal Intensive Care Unit (NICU). In this environment, preterm infants are routinely exposed to atypical amounts, types, and timing of tactile, vestibular, auditory and visual stimulation as a result of their early expulsion from the uterine environment. Even though the NICU provides a range of medical technology to support the survival of the preterm infant (Bennett, 2002), the sensory environment of the NICU (with its elevated light and noise levels) has been an issue of concern in regards to later learning and developmental outcomes (Als, 2004).

Recent reports indicate that the prevalence of preterm births has increased to 12.7% of the 4.3 million live births each year in the United States (Heron, Sutton, Xu, Ventura, Strobino, & Guyer, 2010). This means that more than 10% of infants that are born in the United States will likely spend time in the NICU. Researchers have questioned how the altered sensory environment of the NICU could effect the neural and

behavioral organization of the preterm infant and their subsequent perceptual, cognitive, and behavioral development in the months and years beyond the NICU (e.g., Als, 2013; Rand & Lahav, 2014; Wachman & Lahav, 2010; VandenBerg, 2007). While it is important to investigate these questions, we are severely limited in carrying out manipulations of sensory experience with human fetuses or newborns. Animal based research has proven particularly useful in carrying out experiments in which the timing, amount, and type of sensory experience available during early development is modified or manipulated. In particular, research with animal subjects has helped to extend our understanding of sensory organization and perceptual development during the prenatal period (Lickliter, 2011).

CHAPTER II

LITERATURE REVIEW

Sensory Stimulation in the Uterine Environment

The uterine environment in which a fetus develops is a highly buffered yet rich and complex environment. The amniotic fluid, embryonic membranes, uterus, and maternal abdomen form a barrier of concentric layers and protect the fetus from high levels of sensory stimulation or other disturbances in the external environment (Vandenberg, 2007). In the uterine environment, a fetus receives a variety of tactile, vestibular, chemical, and auditory sensory stimulation. For example, as the mother walks, the sounds of her footsteps correspond with the changes in pressure and tactile feedback experienced by the fetus (Lickliter, 2011). Moreover a fetus exposed to specific speech sounds becomes familiar with the recurrent maternal speech sounds, which has been demonstrated by decreased fetal heart rate to familiar speech (DeCasper, Lecanuet, Granier-Deferre, & Maugeais, 1994), as well as newborns' non-nutritive sucking, (DeCasper & Fifer, 1980), and orientation responses towards the sound source (Clifton, Morrongiello, Kulig, & Dowd, 1981). Further, Moon, Lagercrantz, & Kuhl (2013) demonstrated that newborn infants can show preference for the language they heard in utero (native vs foreign), in addition to recognizing the voice of the speaker (mother's voice vs another female's voice).

Similarly the mother's breathing, her heart-beat, the borborygmi from digestion, and her vocalizations provide auditory stimulation that correspond with tactile stimulation of varying intensities along with specific temporal patterns (Lickliter, 2011). Moreover, fetuses receive tactile stimulation along with proprioceptive feedback through

their spontaneous limb and body movements. A fetus is also exposed to dark-light cycles and circadian rhythms from the mother's sleep-wake cycles (Fifer & Moon, 1988). The uterine environment also provides an ongoing supply of nutrients and provides relatively consistent temperature regulation for the developing fetus.

Premature Birth and Changes in Sensory Organization

Premature birth removes the fetus from the warm, fluid filled and buffered womb to a NICU environment where the newborn is exposed to a range of variable and non-contingent stimuli (Vandenberg, 2007). The stark contrast between the fetus's uterine experience and the NICU environment can create both physical and psychological stress for the neonate. For example, the NICU provides significantly augmented auditory and visual stimulation at a time when the immature infant is struggling to organize its neurobehavioral state. A mismatch between environmental input and brain organization can lead to excessive sensory load and stress, leading to further alterations in neural development (Als, 1986).

Sensory stimulation can have differential effects on the organization, structure, and functional integration of neural systems that in turn can influence development and behavior in preterm infants. Factors such as timing of stimulation (i.e., when they occur in the developmental process), and the nature of stimulation, (i.e., the type and amount of stimulation) can influence early neurological organization. For example, in the NICU, preterm infants experience earlier than normal visual stimulation, varied levels and types of auditory stimulation, and decreased levels of vestibular stimulation. While minimal deviations in sensory input may not affect neural development, changes in the intensity,

timing and amount of stimulation may combine to have significant effects on perceptual, cognitive, and social development (Lickliter, 2000).

Animal based research has demonstrated links between sensory stimulation history and corresponding brain development changes. Since some neurosecretory organs (especially the hypothalamus and the pineal body) respond to light cycles either directly or indirectly, it seems plausible that light could affect metabolic and maturational processes by way of mediating hormone-release prenatally in both birds and mammals (Clendinnen & Eayrs, 1961; Gottlieb, 1971; Ray & Hochhauser, 1969). Moreover, birds that receive prenatal stimulation also show larger number of cells per unit volume of brain tissue in deep optic tectum, a region of the brain that has been implicated for multisensory function (Markham, Shimizu, & Lickliter, 2008). These findings suggest that modified sensory experience in the prenatal period can affect postnatal perceptual preferences, as well the developmental trajectory of brain growth and development.

Contingency Learning in Preterms

Research has shown that preterm infants show a decreased capacity to respond to contingent stimulation when compared to full terms (Gardner, Karmel, Bernard, Magnano, & Catherine, 1992; Gekoski et al., 1984; Haley et al., 2008). The ability to detect contingent stimulation serves as a foundation for early learning and memory (Rochat, 2001). Detecting contingencies can be considered as a primary skill on which other skills develop, including perceptual, behavioral, and cognitive skills. Tarabulsy, Tessier, and Kappas (1996) have argued that the ability to detect contingencies allows predicting events and organizing behaviors in coherent ways, both to attain desirable

outcomes and to avoid aversive consequences. Moreover learning about cause and effect and discovering that one's own actions can influence events provides an important basis for early social responsiveness (Rochat, 2001; Watson, 1979). Contingency learning has thus been considered as a building block that directly or indirectly contributes to various aspects of cognitive, social, and emotional development (Tarabulsky, Tessier, and Kappas, 1996).

A growing body of literature has demonstrated significant differences in learning abilities in preterm infants when compared to full-term infants (e.g., Gekoski et al., 1984; Haley et al., 2006; Haley et al., 2008), suggesting that exposure to an atypical sensory environment may lead to impairment in basic learning skills. For example, a study by Haley et al. (2008) using a conjugate mobile reinforcement paradigm (where the overhead mobile movement is contingent on the infant's foot kicking response) demonstrated that preterm infants differed from the full-terms. Specifically, they showed less evidence of learning, spent less time looking at the mobile, had lower cortisol levels, showed greater heart rate responses to contingency, and dampened heart rate responses to non-reinforcement.

Preterms also show a higher incidence of neurodevelopmental problems. For example, Omizzolo et al. (2014) assessed 'very preterms' at 7 years of age for global, white-matter, cortical grey-matter, deep-grey-matter, and cerebellar abnormalities. They found brain abnormality in the 'very preterms' (<32 weeks) especially with the deep grey-matter and were able to associate these changes with poorer memory and learning performance (immediate memory, working memory, and long-term memory) as compared to full-term Controls. Therefore it is not surprising that there is a higher

probability for preterms to exhibit learning disabilities, below average IQ scores, attention deficit hyperactivity disorder, decreased executive function, neuropsychological deficits, emotional problems, and regulatory disorders (Aylward, 2005; Vohr et al., 2000). Several studies have indicated that very preterm infants born with very low birth weight (<1500 gms) also show deficits in language development apart from deficits in their cognitive ability (Barre, Morgan, Doyle, & Anderson, 2011).

McCormick, Workman-Daniels, and Brooks-Gunn (1996) reported that special education services are required for more than 50% of very low birth weight preterm children by 8 years of age and 15% have repeated at least one grade in school. A ten-year longitudinal study by Barlow and Lewandowski (2000) indicated that preterm infants were at greater risk for cognitive, social, and behavioral problems when compared to full term infants. These findings are troubling and suggest the need to better understand and work toward minimizing or eliminating the risk factors associated with development in the NICU sensory environment.

Historical and Theoretical Views

There have long been opposing views on what types and amounts of sensory environment would be most optimal or appropriate for preterm infants. The prevailing view until the early 1960s was that sensory stimulation in the NICU should be minimized to mimic the attenuated environment of the fetus in utero (see Korner, 1990 for review). During the late 1970s the prevailing view of the importance of sensory deprivation was replaced by the contrasting view that behavioral development could best be promoted by providing additional sensory stimulation to the neonate (Korner, 1990). However, these

positions of sensory deprivation or sensory augmentation are not well supported by either developmental theory or available empirical research (Lickliter, 2000, 2011).

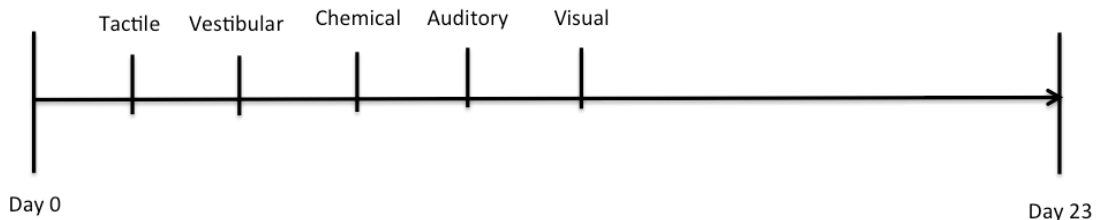
The last several decades have seen a shift in developmental thinking. Earlier, developmental changes were considered to be under the control of a genetic blueprint, where development was thought to unfold as a result of a preexisting program. Developmental research from the last few decades has challenged this theory and it has become clear that changes in ontogeny are not simply due to genes. Development is no longer considered a product of linear relationships or simple cause and effects, but rather as a dynamic, non-linear, multidetermined, and experience dependent process (Ford & Lerner, 1992; Oyama, 1985). This view suggests that ascribing developmental changes to any single factor (genetic or environmental) is a flawed view of development. Reductionistic approaches that aim to understand development by ascribing developmental changes to single or multiple yet independent sources of information downplay the complexity and richness of development and fail to explain how development actually occurs. Therefore a systems perspective is helpful in developmental investigations because it considers multiple variables and contexts that interact over time to support the emergence and maintenance of all phenotypes, including behavior.

Development of Sensory Systems

The development of sensory systems in birds and mammals, including humans, proceeds in a specific and invariant sequence during prenatal development: tactile > vestibular > chemical (taste and smell) > auditory > visual (see Figure 1)(Gottlieb, 1971). The development of sensory systems was once considered a deterministic process where

the maturation of the sensory structures led to the orderly onset of the functioning of those structures. Gottlieb (1971) argued against this view and suggested that sensory systems develop as a result of a bidirectional interactive relationship between structure and function. Gottlieb proposed that development cannot be determined solely through the activity of genes, nor can it be solely due to environmental factors. Instead, development is best viewed as an emergent process which arises from the complex coactions within and between the different levels of an organism's developmental manifold that includes gene activity, neural activity, behavior, and the physical, social and cultural environments (Gottlieb, 2007).

Figure 1. A schematic diagram of the sequence in which sensory systems become functional in bobwhite quail embryos.



Several studies have tried to focus on medical complications and neonatal risk factors such as birth weight and gestational age to better understand the links between preterm birth and developmental disorders (see Aylward, 2002). The findings from these studies have not been very helpful because preterm birth involves several factors (e.g., medical conditions and treatments, altered sensory stimulation) that are interrelated,

which make conclusions about their relative contributions to developmental outcome difficult. Further, developmental processes are polyphasic, where different structures experience different rates of change at different developmental times. This non-linear growth with periods of relatively rapid growth and relatively slow growth, lead to different structural-functional relationships, which become mature and functional at different times (Gollin, 1981; Honeycutt & Lickliter, 2002). The dynamic developmental processes and changes involved in preterm birth are therefore not easy to understand by merely assessing global scores or measures. A pragmatic way of unpacking the processes includes experimentally manipulating the system with different types and amounts of experiences at different times during development. Results from such experiments can help in understanding the relationships among relevant factors and components and the processes that make them functional. Results from these experiments can also help discover principles that govern developmental processes at different time points in the life cycle and across species (Honeycutt & Lickliter, 2002).

Importance of Using Animal Models

Animal-based studies have assessed the role of the timing, amount, and types of sensory stimulation during the perinatal period to subsequent perceptual, cognitive, and social development (Gottlieb & Lickliter, 2004; Lickliter, 2011). Animal studies have especially been useful in understanding the effects of each sensory modality on subsequent development and in understanding what is typical or atypical stimulation during the prenatal period. Experimental modifications of sensory experience are severely limited with human fetuses and neonates, and animal models have proven

particularly useful in assessing the effects of modified perinatal sensory experience on subsequent development. An important advantage of using animal models, especially birds that develop in ovo, is that they can be used to carry out traditional experimental manipulations such as sensory augmentation or sensory deprivation that are generally prohibited with human fetuses and neonates (Lickliter, 2000). Given that preterm infants routinely experience unusually early visual stimulation and increased amounts of unfiltered auditory stimulation compared to full term infants, it is somewhat surprising that few studies have focused on the intramodal (within a specific modality) or intermodal (across different modalities) effects of unusually early and augmented sensory experience (Lickliter, 2000). In order to fill this gap in our knowledge, several comparative studies have used an ‘early exposure’ paradigm to understand the consequences of prenatal sensory stimulation on subsequent perceptual and cognitive development (Lickliter, 2000, 2005).

Effects of Sensory Stimulation on Arousal

Gray (1990), who conducted his experiments with domestic chicks, inferred that responses to sensory manipulations are directly influenced by the organism’s arousal level. Gray demonstrated that high or low arousal levels are associated with poor auditory perceptual performance in domestic chicks, whereas optimal auditory performance is seen in chicks with moderate levels of arousal. Gottlieb (1991, 1993) found that tactile stimulation from broodmates played an important role in mallard duckling’s learning of auditory information (maternal call) presented immediately following hatching. Ducklings that were denied tactile stimulation (reared in isolation)

did not learn an individual maternal call, whereas ducklings reared normally with broodmates readily learned the maternal call. The arousal level of these ducklings was measured by distress vocalizations and the time they spent awake, and the ducklings that were denied tactile stimulation showed significantly higher levels of arousal when compared to Controls.

In studies with human infants, Lewkowicz and Turkewitz (1980) found that newborns prestimulated with a pulse of white noise looked more at a dim light and less at a bright light. Similarly, infants presented with auditory stimulation immediately before or concurrently along with visual stimuli, preferred visual stimulation that was lower in intensity when compared to unstimulated infants (Lewkowicz and Turkewitz, 1981). Garden and Karmel (1995) inferred that the attentional value of a stimulus is directly related to the infant's overall arousal level and the nature of the infant's sensory experience. Hence modifying either sensory experience or arousal level will influence the amount of attention an infant provides to a stimulus. Both full-term and high-risk infants prefer low levels of visual stimulation when more aroused and prefer higher levels of visual stimulation when less aroused (Gardner & Karmel, 1992). These findings demonstrate that developing perceptual capacities are tightly linked to the nature of the stimuli encountered by young organisms, as well as their physiological state and prior experience.

