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Effects of Prenatal and Early Postnatal Exposure to Aversive Stimuli on Fearfulness and Exploratory Behavior in Bobwhite Qauil Neonates (Colinus virginianus)

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

EFFECTS OF PRENATAL AND EARLY POSTNATAL EXPOSURE TO AVERSIVE STIMULI ON FEARFULNESS AND EXPLORATORY BEHAVIOR IN BOBWHITE QUAIL NEONATES (*COLINUS VIRGINIANUS*)

A dissertation submitted in partial fulfillment of
the requirements for the degree of
DOCTOR OF PHILOSOPHY
in
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by
Michael Suarez

2014

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

This dissertation, written by Michael Suarez, and entitled Effects of Prenatal and Early Postnatal Exposure to Aversive Stimuli on Fearfulness and Exploratory Behavior 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.

Anibal Gutierrez

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Date of Defense: October 28, 2014

The dissertation of Michael Suarez is approved.

Interim Dean Michael R. Heithaus
College of Arts and Sciences

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Florida International University, 2014

DEDICATION

I dedicate this dissertation to my family whose support, encouragement, and love has made this achievement possible.

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I wish to thank my committee members for their support and guidance throughout this process. I would also like to thank my major professor, Dr. Robert Lickliter, for his incredible training and invaluable years of mentorship throughout my graduate career. A special thanks to Micheal A. Logan and Natalie Ceballos for their hands-on aid in this project. Finally, I would like to acknowledge the MBRS RISE program for providing me with resources and opportunities that have enhanced my overall research experience.

ABSTRACT OF THE DISSERTATION

EFFECTS OF PRENATAL AND EARLY POSTNATAL EXPOSURE TO AVERSIVE STIMULI ON FEARFULNESS AND EXPLORATORY BEHAVIOR IN BOBWHITE QUAIL NEONATES (*COLINUS VIRGINIANUS*)

by

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Professor Robert Lickliter, Major Professor

Neophobia, the fear of novelty, is a behavioral trait found across a number of animal species, including humans. Neophobic individuals perceive novel environments and stimuli to have aversive properties, and exhibit fearful behaviors when presented with non-familiar situations. The present study examined how early life exposure to aversive novel stimuli could reduce neophobia in bobwhite quail chicks. Experiment 1 exposed chicks to a novel auditory tone previously shown to be aversive to naïve chicks (Suarez, 2012) for 24 hours immediately after hatching, then subsequently tested them in the presence of the tone within a novel maze task. Postnatally exposed chicks demonstrated decreased fearfulness compared to naïve chicks, and behaved more similarly to chicks tested in the presence of a known attractive auditory stimulus (a bobwhite maternal assembly call vocalization). Experiment 2 exposed chicks to the novel auditory tone for 24 hours prenatally, then subsequently tested them within a novel maze task. Prenatally exposed chicks showed decreased fearfulness to a similar degree as those postnatally exposed, revealing that both prenatal and postnatal exposure methods are capable of decreasing fear of auditory stimuli. Experiment 3 exposed chicks to a novel visual stimulus for 24 hours postnatally, then subsequently tested them within a novel emergence box / T-maze apparatus. Chicks exposed to the visual stimulus showed decreased fearfulness compared to naïve chicks, thereby demonstrating the utility of this method across sense modalities. Experiment 4 assessed whether early postnatal exposure to one novel stimulus could generalize and serve to decrease fear of novelty when chicks were tested in the presence of markedly different stimuli. By combining the methods of Experiments 1 and 3, this experiment revealed that chicks exposed to one type of stimulus (auditory or visual) demonstrated decreased fear when subsequently tested in the presence of the opposite type of novel stimulus. These results suggest that experience with novel stimuli can moderate the extent to which neophobia will develop during early development.

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CHAPTER I

INTRODUCTION

Generally speaking, there are two broad categories of stimuli that guide behavior: reinforcing stimuli and aversive stimuli. While reinforcing stimuli generally serve to attract individuals and increase the occurrence of associated behaviors, aversive stimuli do just the opposite by generally repelling individuals and decreasing the occurrence of associated behaviors (Schneirla, 1959, 1965). The potential of coming into contact with aversive stimuli can often lead to fear in individuals, which may manifest itself in a variety of different ways.

One common type of fear found both in human and animal species is neophobia, the fear of novelty. Neophobic individuals tend to display both behavioral and physiological signs of fearfulness when confronted with novel stimuli, as well as generally avoiding or withdrawing from novel objects, places, and situations whenever possible. Neophobic children are often categorized as having behaviorally inhibited temperaments if they display heightened anxious behaviors, sensitivity to novelty, and social withdrawal. Although many children overcome these social difficulties over time, some maintain these patterns through adolescence (Perez-Edgar, Bar-Haim, McDermott, Chronis-Tuscano, Pine, & Fox, 2010).

On the opposite end of the spectrum, neophilic individuals tend to seek out novelty, sometimes taking extreme measures to partake in new and exciting experiences while trying to avoid routines and repetition (Thorpe, 1963). It is unclear what factors may contribute to the development of neophobic or neophilic attributes, but it is generally accepted that these traits emerge early in life and can have a long-lasting influence on individuals' lives.

It is a well-established notion that early-life experiences play important roles in subsequent development, but which experiences are maintained across time and how those experiences are extracted from the environment are not particularly well known. The use of animal models has provided considerable knowledge on these topics, as studies that modify early-life experiences can be difficult to carry out with human infants (Lickliter & Bahrick, 2000). Several studies with precocial birds have demonstrated that not only can auditory learning occur immediately after hatching, it also occurs within the prenatal environment with effects lasting well after hatching (Gottlieb, 1970; 1975; Lickliter, 1989; Lickliter & Hellewell, 1992). Similarly, human children have demonstrated the ability to learn and discriminate between faces and voices as early as 12-36 hours of age (Walton, Bower, & Bower, 1992).

In order to better understand how fearfulness develops, it is critical to understand how early-life experiences can influence how various stimuli gain their reinforcing and aversive properties. More research is needed to explore the role of early life experience with novelty to the development of neophobia. The current study addresses these topics by exploring the extent to which prenatal and postnatal exposure to novel stimuli serves to decrease the subsequent expression of fearful behaviors in the presence of those specific stimuli. Additionally, this study explores the extent to which stimulus generalization is capable of reducing neophobia across a wider range of stimuli than those specifically encountered during early life exposure.

CHAPTER II

LITERATURE REVIEW

Fearfulness has been defined as the general susceptibility of an individual to react to potentially threatening situations (Boissy, 1995). Although this definition is generally accepted, there is some debate as to the stability of fear throughout the lifetime. Some have proposed that fearfulness is a personality trait that remains stable over time, citing research conducted on mammals, birds, amphibians and invertebrates (e.g., Goddard & Beilharz, 1984; Mills & Faure, 1986, 2000; Jones, 1988; Lyons, 1989; Boissy & Bouissou, 1995). Others argue that fearfulness is more context specific and therefore variable over time (Miller, Garner, & Mench, 2006).

Neophobia / Neophilia

Although there are countless stimuli, both learned and unlearned, that can cause fear in individuals, one of the most common types is fear of novelty (i.e., "neophobia") (Thorpe, 1963). In humans, neophobia can emerge as early as 14 months of age and generally remains stable during childhood. Neophobic individuals express fearfulness in the presence of novelty, ranging from behavioral inhibition and shyness to increased extraversion and surgency (Cavigelli & McClintock, 2003; Garcia-Coll, Kagan, & Reznick, 1984). Although this neophobia is most prevalent during childhood and often diminishes as individuals come into contact with additional variable stimuli (thereby reducing the pool of possible novel stimuli in a given environment), it can have cascading effects on development that may persist into adulthood (Perez-Edgar et al., 2010).

In direct contrast to neophobic individuals, neophiles (or neophiliacs) express strong motivation to engage in novel behaviors and to experience novel environments.

Neophilic individuals often forego engaging in routine or traditional behaviors and instead seek to explore novel objects, places, and situations that might normally instill fear in other individuals (Thorpe, 1963). Although both neophobia and neophilia exist within and across species, some species exhibit a pronounced bias toward one or the other. For example, several species of foraging adult birds have been shown to display widespread neophobia in the presence of novelty, thereby potentially limiting their habitat range and the development of innovative behavior (Greenberg & Mettke-Hofmann, 2001; Greenberg, 2003). In contrasts, some species of birds such as the common raven (*Corvus corax*) display an attraction to novel objects over those previously explored (Heinrich, 1995).

Fearfulness and Exploration Motivation

Measures of fearfulness are often used as indicators of animal welfare, with the premise that highly fearful animals have greater risks of developing maladies than their less reactive counterparts (Cavigelli & McClintock, 2003; Mench, 1992). Highly fearful individuals not only show behavioral signs of fear, they also demonstrate increased activity in particular physiological systems, including the amygdala, the autonomic nervous system, and the hypothalamic-pituitary-adrenal axis (Byrne & Suomi, 2002; Schwartz, Wright, Shin, Kagan, & Rauch, 2003). The increased activation of these physiological systems as a result of chronic stress and fearfulness can lead to a myriad of negative health consequences for an individual and can even result in a shortened lifespan (Cavigelli & McClintock, 2003).

A great deal of research has identified reliable behavioral indicators of fearfulness across a variety of species. A widely used method for measuring fearfulness in animal subjects is to conduct behavioral testing in the presence of novel or startling stimuli within unfamiliar environments (Manteca & Deag, 1993; Boissy, 1995; Miller, Garner, & Mench, 2005). In addition to mammalian species, this method for measuring fearfulness has been shown to work well with precocial avian species such as bobwhite quail (*Colinus virginianus*), Japanese quail (*Coturnix japonica*), and domestic chickens (*Gallus gallus*), which are particularly well suited for assessing fear responses soon after birth because of their precocial nature that allows them to be active and responsive within a testing environment almost immediately after hatching (Jones, 2002; Freire, Cheng, & Nicol, 2004; Miller et al., 2005).

Behavioral testing of fearfulness in animal subjects often involves measuring willingness to explore in the face of novelty. Exploration can occur in two distinct forms depending on the stimuli present in an environment, the features or circumstances of the environment, and the state of the organism; these have typically been defined as approach behavior and avoidance or withdrawal behavior (Schneirla, 1959, 1965; Elliot & Covington, 2001). Whereas approach behavior generally involves moving toward positive or desirable stimuli, avoidance behavior is more indicative of fearfulness in that it involves making efforts to distance oneself from negative or aversive stimuli (Elliot & Covington, 2001; Elliot, 2006). Theodore Schneirla (1959, 1965) proposed that during early stages of development, organisms are more likely to approach low-intensity stimuli and withdraw from high-intensity stimuli if they have no prior experience with the stimuli or their potential consequences. This suggests that fear of novelty in early development could be moderated by the intensity of novel stimuli, rather than simply the presence of novel stimuli.

In a variety of mammalian species, diminished explorative tendencies are often associated with increased level of fearfulness (Buss & Plonin, 1984; Einon & Morgan, 1976; Cavigelli & McClintock, 2003). This is also the case with some avian species. Some behavioral measures of fearfulness that have been found to be useful for quail species in particular include measures of latency to explore novel objects, latency to explore novel areas, latency to taste novel foods, and reaction to surprise tests (Miller et al., 2005). Although decreased willingness to explore is usually indicative of increased fearfulness, the opposite has also been found when factors such as habituation to novel objects and differential rearing conditions are considered (Einon & Morgan, 1976).