Effects of Sensory Stimulation on Perceptual Learning

The Effects of Altering the Amount of Premature Stimulation

Studies have been conducted with both avian and mammalian species to examine the effects of perinatal stimulation on subsequent behavioral development. For example, Sleigh and Lickliter (1997) found that presenting continuous augmented prenatal auditory stimulation to bobwhite embryos (40 minutes each hour for the last 24 hours before hatch) interfered with the emergence of species-typical intersensory perception. Whereas control chicks required visual cues (bobwhite hen model) to prefer maternal auditory cues beyond the second day following hatching, chicks that received augmented prenatal auditory stimulation continued to prefer maternal auditory cues presented without visual cues 3 and 4 days following hatching. Sleigh and Lickliter (1997) also found that unlike Controls, bobwhite embryos stimulated with augmented auditory stimulation failed to utilize visual cues and failed to discriminate the bobwhite hen over a scaled hen model in a visual discrimination test at 72 hours following hatching. Further, the auditory-stimulated chicks were found to be more aroused and exhibited higher mortality rates than chicks that were prenatally presented with either no auditory stimulation or relatively less and intermittent prenatal auditory stimulation (10 min/hr versus 40 min/hr).

Radell and Gottlieb (1992) presented augmented amounts of vestibular stimulation (intermittent high frequency waterbed stimulation for 16 cycles per minute) to mallard duck embryos and simultaneously exposed them to a specific variant of a mallard maternal call. After hatching, these hatchlings failed to show a preference for the familiar maternal call when they were tested between the familiar call and a novel maternal call. However, when the amount of the experimental vestibular stimulation was

reduced (8 cycles per minute) to approximate normal levels of prenatal vestibular stimulation and embryos were exposed to an individual maternal call, they showed a preference for the familiar maternal call following hatching. These results indicate that a substantial increase in the overall amount of sensory stimulation present in the prenatal environment can result in subsequent deficits in perceptual discrimination and/or learning.

Lickliter and Lewkowicz (1995) examined the influence of limiting the overall amounts of prenatal sensory stimulation on perceptual learning in bobwhite quail embryos. Embryos were incubated in physical isolation from broodmates (deprived of tactile and vestibular stimulation) and exposed to a bobwhite maternal call for the last 24 hours before hatching. They subsequently failed to show a preference for the familiarized maternal call, suggesting that the sensory deprivation interfered with their prenatal auditory learning. Hence, it appears that some optimal amount of prenatal sensory stimulation may be necessary for guiding normal postnatal development. While slight modifications in sensory stimulation (regardless of modality) might not influence normal development, sensory modifications exceeding the optimal window of stimulation could lead to disruptions in otherwise normal functioning.

Studies have also shown that altering the amount of prenatal stimulation in one sensory modality can influence the development of another sensory modality (Lickliter & Stoumbos, 1991; Radell & Gottlieb, 1992). For example, Lickliter and Stoumbos (1991) examined how prenatal auditory stimulation affected postnatal visual functioning in bobwhite quail hatchlings. Embryos were presented with species-typical auditory stimulation intermittently (recordings of bobwhite embryonic vocalizations) for 10

minutes each hour for the last three days before hatch. After hatching, these chicks showed species-typical auditory responsiveness at 12 and 24 hours of age. However, chicks demonstrated an accelerated visual responsiveness, in that they were able to visually discriminate and prefer a bobwhite hen model paired with the bobwhite maternal call over a scaled hen model paired with a bobwhite maternal call by 24 hours of age, whereas control chicks showed no such visual discrimination at this age. These results suggest that alterations made to one sensory modality cannot be studied in isolation. Modifying sensory experience in one modality not only influences that specific modality but also affects other developing sensory modalities as well.

The Effects of Altering the Type of Premature Stimulation

While the amount of stimulation present in the prenatal environment plays an important role in perceptual development, the type of stimulation has also been shown to be instrumental in postnatal perceptual development. Sleight, Columbus, and Lickliter (1996) showed that when bobwhite embryos heard a specific maternal call intermittently for 10 minutes/hour along with an additional 10 minutes/hour of embryonic contentment vocalizations for 24 hours prior to hatching, chicks preferred the familiar maternal call 24 hours after hatch. However, embryos who received the exact sequence and amount of stimulation but who instead heard embryonic distress vocalizations instead of contentment vocalizations did not prefer the familiar call 24 hours after hatching. Thus, the nature and context in which the stimulation occurs also constitutes a key component in the prenatal environment, further demonstrating the complexity of the prenatal sensory environment and its influence on early attention, learning, and memory.

The Effects of Altering the Timing of Premature Stimulation

The timing of experiences also influences the normal patterns of perceptual development. The fact that the different sensory systems do not become functional at the same time has shown that their developmental histories influence each other during early development. For example, Honeycutt and Lickliter (2003) provided augmented tactile and vestibular stimulation to bobwhite embryos at a time that coincided with the onset of the functioning of the auditory system, bobwhite neonates did not show auditory preference for the bobwhite maternal call, a behavior routinely seen in unmanipulated chicks. In contrast, when the exact same stimulation was given *after* the onset of the functioning of the auditory system, bobwhite neonates showed the species-typical auditory preference response for the bobwhite call at 24 hours of age. These findings illustrate the importance of the timing of sensory stimulation and how it can shape perceptual development.

The sequential development of the various sensory modalities during prenatal development leads to distinct developmental histories at the time of birth. The differential onset of sensory faculties provides structure and organization to the developmental process and has been considered as a major source of early perceptual organization (Turkewitz, & Kenny, 1982). For instance, the tactile and vestibular systems that become functional very early in prenatal development have had more experience at birth or hatching than the auditory or visual systems that became functional much later. Turkewitz and Kenny (1982) have pointed out that the differential timing that the sensory systems become functional allows for the earlier developing sensory

systems to develop without competition or interference from the later developing sensory modalities.

Several studies with both altricial and precocial animal infants have found links between early sensory stimulation and sensory augmentation on subsequent perceptual development. For example, Kenny and Turkewitz (1986) provided early visual stimulation to rat pups and studied its effects on their homing behavior. Homing behavior refers to orientation toward the nest site. Under normal conditions the homing behavior of rat pups shows a consistent developmental trajectory, in which rat pups gradually increase their homing behavior, peaking around 14-15 days post-birth, and then exhibit a gradual decline in this behavior. Thermal and olfactory cues are primarily used to direct homing behavior. Kenny and Turkewitz hypothesized that early visual stimulation (by way of early eye opening) should interfere with the normal development of homing behavior, which is typically mediated by the earlier developing olfactory and tactile systems. Results from this study showed that rat pups that received the unusually early visual stimulation did not show a decrease in homing behavior usually found in normal rat pups and the homing behavior for these atypical rat pups was mediated by visually guided behavior, rather than the typical reliance on thermal and olfactory cues.

In a similar study, Lickliter (1990a) found that bobwhite quail embryos that were presented with continuous patterned visual stimulation during the 24-36 hours before hatching required auditory (maternal call) and visual cues (stuffed bobwhite hen model) to demonstrate a preference for their species typical maternal call when tested at 24 or 48 hours post hatch. In contrast, unmanipulated control chicks were able to show preference for the maternal call at 24 and 48 hours post hatch using only auditory information. In

another study, Lickliter (1990b) found that the acceleration in intersensory functioning in chicks that had received early visual stimulation was related to accelerated visual functioning rather than delayed auditory responsiveness. Chicks who received early visual stimulation appeared more visually oriented than Controls. Moreover, while these chicks did respond to the auditory information alone following hatching, their response to the auditory information declined significantly faster than Controls. Taken together, results of these studies indicate that early visual stimulation can influence both visual as well as auditory responsiveness.

In most avian and mammalian species, including humans, the visual modality does not become functional until birth and opportunities for experiencing visual stimulation is minimal before birth. Therefore earlier developing sensory modalities progress and mature without competition from the later developing visual modality. As reviewed above, providing early visual experience can modify postnatal responsiveness of not just the visual system but also the earlier developing olfactory or auditory systems. Thus, early presentation of sensory stimulation to a later developing sensory modality may not only affect the development of that system but also interfere with the ongoing development of earlier emerging sensory modalities.

In case of preterm infants, due to their ongoing exposure to auditory and visual stimuli in the NICU, hearing and vision are functional at the same time in early development instead of becoming functional sequentially. This can have deleterious effects on sensory functioning and perception. For example, a recent study by Molloy, Wilson-Ching, Doyle, Anderson, and Anderson (2013) showed that very preterm infants demonstrated deficits in visual functioning and perception. Moreover preterm infants

may differ from full-term infants in their neural organization, it is possible that the unusually early sensory stimulation that they experience influences how they employ their attentional, perceptual, and cognitive resources, making them candidates for subsequent learning and cognitive impairment.

On the basis of the studies reviewed here, it seems that modifying the timing and amount of sensory stimulation poses potential threats to arousal regulation, as well as perceptual development. While it is clear that modified auditory or visual stimulation effects perceptual development, it is not clear if these perceptual deficits extend to cognitive skills such as contingency detection and learning. To date, no study has examined the relationship between unusually early or augmented prenatal sensory stimulation and subsequent contingency detection and learning.

CHAPTER III

SCOPE AND DESIGN OF THE STUDY

From the literature reviewed above we know that preterm infants can exhibit impairments in contingency learning. Further, work with the bobwhite quail animal model has demonstrated that preterm sensory stimulation can affect perceptual development and learning. If altered prenatal sensory stimulation creates deficits in perceptual learning, are these learning deficits also extended to impairments in contingency learning? Specifically, can altered visual and auditory sensory stimulation during the late prenatal period affect contingency detection and learning during early postnatal development?

These questions were explored in the current study. The goal of the current project was to systematically alter prenatal auditory and visual stimulation and examine the links between this atypical sensory stimulation and subsequent contingency learning. The bobwhite quail is a useful animal model to pursue these questions because it 1) develops in ovo, making prenatal manipulations possible, 2) is a precocial species, which means it can be tested immediately after hatching, and 3) are known to be sensitive to contingencies (Harshaw & Lickliter, 2007; Harshaw, Tourgeman, & Lickliter, 2008).

The goal of the current study was to assess how altered prenatal auditory and visual sensory stimulation influences postnatal learning abilities. Prior to providing altered visual and auditory stimulation, the visual and auditory stimuli to be used were assessed to ensure they were relatively equivalent in their effects on quail hatchlings. Therefore a preliminary experiment (Experiment 1) was conducted exposing young

hatchlings to light and sound to determine if each had an equivalent effect on the hatchling's behavior.

The main experiments in the study included exploring the effects of augmented prenatal visual stimulation and exploring the effects of augmented prenatal auditory stimulation on contingency detection and learning. The goal of Experiment 2 was to determine a baseline for subsequent experiments. Two control conditions were established. The first condition was comprised of chicks that received no altered prenatal stimulation and were not contingently trained following hatching. The second condition was comprised of chicks that received no prenatal stimulation but were trained contingently following hatching. The goal of Experiment 3 was to assess the impact of prenatal visual stimulation on postnatal contingency learning. The experimental conditions differed in when and how much visual stimulation was experimentally provided. One condition received visual stimulation for 3 days prior to hatch, whereas a second experimental condition received visual stimulation for 6 days prior to hatch. Findings of Experiment 3 led to Experiment 4.

Experiment 4 was designed to assess the impact of prenatal auditory stimulation on postnatal contingency learning. Bobwhite embryos were provided with auditory stimulation prior to hatching. Embryos received auditory stimulation for either 3 days or 6 days before hatching. They thus differed in the amount of stimulation they received and when they received it.

Experiment 5 was carried out to explore whether the total amount of augmented prenatal auditory stimulation influenced the disruptions seen in contingency learning in Experiment 4 or if it was the timing at which the stimulation was provided that was

instrumental in the observed disruptions. Bobwhite embryos therefore received the augmented auditory stimulation starting at 6 days before hatching but for a duration of 3 days, thus evaluating the timing by controlling for duration.

CHAPTER IV

PRELIMINARY EXPERIMENTS

Designing tasks that evaluate contingency detection and learning in animals is challenging. Learning and memory assessments in animals are not direct. Most studies use some form of an operant paradigm, in which an animal exhibits some response (pecking, pushing a pedal, vocalizing) and reinforcement to the response (e.g., food) is usually able to increase the frequency of the response (Pearce, 2013). The first step is to determine if the stimuli presented contingent on the desired response is acting as a reinforcer. In other words, a stimuli can be considered a reinforcer only if its presentation continues to increase the frequency of the desired response. Therefore it is important to ensure that the contingent stimulus is actually a reinforcer. Once that is established, the next step is to measure the frequency of response and measure the time taken by the animal to discriminate the contingent relationship. Thereafter the same situation is presented to the animal after a delay and memory for that event is assessed by observing the time taken to discriminate the contingent relationship along with the frequency of response.

Learning is usually measured by comparing the responses during the different phases within the training session. These phases include a baseline where the frequency of the desired response is observed when the reinforcing stimulus is not present, the training phase where the frequency of the desired response is observed when the reinforcer is present, and the final phase of extinction where frequency of the desired response is observed when no reinforcer is present. An increase in frequency of the response and a shorter latency indicate that there is memory for the contingently learned

response. In the current study several different contingency testing paradigms were assessed in an attempt to find an appropriate test that could meaningfully measure contingency learning in bobwhite quail hatchlings. A review of my preliminary work is described below.

Touch-Screen Paradigm 1:

The operant paradigm consisted of a touch screen computer monitor enclosed in a chamber that was 60.96 cm X 76.2 cm. In front of the touch screen monitor there was a rectangular box with three walls. The fourth side of the rectangular enclosure did not have a wall so that it fit snugly with the touch screen. Thus the touch screen monitor acted as the fourth wall to the rectangular enclosure. This set up allowed a chick to move in the rectangular space and peck on the touch screen monitor. A heat lamp was hung from the ceiling of the chamber over the rectangular area. The chamber was externally cooled before the session started using a fan that blew air into the chamber through a box filled with ice. The temperature of the chamber was brought between 23-25 degree Celsius before each testing session.

The session started with a white rectangular box that appeared on the touch screen against a dark red background. As soon as the chick pecked the white rectangle, the heat lamp would turn itself on for 0.3 seconds and provide heat to the heat challenged chick. The session was 7 minutes long with 5 minutes of a reinforcement period, during which a peck resulted in the turning on of the heat lamp, and the last 2 minutes was a non-reinforcement phase where a peck did not light the heat lamp. An increased frequency in the non-reinforcement period was thought to be a violation of the expectation, indicating

that the chick had learned the expected contingency. A baseline phase of no reinforcement was also introduced initially to check for the pecking response when no reinforcement was present. However, if chicks were not reinforced during the initial pecks, it would not continue to peck on the screen even when the reinforcement phase had begun. Since the majority of the chicks showed a similar behavior, the baseline phase was dropped. The same procedure was repeated the following day and the latency to recognize the contingent relationship along with the frequency of pecking responses in both reinforcement and non-reinforcement phases was measured.

Data were also collected using this paradigm for control chicks. However there was a confound that came into play that made the use of this paradigm less than ideal. As pecking increased, the temperature in the small chamber would increase and it was not clear if the lower frequency of pecking response that was seen towards the end of a trial was due to satiation from the pecking or due to the increase in ambient temperature.

Touch-Screen Paradigm 2:

The next operant paradigm also used retained the touch-screen monitor along with the rectangular enclosure and chamber. However the reinforcement of the heat lamp was replaced by a visual image of a chick accompanied by a burst of a bobwhite maternal call. Each time the chick pecked the white rectangle on the screen it saw a picture of bobwhite chick along with the maternal vocalization. Similar to the earlier paradigm, there was a reinforcement phase and a non-reinforcement phase and chicks were presented with this paradigm at 24 hours of age and then again at 48 hours of age. A visual condition received the augmented prenatal visual stimulation for 3 days before

hatching and the auditory condition received augmented prenatal auditory stimulation for 3 days before hatching. However after the data for the experimental auditory and visual conditions were collected, it was found that all the conditions performed similarly. An additional experimental condition was run in which chicks received auditory stimulation for a week before hatch. The hatch rate for this condition was very low. More than 50% of the chicks did not hatch, most likely due to prolonged augmented visual stimulation. However, the ones that did survive performed similar to the other conditions above. This led to the conclusion that there was a ceiling effect across all the conditions, and that the testing paradigm was not adequately challenging to the young chicks.

Touch-Screen Paradigm 3:

Prior testing made it clear that the contingency test should be a task that the chicks are capable of performing and at the same time it should be challenging enough to identify any differences in learning that may be present due to the experimental procedures. The touch-screen paradigm was thus modified to make the task more difficult. Two rectangles were presented and only one would provide reinforcement. When chicks were tested using this paradigm, it appeared that chicks were not able to learn that they had to be on one side of the touch screen to receive reinforcement. Chicks pecked on both rectangles throughout the trial. Control chicks performed poorly on this test as well, indicating that the test was likewise overly challenging for them.

Vocalization Contingency Paradigm with bobwhite maternal call:

A contingency learning paradigm that had been previously successful with bobwhite hatchlings (Harshaw & Lickliter, 2007) was used in which a day-old chick was placed in a circular arena and its vocalizations were reinforced with a specific variant of the bobwhite maternal call. On the following day, the chicks were tested for their auditory preference between the familiarized bobwhite maternal call and a novel bobwhite maternal call. In this paradigm, chicks from the experimental auditory and visual conditions performed similarly to the Controls. Thus, this test also seemed less than ideal to assess the effects of prenatal sensory stimulation manipulations.

Vocalization Contingency Paradigm with Japanese quail maternal call:

Harshaw, Tourgeman, and Lickliter (2008) developed a vocalization contingency paradigm in which a Japanese quail call was presented contingent on a chick's vocalization. Results from this study suggested that chicks that were trained with this paradigm showed a shift in their preference for the bobwhite maternal call after the contingency training. However the training did not influence the chicks to prefer the Japanese quail. When naïve chicks that were not trained with the Japanese quail were presented with a simultaneous choice test between a bobwhite quail call and a Japanese quail call, they preferred the bobwhite call as expected. However, chicks that received the contingency training with Japanese quail call did not prefer the bobwhite call, showing a shift in preference for their own species' maternal call.

This paradigm was deemed appropriate for the contingency test because of the following reasons: 1) there was a clear difference between the naïve Controls that had not

been trained and the unstimulated chicks that were trained. This difference clearly showed that the contingency training paradigm had an effect and chicks learned and remembered the maternal call from a different species, 2) since the test was to learn and remember a different species call, it was likely that we would not have a ceiling effect in terms of preference. This made the test challenging enough to assess potential nuances in contingency detection and learning.

CHAPTER V

HYPOTHESES AND PREDICTIONS

Previous studies have shown that augmented visual and auditory stimulation presented prenatally can disrupt species-typical perceptual functioning (Lickliter & Stoumbus, 1991; Sleigh & Lickliter, 1997; Turkewitz & Kenny, 1982). No studies have assessed the effects of augmented prenatal stimulation on postnatal contingency detection and learning. Therefore it was difficult to hypothesize expected results based on previous studies.

A preliminary study was carried out, which showed that chicks' contingency learning was not disrupted when altered visual or auditory stimulation was provided for only 2 days prior to hatching. Based on these preliminary findings, I hypothesized that:

1. Bobwhite embryos that do not receive altered prenatal sensory stimulation and who are not contingently trained following hatching with a Japanese quail maternal call will prefer the species-specific bobwhite maternal call over the Japanese maternal call during testing.
2. Bobwhite embryos that do not receive altered prenatal sensory stimulation but who are contingently trained following hatching with the Japanese quail maternal call will not prefer either the bobwhite maternal or the Japanese quail call during testing, thus showing a shift in their preference for the species-typical bobwhite call as a result of the contingency training.
3. Bobwhite embryos that receive early visual stimulation for three days prior to hatching and are contingently trained following hatching with the Japanese

quail maternal call will not prefer either the bobwhite maternal or the Japanese quail call during testing, thus showing that shorter durations of prenatal visual stimulation do not disrupt contingency learning abilities.

4. Bobwhite embryos that receive early visual stimulation for six days prior to hatching and are contingently trained following hatching with the Japanese quail call will prefer the bobwhite maternal call over the Japanese maternal call during testing, showing a failure to learn the contingently trained call as a result of the altered prenatal visual stimulation.
5. Bobwhite embryos that receive augmented auditory stimulation for three days prior hatching and are contingently trained following hatching with the Japanese quail call will not prefer either the bobwhite maternal or the Japanese quail call during testing, thus showing that shorter durations of altered prenatal auditory stimulation does not disrupt contingency learning abilities.
6. Bobwhite embryos that receive early auditory stimulation for six days prior to hatching and are contingently trained following hatching with the Japanese quail call will prefer the bobwhite maternal call over the Japanese maternal call during testing, showing a failure to learn the contingently trained call as a result of the altered prenatal auditory stimulation.

Below are listed the different conditions used in this study and the results that were predicted. All testing was done at 48 hours following hatching to assess chicks' preference between a non-conspecific (Japanese quail) maternal call, which they were

familiarized with during the contingency training, versus a maternal call of their own species (bobwhite) for which no familiarization or training was provided.

Experiment 2 (Controls)

Control Condition	Prediction
Not stimulated, and not trained	Preference for Bobwhite Call
Not stimulated, and trained	No Preference for either BW Call or Japanese Quail Call

Experiment 3 (Augmented Visual Stimulation)

Visual Condition	Prediction
Stimulated for 3 days, and trained	No Preference for either BW Call or Japanese Quail Call
Stimulated for 6 days, and trained	Preference for Bobwhite Call

Experiment 4 (Augmented Auditory Stimulation)

Auditory Condition	Prediction
Stimulated for 3 days, and trained	No Preference for either BW Call or Japanese Quail Call
Stimulated for 6 days, and trained	Preference for Bobwhite Call

CHAPTER VI

GENERAL METHODS

The experimental design had several features that were common across most of the experiments. Below is a description of the shared features of the experimental design before going into the particular details of each experiment.

Subjects

Subjects were 428 incubator-reared bobwhite quail (*Colinus virginianus*) embryos. Fertile unincubated eggs were received weekly from a commercial supplier and incubated in an incubator maintained at 37.5 °C and 65-70% relative humidity. An embryo's age was calculated such that the first day of incubation was Day 0, and so forth. Two days before hatching, the embryos were transferred to a different hatcher that did not have a turning mechanism, as the embryos no longer needed egg turning. On Day 21 of the incubation period the eggs were transferred to a hatching tray in a separate hatcher. The possible influence of between-batch variation was minimized by drawing subjects from two or more batches (i.e., weeks) of eggs. Chicks from the experimental conditions were moved to portable hatchers, where they continued to receive sensory stimulation until they hatched. Following hatching, 12-15 chicks were reared together in standard plastic tubs in a rearing room where the ambient air temperature was maintained at approximately 35° C. Chicks had constant access to food and water, except during training and testing sessions.

Apparatus

The training and testing were carried out in a large circular arena measuring 130 cm in diameter and a wall that was 60 cm in height surrounded it. The floor of the arena surface was painted white, and a white curtain covered the walls of the arena. A video camera was mounted directly above the arena and it was used to observe the chicks and for logging the training and testing data. Data were collected remotely from a computer. The Noldus Ethovision XT tracking software was used to measure each chick's movements within the arena. Chicks' vocalizations were manually recorded using a program written in Visual Basic. The speakers broadcasting the calls were located behind the arena wall and were hidden behind the white curtain.

Contingency Training

The contingency training paradigm used in the current study was based on the paradigm developed by Harshaw and Lickliter (2007). Harshaw and Lickliter (2007) found that one-day-old bobwhite hatchlings can learn contingencies and show a preference for contingently learned auditory information the following day. In their contingency training paradigm chicks heard a specific variant of the bobwhite maternal call in response to each of their own vocalization. What is noteworthy is that these chicks were presented with the contingency training for only 5 minutes and only heard approximately 30 maternal vocalizations. In contrast, previous studies have shown that chicks require at least 24 hours of intermittent non-contingent passive exposure to a specific variant of the maternal call (10 min/hour = 240 minutes of exposure and

approximately 3600 vocalizations) to show a significant preference for the familiarized call in subsequent testing.

In a follow-up study, Harshaw, Tourgmann, and Lickliter (2008) demonstrated that bobwhite chicks that typically show a strong species-typical preference for the bobwhite maternal call over maternal calls of other quail species (Banker & Lickliter, 1993; Lickliter & Virkar, 1989) no longer showed a preference for the bobwhite maternal call when they were contingently trained on a FR1 schedule with a Japanese quail (JQ) maternal call for just 5 minutes at 24 hours of age. Further, when the contingency training with the JQ call was carried out with a VR2 schedule (where chicks vocalize between one and three times to hear the JQ maternal call), chicks significantly preferred the Japanese quail call over the bobwhite call. These results with bobwhite hatchlings indicate their sensitivity to detecting contingencies and the subsequent alteration of their species-typical perceptual preference and suggested the robustness of this paradigm to test contingency detection abilities. Therefore this mode of training was used in the current study to assess chick's contingency detection and learning abilities.

In the current study, the contingency training procedure took place 24 hours after hatching. The training involved exposure to an unfamiliar non-conspecific maternal call (Japanese quail). Each chick was presented with a JQ call contingent on its own vocalization (FR1 schedule) for 5 minutes. The vocalizations that were reinforced did not include the distress call that quail chicks make occasionally. Chick distress calls, which generally consist of a string of rapid "peeps" can be easily differentiated from chicks' usual vocalizations. Once a chick was placed in the arena it either vocalized on its own or it did not vocalize. If the chick vocalized on its own, it would hear the Japanese

quail call contingent on its vocalization. If the chick did not vocalize on its own, the Japanese quail call was played non-contingently for a maximum of 5 times within a 30 second period to entice the chick to vocalize. If a chick did not vocalize within 150 seconds, it was considered a non-responder and was excluded from the study. A count was maintained for all chick vocalizations during the training session. There was an occasional example observed in some chicks in which they began vocalizing before the end of the stimulation (JQ call). Instances in which the chick vocalized before the auditory stimulation ended were not reinforced. This prevented a precise tally of vocalizations; hence the count of vocalizations that were recorded should be considered an estimate.

Testing

Testing took place 24 hours after training. All chicks from the different control and experimental conditions were tested individually for 5 minutes. Testing involved a simultaneous choice test of preference. A chick could choose between the familiarized JQ maternal call or a bobwhite maternal call by spending time in the approach area close to either of the maternal calls. The approach areas constituted 5% of the total area of the arena. The position of the two maternal calls was counterbalanced across testing sessions to avoid any side bias. The volumes of the calls were calibrated to play at 65db. Both maternal calls were played simultaneously and preference for the familiarized call was determined by recording how much time the chick spent in each of these approach areas during the testing trial. The number of times a chick vocalized during the testing session was also recorded.

Data Analysis

Chicks' vocalizations during training and testing were compared using a paired-samples *t*-test. This test determined if the number of vocalizations during training, when the contingent stimulus was present, exceeded the number of vocalizations during testing when the contingent stimulus was absent. This would demonstrate that the contingency was indeed operating during the training session.

Naïve bobwhite quail show a strong preference for their species-typical maternal call. Therefore the testing paradigm involved evaluating if the chicks' would utilize the contingency training and demonstrate a shift in their strong species-typical preference or if the chicks would continue to maintain their species-typical bobwhite preference. The dependent variable of interest was the measure of preference for the novel bobwhite maternal call during the testing session. The measures of preference used were 1) the total duration of time spent within the trained versus the novel approach area, 2) the latency of the chicks to enter the approach area with the trained maternal call versus the approach area with the novel call, and 3) the total number of entries into the familiar approach area versus the novel approach area. These measures of preference were converted into proportions to evaluate the relative preference of the chick for the trained approach area over the other.

The duration scores were converted into proportion of total duration (PTD) by dividing the duration of time spent in the area with the novel bobwhite maternal call by the total duration spent in the area with the trained maternal call and the area with the novel bobwhite maternal call. The number of entries into the area with the trained Japanese maternal call was converted into proportion of total entries (PTE) by dividing

the number of times the chick entered into the area with the novel bobwhite maternal call by the total number of the chick's entries into the area with the trained Japanese maternal call and into the area with the novel Bobwhite maternal call. The latency scores were converted into proportion of total latency (PTL) by dividing the latency of the chick's entering the area with the novel bobwhite maternal call with the total latency of the chick's entering the area with the trained Japanese maternal call and into the area with the novel bobwhite maternal call.

Within-condition preferences were calculated by evaluating the PTD, PTL, and PTE scores using the one-sample *t*-test. The .5 mark would suggest that the chicks had no preference for either the Japanese quail call or the bobwhite quail call. If the PTD scores for a condition were significantly lower than .05, it would mean that the chicks preferred the bobwhite quail call, whereas if the PTD scores for a condition would be significantly more than .05, it would mean that the chicks in that condition preferred the Japanese quail call. The effect size or the strength of preference for all the conditions was calculated using Cohen's *d*. An effect size of .2 was considered small, an effect size of .5 was considered medium, and an effect size of .8 was considered large (Cohen, 2013).

Between-condition preferences were calculated using an independent samples *t*-test in experiments where only two groups were present. In experiments where there were more than two groups, a linear contrast was performed to evaluate if a trend existed among the different groups (Rosenthal, Rosnow, & Rubin, 2000). A significance level of $p < .05$ was used in all analyses.

The vocalizations during the contingency training were also compared with the vocalizations during testing, which was a non-contingent paradigm, to assess the effect of

contingency. The vocalizations recorded during training and testing were also compared between the control condition and each of the experimentally manipulated conditions to determine if there was any difference in the way the chicks responded during the contingency training trials.

CHAPTER VII

EXPERIMENT 1: EQUATING VISUAL AND AUDITORY STIMULI

The goal of this initial experiment was to ensure that the light and sound employed in the prenatal augmentation procedures were relatively matched in intensity, as assessed by the behavioral responsiveness of chicks in the presence of the stimuli following hatching.