Differential Rearing

A great deal of research has focused on how different rearing conditions could affect fearfulness (neophobia) and willingness to explore novel objects, environments, or situations (neophilia) across a variety of animal species. High levels of fearfulness in individuals is often produced as a result of intense and prolonged arousal brought on by stressful living conditions (Mench, 1992; Vanderheed & Bouissou, 1998). Some stressful living conditions that have been shown to increase fear and stress in animals are socially isolated rearing (Einon & Morgan, 1976; Gamallo, Villanua, Trancho, & Fraile, 1986; Molina-Hernandez, Tellez-Canatara, & Perez-Garcia, 2001), over-crowding (Brown & Grunberg, 1995; Gamallo et al., 1986), resource shortage, and predator pressure (Clinchy, Zanette, Boonstra, Wingfield, & Smith, 2004). Subjects raised under these conditions are generally less willing to explore novel objects and environments, have increased defecation rates, display depressive-like behaviors, and demonstrate physiological signs of stress, such as elevated heart rate or cortisol levels.

One of the most thoroughly investigated methods of reducing fearfulness and increasing exploratory behavior in animals has been the enrichment of rearing conditions. Research has shown that rhesus monkeys living in stimulus-deprived rearing conditions are generally more inactive, do not seek complex visual and manipulatory stimuli, show little exploration of their environments, and are more likely to withdraw from social contact than monkeys living under more normative conditions (Sackett, 1965). In addition, mammals such as piglets (Bolhuis, Schouten, de Leeuw, Schrama, & Wiegant, 2004) and ewes (Vandenheede & Bouissou, 1998) demonstrate less fearful behavior and more explorative tendencies when reared under enriched conditions. Studies on avian species including domestic chickens (Freire et al., 2004; Jones, 2002), crimson-bellied conures (van Hoek & King, 1997), Japanese quail (Miller & Mench, 2005), and bobwhite quail (Lazic, Schneider, & Lickliter, 2007) have also shown that enriched rearing can reduce fearful behavior in young chicks and increase exploratory behavior during subsequent testing.

Exposure Therapy

Several useful techniques have also been developed to diminish existing effects of fear-inducing stimuli. One technique in particular that has demonstrated significant utility in this regard is exposure therapy. Stemming procedurally from the fear extinction paradigm research conducted on rodents, exposure therapy involves exposing an individual to stimuli that evoke fearful behavior until those fears eventually dissipate (Marks, 1979, Myers & Davis, 2006; Rothbaum & Schwartz, 2002). In humans, this exposure is usually carried out in a step-wise motion in which the intensity of the stimulus is steadily escalated until the terminal stimulus has been reached and its fear-inducing properties have been attenuated. This technique has been shown to be effective in the treatment of specific phobias, posttraumatic stress disorder, obsessive-compulsive disorder, and generalized anxiety disorder (Abramowitz, 1996; Marks, 1979; Myers & Davis, 2006; Rothbaum & Schwartz, 2002). Although clearly effective, with an estimated 75% of cooperative patients being restored to normal functioning, exposure therapy does not come without its limitations (Marks, 1979). Exposure therapy can often take a great deal of time and resources to properly implement because of the step-wise manner in which it deals with progressively stronger stimuli to ultimately address a target stimulus. This technique also sometimes relies on the use of imaginative stimuli in which an individual is asked to imagine a situation that would normally cause them fear; for obvious reasons, this portion of the technique lacks utility for the attenuation of fear in non-verbal humans and animals.

Generalization

A behavior change is said to have generalizability if it persists across time, is displayed in a variety of settings, and/or if it spreads to different related behaviors (Cooper, Heron, & Heward, 2007). The principle of generalization refers to instances in which an individual emits a conditioned response in the presence of different stimuli than the original conditioned stimulus. This is said to occur when an organism fails to adequately discriminate between the original conditioned stimulus and other similar stimuli (Philip, 1947). The likelihood that generalization will occur across stimuli is said to be determined by the degree of similarity between the conditioned stimulus and the unconditioned stimulus. This is shown in a generalization curve (or gradient of generalization) which displays the rate at which generalization falls off as different stimuli become less and less similar to the original stimulus (Philip, 1947; Hanson, 1959).

Research linking stimulus generalization with approach and avoidance behaviors has shown mixed results in regards to the extent to which generalization is found among different measures. Brown (1942) studied how rats behaved when tested in the presence of lights of varying brightness from those previously trained. He found that a high degree of generalization can be expected for approach responses, even when the stimuli present during testing (light intensity) differed significantly from those previously encountered. However, he also found that generalized approach responses tend to extinguish more rapidly than those previously trained.

In summation, a great deal is known about neophilia and its effects on humans and animals, as well as effective procedures for treating this condition; however, relatively little is known regarding how or why neophobia develops to begin with. Understanding the mechanisms involved in the development of this condition is fundamental to learning how it can potentially be prevented or modified in early development.

CHAPTER III

PREDICTING EXPLORATIVE BEHAVIOR BY LEVEL OF EMOTIONAL REACTIVITY IN BOBWHITE QUAIL NEONATES (*Colinus virginianus*)

Various postnatal studies have supported the notion that level of emotional reactivity can be influenced by such factors as environmental enrichment and other forms of differential rearing conditions, but little is known about how level of emotional reactivity may be influenced by factors present solely during testing procedures. In order to investigate the role of differential auditory stimuli on emotional reactivity, Suarez (2012) designed a study to determine how explorative tendencies differed as a function of the subjects' motivation to traverse a novel maze toward the direction of an attractive auditory stimulus, or in the opposite direction of an aversive auditory stimulus. It was hypothesized that chicks tested in the presence of an attractive auditory stimulus would demonstrate decreased levels of emotional reactivity, as well as increased willingness to explore a novel maze environment when compared to chicks tested in the presence of an aversive auditory stimulus. To test these hypotheses, bobwhite quail chicks (*Colinus virginianus*) were either tested in the presence of a bobwhite maternal assembly call vocalization, or in the presence of a novel auditory tone 24 hours after hatching.

Procedure

Bobwhite quail chicks were separated into six experimental conditions: an Attraction Test condition (n = 17) used to demonstrate the attractive properties of the bobwhite maternal vocalization, an Aversive Test condition (n = 17) used to demonstrate the aversive properties of a novel auditory tone, an Approach condition (n = 30) in which chicks were tasked with exploring a novel maze environment toward the direction of the maternal vocalization, an Avoidance condition in which chicks were tasked with exploring a novel maze environment in the opposite direction of the novel auditory tone stimulus, and two control conditions (n = 30 per condition) during which no sounds were played and whose purpose was to ensure the absence of starting biases within the maze. Chicks were randomly allocated into each experimental group and received identical rearing conditions prior to testing.

Postnatal behavioral tests were conducted within a 58.42cm by 58.42cm square maze set inside a sound-attenuated room (see Appendix A). The maze was divided into 5 parallel chambers of equal area, each of which had 8cm by 9.5cm openings at each end to allow subjects to enter and exit each chamber. One end of the maze was replaced with a wire screen to allow sound to pass more easily through the maze. A speaker concealed behind the wire screen on the outside of the maze was used to present auditory stimuli throughout the extent of each experimental trial.

The extent of each trial was recorded using a ceiling mounted video camera and was simultaneously assessed using such measures as immobile duration, latency to exit the first chamber of the maze, mean velocity, total distance traveled, latency to complete the maze, and percentage of the maze explored using Noldus Ethovision XT tracking software.

Results

Chicks' naive postnatal preferences to stay at a close proximity or at an extended proximity of each auditory stimulus were assessed. This revealed that chicks whose starting position was near the maternal vocalization preferred to stay at a close proximity of that auditory stimulus ($Z = -3.65$, $p < .001$, two-tailed) rather than move away from it, thereby demonstrating the attractive properties of that auditory stimulus. Contrarily, chicks whose starting position was opposite the novel tone preferred to stay at an extended proximity of that stimulus ($Z = -2.68$, $p < .01$, two-tailed) rather than move toward it, thereby demonstrating the aversive properties of that stimulus.

Chicks tested in the Approach condition differed significantly from those in the Avoidance condition across several different behavioral measures. Chicks in the Approach condition demonstrated significantly shorter immobile duration, $U(58) = 331.00$, $Z = -1.76$, $p \leq .05$ (one-tailed), decreased latency to emerge from the initial chamber of the maze, $U(58) = 330.50$, $Z = -1.77$, $p \leq .05$ (one-tailed), increased mean velocity of movement, $U(58) = 326.00$, $Z = -1.83$, $p \leq .05$ (one-tailed), a greater percentage of the maze explored, $U(58) = 339.50$, $Z = -1.63$, $p \leq .05$ (one-tailed), decreased latency to complete the maze, $U(58) = 274.50$, $Z = -2.63$, $p \leq .01$ (one-tailed), decreased latency to complete the maze after emerging from the initial chamber of the maze, $U(58) = 331.00$, $Z = -1.76$, $p \leq .05$ (one-tailed), and a greater total distance traveled throughout the maze, $U(58) = 321.00$, $Z = -1.91$, $p \leq .05$ (one-tailed), than chicks tested in the Avoidance condition.

Summary

The purpose of this study was to investigate how level of emotional reactivity could be altered within testing trials through the introduction of different auditory stimuli and to assess how these alteration may influence motivation to explore in a manner as to approach or withdraw from these auditory stimuli. The results of the study identified several viable behavioral indicators for measuring this phenomenon which revealed that exploration motivation increases when chicks are tasked with approaching an attractive auditory stimulus, and decreases when they are expected to withdraw from an aversive auditory stimulus. Perhaps more importantly, this study revealed that naïve chicks, having never previously heard either sound, demonstrate increased levels of emotional reactivity when tested in the presence of the novel tone auditory stimulus compared to those tested in the presence of the maternal vocalization. Additional experimentation is required to determine what experiential factors may be influencing this differentiation of behavior so early in development.

CHAPTER IV

RESEARCH DESIGN

The current study was designed to provide insight into how early exposure to aversive stimuli may influence the expression of fearfulness later in life when individuals are presented with situations in which those previously exposed aversive stimuli may be present. Further, the study also aimed to examine how exposure to specific aversive stimuli early in life may subsequently influence level of fearfulness in the presence of aversive stimuli that are markedly different from those which subjects had previously experienced. On the basis of previous research (Suarez, 2012), which demonstrated that bobwhite quail neonates are capable of differentiating between novel auditory stimuli, as well as exhibiting differential levels of fearfulness and motivation for exploration in the presence of these different auditory stimuli, the current study investigated how prenatal and early postnatal exposure may serve to reduce the aversive properties of novel stimuli, as well as to potentially simultaneously increase their attractiveness (see Chapter III).

In the current study, the effect of early-life exposure to aversive auditory and visual stimuli on subsequent level of fearfulness was explored in bobwhite quail chicks (*Colinus virginianus)*. Prior experiments have demonstrated that chicks demonstrate a preference to approach and stay in close proximity to their species-specific maternal assembly call without having received any discrete form of exposure to that specific auditory stimulus earlier in life (Lickliter, 1989). This is thought to occur because of similarities in tone and frequency between this type of maternal call vocalization and the vocalizations chicks come in contact with prenatally and postnatally by means of their own self-stimulatory vocalizations and those produced by siblings (Gottlieb, 1970, 1975;

Lickliter, 1989). The current study aimed to determine how novelty plays a role in influencing how specific stimuli gain or lose aversive and attractive properties. It was hypothesized that eliminating the novelty of specific stimuli would decrease the aversive properties of those stimuli, thereby decreasing the level of fearfulness displayed when subsequently tested in the presence of those stimuli, when compared to naïve subjects. It was also hypothesized that exposure to one type of stimulus could potentially reduce the level of fearfulness displayed in the presence of different novel stimuli as a result of generalization effects, resulting in an overall decrease in propensity to fear or avoid novelty.