Method

Subjects were 78 bobwhite quail chicks that were divided into four conditions. Embryos were presented with either augmented prenatal visual or auditory stimuli for three days prior to hatching. Specifically, embryos were assigned to one of the following conditions: 1) Visual condition, 2) Auditory condition I (1000 Hz), and 3) Auditory condition II (250 Hz), and 4) a Control condition. Embryos assigned to the control condition did not receive any modified prenatal stimulation prior to hatching. Two auditory conditions were compared to determine which tone had a similar effect on chick's behavior as the visual stimuli. After hatching chicks were presented with a behavioral test in which the following responses were measured: 1) the mean distance travelled during the testing trial, 2) the mean velocity of movement, and 3) the mean number of vocalizations. The responses of the chicks assessed if the different types of prenatal sensory stimulation produced similar behavioral responsiveness.

Each trial consisted of placing a chick in an enclosure that was 2.32cm x 2.32cm x 2.32cm. A video camera that was mounted on the ceiling recorded the chick's behavior during the trials. The behavioral measures of mean distance travelled and the

mean velocity of movement were calculated using Noldus Ethovision XT tracking software. The mean vocalizations were scored manually using a program written in Visual Basic. All trials were 2 minutes long and took place when the chicks were 24 hours of age.

Results and Discussion

A one-way ANOVA compared the mean distance traveled, the mean velocity of movement, and the mean vocalizations for the visual, auditory, and the control conditions. Results revealed a significant difference in the distance traveled [$F(3,74) = 3.92, p < .05$] and the velocity [$F(3,74) = 3.90, p < .05$] between the different conditions but not for vocalizations [$F(3,74) = .82, p = .48$]. A post-hoc Tukey HSD performed on the 'distance traveled' measure indicated that Controls did not differ from the Visual condition ($p = .22$). However the Controls differed significantly from the Auditory 1000 Hz condition ($p < .05$) and from the Auditory 250 Hz condition ($p < .05$). Moreover the Visual condition did not differ significantly from the Auditory 1000 Hz condition ($p = .71$) or the Auditory 250 Hz condition ($p = .82$). Similarly, a post-hoc Tukey HSD performed on the 'velocity' measure demonstrated that Controls did not differ from the Visual condition ($p = .21$). However the Controls differed significantly from the Auditory 1000 Hz condition ($p < .05$) and from the Auditory 250 Hz condition ($p < .05$). Moreover the Visual condition did not differ significantly from the Auditory 1000 Hz condition ($p = .72$) or the Auditory 250 Hz condition ($p = .86$). Table 1 lists the mean scores for the behavioral measures of distance traveled, velocity, and vocalizations.

Table 1. Mean and Standard Deviations of Behavioral Measures of Distance Traveled, Velocity, and Vocalizations

Condition	Distance Traveled		Velocity		Vocalizations	
	Mean	SD	Mean	SD	Mean	SD
Controls	281.84	200.85	2.35	1.68	14.25	10.74
Visual	451.04	247.133	3.82	2.11	13.66	8.96
Auditory 1 k Hz	543.64	308.43	4.60	2.69	10.2	8.31
Auditory 250 Hz	526.48	305.66	4.40	2.55	13.45	7.49

Figure 2. Mean distance traveled for the Control, Visual, Auditory 1000 Hz, and the Auditory 250 Hz Conditions.

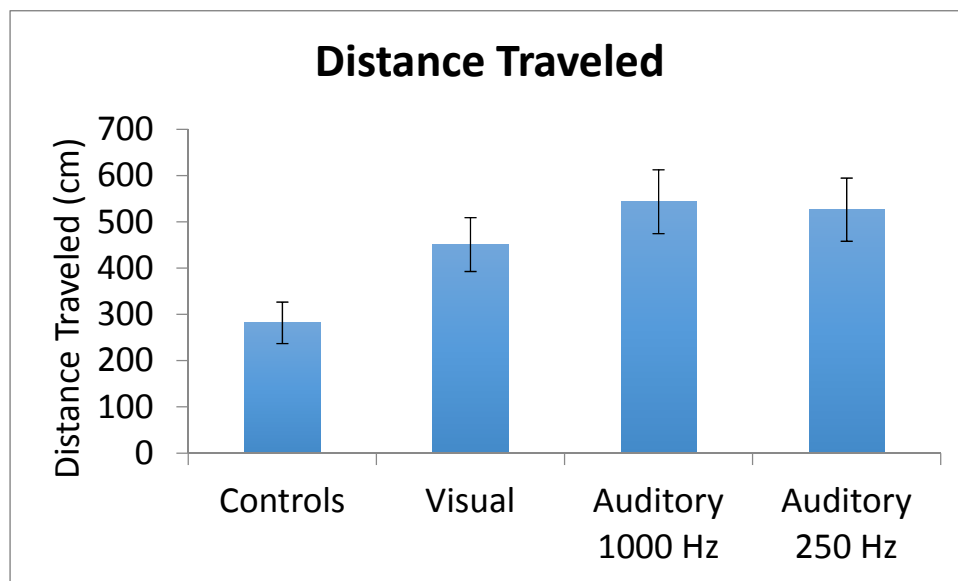


Figure 3. Mean velocity for the Control, Visual, Auditory 1000 Hz, and the Auditory 250 Hz Conditions.

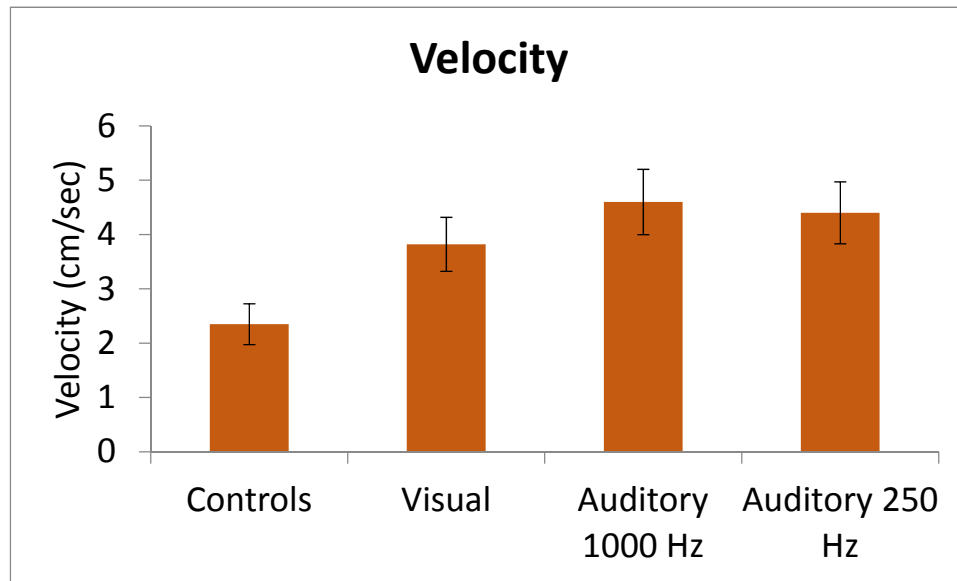
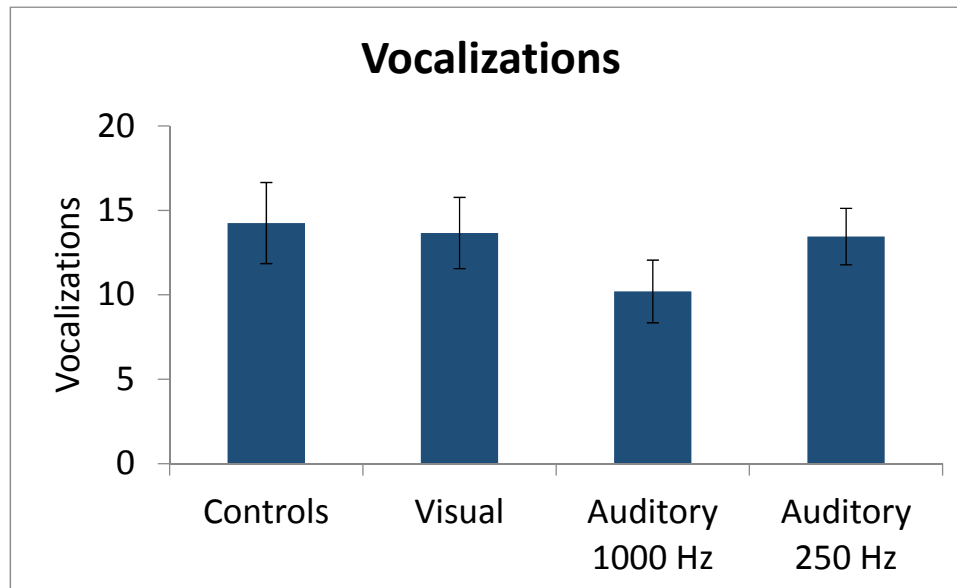


Figure 4. Mean vocalizations for the Control, Visual, Auditory 1000 Hz, and the Auditory 250 Hz Conditions.



Figures 2, 3, and 4 display the mean distance traveled, mean velocity, and mean vocalizations. The results of this experiment suggested that the controls differed significantly from both the auditory groups but it did not differ significantly from the visual group, making the results inconclusive. The 'Auditory 1000 Hz' sound stimuli was thus used in the auditory experimental conditions, which is part of the mid range band of frequencies (500 Hz to 2 kHz).

CHAPTER VIII

EXPERIMENT 2: SETTING UP CONTROLS

In order to determine whether augmented prenatal stimulation affected bobwhite hatchling's contingency detection and learning, it was necessary to first understand the normal pattern of postnatal responsiveness in naïve chicks that were not subject to altered prenatal stimulation. I therefore tested chicks that received neither altered prenatal stimulation nor the contingency training with the Japanese quail maternal call to assess their naïve auditory preference when simultaneously tested between their species-typical bobwhite maternal call and a non-conspecific Japanese quail maternal call. I called this control condition 'Untrained Controls'. Another control condition likewise did not receive altered prenatal stimulation, but did receive contingency training with the Japanese quail maternal call 24 hours following hatching. This control condition was necessary to determine if naïve chicks that did not experience altered prenatal stimulation would respond to the contingency training. I called this control condition 'Trained Controls'.

Method

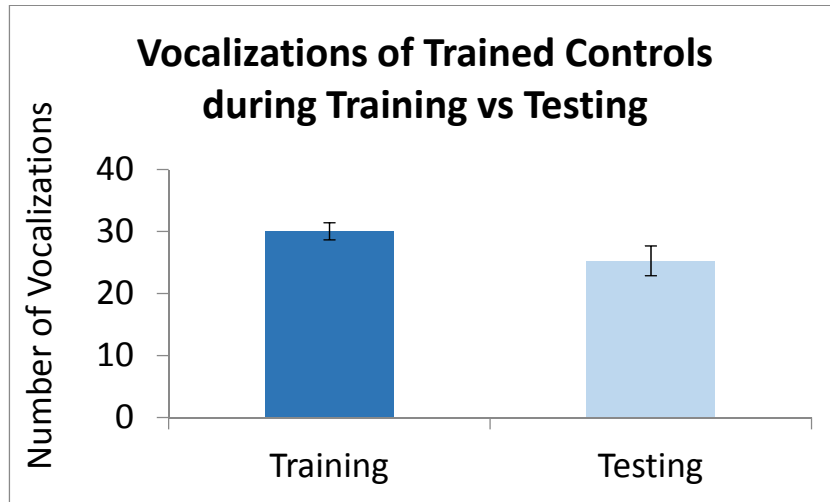
100 bobwhite quail embryos were divided into the two Control conditions. Embryos were originally incubated in a common incubator with no altered sensory stimulation. Chicks from both Control conditions did not receive any altered prenatal stimulation. However, chicks from one of the Control conditions received the contingency training. Training involved providing each chick with a Japanese quail maternal call contingent on its own vocalizations at 24 hours following hatching. The

training setup involved hearing the Japanese quail call from either speaker in the arena when the chick vocalized. Testing involved a simultaneous choice test to evaluate the preference of the chick for either the bobwhite maternal call or the familiarized Japanese quail maternal call. During testing each chick heard the bobwhite quail maternal call from one speaker and the Japanese quail maternal call from the other speaker and its auditory preference was recorded by observing how much time it spent in either of the approach areas that were broadcasting the calls.

Results and Discussion

The goal of the first analyses was to evaluate 1) if the stimulus presented during the contingency paradigm acted as a reinforcer and 2) to confirm contingency detection was operating during the training. This analysis was only conducted for the Trained Controls, since they were the control group that received the contingency training. A correlated groups *t*-test compared the mean vocalization responses during the training phase when the Japanese quail call was contingent at 24 hours of age with the mean vocalization responses during the testing phase when the Japanese quail call was not contingent at 48 hours of age. Results indicated that the test was statistically significant for Trained Controls at an alpha level of .05, $t(32) = 2.24$, $p = .03$, suggesting that the Japanese quail call elicited significantly higher number of vocalizations when the contingency was operating ($M = 30.03$; $SD = 7.95$) as compared to when the contingency was not operating ($M = 25.27$; $SD = 13.78$). Figure 5 shows the mean vocalizations in the Trained Controls condition during their training and their testing.

Figure 5. Mean Vocalizations for Trained Controls
During Training and Testing



A one-sample *t*-test was conducted on the PTD scores recorded during testing for the Control conditions to evaluate whether the mean was significantly different from .5. On a scale of 0 to 1, a score of .5 would mean that the chicks did not show preference for either the bobwhite call or the familiarized Japanese quail call. A score less than .5 would indicate that chicks preferred the bobwhite quail call and a score greater than .5 would indicate that the chicks preferred the Japanese quail call.

The naïve control condition that received no prenatal stimulation and no postnatal training showed a significant preference for their species-specific bobwhite maternal call. This finding showed that naïve chicks show a preference for the maternal call of their own species when a competing maternal call from a different quail species is present. The control condition that received no prenatal stimulation and postnatal contingency training showed a shift in this preference for its own species-typical bobwhite maternal call. This

condition showed a preference for neither the bobwhite nor the Japanese quail maternal call, indicating that the contingency training resulted in a shift in their typical auditory preference. Table 2 lists the mean proportion of time the chicks spent in the untrained species-typical bobwhite maternal call approach area.

Table 2. Mean Proportion of Total Duration (PTD) across the Trained and Untrained Control Conditions

Condition	Proportion of time spent in BW Call area	SD	<i>t</i> value (one sample t-test)	<i>p</i> value
Untrained Controls	.70	.30	$t(42) = 4.48$.00*
Trained Controls	.53	.36	$t(39) = .54$.59

A one-sample *t*-test was conducted on the proportion of total entries (PTE) scores for the Control conditions to assess how often the chicks in the various conditions entered the approach area that broadcast the species-typical (untrained) bobwhite call. The assumption was that if a chick entered the familiar area (with the trained JQ maternal call) more often than the novel area (with the untrained bobwhite maternal call), this would indicate a preference for the contingently trained maternal call. Table 3 lists the PTE scores for all conditions. Results revealed that the naïve control condition that received no stimulation prenatally and which did not receive postnatal contingency training did not prefer the area where the Japanese quail call was broadcast. Rather, this

condition preferred the species-typical bobwhite call. The control condition that did not receive any prenatal stimulation but which received training with the Japanese quail call showed no difference in their number of entrances to either approach areas (the trained Japanese quail call or bobwhite call). This finding indicated a shift from their species-typical preference for the bobwhite call, and suggested that the contingency training did influence their preference for the Japanese quail call.