Experiment 1 tested the first of these hypotheses by exposing bobwhite quail chicks to a novel auditory tone for 24 hours immediately after hatching. This postnatal exposure, present continually at a rate of 15 tones per minute was predicted to decrease the aversive properties of the auditory stimulus by demonstrating to the chicks that no negative effects were associated with the sound. Furthermore, it was hypothesized that beyond only decreasing the aversive properties of the tone, postnatal exposure to the novel auditory stimulus would also act to increase the attractive properties of the tone because of possible pairings made between the comfortable rearing environment (access to food, water, siblings, etc.) in which the tone was presented, and the tone itself. The effects of postnatal exposure to the auditory tone stimulus were tested at 24 hours of age, at which time chicks' motivation to explore a novel maze task in the presence of the auditory tone was assessed.

Experiment 2 was designed to extend the findings of Experiment 1 to the prenatal period. Similar to Experiment 1, prenatal exposure to a novel auditory stimulus was predicted to decrease the aversive properties of the auditory stimulus, while potentially increasing its attractive properties due to pairings that may have been created between the auditory tone and the chicks' prenatal environment. The findings of this experiment were compared with those of Experiment 1 to determine if there were any different effects of prenatal vs. postnatal auditory exposure. The effects of prenatal exposure to the auditory tone stimulus were tested at 24 hours of age using identical methods to those employed in Experiment 1.

Experiment 3 was designed to determine whether exposure to novelty could serve to decrease the aversive properties of not only auditory stimuli, but to stimuli perceived by a different sense modality. Experiment 3 exposed chicks to a novel visual stimulus resembling a hawk in flight for 24 hours immediately following hatching. This type of exposure was predicted to decrease the aversive properties of the visual stimulus by familiarizing the chicks with the stimulus, revealing that it poses no imminent threat. The effects of postnatal exposure to the visual stimulus were assessed at 24 hours of age by testing chicks' motivation to exit an emergence box and explore a novel T-maze while in the presence of the visual stimulus.

Experiment 4 tested the hypothesis that postnatal exposure to one type of stimulus may reduce the level of fearfulness displayed in the presence of a different novel stimulus. It was predicted that 24 hours of postnatal exposure to a novel auditory tone would result in chicks demonstrating decreased fearfulness when subsequently tested in the presence of a novel visual stimulus. Similarly, it was hypothesized that 24 hours of postnatal exposure to the novel visual stimulus would result in decreased fearfulness when subsequently tested in the presence of the novel auditory stimulus. The results of this experiment were compared to those of Experiments 1, 2, and 3 to determine the degree to which fear of novelty is capable of being decreased across audio/visual stimuli, relative to the levels of change found when more direct exposure methods were utilized.

Research Aims & Hypotheses

The following research questions were addressed in this study (predicted results are shown in Table 1):

Research Aim 1: Having been postnatally exposed to a novel auditory stimulus for 24 hours, will subjects demonstrate decreased levels of fearfulness as measured by increased willingness to explore a novel maze environment, while in the presence of the previously exposed auditory stimulus?

Hypothesis 1: Chicks postnatally exposed to the auditory tone stimulus for 24 hours will demonstrate increased motivation to explore a novel maze environment when tested in the presence of the auditory tone, thereby indicating that they are less fearful in the presence of that auditory stimulus when compared to naïve chicks. If the auditory tone stimulus has had its aversive properties reduced, as well as its attractive properties increased, then we should expect that these exposed chicks would demonstrate similar behavioral patterns to those chicks tested in the presence of the maternal assembly call auditory stimulus.

Research Aim 2: Having been prenatally exposed to a novel auditory stimulus for 24 hours, will subjects demonstrate decreased levels of fearfulness by means of increased willingness to explore a novel maze environment, while in the presence of the previously exposed auditory stimulus?

Hypothesis 2: Chicks prenatally exposed to the auditory tone stimulus for 24 hours will demonstrate increased motivation to explore a novel maze environment when tested in the presence of the auditory tone, thereby indicating that they are less fearful in the presence of that auditory stimulus when compared to naïve chicks. If the auditory tone stimulus has had its aversive properties reduced, as well as its attractive properties increased, then we should expect that these exposed chicks will demonstrate similar behavioral patterns to those chicks tested in the presence of the maternal assembly call auditory stimulus.

Research Aim 3: Is one method of exposure, either prenatal or postnatal, more effective than the other in reducing fearfulness in the presence of the previously exposed auditory stimulus?

Hypothesis 3: Both prenatal and postnatal exposure will serve to decrease chicks' fearfulness when subsequently tested in the presence of the auditory tone stimulus. The extent to which effects differ between exposure groups is difficult to predict. If any significant differences are detected, they will likely be due to the environment present during the time of exposure, suggesting that postnatal exposure may demonstrate more pronounced effects as a result.

Research Aim 4: Having been postnatally exposed to a novel visual stimulus for 24 hours, will subjects demonstrate decreased levels of fearfulness by means of increased willingness to exit from an emergence box and subsequently explore a novel T-maze, while in the presence of the previously exposed visual stimulus?

Hypothesis 4: Chicks postnatally exposed to the novel visual stimulus for 24 hours will demonstrate increased motivation to exit from an emergence box and explore a novel T-maze when tested in the presence of the visual stimulus, thereby indicating that they are less fearful in the presence of that stimulus when compared to naïve chicks.

Research Aim 5: Having been postnatally exposed to a novel auditory stimulus for 24 hours, will subjects demonstrate decreased levels of fearfulness by means of increased willingness to exit from an emergence box and subsequently explore a novel T-maze, while in the presence of a novel visual stimulus?

Hypothesis 5: Chicks postnatally exposed to the novel auditory tone stimulus for 24 hours will make a generalization from that auditory tone that will extend into other domains, thereby potentially decreasing the amount of fearfulness displayed in the presence of any novel stimuli, including the novel visual stimulus.

Research Aim 6: Having been postnatally exposed to a novel visual stimulus for 24 hours, will subjects demonstrate decreased levels of fearfulness by means of increased willingness to explore a novel maze task, when in the presence of a novel visual stimulus?

Hypothesis 6: Chicks postnatally exposed to the novel visual stimulus for 24 hours will make a generalization from that stimulus that will extend to other domains, thereby potentially decreasing the amount of fearfulness displayed in the presence of any novel stimuli, including a novel auditory stimulus.

Table 1

Outline of Hypotheses Tested

Relative Willingness to Explore Novel Environment		
Postnatal Auditory Tone Exposed - Auditory Tone Tested	>	Naïve Subjects - Auditory Tone Tested
Postnatal Auditory Tone Exposed - Auditory Tone Tested	=/<	Naïve Subjects - Maternal Assembly Call Tested
Prenatal Auditory Tone Exposed - Auditory Tone Tested	>	Naïve Subjects - Auditory Tone Tested
Prenatal Auditory Tone Exposed - Auditory Tone Tested	=/<	Naïve Subjects - Maternal Assembly Call Tested
Prenatal Auditory Tone Exposed - Auditory Tone Tested	=/<	Postnatal Auditory Tone Exposed - Auditory Tone Tested
Postnatal Visual Stimulus Exposed - Visual Stimulus Tested	>	Naïve Subjects - Visual Stimulus Tested
Postnatal Auditory Tone Exposed - Visual Stimulus Tested	>	Naïve Subjects - Visual Stimulus Tested
Postnatal Visual Stimulus Exposed - Auditory Tone Tested	>	Naïve Subjects - Auditory Tone Tested
Postnatal Visual Stimulus Exposed - Auditory Tone Tested	=/<	Naïve Subjects - Maternal Assembly Call Tested
Postnatal Auditory Tone Exposed - Visual Stimulus Tested	=/<	Postnatal Auditory Tone Exposed - Auditory Tone Tested
Postnatal Visual Stimulus Exposed - Auditory Tone Tested	=/<	Postnatal Visual Stimulus Exposed - Visual Stimulus Tested

“=/<” : No significant difference expected, if it does exist, it would be predicted in the direction outlined.

CHAPTER V

GENERAL METHOD

Subjects

Subjects were 450 incubator reared bobwhite quail chicks (*Colinus virginianus*). Fertilized, unincubated eggs were received weekly from a commercial game bird supplier (Stickland) and set in an incubator maintained at 37.5˚C, with a relative humidity of 75-80%. Embryonic age was calculated on the basis of the first day of incubation as Day 0, and so forth. To control for possible variations in developmental age, only those birds that hatched on Day 23 were used as subjects. Following hatching, chicks were transferred into standard clear plastic rearing tubs in groups of 12-15 to replicate typical brood conditions, and placed in a sound-attenuated rearing room maintained at approximately 30˚ C. Chicks were given constant access to food and water, except during testing sessions.

Apparatuses

Behavioral tests were conducted using two different apparatuses. Experiments 1 and 2 were conducted within a 58.42cm by 58.42cm square maze set inside a sound attenuated room (see Appendix A). This maze was divided into 5 parallel chambers of equal area, each measuring 58.42cm by 11.74cm. Each dividing panel included an 8cm by 9.5cm opening cut out at the end opposite of where the subject could enter each chamber. One wall of the maze was removed and replaced with a wire screen to allow sound to pass through the maze more easily. A speaker concealed behind the wire screen on the outside of the maze was used to present auditory stimuli throughout the extent of each experimental trial.

Experiment 3 was conducted using an emergence box (20.32cm x 20.32cm x 20.32cm) attached to a T-maze (Start Arm: 47cm (L) x 11.43cm (W) x 20.32cm (H) Cross Arms: 71cm (L) x 11.43cm (W) x 20.32cm (H)) placed inside a sound attenuated room (see Appendix B). The emergence box includes a doorway that could be opened and closed remotely without entering the testing room, thereby minimizing direct human influence on subjects during testing. This doorway was used to either grant or deny passage between the emergence box and the T-maze. A predatory hawk visual stimulus (34.25cm (L) x 80cm (W) x 30.50cm (H)) was suspended above the emergence box and its wings spun to simulate flight when testing visual experimental conditions (see Appendix C).

Experiment 4 was conducted using both apparatuses described previously.

A video camera was mounted directly above each testing apparatus and connected to a computer located outside of the testing room. Noldus Ethovision XT tracking software was used to automatically record behavioral measures in real time during the course of each trial.

Auditory / Visual Stimuli

Two auditory stimuli and one visual stimulus were presented across different experimental conditions. Select experimental conditions utilized a bobwhite maternal assembly call vocalization, calibrated to 65dB at the end of the maze closest to the speaker, which played continually throughout each testing trial. Experimental conditions utilizing a novel tone auditory stimulus (120 Hz) during testing played the sound continually at a rate of 15 tones/minute, calibrated to 65dB at the end of the maze closest to the speaker, for the entire duration of each trial. Postnatal exposure to the novel tone presented in select experimental conditions was accomplished by placing a small speaker within the chicks' rearing tubs and playing the auditory stimulus, calibrated to 65 dB at a 10cm distance from the speaker, continually for 24 hours prior to testing. Prenatal exposure to the novel tone was presented in select experimental conditions by placing a small speaker within the chicks' incubator and continually playing the auditory stimulus, calibrated to 75dB (to account for the attenuating effect of egg shells on sound) at a 10cm distance from the eggs, continually for 24 hours prior to hatching.

Experimental conditions requiring the use of a novel visual stimulus utilized a predatory hawk visual stimulus (34.25cm (L) x 80cm (W) x 30.50cm (H) which was suspended above the emergence box while its wings spun in a circular manner, to simulate flight. Similarly, conditions requiring postnatal exposure to this stimulus were carried out by suspending the moving predatory hawk visual stimulus above the chicks' rearing tubs for 24 hours prior to testing.

Procedure

Bobwhite quail chicks were divided into 15 experimental conditions, of which 11 were tested within the square maze (Appendix A) and four were tested using the emergence box / T-maze (Appendix B). The conditions tested within the square maze consisted of: two control conditions in which chicks received no prior exposure to any auditory stimuli before testing and were tested without the presence of any auditory stimuli (n = 30 per condition), two conditions in which chicks received no exposure to any auditory stimuli prior to testing and were tested in the presence of a bobwhite maternal assembly call vocalization (n = 30 per condition), two conditions in which chicks received no exposure to any auditory stimuli prior to testing and were tested in the presence of a novel tone auditory stimulus (n = 30 per condition), two conditions in which chicks received 24 hours of postnatal exposure to the novel tone auditory stimulus and were subsequently tested in the presence of that same auditory stimulus (n = 30 per condition), two conditions in which chicks received 24 hours of prenatal exposure to the novel tone auditory stimulus and were subsequently tested in the presence of that same auditory stimulus (n = 30 per condition), and one condition in which chicks received 24 hours of postnatal exposure to a novel visual stimulus and were subsequently tested in the presence of the novel tone auditory stimulus (n = 30).

Square maze testing consisted of 20 min (1200s) trials in which individual chicks were evaluated for behavioral indicators of fearfulness by means of willingness to explore their novel maze environment under differing experimental conditions. Behavioral tracking was set to commence immediately after a subject was placed in the testing apparatus and continued for the extent of each testing trial. Half of the conditions tested in this maze consisted of chicks starting each of their testing trials at an extended proximity of the speaker located at one end of the maze, while the other half started each of their testing trials at a close proximity of the speaker (with the exception of those chicks who received 24 hours of postnatal exposure to the novel visual stimulus which always started at an extended proximity of the speaker) (see Appendix A).

The conditions tested using the emergence box / T-maze consisted of: a condition in which chicks received no prior exposure to either visual or auditory stimuli and were also tested without the presence of any stimuli (n = 30), a condition in which chicks received no prior exposure to either visual or auditory stimuli and were tested in the presence of the novel visual stimulus (n = 30), a condition in which chicks received 24 hours of postnatal exposure to the novel visual stimulus and were subsequently tested in the presence of that same visual stimulus (n = 30), and a condition in which chicks received 24 hours of postnatal exposure to the novel tone auditory stimulus and were subsequently tested in the presence of the novel visual stimulus (n = 30).

Emergence box / T-maze testing consisted of 6 min (360s) trials in which individual chicks were evaluated for behavioral indicators of fearfulness by means of willingness to emerge into a novel environment and propensity to explore a novel T-maze under differing experimental conditions. Each testing trial began with an individual chick being placed into the closed emergence box and allowed to become accustomed to its novel environment for 1 min (60s). After this time, a door on one side of the box was remotely opened providing the chick with the opportunity to explore an attached T-maze for the remaining 5 min (300s) of each testing trial. Upon initial emergence into the T-maze, the emergence box was promptly closed to prohibit re-entry as well as to encourage further exploration of the T-maze.

All subjects were tested individually 24 hours after hatch, starting at approximately 12pm each week to control for developmental age and other potential daily rhythm variables. To avoid any effects that may arise from social isolation prior to testing, the last four birds in each rearing tub remained untested. Each subject was transferred from the rearing room by hand and placed at its corresponding starting point, at which time Noldus Ethovision XT automatically began recording all subsequent movement throughout the session. Following testing, subjects were transferred back to the rearing room and placed in a separate rearing tub from those chicks that had yet to be tested.

Data Analyses

Results from Suarez (2012) were expanded upon and reanalyzed using different statistical methods so as to allow direct comparisons between findings from that study and the present study.

Relevant dependent variables in each experiment were measures of motivation to explore either a novel square maze, or a novel emergence box / T-maze, from which it is possible to deduce relative levels of fearfulness between experimental conditions. Experimental conditions utilizing the square maze during testing relied on the following measures of exploration motivation: latency to emerge from the initial chamber of the maze, latency to maze completion (90% of maze explored), latency to maze completion excluding initial time spent in the first chamber of the maze prior to emerging into the second, percentage of the maze explored, distance traveled throughout testing trials, duration of time spent immobile (subject moving < 1cm per second) throughout testing trials, and mean velocity of movement throughout testing trials. Duration of time spent in close proximity of the sound playing (time spent within the 2 chambers of the maze closest to the origin of the sound) and duration of time spent at an extended proximity of the sound playing (time spent within the 2 chambers of the maze furthest from the origin of the sound) were also measured.

Experimental conditions utilizing the emergence box / T-maze during testing relied on the following measures of exploration motivation: latency to emerge from the box, percentage of the T-maze explored, distance traveled throughout testing trials, duration of time spent immobile (subject moving < 1cm per second) throughout testing trials, and mean velocity of movement throughout testing trials.

As a result of highly variable sample distributions and ceiling effects, non-parametric analyses were used across all experimental conditions for each measure. The Kruskal-Wallis one-way analysis of variance was used to evaluate overall between-group differences for each measure. Post-hoc Kruskal-Wallis tests were performed for every possible 1:1 group comparison within each measure, regardless of whether the initial Kruskal-Wallis detected overall between-group differences or not. One-tailed or two-tailed significance levels of $p < .05$ were used to evaluate all results depending on whether or not directional hypotheses were made at the onset of the study.

On the basis of preliminary findings, a criterion for detecting a difference in level of fearfulness between groups was set. The criterion states that two groups must demonstrate statistically significant differences in at least three measures of exploration motivation to be considered as having differing levels of fearfulness. Groups that did not meet this standard were not said to differ in level of fearfulness.

CHAPTER VI

PRELIMINARY ANALYSES

Kruskal-Wallis One-Way Analysis of Variance

In order to make comparisons across experimental groups, Kruskal-Wallis tests were performed across all experimental groups sharing the same experimental apparatus (11 conditions tested using the square maze & 4 conditions tested using the emergence box / T-maze), for each measure of exploration motivation. These tests were also performed on measures of time spent in close proximity of auditory stimulus, and time spent at an extended proximity of auditory stimulus during testing. Following these tests, post-hoc Kruskal-Wallis tests were performed to compare individual conditions to one another for each measure.

A Kruskal-Wallis H test revealed that there was a statistically significant difference in latency to complete the novel maze task between the different experimental conditions tested within the square maze ($H(10) = 32.585$, $p < .001$). Among these groups, Kruskal-Wallis H tests also revealed significant differences in latency to complete the novel maze task after emerging from the first chamber of the maze ($H(10) = 30.214$, $p = .001$), percentage of the maze explored ($H(10) = 33.912$, $p < .001$), total distance traveled throughout testing trials ($H(10) = 22.779$, $p = .012$), and mean velocity ($H(10) = 22.330$, $p = .014$). Significant differences were also found in measures of time spent in close proximity of the auditory stimulus ($H(8) = 97.599$, $p < .001$) and time spent at an extended proximity of the auditory stimulus ($H(8) = 100.066$, $p < .001$) during testing.

No significant differences were detected among these groups for measures of latency to emerge from the first chamber of the maze (*H*(10) = 14.554, *p* = .149) and immobile duration (*H*(10) = 13.906, *p* = .177). Despite these insignificant findings, post-hoc tests were still carried out on these measures to address potential problems with detection caused by high levels of variability, ceiling effects, and the directional hypotheses employed in this study.

Kruskal-Wallis H tests were also carried out on the experimental conditions tested within the emergence box / T-maze. These tests revealed significant differences in total distance traveled (*H*(3) = 8.62, *p* = .035), latency to exit the emergence box (*H*(3) = 8.42 *p* = .038), immobile duration (*H*(3) = 7.799 *p* = .050), and mean velocity of movement (*H*(3) = 8.55 *p* = .036). A Kruskal-Wallis test performed on the measure of percentage of the T-maze explored did not reveal any significant differences between groups (*H*(3) = 7.63, *p* = .054), however, due to the very close approximation to the significance value (*p* ≤ .05), post-hoc tests were performed on this measure as well.

Determining Fearfulness Criterion

Level of fearfulness is often assessed behaviorally in animal species by analyzing subjects' willingness to explore novel environments (Miller et al., 2005; Suarez, 2012). Although several different explorative measures have been shown to be effective indicators of fearfulness in different quail species, no single behavioral measure is capable of reliably measuring level of fearfulness across different individual subjects or across different testing procedures (Miller et al., 2005; 2006). For this reason, it is good practice to put in place specific criteria, based on a wide variety of behavioral measures, for assessing level of fearfulness.

A criterion for assessing relative level of fearfulness is necessary to more confidently identify differences between experimental conditions in this study. In order to most fairly create this criterion, a preliminary comparison was made between naïve chicks tasked with approaching the maternal assembly call vocalization, and naïve chicks tasked with approaching the novel auditory stimulus. This comparison was chosen because these groups were hypothesized to demonstrate significant differences in willingness to explore their novel maze environment, while at the same time controlling for starting side biases.

Comparisons between the Naïve Subjects – Maternal Call Tested – Extended Proximity condition and the Naïve Subjects – Auditory Tone Tested – Extended Proximity condition revealed that chicks tested in the presence of the maternal assembly call completed the novel maze task more quickly than those tested in the presence of the auditory tone stimulus ($H(1) = 8.673$, $p < .005$ (one-tailed)), as well as having a shorter latency to complete the maze after emerging from the first chamber of the maze ($H(1) = 5.362$, $p < .05$ (one-tailed)). However, these two groups did not significantly differ in measures of latency to emerge from the first chamber of the maze ($H(1) = 2.337$, $p = .063$ (one-tailed)), percentage of the maze explored ($H(1) = 1.434$, $p = .116$ (one-tailed)), total distance traveled ($H(1) = 0.003$, $p = .480$ (one-tailed)), immobile duration ($H(1) = .283$, $p = .297$ (one-tailed)), or mean velocity of movement ($H(1) = 0.003$, $p = .480$ (one-tailed)).

On the basis of this comparison, a criterion for assessing relative level of fearfulness between experimental conditions was established. The criterion states that in order for a group to be considered less fearful than another group, that group must demonstrate significantly greater willingness to explore across *three* or more behavioral measures in comparison with the other group (one more than in the comparison groups used as a standard). Comparison groups not meeting this standard were considered to have shown no significant overall difference in relative level of fearfulness.

CHAPTER VII

EXPERIMENT 1

Effects of Postnatal Auditory Exposure on Fearfulness

Prior experiments have demonstrated that level of fearfulness and willingness to explore can be differentially influenced by exposure to different types of auditory stimuli (Suarez, 2012). In this previous study, bobwhite quail chicks were tested within a novel maze environment at 24 hours of age in a variety of experimental conditions. Naïve chicks were either tested within the maze in the presence of a bobwhite maternal assembly call vocalization or in the presence of a novel auditory tone and assessed for differing levels of fearfulness and willingness to explore. The results of this study demonstrated that chicks found the bobwhite maternal assembly call vocalization to be generally attractive, whereas chicks tested in the presence of the novel auditory tone generally found it to be aversive. Furthermore, chicks tested in the presence of the bobwhite maternal call displayed increased willingness to explore and reduced fearfulness when compared to chicks tested in the presence of the novel auditory tone. These results suggest that the novel properties of the auditory tone likely played a significant role in increasing its aversiveness to chicks, compared to the maternal assembly call vocalization which, although also novel to the chicks, shares similarities in tone and frequency with chicks' own vocalizations and those of siblings.