Table 3. Mean Proportion of Total Entries (PTE) across the Trained and Untrained Control Conditions

Condition	Proportion of Entries to BW Call area	SD	<i>t</i> value (one sample t-test)	<i>p</i> value
Untrained Controls	.68	.26	$t(42) = 4.6$.00*
Trained Controls	.52	.31	$t(39) = .45$.65

Apart from evaluating the time spent in the species-typical untrained bobwhite area and the frequency of entries into the untrained bobwhite area, latency to enter the untrained bobwhite area was also assessed. If chicks preferred the species-typical bobwhite approach area, their latency score should be lower than 50% for that area. Table 4 lists the PTL scores for Trained and Untrained Control Conditions. Results showed that chicks from both the Control conditions looked similar in their latency to approach the area that was broadcasting the bobwhite maternal call. Moreover, the

latency of the chicks to enter the familiar area (trained Japanese quail call) was not significantly different from the latency to enter the novel area (bobwhite maternal call). The naive control condition that received no contingency training clearly had a preference for the bobwhite maternal call as seen in the PTD and PTE scores. However the PTL scores for this condition did not match these results.

Table 4. Mean Proportion of Total Latency (PTL) across the Trained and Untrained Control Conditions

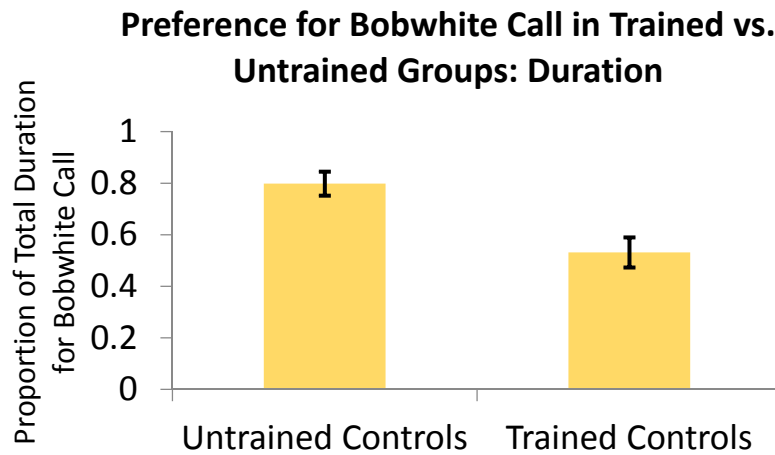
Condition	Proportion of time spent in BW Call area	SD	<i>t</i> value (one sample t-test)	<i>p</i> value
Untrained Controls	.40	.31	$t(42) = 1.98$.053
Trained Controls	.46	.36	$t(39) = .55$.58

After the within condition preference was calculated for the PTD, PTL, and PTE for all four conditions, a between-conditions comparison was carried out between conditions with the PTD and the PTE scores. The PTL scores were not used for the between condition comparison because they did not show any difference between any of the conditions in the preliminary within-condition tests.

A between-subject comparison was run between the Trained Controls and the Untrained Controls, using an independent samples *t*-test. Results for PTD scores showed a significant difference between the Untrained Controls and the Trained Controls; $t(81) =$

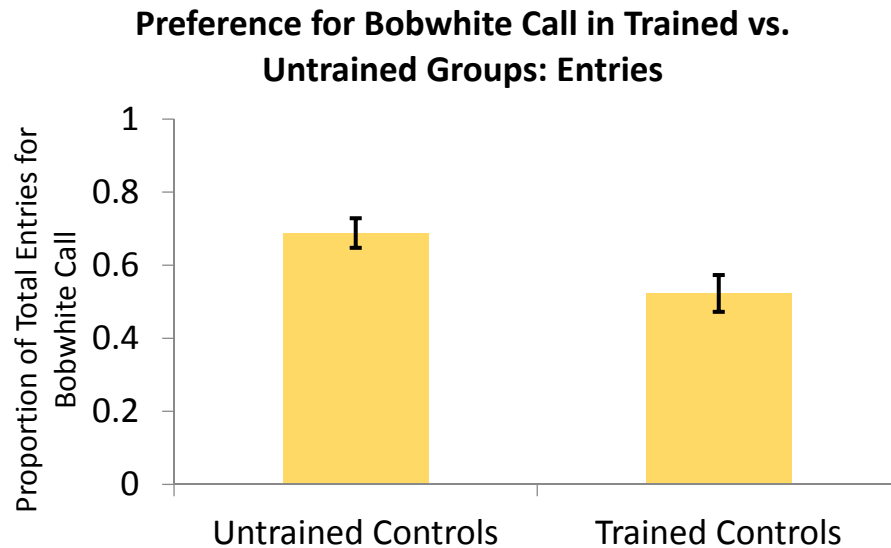
2.3, $p < .05$, $d = .51$, which was expected. Figure 6 depicts the proportion of total time the Trained and Untrained chicks spent with the untrained species-typical bobwhite call.

Figure 6. Mean Proportion Scores of Duration for the Control Conditions



Similarly the PTE scores also showed a significant difference between the Untrained Controls and the Trained Controls; $t(81) = 2.5$, $p < .05$, $d = .40$. Figure 7 depicts the number of times the Trained and Untrained chicks entered the approach area of the untrained species-typical bobwhite call.

Figure 7. Mean Proportion Scores of Total Entries for the Control Conditions



These results successfully replicated the findings of Harshaw, Tourgeman, and Lickliter (2008) where it was found that bobwhite quail chicks exposed to a Japanese quail call contingent on their own vocalization for 5 minutes no longer showed their species-typical preference for the bobwhite maternal call. Results from this experiment also provided a basis for future experiments. I hypothesized that if the augmented prenatal stimulation influenced the contingency learning abilities in chicks, then they would resemble Untrained Controls that did not receive any training. On the other hand, if the augmented prenatal stimulation did not influence the contingency learning abilities in chicks, then they would resemble Trained Controls and show a shift in their species-typical auditory preference.

CHAPTER IX

EXPERIMENT 3: ALTERING PRENATAL VISUAL STIMULATION

The goal of the current experiment was to evaluate if altered prenatal visual stimulation would interfere with the ability of quail neonates to learn contingent relationships. Specifically, this experiment examined the effects of unusually early prenatal visual stimulation on chicks' preference for a specific maternal call following contingent training with that call. Embryos were experimentally exposed to non-patterned light for three days prior to hatching (Short-Duration Visual Condition) or for six days prior to hatching (Long-Duration Visual Condition).

The Short-Duration Visual Condition, and the Long-Duration Visual Condition received contingency training with the Japanese quail call at 24 hours following hatching in the exact same procedure as the Trained Controls. All chicks were subsequently individually tested in a simultaneous choice test between the trained maternal call and a novel maternal call at 48 hours after hatching.

Method

Subjects were 100 bobwhite quail embryos that were divided into two conditions as outlined above. Embryos were originally incubated in a common incubator with no altered sensory stimulation and they were then moved to a different incubator on the day their experimental manipulation began. The Short Visual Condition received the altered visual stimulation from day 20 of the 23-day incubation period. The Long Visual Condition received the same stimulation but from day 17 of the 23-day incubation period. The Short Visual and the Long Visual Conditions were both exposed to non-patterned

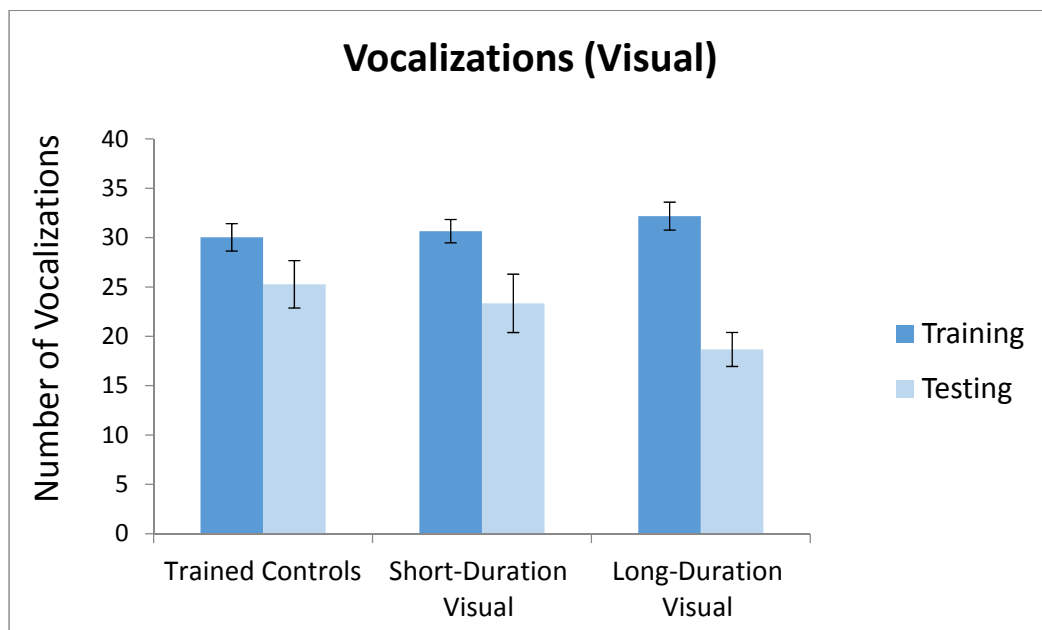
light (1600 Lumens, 60 Hz) continuously for 45 minutes every hour. This light bulb was suspended 5 inches above the embryos inside a portable incubator. Contingency training was provided to chicks on Day 1 after hatch as explained in the earlier experiment and were tested between the trained Japanese quail call and an untrained species-specific bobwhite call the next day. Chick's vocalizations during the training and testing were recorded.

Results and Discussion

The first analyses were performed to evaluate and confirm that the stimulus presented during the contingency paradigm acted as a reinforcer and that contingency detection was operating during the training. I compared the the Short-Duration Visual condition and the Long-Duration Visual condition, based on their vocalizations during the training trial. The frequency of vocal responses during training for the group of chicks (when the Japanese maternal quail call was contingent) was compared with the frequency of vocal responses during testing (when the same stimuli was non-contingent) to confirm that the contingency was operating during the training session. A 2 x 3 repeated measures ANOVA revealed a main effect of Training versus Testing, such that fewer vocalizations were found at Testing, $F(1,97) = 39.81, p < .001$ (Greenhouse-Geisser corrected). This provides support for the idea that the contingency was operating because they vocalized more when the contingency was present as opposed to when the contingency was absent during testing. Moreover, this was qualified by a significant interaction, $F(2,97) = 4.14, p < .05$ (Greenhouse-Geisser corrected). The interaction was driven by a linear decrease, across conditions, in vocalization during the Testing phase $t(97) = 2.07, p < .05$, but not

the training phase $t(97) = 1.15, p = .25$. This pattern suggested that during training chicks' performed similarly across the different conditions. However during testing the linear pattern suggested a trend where decline in vocalizations was a function of the increasing prenatal visual stimulation. Figure 8 illustrates the mean vocalizations during training and testing for the Trained Controls, Short-Duration Visual, and the Long-Duration Visual conditions.

Figure 8. Mean Vocalizations for Trained Controls, Short-Duration Visual, and Long-Duration Visual Conditions during Training and Testing



A one-sample t -test was used to analyze the Proportion of Total Duration (PTD) scores during testing for the experimental conditions to evaluate whether the mean was significantly different from 50%. Table 5 lists the PTD scores for all conditions. Results from the Short-Duration Visual and the Long-Duration Visual conditions indicated that chicks benefitted from the contingency training, demonstrating a shift in their

preference from the bobwhite quail call to no preference between the two calls, identical to the Trained Controls.

Table 5. Mean Proportion of Total Duration (PTD) scores across the Trained Controls and the Visual Conditions

Condition	Proportion of time spent in BW Call area	SD	<i>t</i> value (one sample t-test)	<i>p</i> value
Trained Controls	.53	.36	$t(39) = .54$.59
Short-Duration Visual	.55	.32	$t(42) = 1.03$.30
Long-Duration Visual	.58	.27	$t(41) = 1.90$.06

Similarly, the Proportion of Total Entries (PTE) scores were analyzed using a one-sample *t*-test for the Visual conditions to see how often the chicks entered the approach area that was broadcasting the species-typical bobwhite call over the approach area that was broadcasting the Japanese quail call. Table 6 lists the PTE scores for all conditions. The Short-Duration Visual condition and the Long-Duration Visual condition resembled the Trained Controls that received the contingency training, showing no differences in number of entrances to either call approach area. The PTE results were similar to the PTD results for all conditions, thus supporting the finding that the augmented prenatal visual stimulation, irrespective of its duration (3 days or 6 days), did

not disrupt the ability of chicks to learn and remember a familiarized call as a result of the contingency training.

Table 6. Mean Proportion of Total Entries (PTE) across the Trained Control and Visual Conditions

Condition	Proportion of Entries to BW Call area	SD	<i>t</i> value (one sample t-test)	<i>p</i> value
Trained Controls	.52	.31	$t(39) = .45$.65
Short-Duration Visual	.54	.28	$t(42) = .99$.32
Long-Duration Visual	.56	.22	$t(41) = 1.81$.07

The third measure, Proportion of Total Latency (PTL) evaluated latency to enter the species-typical untrained bobwhite approach area. If chicks showed a preference for their species-typical bobwhite (untrained) approach area, their latency was expected to be lower than 50% for that area. Table 7 lists the PTL scores for the Trained Controls and the Visual conditions. Results showed that chicks from these conditions looked similar in their latency to approach the area that played the novel (untrained) bobwhite maternal call. Specifically, the latency of the chicks to enter the novel area (bobwhite maternal call) was not different from the latency to enter the familiar area (contingently trained

Japanese quail call). Since the PTL scores did not correlate with the PTD or PTE measures, it was not used in further analyses.

Table 7. Mean Proportion of Total Latency (PTL) across the Trained Control and Visual Conditions

Condition	Proportion of time spent in BW Call area	SD	<i>t</i> value (one sample t-test)	<i>p</i> value
Trained Controls	.46	.36	$t(39) = .55$.58
Short-Duration Visual	.48	.35	$t(42) = .20$.83
Long-Duration Visual	.45	.29	$t(41) = .96$.34

The next step was to compare the PTD and the PTE scores across the Trained Controls, the Short-Duration Visual, and the Long-Duration Visual conditions. A linear contrast (using weights -1, 0, 1) for the Trained Controls, the Short-Duration Visual, and the Long-Duration Visual conditions showed no significant linear trend in the data for either PTD or PTE, $t(122) = .69, p = .495$ and $t(122) = .65, p = .515$, respectively. A bootstrap was performed (1000 iterations) to determine the standard errors. These results suggest that there is no effect of visual prenatal exposure, irrespective of the amount of stimulation presented (3 days vs 6 days), on postnatal contingency learning.

The Short Duration Visual condition performed similar to the Trained Controls that did not receive any altered prenatal stimulation. Altered visual stimulation for three days before hatching did not appear to disrupt the chicks' ability to detect contingent relationships, as the Short-Duration Visual chicks did not maintain preference for their species-typical bobwhite call after the contingency training. However, what was unexpected was that increasing the duration of the prenatal visual stimulation to 6 days also did not impair contingency learning abilities in quail hatchlings. Figure 9 shows the mean PTD for the Trained Controls and the Visual conditions and Figure 10 shows the mean PTE for the Trained Controls and the Visual conditions.