This experiment aimed to investigate the role of novelty on how an auditory stimulus would influence level of fearfulness during subsequent testing. Therefore, the same testing procedures (i.e., apparatus used, auditory tone presented during testing) were used for this experiment as were used for the previous study (Suarez, 2012).

However, bobwhite quail chicks were now exposed *postnatally* to the auditory tone for the first 24 hours of development following hatching. It was hypothesized that this postnatal exposure to the auditory tone would effectively eliminate the novelty of the tone, thereby decreasing fearfulness during subsequent testing in the presence of the tone.

Method

Sixty bobwhite quail chicks, divided into 2 experimental conditions, (a) Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity and (b) Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity Tested, served as subjects and were compared with previous experimental findings. All chicks were exposed in groups of 12-15 to a 120 Hz novel auditory tone calibrated to 65dB continually for 24 hours at a rate of 15 tones/minute, immediately after hatching. At 24 hours of age, all chicks were tested individually within a novel maze task in the presence of the postnatally exposed auditory tone, utilizing the same protocol employed in our previous study (Suarez, 2012).

Results and Discussion

Results are shown in Tables 2-8 and Appendices D-L. Post-hoc Kruskal-Wallis tests revealed significant differences between experimental conditions among several different measures of exploratory behavior. Comparing measures of exploration between the Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity condition and the Naïve Subjects – Auditory Tone Tested – Extended Proximity condition demonstrated that chicks postnatally exposed to the auditory tone were generally less fearful during subsequent testing than naïve chicks. Exposed chicks displayed significantly shorter latencies to complete the novel maze ($H(1) = 6.454$, $p =$

.006 (one-tailed)), significantly shorter latencies to complete the novel maze after emerging from the initial chamber of the maze ($H(1) = 10.423$, $p < .001$ (one-tailed)), and significantly greater percentage of the maze explored ($H(1) = 4.193$, $p = .021$ (one-tailed)), when compared to naïve chicks tested under identical conditions. Measures of latency to emerge from the first chamber of the maze ($H(1) = 0.317$, $p = .287$ (one-tailed)), total distance traveled ($H(1) = 1.850$, $p = .087$ (one-tailed)), immobile duration ($H(1) = 0.443$, $p = .253$ (one-tailed)), and mean velocity of movement ($H(1) = 1.810$, $p = .090$ (one-tailed)) were not found to be significantly different between the two conditions.

Comparing measures of exploration between the Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity condition and the Naïve Subjects – Auditory Tone Tested – Close Proximity condition also demonstrated that chicks postnatally exposed to the auditory tone were generally less fearful during subsequent testing than naïve chicks. Exposed chicks traveled significantly greater distances throughout testing trials ($H(1) = 8.397$, $p = .002$ (one-tailed)), spent significantly less time immobile during testing trials ($H(1) = 3.638$, $p = 0.028$ (one-tailed)), and had significantly greater mean velocities ($H(1) = 7.725$, $p = .003$ (one-tailed)), compared to naïve chicks tested under identical conditions. Measures of latency to emerge from the first chamber of the maze ($H(1) = .197$, $p = .329$ (one-tailed)), latency to complete the maze ($H(1) = .497$, $p = .241$ (one-tailed)), latency to complete the maze after emerging from the first chamber of the maze ($H(1) = .484$, $p = .244$ (one-tailed)), and percentage of the maze explored ($H(1) = .001$, $p = .486$ (one-tailed)) were not found to be significantly different between the two conditions.

Comparing measures of exploration between the Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity condition and the Naïve Subjects – No Sound Control Tested – Extended Proximity condition revealed that exposed chicks tested in the presence of the auditory tone were less fearful than naïve chicks when tested within the novel maze without any sound present. Exposed chicks displayed significantly shorter latencies to complete the novel maze ($H(1) = 3.847$, $p = .050$ (two-tailed)), significantly shorter latencies to complete the novel maze after emerging from the initial chamber of the maze ($H(1) = 4.440$, $p = .035$ (two-tailed)), and significantly greater percentage of the maze explored ($H(1) = 4.284$, $p = .038$ (two-tailed)), compared to naïve chicks tested within a no-sound control condition. Measures of latency to emerge from the first chamber of the maze ($H(1) = 1.561$, $p = .211$ (two-tailed)), total distance traveled ($H(1) = 1.041$, $p = .308$ (two-tailed)), immobile duration ($H(1) = .087$, $p = .767$ (two-tailed)), and mean velocity of movement ($H(1) = 1.041$, $p = .308$ (two-tailed)) were not found to be significantly different between the two conditions.

Comparing measures of exploration between the Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity condition and the Naïve Subjects – No Sound Control Tested – Close Proximity condition did not reveal a significant overall difference in level of fearfulness between groups. Although exposed chicks tested in the presence of the auditory tone explored significantly more of the maze ($H(1) = 4.596$, $p = .032$ (two-tailed)) than naïve chicks tested in the no-sound control condition, they did not differ significantly in measures of latency to emerge from the first chamber of the maze ($H(1) = 1.771$, $p = .183$ (two-tailed)), latency to complete the maze ($H(1) = .000$, $p = .988$ (two-tailed)), latency to complete the maze after emerging from the first chamber of the maze (*H*(1) = .350, *p* = .554 (two-tailed)), total distance traveled throughout testing trials (*H*(1) = 2.230, *p* = .135 (two-tailed)), immobile duration (*H*(1) = .591, *p* = .442 (two-tailed)), or mean velocity of movement throughout testing trials (*H*(1) = 2.142, *p* = .143 (two-tailed)).

Comparing measures of exploration between the Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity condition and the Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity condition also did not reveal a significant difference in fearfulness between groups. No significant differences were found for any measures between groups. These measures included latency to emerge from the first chamber of the maze (*H*(1) = 1.543, *p* = .214 (two-tailed)), latency to complete the maze (*H*(1) = .343, *p* = .558 (two-tailed)), latency to complete the maze after emerging from the first chamber of the maze (*H*(1) = .404, *p* = .525 (two-tailed)), percent of the maze explored (*H*(1) = 1.617, *p* = .204 (two-tailed)), total distance traveled (*H*(1) = 1.041, *p* = .308 (two-tailed)), immobile duration (*H*(1) = .014, *p* = .906 (two-tailed)), and mean velocity of movement (*H*(1) = 1.071, *p* = .301 (two-tailed)). These groups also did not differ significantly in duration of time spent in close proximity (*H*(1) = 1.106, *p* = .293 (two-tailed)) or at an extended proximity (*H*(1) = .525, *p* = .469 (two-tailed)) of their respective auditory stimuli during testing.

Comparing measures of exploration between the Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity condition and the Naïve Subjects – Maternal Assembly Call Tested – Close Proximity condition did not reveal a significant difference in fearfulness between groups. Although exposed chicks demonstrated a shorter latency to complete the maze after emerging from the first chamber (*H*(1) =

4.412, $p = .036$ (two-tailed)) than naïve chicks tested in the presence of the maternal assembly call, they did not differ significantly differ in measures of latency to emerge from the first chamber of the maze ($H(1) = 1.656$, $p = .198$ (two-tailed)), latency to complete the maze ($H(1) = 3.629$, $p = .057$ (two-tailed), percentage of the maze explored ($H(1) = 2.186$, $p = .139$ (two-tailed)), total distance traveled ($H(1) = 1.693$, $p = .193$ (two-tailed)), immobile duration ($H(1) = .744$, $p = .379$ (two-tailed)), and mean velocity of movement ($H(1) = 1.693$, $p = .193$ (two-tailed)).

Comparing measures of exploration between the Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity condition and the Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity condition revealed increased motivation to explore the novel maze toward the direction of the auditory tone stimulus (greater in chicks starting at an extended proximity of the tone) rather than in the opposite direction of the stimulus. Chicks approaching the auditory tone stimulus demonstrated shorter latencies to complete the maze ($H(1) = 5.014$, $p = .025$ (two-tailed)), shorter latencies to complete the maze after emerging from the first chamber of the maze ($H(1) = 4.788$, $p = .029$ (two-tailed)), and greater percentages of the maze explored ($H(1) = 13.991$, $p < .001$ (two-tailed)) compared to chicks starting their testing trials at a close proximity of the tone. Measures of latency to emerge from the first chamber of the maze ($H(1) = 0.404$, $p = .525$ (two-tailed)), total distance traveled ($H(1) = 0.026$, $p = .871$ (two-tailed)), immobile duration ($H(1) = .171$, $p = .679$ (two-tailed)), and mean velocity of movement ($H(1) = 0.026$, $p = .871$ (two-tailed)) did not differ significantly between the two conditions.

Table 2

Experiment 1: Latency to Emerge from First Chamber of Maze

Experimental Conditions	Mean (in seconds)	Standard Deviation
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	269.86	307.91
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	293.34	303.35
Naïve Subjects – Auditory Tone Tested – Extended Proximity	451.59	498.27
Naïve Subjects – Auditory Tone Tested – Close Proximity	400.90	417.07
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	263.83	385.71
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	315.93	421.04
Naïve Subjects – No Sound Control Tested – Extended Proximity	277.08	354.51
Naïve Subjects – No Sound Control Tested – Close Proximity	334.59	345.01

Table 3

Experiment 1: Latency to Complete Maze

Experimental Conditions	Mean (in seconds)	Standard Deviation
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	698.01	395.31
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	510.99	411.55
Naïve Subjects – Auditory Tone Tested – Extended Proximity	800.93	438.69
Naïve Subjects – Auditory Tone Tested – Close Proximity	794.47	450.79
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	463.17	412.21
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	898.02	408.93
Naïve Subjects – No Sound Control Tested – Extended Proximity	697.43	390.70
Naïve Subjects – No Sound Control Tested – Close Proximity	686.06	407.36

Table 4

Experiment 1: Latency to Complete Maze After Emerging from First Chamber

Experimental Conditions	Mean (in seconds)	Standard Deviation
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	484.76	415.31
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	283.09	354.69
Naïve Subjects – Auditory Tone Tested – Extended Proximity	669.27	479.44
Naïve Subjects – Auditory Tone Tested – Close Proximity	593.74	457.18
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	362.01	449.24
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	744.213	434.53
Naïve Subjects – No Sound Control Tested – Extended Proximity	540.35	434.31
Naïve Subjects – No Sound Control Tested – Close Proximity	473.60	411.73

Table 5

Experiment 1: Percentage of Maze Explored

Experimental Conditions	Mean (%)	Standard Deviation
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	83.79	27.57
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	89.87	24.46
Naïve Subjects – Auditory Tone Tested – Extended Proximity	70.74	39.64
Naïve Subjects – Auditory Tone Tested – Close Proximity	70.04	37.70
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	85.55	32.85
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	63.92	34.94
Naïve Subjects – No Sound Control Tested – Extended Proximity	85.69	28.67
Naïve Subjects – No Sound Control Tested – Close Proximity	85.98	27.95

Table 6

Experiment 1: Total Distance Traveled

Experimental Conditions	Mean (in centimeters)	Standard Deviation
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	9614.69	5043.90
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	9668.91	5750.42
Naïve Subjects – Auditory Tone Tested – Extended Proximity	7557.55	5730.41
Naïve Subjects – Auditory Tone Tested – Close Proximity	5616.92	4693.34
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	8105.50	4324.84
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	8003.94	5485.34
Naïve Subjects – No Sound Control Tested – Extended Proximity	7696.71	4276.52
Naïve Subjects – No Sound Control Tested – Close Proximity	8071.27	4460.56

Table 7

Experiment 1: Immobile Duration

Experimental Conditions	Mean (in seconds)	Standard Deviation
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	764.23	216.21
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	782.45	232.43
Naïve Subjects – Auditory Tone Tested – Extended Proximity	836.98	268.82
Naïve Subjects – Auditory Tone Tested – Close Proximity	893.12	235.66
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	792.17	216.39
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	817.72	232.64
Naïve Subjects – No Sound Control Tested – Extended Proximity	814.42	215.15
Naïve Subjects – No Sound Control Tested – Close Proximity	800.48	219.34

Table 8

Experiment 1: Mean Velocity of Movement

Experimental Conditions	Mean (in centimeters / second)	Standard Deviation
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	8.46	4.22
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	8.02	4.79
Naïve Subjects – Auditory Tone Tested – Extended Proximity	6.30	4.78
Naïve Subjects – Auditory Tone Tested – Close Proximity	4.79	3.95
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	6.75	3.60
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	6.69	4.58
Naïve Subjects – No Sound Control Tested – Extended Proximity	6.42	3.57

Consistent with the hypothesis that fearfulness of a novel auditory stimulus could be decreased through postnatal exposure to that stimulus, I predicted that chicks tested in the presence of the auditory stimulus after having been exposed to that stimulus for 24 hours after hatching would demonstrate decreased fearfulness when compared to naïve chicks tested under identical parameters. Results revealed that chicks previously exposed to the auditory tone and then subsequently tested in the presence of that tone in a novel maze task were generally less fearful when starting in close proximity of the tone (three measures indicating increased exploration motivation) and at an extended proximity to the tone (also three measures indicating increased exploration motivation).

Comparisons between chicks postnatally exposed to the auditory tone, then subsequently tested in the presence of that tone, and naïve chicks tested without any sound present revealed mixed results. Comparisons made between these groups in which both groups started at a close proximity of the speaker did not reveal a significant relative difference in level of fearfulness (differing significantly in only one measure of exploration motivation). However, when these groups were compared sharing a starting position at an extended proximity to the speaker, exposed chicks tested in the presence of the tone demonstrated significantly decreased relative fearfulness when compared to no-sound controls (three measures indicating increased exploration motivation). These comparisons suggest that postnatal exposure to the auditory stimulus may have not only decreased its aversive properties, but also increased its attractive properties, thereby increasing motivation for chicks to explore toward the direction of the sound and decreasing motivation to explore in the opposite direction of the sound.

Comparisons between chicks postnatally exposed to the auditory tone, then subsequently tested in the presence of that tone, and naïve chicks tested in the presence of the maternal assembly call vocalization did not reveal a significant relative difference in level of fearfulness between groups. These results suggest that postnatal exposure to the auditory tone effectively worked to shift the aversive and attractive properties of the auditory tone to be equivalent to those of the maternal assembly call vocalization (a known attractive stimulus).

Lastly, comparisons made between chicks postnatally exposed to the auditory tone, then subsequently tested starting at either a close proximity or at an extended proximity of the tone revealed a significant difference in willingness to explore the novel maze environment. Postnatally exposed chicks starting their testing trials at an extended proximity of the auditory tone demonstrated increased willingness to explore the novel maze environment when compared to chicks beginning their testing trials at a close proximity to the auditory tone. These results suggest that chicks starting at an extended proximity to the auditory tone were either less fearful than their counterparts (three measures indicating increased exploration motivation), or more likely demonstrated increased motivation to explore in the direction of the auditory tone stimulus because of its increased attractiveness as a result of the postnatal exposure. In contrast, chicks starting near the auditory stimulus demonstrated decreased motivation to explore in the opposite direction of the auditory stimulus, likely because of its attractive properties.

CHAPTER VIII

EXPERIMENT 2

Effects of Prenatal Auditory Exposure on Fearfulness

The previous experiment in the present study investigated how 24 hours of early postnatal exposure to a novel auditory tone could decrease the aversive properties of that stimulus, in turn reducing the expression of fear-related behaviors during subsequent testing in the presence of the same auditory stimulus. Findings suggest that addressing novelty by means early postnatal exposure to the auditory stimulus effectively reduced chicks' expression of fearfulness during subsequent testing in the presence of the same auditory stimulus. Further indicators suggest that postnatal exposure may have not only reduced the aversive properties of the novel tone stimulus, but may have additionally increased its attractive properties to the same level as that of the bobwhite maternal assembly call vocalization (a known attractive stimulus).

Experiment 2 was designed to explore the extent to which *prenatal* exposure to a novel auditory stimulus reduces fearfulness during subsequent testing, and to determine the relative utility of prenatal exposure to postnatal exposure for this purpose. Therefore, the same testing procedures (i.e., apparatus used, auditory tone presented during testing) were used for this experiment as those employed in Experiment 1. However, bobwhite quail chicks were now exposed prenatally to the auditory tone for 24 hours immediately prior to hatching, as opposed to 24 hours immediately after hatching. It was hypothesized that this prenatal exposure to the auditory tone would effectively eliminate the novelty of the tone to a similar degree as previous findings on postnatal exposure, thereby decreasing fearfulness during subsequent testing in the presence of the tone.

Method

Sixty bobwhite quail chicks, divided into 2 experimental conditions, (a) Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity and (b) Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity Tested, served as subjects and were compared with previous experimental findings. All chicks were exposed in groups of 12-15 to a 120 Hz novel auditory tone calibrated to 75dB (to account for egg shell attenuation) continually for 24 hours at a rate of 15 tones/minute, immediately prior to hatching. At 24 hours of age, all chicks were tested individually within a novel maze task in the presence of the prenatally exposed auditory tone, utilizing the same protocol employed in Experiment 1.

Results and Discussions

Results are shown in Tables 9-15 and Appendices D-L. Post-hoc Kruskal-Wallis tests revealed significant differences between experimental conditions among several different measures of exploratory behavior. Comparing measures of exploration between the Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity condition and the Naïve Subjects – Auditory Tone Tested – Close Proximity condition demonstrated that chicks prenatally exposed to the auditory tone were generally less fearful during subsequent testing than naïve chicks. Prenatally exposed chicks had significantly shorter latencies to complete the novel maze task ($H(1) = 3.538$, $p = .030$ (one-tailed)), traveled significantly greater distances throughout testing trials ($H(1) = 3.200$, $p = .037$ (one-tailed)), and had significantly greater mean velocities ($H(1) = 2.742$, $p = .049$ (one-tailed)), compared to naïve chicks tested under identical conditions. Measures of latency to emerge from the first chamber of the maze ($H(1) = .121$, $p = .364$

(one-tailed)), latency to complete the maze after emerging from the first chamber of the maze (*H*(1) = 2.579, *p* = .054 (one-tailed)), percentage of the maze explored (*H*(1) = 1.133, *p* = .144 (one-tailed)), and immobile duration (*H*(1) = 1.471, *p* = .113 (one-tailed)) were not found to be significantly different between the two conditions.

Comparing measures of exploration between the Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity condition and the Naïve Subjects – Auditory Tone Tested – Extended Proximity condition did not reveal a significant overall difference in level of fearfulness between groups. Although prenatally exposed chicks tested in the presence of the auditory tone traveled significantly greater distances (*H*(1) = 2.742, *p* = .049 (one-tailed)) and had greater mean velocities (*H*(1) = 2.941, *p* = .043 (one-tailed)) than naïve chicks tested under the same conditions, they did not differ significantly in measures of latency to emerge from the first chamber of the maze (*H*(1) = 2.467, *p* = .058 (one-tailed)), latency to complete the maze (*H*(1) = 2.018, *p* = .078 (one-tailed)), latency to complete the maze after emerging from the first chamber of the maze (*H*(1) = 1.476, *p* = .112 (one-tailed)), percentage of the maze explored (*H*(1) = 1.911, *p* = .089 (one-tailed)), or immobile duration (*H*(1) = 2.143, *p* = .072 (one-tailed)).

Comparing measures of exploration between the Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity condition and the Naïve Subjects – No Sound Control Tested – Extended Proximity condition did not reveal a significant overall difference in level of fearfulness between groups. Although prenatally exposed chicks tested in the presence of the auditory tone had shorter latencies to emerge from the first chamber of the maze (*H*(1) = 8.231, *p* = .004 (two-tailed)) than naïve chicks tested in the no-sound control condition, they did not differ significantly in measures of latency to complete the maze (*H*(1) = .356, *p* = .550 (two-tailed)), latency to complete the maze after emerging from the first chamber of the maze (*H*(1) = .299, *p* = .584 (two-tailed)), percentage of the maze explored (*H*(1) = 1.263, *p* = .131 (one-tailed)), total distance traveled (*H*(1) = 2.597, *p* = .107 (two-tailed)), immobile duration (*H*(1) = 1.655, *p* = .198 (two-tailed)), or mean velocity of movement (*H*(1) = 2.841, *p* = .092 (two-tailed)).

Comparing measures of exploration between the Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity condition and the Naïve Subjects – No Sound Control Tested – Close Proximity condition also did not reveal a significant overall difference in level of fearfulness between groups. No significant differences were found among any measures of exploration between the two groups including latency to emerge from the first chamber of the maze (*H*(1) = .801, *p* = .371 (two-tailed)), latency to complete the maze (*H*(1) = 1.671, *p* = .196 (two-tailed)), latency to complete the maze after emerging from the first chamber of the maze (*H*(1) = 2.166, *p* = .141 (two-tailed)), percentage of the maze explored (*H*(1) = .022, *p* = .882 (two-tailed)), total distance traveled (*H*(1) = .002, *p* = .965 (two-tailed)), immobile duration (*H*(1) = .026, *p* = .871 (two-tailed)), and mean velocity of movement (*H*(1) = .003, *p* = .953 (two-tailed)).

Comparing measures of exploration between the Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity condition and the Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity condition also did not reveal a significant difference in fearfulness between groups. No significant differences were found for any measures between groups. These measures included latency to emerge from the first chamber of the maze (H(1) = .032, *p* = .859 (two-tailed)), latency to complete the maze (*H*(1) = 2.647, *p* = .104 (two-tailed)), latency to complete the maze after emerging from the first chamber of the maze (*H*(1) = 3.098, *p* = .078 (two-tailed)), percentage of the maze explored (*H*(1) = .423, *p* = .515 (two-tailed)), total distance traveled (*H*(1) = 2.550, *p* = .110 (two-tailed)), immobile duration (*H*(1) = 1.149, *p* = .284 (two-tailed)), and mean velocity of movement (*H*(1) = 2.645, *p* = .104 (two-tailed)). These groups also did not differ significantly in duration of time spent in close proximity (*H*(1) = 2.257, *p* = .133 (two-tailed)) or at an extended proximity (*H*(1) = .184, *p* = .668 (two-tailed)) of their respective auditory stimuli during testing.