Figure 9. Mean Proportion Scores of Duration for the Trained Control and Visual Conditions

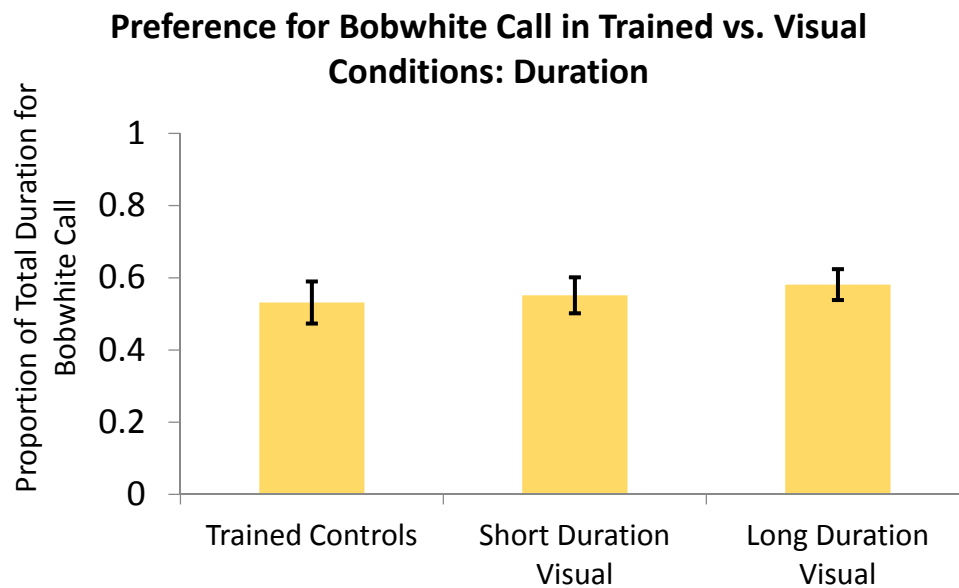
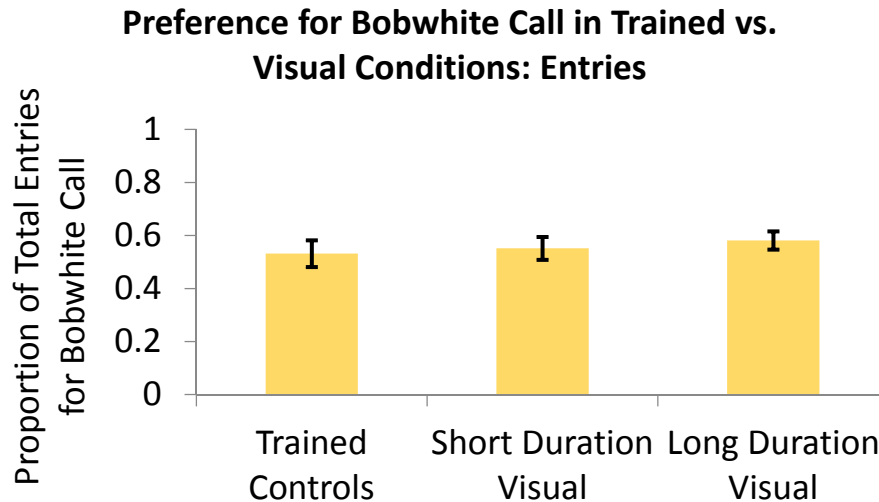


Figure 10. Mean Preference Scores of Total Entries for the Trained Control and Visual Conditions



Previous studies have indicated that bobwhite quail embryos presented with patterned visual stimulation during the last 2-3 days before hatching do not demonstrate species-typical perceptual development (Lickliter, 1990a). While unmanipulated control chicks showed preference for the bobwhite maternal call at 24 or 48 hours post hatch using only auditory information, chicks that were presented with visual stimulation prenatally needed both bobwhite auditory and visual cues to make the same discrimination. Even though visual stimulation provided during the last 3 days before hatching affects perceptual learning, results from the present experiment suggest that these deficits in perceptual learning do not extend to contingency detection and learning, even if the prenatal visual stimulation is presented for as long as 6 days. These findings suggest that contingent learning, which is a fundamental form of learning, and supports cognitive, social, and behavioral growth, is not readily disrupted.

Collectively, these findings suggest that the altered prenatal visual stimulation, irrespective of its duration, failed to disrupt contingency detection and contingency learning as measured in the simultaneous choice test. In spite of receiving prenatal visual stimulation for three days or six days prior to hatching, chicks were able to benefit from the contingency training and shift their auditory preference away from their species-specific maternal call. There are several possibilities that could explain this finding. One is when the visual modality becomes functional. The visual modality in bobwhite quail develops between Days 14-19 (Lickliter & Honeycutt, 2003). In the current experiment the visual stimulation was provided from Day 17. It is possible that when the augmented visual stimulation started at Day 17, the visual modality, being a newly emerging sensory modality, may not yet have been able to discriminate and process the visual stimuli as effectively, as would be the case later in development. The stimulation would thus be minimally effective. Moreover unlike ears that cannot be covered, eyes can be closed by embryos to attenuate any stimulation that is aversive. This may also contribute to minimizing the effects of prenatal visual stimulation.

Another possibility could be that augmented visual stimulation during the prenatal period may not cause impairments in an auditory-contingency task. This modality-specific assumption is suggested by the fact that when the training and testing vocalizations for the Trained Controls and the Visual conditions were compared, a statistically significant linear trend showed that testing vocalizations decreased as the amount of augmented prenatal visual stimulation increased. The decreased vocalizations observed in the Visual experimental conditions during testing suggest that augmented visual stimulation prior to hatching affects the chicks' overall state. Results from the

current experiment showed that even though the chick's behavioral responses (vocalizations) were influenced by augmented visual stimulation presented prior to hatching, they showed no significant difference in their *auditory* contingency abilities when compared to Trained Controls. Therefore it seemed likely that augmented auditory stimulation prior to hatching might affect bobwhite chick's ability to learn and remember contingent auditory information. This led to the next experiment, in which augmented auditory stimulation was presented to embryos prior to hatching.

CHAPTER X

EXPERIMENT 4: ALTERING PRENATAL AUDITORY STIMULATION

This experiment evaluated the effects of augmented auditory stimulation during the late prenatal period on postnatal contingency learning. The previous experiment showed that altering visual stimulation prenatally did not appear to affect chicks' ability to learn contingencies and shift their normal preference for their species-typical maternal call. It is possible that the effects of augmented visual stimulation were not evident in an auditory-contingency task. This could mean that there might be a link between the type (visual or auditory) of augmented prenatal stimulation presented and the modality (visual or auditory) used for the contingency task. To explore this possibility, embryos in the current experiment were provided with augmented auditory stimulation prior to hatching and their postnatal ability to learn about contingent relations were evaluated.

Method

100 bobwhite embryos served as subjects. Two experimental conditions were formed in this experiment. The same control condition of 'Trained Controls' was used for comparison with the altered prenatal auditory condition. Embryos were exposed to a tone for three days prior to hatching (Short-Duration Auditory Condition), or for six days before hatching (Long-Duration Auditory Condition). The auditory stimulation began on Day 20 of the 23-Day incubation cycle for the Short-Duration Auditory Condition, whereas the Long-Duration Auditory Condition received the auditory stimulation from Day 17 of incubation until hatch at Day 23. A speaker was attached inside a portable incubator to provide the auditory stimuli. Both the Short-Duration Auditory Condition

and the Long-Duration Auditory Condition were exposed to a 1000 Hz tone continuously for 45 minutes every hour during the days prior to hatching. The volume of the tone was calibrated at 65 dB.

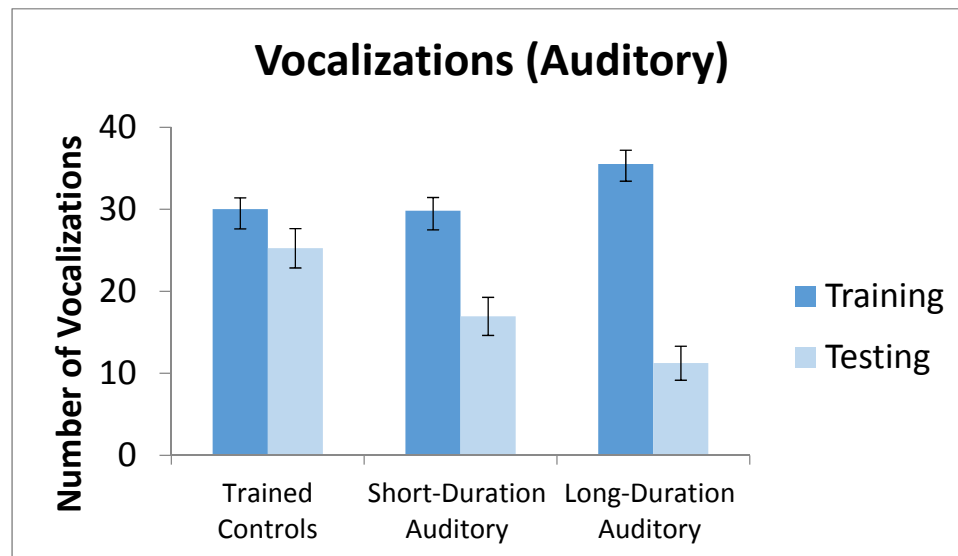
The contingency training was conducted 24 hours after hatching; each chick was presented a burst of the Japanese quail call contingent on its own vocalization as described in Experiment 2. Chicks were then tested in a simultaneous choice test at 48 hours of age between the Japanese quail maternal call and a bobwhite maternal call. The preference for the untrained species-typical bobwhite call was measured by evaluating the duration of time spent in the bobwhite approach area, the latency to enter the bobwhite area, and the number of times it entered the bobwhite area. The vocalizations of the chicks during training and testing were also recorded.

Results and Discussion

Vocalizations of the Short-Duration Auditory condition and the Long-Duration Auditory condition during training and testing were compared to ensure that contingency detection was operating during the training. A 2 x 3 repeated measures ANOVA indicated a main effect of Training versus Testing, with lower number of vocalizations during Testing, $F(1,86) = 56.69, p < .001$ (Greenhouse-Geisser corrected). This demonstrated that the contingency was operating since the chicks' vocalized more during training when the contingency was present as compared to testing when the contingency was absent. Further, this was qualified by a significant interaction, $F(2,86) = 4.83, p < .05$ (Greenhouse-Geisser corrected). The interaction was driven by a linear decrease, across conditions, in vocalization during the Testing phase $t(86) = 2.27, p < .05$, but not the

training phase $t(86) = .95, p = .34$. The pattern suggests that chicks from the Auditory conditions behaved similar to the Trained Controls and showed an increase in their vocalizations during training, indicating that they actively participated in the contingency learning task. This is an important finding because it rules out suspicions of hearing loss in the auditory-stimulated chicks during training. The testing vocalizations in the Auditory conditions also showed a decline as a function of augmented auditory stimulation prior to hatching, similar to the Visual conditions. Figure 11 illustrates the mean vocalizations during training and testing for the Trained Controls and the Auditory conditions.

Figure 11. Mean Vocalizations for Trained Controls, Short-Duration Auditory, and Long-Duration Auditory Conditions during Training and Testing



A one-sample t -test assessed the within-condition preference of chicks for the untrained species-typical bobwhite quail maternal call. Results for the mean PTD scores

are shown in Table 8. As was the case with Experiment 2, the results from the Control conditions showed that the Untrained Controls preferred the species-typical bobwhite call, whereas the Trained Controls did not show a preference for either the bobwhite quail call or the Japanese quail call. Results from the experimental auditory conditions revealed that the contingency training influenced the Short-Duration Auditory condition, shifting their species-typical auditory preference. However, the Long-Duration Auditory condition was significantly less than .05, indicating that chicks in this condition did not show a shift in their typical preference for the bobwhite quail call. Chicks in the Long-Duration Auditory condition (in spite of being trained) did not appear to remember the contingently trained Japanese maternal call in the testing trial.

Table 8. Mean Proportion of Total Duration (PTD) scores across the Trained Controls and the Auditory Conditions

Condition	Proportion of time spent in BW Call area	SD	<i>t</i> value (one sample t-test)	<i>p</i> value
Trained Controls	.53	.36	$t(39) = .54$.59
Short-Duration Auditory	.60	.35	$t(39) = 1.80$.07
Long-Duration Auditory	.68	.22	$t(41) = 5.01$.00*

The PTE scores for the Trained Control and Auditory conditions are shown in Table 9. The PTE scores for the Trained Controls indicated that chicks entered both the areas (with the Japanese quail call and the Bobwhite quail call) in similar frequency. The PTE scores for the Short-Duration auditory condition resembled the Trained Controls in that there was no significant difference in the number of entries to either approach areas. Chicks in Long-Duration Auditory condition entered the approach area with the bobwhite call significantly more often than the approach area with the trained Japanese quail call. These results suggest that chicks in the Long-Duration Auditory condition preferred the untrained species-typical bobwhite call, indicating that they did not learn the contingent relationship effectively, and did not remember or prefer the familiarized Japanese quail call on the day following training.

Table 9. Mean Proportion of Total Entries (PTE) across the Trained Control and Auditory Conditions

Condition	Proportion of Entries to BW Call area	SD	<i>t</i> value (one sample t-test)	<i>p</i> value
Trained Controls	.47	.31	$t(39) = .45$.65
Short-Duration Auditory	.58	.31	$t(39) = 1.63$.11
Long-Duration Auditory	.68	.20	$t(40) = 5.74$.00*

The PTL scores (shown in Table 10) showed no difference between the Control conditions or the Auditory conditions, unlike the PTD and the PTE scores. As was the case in the previous experiment, the latency of chicks to enter the familiar approach area was not indicative of their preference for a specific maternal call. It is known from the PTD and PTE scores that the Untrained Controls and the Long-duration Auditory condition preferred the bobwhite call. However this preference was not evident in terms of latency to approach the area broadcasting the bobwhite call. Chicks that showed preference for the bobwhite call did not necessarily move into the area at the onset of the testing trial, but did maintain proximity to that call over the course of the 5 minute test.

Table 10. Mean Proportion of Total Latency (PTL) across the Trained Control and Auditory Conditions

Condition	Proportion of time spent in BW Call area	SD	<i>t</i> value (one sample t-test)	<i>p</i> value
Trained Controls	.53	.36	$t(39) = .55$.58
Short-Duration Auditory	.45	.35	$t(39) = .83$.40
Long-Duration Auditory	.47	.28	$t(40) = .67$.50

The PTD and the PTE scores were compared across the Trained Controls, the Short-Duration Auditory, and the Long-Duration Auditory conditions. A linear contrast

(using weights -1, 0, 1) showed a significant linear trend in the data for both PTD or PTE, $t(118) = 2.12, p = .038$ and $t(118) = 2.68, p = .009$, respectively. A bootstrap was performed (1000 iterations) to determine the standard errors. The results suggest that there is an effect of auditory prenatal exposure, such that greater prenatal auditory stimulation is associated with greater preference for the bobwhite maternal call. This indicates impaired contingency learning. Figures 12 and 13 display the PTD and PTE scores for the Trained Controls and the Auditory conditions.