Alternatively, comparing measures of exploration between the Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity condition and the Naïve Subjects – Maternal Assembly Call Tested – Close Proximity condition did reveal significant differences in motivation to explore the novel maze. Chicks exposed to the auditory tone, then tested in its presence demonstrated shorter latencies to complete the maze task (*H*(1) = 8.255, *p* = .004 (two-tailed)), shorter latencies to complete the maze after emerging from the first chamber of the maze (*H*(1) = 8.070, *p* = .004 (two-tailed)), and greater percentage of the maze explored (*H*(1) = 6.391, *p* = .011 (two-tailed)). These groups did not differ significantly in latency to emerge from the first chamber of the maze (*H*(1) = 1.366, *p* = .242 (two-tailed)), total distance traveled (*H*(1) = .005, *p* = .941 (two-tailed)), immobile duration (*H*(1) = .009, *p* = .923 (two-tailed)), or mean velocity of movement (*H*(1) = .014, *p* = .906 (two-tailed). These groups also differed significantly in duration of time spent in close proximity and at an extended proximity of their respective auditory stimuli. Chicks tested in the presence of the maternal assembly call vocalization spent significantly more time at a close proximity of the maternal call in comparison with the duration of time prenatally exposed chicks spent in close proximity of the auditory tone during testing ($H(1) = 20.747$, $p = .000$ (two-tailed)). Alternatively, prenatally exposed chicks spent significantly more time at an extended proximity of the auditory tone in comparison with the duration of time naive chicks spent at an extended proximity of the maternal assembly call during testing ($H(1) = 16.157$, $p = .003$ (two-tailed)).

Comparing measures of exploration between the Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity condition and the Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity condition did not reveal a significant overall difference in motivation to explore the novel maze between groups. Although chicks starting at a close proximity of the auditory tone stimulus demonstrated increased latencies to emerge from the initial chamber of the maze ($H(1) = 3.363$, $p = .067$ (two-tailed)) compared to chicks starting at an extended proximity of the tone, both groups did not differ significantly in measures of latency to complete the maze task ($H(1) = .255$, $p = .613$ (two-tailed)), latency to complete the maze after emerging from the first chamber of the maze ($H(1) = 1.618$, $p = .203$ (two-tailed)), percentage of the maze explored ($H(1) = 1.617$, $p = .204$ (two-tailed)), total distance traveled ($H(1) = 1.434$, $p = .231$ (two-tailed)), immobile duration ($H(1) = 1.953$, $p = .162$ (two-tailed)), or mean velocity of movement ($H(1) = 1.579$, $p = .209$ (two-tailed)).

Comparing measures of exploration between the Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity condition and the Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity condition did not reveal a significant overall difference in fearfulness between groups. Although prenatally exposed chicks had greater latencies to complete the maze after emerging from the initial chamber of the maze ($H(1) = 6.771$, $p = .009$ (two-tailed)) compared to postnatally exposed chicks, both groups did not differ significantly in latency to emerge from the first chamber of the maze (*H*(1) = 1.693, *p* = .193 (two-tailed)), latency to complete the maze (*H*(1) = 1.177, *p* = .278 (two-tailed)), percentage of the maze explored (*H*(1) = .197, *p* = .657 (two-tailed)), total distance traveled (*H*(1) = .253, *p* = .615 (two-tailed)), immobile duration (*H*(1) = 1.102, *p* = .294 (two-tailed)), or mean velocity of movement (*H*(1) = .283, *p* = .595 (two-tailed)).

Comparing measures of exploration between the Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity condition and the Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity condition did not reveal a significant overall difference in fearfulness between groups. Although prenatally exposed chicks explored a significantly greater percentage of the maze (*H*(1) = 4.532, *p* = .033 (two-tailed)) compared to postnatally exposed chicks, both groups did not differ significantly in measures of latency to emerge from the first chamber of the maze (*H*(1) = .001, *p* = .976 (two-tailed)), latency to complete the maze task (*H*(1) = 1.364, *p* = .243 (two-tailed)), latency to complete the maze after emerging from the first chamber of the maze (*H*(1) = 1.041, *p* = .308 (two-tailed)), total distance traveled (*H*(1) = .735, *p* = .391 (two-tailed)), immobile duration (*H*(1) = .341, *p* = .559 (two-tailed)), or mean velocity of movement (*H*(1) = .710, *p* = .399 (two-tailed)).

Table 9

Experiment 2: Latency to Emerge from First Chamber of Maze

Experimental Conditions	Mean (in seconds)	Standard Deviation
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	207.36	291.93
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	309.33	337.01
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	269.86	307.91
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	293.34	303.35
Naïve Subjects – Auditory Tone Tested – Extended Proximity	451.59	498.27
Naïve Subjects – Auditory Tone Tested – Close Proximity	400.90	417.07
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	263.83	385.71
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	315.93	421.04
Naïve Subjects – No Sound Control Tested – Extended Proximity	277.08	354.51
Naïve Subjects – No Sound Control Tested – Close Proximity	334.59	345.01

Table 10

Experiment 2: Latency to Complete Maze

Experimental Conditions	Mean (in seconds)	Standard Deviation
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	646.91	440.94
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	572.71	382.15
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	698.01	395.31
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	510.99	411.55
Naïve Subjects – Auditory Tone Tested – Extended Proximity	800.93	438.69
Naïve Subjects – Auditory Tone Tested – Close Proximity	794.47	450.79
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	463.17	412.21
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	898.02	408.93
Naïve Subjects – No Sound Control Tested – Extended Proximity	697.43	390.70
Naïve Subjects – No Sound Control Tested – Close Proximity	686.06	407.36

Table 11

Experiment 2: Latency to Complete Maze After Emerging from First Chamber

Experimental Conditions	Mean (in seconds)	Standard Deviation
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	519.45	416.47
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	383.37	388.70
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	484.76	415.31
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	283.09	354.69
Naïve Subjects – Auditory Tone Tested – Extended Proximity	669.27	479.44
Naïve Subjects – Auditory Tone Tested – Close Proximity	593.74	457.18
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	362.01	449.24
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	744.213	434.53
Naïve Subjects – No Sound Control Tested – Extended Proximity	540.35	434.31
Naïve Subjects – No Sound Control Tested – Close Proximity	473.60	411.73

Table 12

Experiment 2: Percentage of Maze Explored

Experimental Conditions	Mean (%)	Standard Deviation
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	86.65	26.40
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	86.60	29.94
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	83.79	27.57
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	89.87	24.46
Naïve Subjects – Auditory Tone Tested – Extended Proximity	70.74	39.64
Naïve Subjects – Auditory Tone Tested – Close Proximity	70.04	37.70
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	85.55	32.85
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	63.92	34.94
Naïve Subjects – No Sound Control Tested – Extended Proximity	85.69	28.67
Naïve Subjects – No Sound Control Tested – Close Proximity	85.98	27.95

Table 13

Experiment 2: Total Distance Traveled

Experimental Conditions	Mean (in centimeters)	Standard Deviation
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	10069.81	5345.40
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	8829.84	6228.17
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	9614.69	5043.90
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	9668.91	5750.42
Naïve Subjects – Auditory Tone Tested – Extended Proximity	7557.55	5730.41
Naïve Subjects – Auditory Tone Tested – Close Proximity	5616.92	4693.34
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	8105.50	4324.84
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	8003.94	5485.34
Naïve Subjects – No Sound Control Tested – Extended Proximity	7696.71	4276.52
Naïve Subjects – No Sound Control Tested – Close Proximity	8071.27	4460.56

Table 14

Experiment 2: Immobile Duration

Experimental Conditions	Mean (in seconds)	Standard Deviation
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	726.90	240.59
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	798.16	253.35
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	764.23	216.21
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	782.45	232.43
Naïve Subjects – Auditory Tone Tested – Extended Proximity	836.98	268.82
Naïve Subjects – Auditory Tone Tested – Close Proximity	893.12	235.66
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	792.17	216.39
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	817.72	232.64
Naïve Subjects – No Sound Control Tested – Extended Proximity	814.42	215.15
Naïve Subjects – No Sound Control Tested – Close Proximity	800.48	219.34

Table 15

Experiment 2: Mean Velocity of Movement

Experimental Conditions	Mean (in centimeters / second)	Standard Deviation
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	8.46	4.43
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	7.38	5.17
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	8.46	4.22
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	8.02	4.79
Naïve Subjects – Auditory Tone Tested – Extended Proximity	6.30	4.78
Naïve Subjects – Auditory Tone Tested – Close Proximity	4.79	3.95
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	6.75	3.60
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	6.69	4.58
Naïve Subjects – No Sound Control Tested – Extended Proximity	6.42	3.57

Consistent with the hypothesis that fearfulness of a novel auditory stimulus could be decreased through prenatal exposure to that stimulus, I predicted that chicks tested in the presence of the auditory stimulus after having been exposed to that stimulus for 24 hours immediately prior to hatching would demonstrate decreased fearfulness when compared to naïve chicks tested under identical parameters. Results revealed that prenatally exposed chicks were generally less fearful during testing when starting at a close proximity of the auditory tone compared to naïve chicks (three measures indicating increased exploration motivation). However, prenatally exposed chicks starting at an extended proximity of the auditory tone did not meet the preset criterion to be able to confidently state that they differed from naïve chicks in level of fearfulness (only two measures indicating increased exploration motivation).

Comparisons between prenatal exposure conditions and no-sound control conditions did not yield any significant relative differences between groups suggesting that comparisons between experimental groups and no-sound control groups have limited utility within this testing paradigm.

Comparisons between chicks prenatally exposed to the auditory tone, then subsequently tested in the presence of that tone, and naïve chicks tested in the presence of the maternal assembly call vocalization yielded mixed results. Comparisons between these groups in which chicks began testing trials at an extended proximity of their respective auditory stimuli revealed no relative difference in level of fearfulness (not differing significantly in any measures of exploration motivation). However, when these groups were compared sharing a starting position at a close proximity of their respective auditory stimuli, prenatally exposed chicks tested in the presence of the auditory tone demonstrated increased willingness to explore the novel maze environment compared to naïve chicks tested in the presence of the maternal assembly call (three measures indicating increased exploration motivation). These results suggest that exposed chicks were either less fearful in the presence of the auditory tone than naïve chicks tested in the presence of the maternal call, or more likely demonstrated decreased motivation to stay at a close proximity of the auditory tone while simultaneously experiencing decreased fearfulness allowing exploratory behavior in the opposite direction of the auditory tone.

Comparisons between prenatally exposed conditions revealed no relative difference in level of fearfulness based on starting location in the maze (differing in only one measure of exploration motivation). These results suggest that level of fearfulness was consistent among prenatally exposed chicks whether they were started at either a close proximity or at an extended proximity of the auditory tone during testing. More importantly, these results reveal that chicks were not particularly motivated to approach or avoid the auditory tone (unlike postnatally exposed chicks) suggesting that although prenatal exposure effectively reduced the aversive properties of the auditory tone, it did not necessarily increase its attractive properties.

Lastly, comparisons between prenatal exposure groups and postnatal exposure groups did not reveal any significant relative differences in level of fearfulness. These results suggest that both prenatal and postnatal exposure to a novel auditory stimulus share similar utility in reducing the auditory stimulus’s aversive properties during subsequent testing, thereby reducing the expression of fearful behaviors to a similar extent among both exposure types.

CHAPTER IX

EXPERIMENT 3

Effects of Postnatal Visual Exposure on Fearfulness

Previous results have demonstrated that fear of a novel auditory stimulus can be diminished by means of prenatal exposure or early postnatal exposure to the auditory stimulus. Findings suggest that 24 hours of prenatal or postnatal exposure can decrease aversive properties associated with a novel auditory stimulus thereby allowing bobwhite quail (*Colinus virginianus*) chicks to exhibit fewer fearful behaviors during subsequent contact with the auditory stimulus. Although these results provide valuable information as to how fear of specific novel auditory stimuli can be diminished during early development, they provide little detail into the potential generalizability of this method across different sense modalities.