Further, in an earlier analysis when vocalizations were compared between training and testing, a significant linear trend was confirmed where vocalizations declined as a function of increased prenatal stimulation, as was seen in the previous Visual experiment. Moreover it is interesting that an increase in prenatal auditory stimulation similarly impairs contingency learning abilities. Together, these results indicate that the augmented stimulation possibly affects not only learning processes but also the overall state of the organism during training and testing.

Figure 12. Mean Proportion Scores of Total Duration for the Trained Control and Auditory Conditions

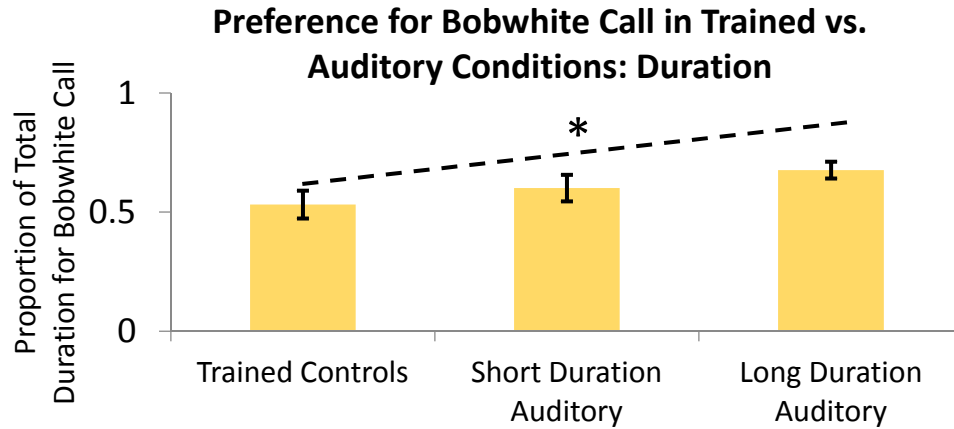
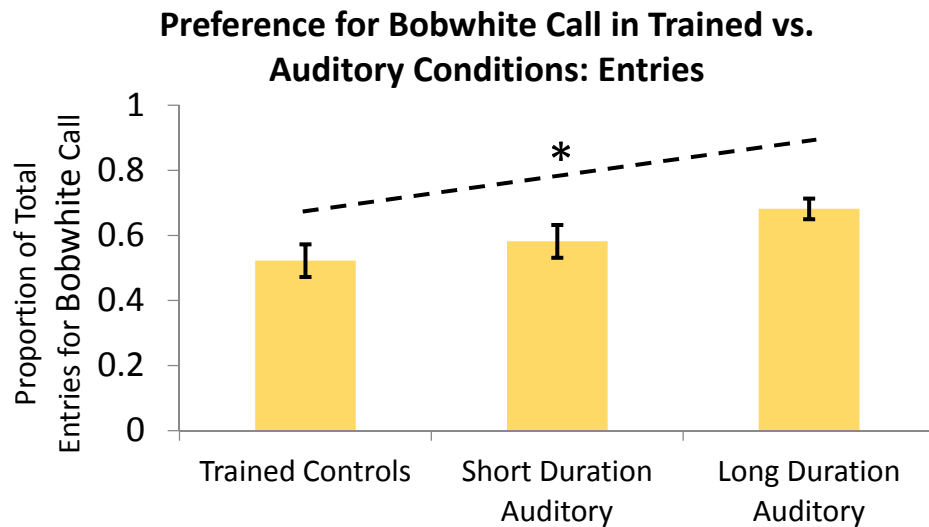


Figure 13. Mean Proportion Scores of Total Entries for the Trained Control and Auditory Conditions



It was predicted that augmented auditory stimulation presented to embryos prior to hatching would impair their auditory-contingency abilities. Results from this

experiment confirmed this prediction. Based on results from this experiment and the previous Visual experiment it is possible that early contingency learning abilities are not domain general, but rather modality specific in nature. However, it is not yet clear how embryos receiving augmented auditory stimulation would perform on a visual-contingency task.

The disruption in contingency learning that was seen in the Auditory conditions are interesting and it is useful to explore possible scenarios that could explain this phenomenon. Compared to the visual system, an embryo typically experiences auditory stimulation relatively early during the prenatal period and therefore the auditory modality is likely more functional than the visual modality during the late stages of prenatal development. If augmented auditory stimulation is made available to embryos after the auditory modality has developed, the prolonged processing of auditory information could require the young organism to use resources that would typically be allocated for other sensory systems, thus modifying perceptual and cognitive functioning. Moreover, the organism cannot effectively attenuate the augmented auditory stimulation provided during the prenatal period. Unlike the eyelids, there is no ear-mechanism to attenuate the auditory stimulation. Thus augmented prenatal auditory stimulation may affect the organism more so than augmented prenatal visual stimulation.

Even though results from this experiment suggest that the augmented auditory stimulation effects contingency learning abilities, the maximum impairment was seen in the condition that received the 6-day augmented auditory stimulation regimen. Therefore it was not clear if the Long-Duration Auditory condition showed impairments in remembering the contingent information due to the prolonged duration of stimulation or

because the stimulation was presented earlier in development than in the 3-day condition. In a study by Honeycutt and Lickliter (2003), bobwhite embryos were provided augmented tactile and vestibular stimulation at a time that coincided with the onset of the auditory and visual functioning. They found that augmented tactile and vestibular stimulation to bobwhite embryos at a time that coincided with the onset of the functioning of auditory system led to atypical perceptual performance during the postnatal period. However when the augmented tactile and vestibular stimulation did not coincide with the onset of the functioning of the auditory modality, the postnatal perceptual performance was not affected, even though the amount of tactile and vestibular stimulation they received during the prenatal period was the same. This showed that the timing at which stimulation was provided played an important role in subsequent perceptual functioning.

Since the Long-Duration Auditory condition received the augmented auditory stimulation starting on Day 17 of incubation, unlike the Short-Duration Auditory condition that received the stimulation from Day 20, it was not clear if it was the *duration* (6 days vs 3 days) or if it was the *timing* when stimulation started relative to the embryo's developmental stage that was responsible for the disruption in contingency detection and learning. Therefore it seemed important to explore if the Long-Duration Auditory group was impaired in their contingency detection and learning because of when the stimulation began (timing) or because of the duration of exposure. To assess this, an additional experiment provided prenatal auditory stimulation to embryos from the same onset time as the Long-Duration Auditory condition, but for a shorter overall duration.

CHAPTER XI

EXPERIMENT 4: ALTERING TIMING OF AUDITORY STIMULATION

The goal of the current experiment was to explore the role of timing of prenatal sensory stimulation and assess if it affects contingency learning during early postnatal development. Results from the previous experiment suggested that a disruption in contingency learning could be due to the timing of the stimulation or due to the duration, or due to both the factors. To better understand this developmental process, the current experiment provided auditory stimulation that started at the same time as the Long-Duration Auditory condition (Day 17) in the previous experiment, but for a shorter duration (3 days). The objective of this experiment was to evaluate if the performance of the bobwhite hatchlings that received stimulation starting at the same time as the Long-Duration condition, but for a shorter duration (3 days) would show impairments like the Long-Duration Auditory condition chicks. Moreover, since the Short-Duration Auditory condition matched the Short-Early Auditory condition with respect to duration of augmented stimulation, it would be important to compare these conditions. Therefore in this experiment all the Auditory conditions and the Trained Controls were examined to understand the relative affects on contingency learning.

Method

Subjects were 50 bobwhite embryos that received augmented prenatal auditory stimulation. The embryos were transferred from a large communal incubator to a portable incubator in which the experimental auditory stimulus was presented. The auditory stimulus was provided via a speaker that was attached inside the incubator. The auditory

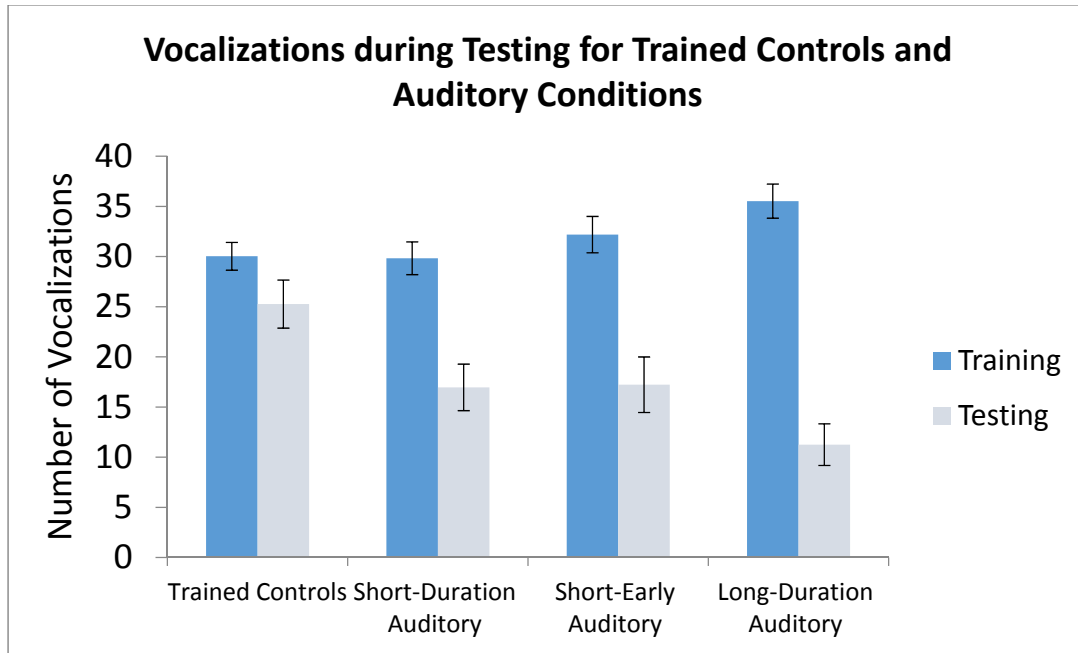
stimulation began on Day 17 of the 23-Day incubation cycle and ended on Day 20. Similar to the Short-Duration Auditory Condition and the Long-Duration Auditory Condition, this Short-Early Auditory condition was exposed to a 1000 Hz tone continuously for 45 minutes every hour, calibrated to 65 dB. These embryos did not receive any augmented auditory stimulation from Day 20 of incubation until hatching on Day 23. The chicks were provided the contingency training at 24 hours of age and were tested at 48 hours of age to evaluate their preference for the trained maternal call. Because the Short-Duration Auditory and Short-Early Auditory conditions were experimentally presented with the same type and amount of sensory stimulation, any observed differences between the Short-Duration Auditory and Short-Early Auditory conditions should be due to a timing-based effect.

Results and Discussion

Vocalizations of the Short-Early Auditory condition during the training and testing trials were compared to ensure that contingency detection was operating during training. A 2 x 4 repeated measures ANOVA revealed a main effect of Training versus Testing, with lower vocalizations during testing, $F(1,112) = 131.99, p < .001$ (Greenhouse-Geisser corrected). This result indicated that the contingency was operating because the vocalizations were greater when the contingency was present as opposed to when it was absent. Moreover, there was a significant interaction, $F(2,112) = 10.79, p < .001$ (Greenhouse-Geisser corrected). The interaction showed a linear decrease across conditions for vocalization during testing $t(112) = 2.62, p < .05$, but not the training phase $t(112) = 1.65, p = .10$. This pattern suggests that the vocalizations during testing decrease

as a function of increased prenatal auditory stimulation. Figure 14 illustrates the mean vocalizations during testing and training in the Trained and the Auditory conditions.

Figure 14. Mean Vocalizations in Trained Controls, Short-Duration Auditory, Short-Early Auditory and Long-Duration Auditory Conditions



A one-sample *t*-test revealed that chicks that received augmented auditory stimulation for 3 days starting from Day 17 to 20 of the 23-day incubation cycle showed a significant preference for the bobwhite maternal call. The PTD for the untrained species-typical bobwhite call was significantly more than .05 (shown in Table 11 below), indicating that they preferred the untrained species-typical bobwhite quail call over the familiarized Japanese quail maternal call, in spite of receiving the contingent training. This finding was similar to the findings of the Long-Duration Auditory condition. The

Controls that received training did not show a preference for either the bobwhite quail maternal call or the Japanese quail maternal call, thus showing a shift in their species-typical preference for the bobwhite maternal call.

Table 11. Mean Proportion of Total Duration (PTD) for the Trained Control, Short-Early, and Long-Duration Auditory Conditions

Condition	Proportion of time spent in BW Call area	SD	<i>t</i> value (one sample t-test)	<i>p</i> value
Trained Controls	.53	.36	$t(39) = .54$.59
Short-Early Auditory	.61	.26	$t(39) = 2.51$.01*
Short-Duration Auditory	.60	.35	$t(39) = 1.80$.07
Long-Duration Auditory	.68	.20	$t(40) = 5.74$.00*

A one-sample *t*-test showed that the chicks in the Short-Early Auditory condition entered the approach area of the familiar JQ call significantly less than the bobwhite call approach area. This PTE data was similar to the PTD data (shown in Table 12 below), in that chicks from the Long-Duration Auditory and Short-Early Auditory conditions spent more time in the approach area broadcasting the bobwhite maternal call and entered it more often than the approach area broadcasting the Japanese quail maternal call.

Table 12. Mean Proportion of Total Entries (PTE) for the Trained Control, Short-Early, and Long-Duration Auditory Conditions

Condition	Proportion of Entries to BW Call area	SD	<i>t</i> value (one sample t-test)	<i>p</i> value
Trained Controls	.47	.31	$t(39) = .45$.65
Short-Early Auditory	.61	.22	$t(39) = 3.01$.00*
Short-Duration Auditory	.58	.31	$t(39) = 1.63$.11
Long-Duration Auditory	.68	.20	$t(40) = 5.74$.00*

The PTL data was evaluated using the one sample *t*-test (listed below in Table 13) and revealed that the latency scores were not different for entering either of the approach areas for any of the three conditions listed below. Even though chicks from the Long-Duration Auditory and Short-Early Auditory conditions spent more time in the area broadcasting the bobwhite maternal call and entered it more times than the other approach area, they did not show a significantly shorter latency in entering the area

broadcasting the bobwhite maternal call as compared to the competing Japanese maternal call.

Table 13. Proportion of Total Latency (PTL) scores for the Trained Control, Short-Early, and Long-Duration Auditory Conditions

Condition	Proportion of time spent in BW Call area	SD	<i>t</i> value (one sample t-test)	<i>p</i> value
Trained Controls	.53	.36	$t(39) = .55$.58
Short-Early Auditory	.48	.29	$t(39) = .43$.66
Short-Duration Auditory	.45	.35	$t(39) = .83$.40
Long-Duration Auditory	.47	.28	$t(40) = .67$.50

To evaluate the Trained Controls with the Short-Early Auditory, Short-Duration Auditory and the Long-Duration Auditory conditions, a linear contrast was used. If the Short-Early Auditory condition fit the linear trend, then it would mean that the time at which the augmented stimulation was provided was instrumental in the postnatal impairment that was observed. A linear contrast (using weights -3, -1, 1, 3) showed a significant linear trend in the data for both PTD or PTE, $t(157) = 2.03$, $p < .05$ and $t(157) = 2.66$, $p < .05$, respectively. Figure 15 and 16 illustrate the linear trend for the mean

proportion scores of total duration and entries for the Trained Control, Short-Duration Auditory, Short-Early Auditory and Long-Duration Auditory Conditions. The results indicate a clear effect of auditory prenatal exposure, where increased prenatal auditory stimulation was associated with greater preference for the untrained species-typical bobwhite maternal call. The linear trend showed that the Short-Duration Auditory condition and the Short-Early Auditory condition were similar in their performance when compared to the Long-Duration Auditory condition. Chicks in the Short-Early Auditory condition did not show similar impairments as chicks in the Long-Duration Auditory condition, indicating that the time window (Day 17-Day 20) during which the augmented stimulation was available was not solely responsible for the impairments in contingency learning seen in the Long-Duration Auditory group.

Figure 15. Mean Proportion Scores of Total Duration for the Trained Control, Short-Duration Auditory, Short-Early Auditory and Long-Duration Auditory Conditions

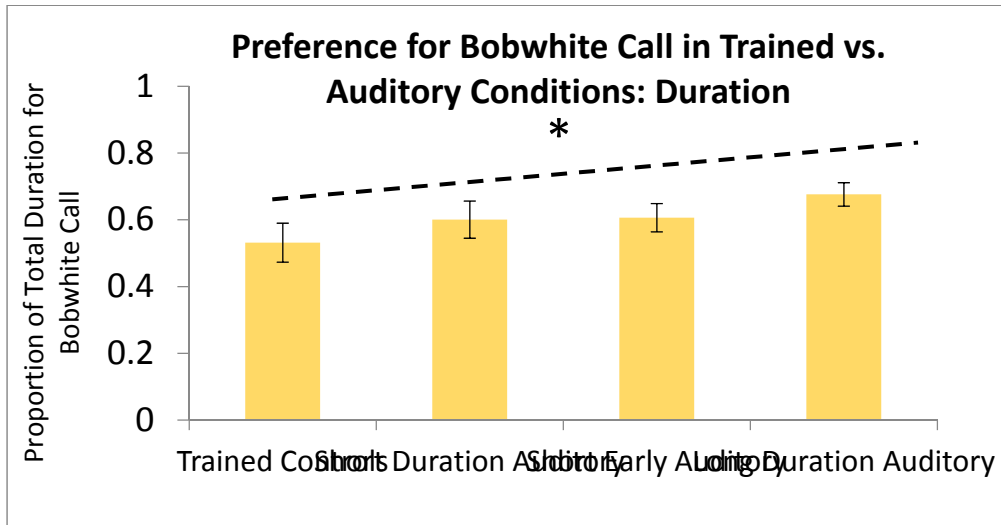
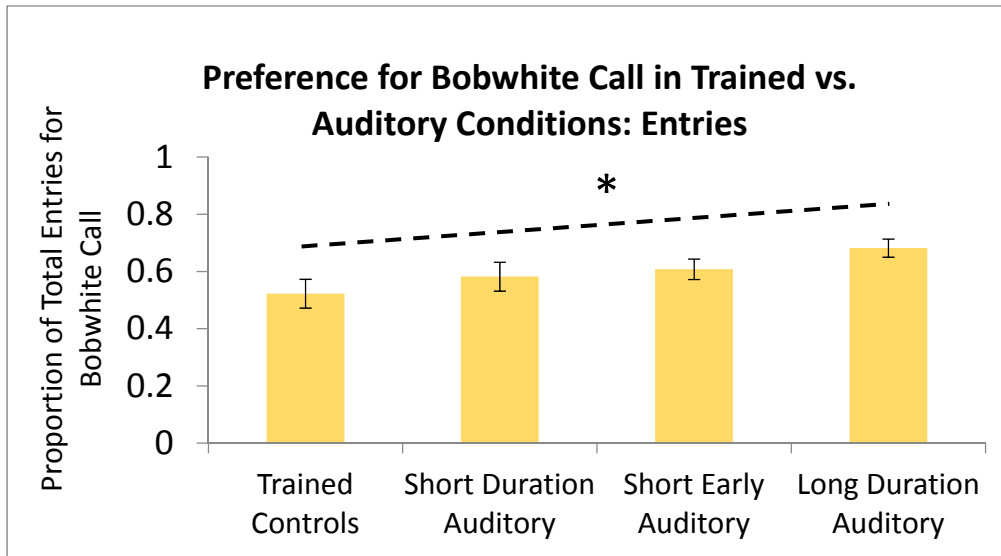


Figure 16. Mean Proportion Scores of Total Entries for the Trained Control, Short-Duration Auditory, Short-Early Auditory and Long-Duration Auditory Conditions



It seems more likely that the prolonged (6 days) duration of stimulation was primarily responsible for the observed deficits in contingency learning. However, since the Short-Early Auditory condition responded similar to the Short-Duration Auditory condition, it is possible that both the alteration in timing *and* the duration of prenatal auditory stimulation acted together to contribute to the observed interference in learning and remembering contingent relationships seen in the Long-Duration Auditory condition.

CHAPTER XII

GENERAL DISCUSSION

The current study was designed to explore how altered sensory stimulation during the late prenatal period would affect postnatal contingency learning and memory in bobwhite quail hatchlings. Modifying sensory experience can yield valuable information about the mechanisms of early perceptual, cognitive, and behavioral development. Prior research with bobwhite quail neonates has demonstrated that early perceptual and behavioral development are influenced by several related factors, including the amount of sensory stimulation provided or denied to young organism, the type of stimulation that is provided, and the timing of the presented stimulation in relation to the developmental age of the organism (Lickliter, 2000). The current study built on these findings and was the first study to evaluate postnatal contingency detection and learning as a consequence of augmented prenatal sensory stimulation. Results of this study add a novel contribution to the existing literature on early development. The novel aspect of this contribution lies in 1) assessment of early postnatal contingency detection and learning, 2) assessment of how augmented prenatal stimulation to the auditory and visual sensory modalities affect contingency learning.

Results indicated that bobwhite embryos given augmented prenatal visual stimulation for 3 days (Short-Duration Visual condition) or 6 days (Long-Duration Visual condition) before hatching did not show a disruption in their postnatal ability to learn and remember contingent relationships. It is known that contingency learning abilities provide a key basis for behavioral, cognitive, social, and emotional learning (Rochat, 2001). Therefore it seemed likely that this fundamental skill (contingency learning) may

not be easy to disrupt. It was however surprising that prolonged visual stimulation for as long as 6 days also did not disrupt contingency learning. Embryos that received prolonged visual stimulation continued to exhibit contingency learning abilities similar to Controls. Thus, findings from both the Short-Duration Visual as well as the Long-Duration Visual conditions support the robustness of contingency learning, a skill that most likely develops early in the prenatal period and one that is not easy to disrupt.

Results from the Visual experiment generated several alternate theories to explain why the visual stimulation did not cause any disruptions in contingency learning. Since the visual modality in bobwhite quail develops later in the incubation cycle as compared to other sensory modalities, it is likely that the visual modality may not be fully functional, thereby limiting the ability to efficiently process the augmented visual stimulation provided. Further, visual stimulation can be regulated by quail embryos by use of their eyelids. By keeping eyelids closed, the young organism can attenuate visual stimulation, thus potentially minimizing the aversive effects of the augmented stimulation during the prenatal period.

Another alternative theory is that contingency learning is probably not a domain general skill but modality-specific in nature. This would mean that embryos that received visual stimulation had decreased opportunity to experience contingencies in the visual domain because of continuous visual stimulation. Therefore they would most likely show impairments in a visual-contingency task but might not show impairments in an auditory-contingency task. To test this theory, embryos were given augmented auditory stimulation for the same duration and regime as embryos that received the visual

stimulation. It was expected that chicks receiving augmented auditory stimulation prenatally would show impairments in the auditory-contingency task.

Results from the auditory experiment indicated that augmented prenatal auditory stimulation prior to hatching could disrupt auditory-contingency learning abilities in bobwhite neonates. While these results seem to conform to the idea that contingency learning skill is modality specific, further evidence is needed. If visually stimulated chicks do show impairments in visual-contingency task, and/or if olfactory stimulated chicks do show impairments in olfactory-contingency task, we can be more confident of the modality specific nature of early contingency learning.

Results from the Auditory experiments indicated that the duration of augmented prenatal auditory stimulation was instrumental in impairments to learn and remember the contingent information (Japanese quail call). These findings emphasize the connection between noise exposure during the prenatal period and deficits in learning abilities during the postnatal period. The disruption in learning due to prolonged auditory prenatal stimulation could be the result of the fact that the auditory system begins to develop relatively early in the prenatal period (around day 9 of incubation cycle) and thus is likely more mature and functional during later prenatal development. This prolonged processing of auditory information may organize the overall state of the organism as well as the brain in different ways than usual, leading to subsequent learning deficits during the postnatal period.

A review by Wachman and Lahav (2010) highlighted the deleterious effects of noise in the NICU that can potentially cause physiological changes, including increased heart rate, blood pressure, and respiratory rate, as well as decreased oxygen saturation.

These physiological changes are likely to affect a young organism's ability to learn and process information. In the Auditory experiment, vocalizations of the chicks during testing declined as a function of increased prenatal exposure. Moreover chicks from the Auditory condition showed impairments in contingency learning as a function of augmented prenatal stimulation. Taken together, these findings suggest that increased auditory stimulation prior to hatching not only affects subsequent cognitive functioning but also affects the overall state and arousal of the organism indexed by the reduced number of vocalizations.

The current study found that contingency learning deficits increased as a function of increased prenatal auditory stimulation. These results potentially shed light on why 'very preterms', which receive prolonged altered stimulation (including augmented auditory stimulation), are more likely to be susceptible to later developmental problems than preterms who do not spend as much time in the NICU.

The current study demonstrated links between prolonged augmented auditory stimulation prior to hatching and postnatal impairments in contingency learning. These findings share similarities with human findings, where there is a strong correlation between a preterm's stay in the NICU and subsequent neurodevelopmental problems (Als, 2004; Als, 2013; Aylward, 2005; VandenBerg, 2007). For example, Omizzolo et al. (2014) reported brain-matter organization abnormalities with corresponding deficits in immediate memory, working memory, and long-term memory in children born very prematurely.

Previous studies have shown that augmented visual and auditory stimulation for the last 2-3 days of incubation (Sleigh & Lickliter, 1995, 1997) can cause disruptions in

perceptual development in bobwhite quails. However it was not clear if these perceptual deficits would also extend to contingency learning abilities. Even though the current study demonstrated deficits in contingency learning as a result of augmented auditory stimulation, it was difficult to infer that perceptual deficits contributed to this impairment. For instance, in the current study, stimulation that was presented was continuous and not intermittent, unlike previous research with bobwhite quail that have examined perceptual learning (Lickliter & Stoumbos, 1991). The Visual experiment in the current study used a visual stimulus that was non-patterned, whereas previous studies have typically used patterned light (Lickliter 1990a, 1990b, Sleigh & Lickliter, 1995). The Auditory experiment in the current study used an auditory stimulus which was an artificial tone, whereas prior studies with bobwhite quail have used normally occurring chick or maternal vocalizations (Sleigh & Lickliter, 1996). Moreover, the duration of stimulation in the current study was as long as 6 days prior to hatching, whereas previous studies have not provided altered stimulation for more than 3 days prior to hatching. These factors made it difficult to compare the results of the current study with previous perceptual learning studies.

Chicks receiving the augmented stimulation were able to *perceive* the contingency during the training session. Chicks from experimental conditions that received augmented stimulation prior to hatching vocalized to hear the Japanese quail maternal call and the frequency of their vocalizations was similar to the Trained Controls. This demonstrated that they were able to perceive the contingent stimuli during the training session. However there was a clear deficit in their ability to process and remember the

contingently presented information, which was evident by their performance during the testing session.

The parameters within which the current study was conducted in terms of timing of stimulation (after the sensory modalities are functional), type of stimulation (auditory and visual), duration of stimulation (3 and 6 days), and the quality of stimulation (non-patterned white light, and 1000 Hz tone), limit how the results of the study can be generalized to other contexts where the timing, type, duration, and quality of stimulation are different. Nevertheless the findings from the current study do inform us about the effects of augmented auditory stimulation on contingency learning abilities and can provide testable hypothesis to the infant research community for further exploration.

Development is a relational process and therefore the *amount* of stimulation cannot be separated from its referents. In the current study, visual stimulation for as long as 6 days did not appear to affect subsequent contingency detection and learning. In contrast, auditory stimulation for as long as 6 days did disrupt contingency learning. This indicated that the type of stimulation did matter. The *amount* of stimulation was tightly linked to the *type* of stimulation and the type of stimulation was tied to the *timing* (earlier vs later) at which a sensory modality became functional. These interrelated factors underscore that development is a result of present and historical experiences, all aspects of which play a role subsequent developmental outcome.

Limitations and Future Directions

A major limitation of this study was the lack of a physiological measure such as a heart rate measure to evaluate the overall arousal level of embryos that received the augmented prenatal stimulation. This measure could have been helpful to assess the

overall arousal level of the embryo and neonate across the various experimental conditions. Future work along these lines could explore links between prenatal and postnatal physiological measures and subsequent contingency learning abilities.

Further, it was surprising that embryos presented with the prolonged 6-day visual stimulation did not exhibit disruptions in contingency learning. Previous research has shown that augmented visual stimulation alters perceptual development postnatally (Sleigh & Lickliter, 1995). It would be interesting to prolong visual stimulation for more than 6 days to see if and when the visual stimulation can interfere with postnatal contingency learning abilities.

The majority of previous studies with bobwhite quail that have evaluated perceptual learning and development have provided lower amounts of stimulation (10min/hour for 2-3 days) and different types of stimuli (bobwhite chick vocalizations, maternal vocalizations, tones). It would be useful to standardize the stimuli and the amount of stimulation to systematically compare the impact of different types of sensory stimulation on perceptual and cognitive development.

Since our sensory systems develop sequentially, the differential experience with different sensory systems could suggest that contingency learning is modality specific in nature during early development. To explore this theory further, future studies could develop a visual-contingency task to evaluate if augmented prenatal visual stimulation impairs contingency learning in chicks.

As previously mentioned, the kind of stimuli used in these types of experiment forms a critical component of the experimental results. Rand and Lahav (2014) have suggested that synchronous infant-directed speech in the NICU for ‘very preterms’ can be

critical for the initial wiring of the brain, promoting language development. It is not clear if infant-directed contingent speech stimulation could also influence other learning abilities. Further experiments with bobwhite quail could rerun a condition similar to the Long-Duration Auditory condition by replacing the 1000 Hz tone with more ecologically valid auditory stimuli (such as maternal vocalizations that are not continuous but intermittent) to determine if chicks show similar deficits in early contingency detection and learning.

Summary

Sensory stimulation that is experienced by the premature infant in the NICU is a mix of typical and atypical sensory experiences, including low levels of tactile and vestibular experience and increased levels of auditory and visual experience. While it is known that this mix of altered sensory stimulation can affect postnatal cognitive abilities (Als, 2013), it is not clear what type, amount, or timing of sensory stimulation is more disruptive to the young organism. The current study was a step forward in the direction of addressing some of these questions. Results from the current study with bobwhite quail indicated that prolonged auditory stimulation during the prenatal period could cause disruptions in contingency learning abilities during early postnatal period. This is a first step and more work is needed to fully understand the complex mechanisms underlying the processes that govern sensory development and its effects on subsequent cognitive development.

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