Experiment 3 was designed to explore the extent to which early postnatal exposure to a novel visual stimulus can reduce fearfulness during subsequent testing in the presence of the visual stimulus. Unlike previous experiments which utilized an artificial tone to assess fear of novelty, the present experiment utilized a more ecologically valid visual stimulus; a moving decoy mimicking a predatory hawk in flight. Although this type of visual stimulus is often believed to cause an “innate” fear within prey species, I hypothesized that fearful behaviors exhibited in the presence of the stimulus would stem from the novelty of the stimulus, rather than from any “evolutionarily inherited” properties of the stimulus. Therefore, it was hypothesized that postnatal exposure to the visual stimulus would effectively eliminate the novelty of the stimulus, thereby decreasing fearfulness during subsequent testing in its presence.

Method

Subjects consisted of ninety bobwhite quail chicks divided into 3 experimental conditions, (a) Naïve Subjects – No Visual Stimulus Tested, (b) Naïve Subjects – Visual Stimulus Tested, and (c) Postnatal Visual Stimulus Exposed – Visual Stimulus Tested. All chicks were reared in groups of 12-15 to mimic natural brooding conditions. Chicks in conditions A and B served as controls receiving no exposure to the visual stimulus prior to testing whereas chicks in condition C were postnatally exposed to the novel visual stimulus for 24 hours immediately following hatching (depicted in Appendix C). At 24 hours of age, all chicks were tested individually within a novel emergence box / T-maze task. During testing, conditions B and C were exposed to the visual stimulus, whereas chicks in condition A were tested in the absence of the visual stimulus.

Results and Discussion

Results are shown in Tables 16-20 and Appendices M-Q. Post-hoc Kruskal-Wallis tests revealed significant differences between groups tested in the presence of the visual stimulus. Comparing measures of exploration between the Naïve Subjects – Visual Stimulus Tested and the Postnatal Visual Stimulus Exposed – Visual Stimulus Tested conditions demonstrated that chicks postnatally exposed to the novel visual stimulus were generally less fearful during subsequent testing than naïve chicks. Exposed chicks displayed significantly greater distances traveled throughout testing ($H(1) = 5.390$, $p = .010$ (one-tailed)), greater mean velocity of movement ($H(1) = 5.388$, $p = .010$ (one-tailed)), and decreased immobile duration ($H(1) = 3.751$, $p = .027$ (one-tailed)), when compared to naïve chicks tested under identical conditions. Measures of latency to exit the emergence box ($H(1) = 1.676$, $p = .098$ (one-tailed) and percentage of the T-maze explored (*H*(1) = 2.037, *p* = .077 (one-tailed)) were not found to be significantly different between the two conditions.

Comparing measures of exploration between the Naïve Subjects – No Visual Stimulus Tested and the Naïve Subjects – Visual Stimulus Tested revealed no general difference in fearfulness between groups. Chicks in these conditions did not differ in measures of total distance traveled (*H*(1) = 2.057, *p* = .152 (two-tailed)), latency to exit the emergence box (*H*(1) = 1.485, *p* = .223 (two-tailed)), percentage of the T-maze explored (*H*(1) = 0.085, *p* = .771 (two-tailed)), immobile duration (*H*(1) = 0.895, *p* = .344 (two-tailed)), or mean velocity of movement (*H*(1) = 1.973, *p* = .160 (two-tailed)).

Comparing measures of exploration between the Naïve Subjects – No Visual Stimulus Tested and Postnatal Visual Stimulus Exposed – Visual Stimulus Tested conditions also revealed no difference in fearfulness between groups. Chicks in these conditions did not differ in measures of total distance traveled (*H*(1) = 0.920, *p* = .337 (two-tailed)), latency to exit the emergence box (*H*(1) = 0.193, *p* = .660 (two-tailed)), percentage of the T-maze explored (*H*(1) = 1.996, *p* = .158 (two-tailed)), immobile duration (*H*(1) = 1.133, *p* = .287 (two-tailed)), or mean velocity of movement (*H*(1) = 0.952, *p* = .329 (two-tailed)).

Table 16

Experiment 3: Latency to Exit from Emergence Box into T-Maze

Experimental Conditions	Mean (in seconds)	Standard Deviation
Naïve Subjects – No Visual Stimulus Tested –	179.05	114.62
Naïve Subjects – Visual Stimulus Tested –	207.93	114.85
Postnatal Visual Stimulus Exposed – Visual Stimulus Tested –	163.00	123.99

Table 17

Experiment 3: Total Distance Traveled

Experimental Conditions	Mean (in centimeters)	Standard Deviation
Naïve Subjects – No Visual Stimulus Tested –	1615.74	1338.63
Naïve Subjects – Visual Stimulus Tested –	1132.31	1111.19
Postnatal Visual Stimulus Exposed – Visual Stimulus Tested –	1982.42	1481.27

Table 18

Experiment 3: Percentage of T-Maze Explored

Experimental Conditions	Mean (%)	Standard Deviation
Naïve Subjects – No Visual Stimulus Tested –	35.71	44.91
Naïve Subjects – Visual Stimulus Tested –	42.24	48.22
Postnatal Visual Stimulus Exposed – Visual Stimulus Tested –	57.88	48.16

Table 19

Experiment 3: Immobile Duration

Experimental Conditions	Mean (in seconds)	Standard Deviation
Naïve Subjects – No Visual Stimulus Tested –	135.20	96.06
Naïve Subjects – Visual Stimulus Tested –	163.15	107.06
Postnatal Visual Stimulus Exposed – Visual Stimulus Tested –	116.65	103.02

Table 20

Experiment 3: Mean Velocity of Movement

Experimental Conditions	Mean (in centimeters / second)	Standard Deviation
Naïve Subjects – No Visual Stimulus Tested –	5.43	4.47
Naïve Subjects – Visual Stimulus Tested –	3.82	3.74
Postnatal Visual Stimulus Exposed – Visual Stimulus Tested –	6.66	4.97

Consistent with the hypothesis that fearfulness of a novel visual stimulus could be decreased through postnatal exposure to that stimulus, I predicted that chicks tested in the presence of the visual stimulus after having been exposed to that stimulus for 24 hours after hatching would demonstrate decreased fearfulness when compared to naïve chicks tested under identical parameters. Results revealed that chicks previously exposed to the novel visual stimulus and then subsequently tested in the presence of that visual stimulus were generally less fearful than naïve chicks tested in the presence of the novel visual stimulus. These results suggest that fearful behaviors exhibited in the presence of the visual stimulus were due to the novel properties of the visual stimulus as opposed to the specific physical properties of the stimulus. These results also support the prediction that early life exposure to novel stimuli can be utilized to decrease fear of not only auditory stimuli, but also visual stimuli and potentially those of other sense modalities.

CHAPTER X

EXPERIMENT 4

Generalizability of Postnatal Exposure across Novel Auditory and Visual Stimuli and its Effects on Fearfulness

Previous experiments have shown that early-life exposure to specific novel auditory and visual stimuli can reduce fearfulness during subsequent testing in the presence of those stimuli. Findings suggest that 24 hours of prenatal exposure to a novel auditory stimulus can effectively decrease its aversive properties thereby allowing bobwhite quail (*Colinus virginianus*) chicks to exhibit fewer fearful behaviors during subsequent contact with the auditory stimulus. Similarly, 24 hours of postnatal exposure to either a novel auditory stimulus or a novel visual stimulus has been shown to effectively decrease subsequent fearfulness in chicks when subsequently tested in the presence of the previously exposed stimulus. Although these results provide valuable insight as to how early-life exposure can be used to reduce fear of specific stimuli, more information is required to determine how this type of exposure can potentially influence the development of neophobia.

Experiment 4 was designed to explore the extent to which early postnatal exposure to a specific novel stimulus could generalize to subsequently decrease fear of a markedly different novel stimulus. This experiment tested this by combining the methods and testing procedures of the previous experiments. Bobwhite quail chicks were either exposed to a novel auditory stimulus, then tested in the presence of a novel visual stimulus, or exposed to a novel visual stimulus, then tested in the presence of a novel auditory stimulus. It was hypothesized that postnatal exposure to one type of novel stimulus would not only affect subsequent behavior in the presence of that specific stimulus, but would generally influence a wide range of novel stimuli, thereby decreasing fearfulness in the presence of a markedly different novel stimulus.

Method

Sixty bobwhite quail chicks, divided into 2 experimental conditions, (a) Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity and (b) Postnatal Auditory Tone Exposed – Visual Stimulus Tested, served as subjects and were compared with previous experimental findings. All chicks were reared in groups of 12-15 to mimic natural brooding conditions. Chicks in condition A were exposed to a novel visual stimulus (see Experiment 3) for 24 hours immediately following hatching, then tested within a novel maze task in the presence of a novel auditory tone stimulus (see Experiment 1-2). Chicks in condition B were exposed to a novel auditory tone stimulus (see Experiment 1-2) for 24 hours immediately following hatching, then tested within a novel emergence box / T-maze apparatus in the presence of a novel visual stimulus (see Experiment 3).

Results and Discussion

Results are shown in Tables 21-32 and Appendices D-Q. Post-hoc Kruskal-Wallis tests revealed significant differences between postnatally exposed groups and naïve groups tested in the presence of either novel visual or auditory stimulus. Comparisons between the Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity condition and the Naïve Subjects – Auditory Tone Tested – Extended Proximity condition demonstrated that chicks postnatally exposed to the novel visual stimulus, then subsequently tested in the presence of the novel tone auditory stimulus were generally less fearful compared to naïve chicks tested under identical conditions. Compared to naïve chicks, chicks exposed to the novel visual stimulus prior to being tested in the presence of the novel tone displayed significantly greater distance traveled throughout testing trials ($H(1) = 5.808$, $p = .008$ (one-tailed)), decreased latency to complete the maze task ($H(1) = 5.774$, $p = .008$ (one-tailed)), decreased latency to complete the maze after emerging from the first chamber of the maze ($H(1) = 6.145$, $p = .007$ (one-tailed)), decreased immobile duration ($H(1) = 3.152$, $p = .038$ (one-tailed)), and greater mean velocity of movement ($H(1) = 5.808$, $p = .008$ (one-tailed)). Measures of latency to emerge from the first chamber of the maze ($H(1) = .004$, $p = .477$ (one-tailed)) and percentage of the maze explored ($H(1) = 2.410$, $p = .061$ (one-tailed)) did not differ significantly between the two conditions.

Comparing measures of exploration between the Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity condition and the Naïve Subjects – No Sound Control Tested – Extended Proximity condition demonstrated that chicks postnatally exposed to the novel visual stimulus, then subsequently tested in the presence of the novel tone auditory stimulus were generally less fearful compared to naïve chicks tested without any novel stimuli present. Chicks exposed to the novel visual stimulus prior to being tested in the presence of the novel tone displayed shorter latencies to complete the maze task ($H(1) = 3.987$, $p = .046$ (two-tailed)), greater distances traveled throughout testing trials ($H(1) = 6.318$, $p = .012$ (two-tailed)), and a greater mean velocity of movement ($H(1) = 6.543$, $p = .011$ (two-tailed)) compared to control chicks. Measures of latency to emerge from the first chamber of the maze ($H(1) = 1.508$, $p =$

.219 (two-tailed)), latency to complete the maze after emerging from the first chamber (*H*(1) = 1.619, *p* = .203 (two-tailed)), percentage of the maze explored (*H*(1) = 2.503, *p* = .114 (two-tailed)), and immobile duration (*H*(1) = 3.149, *p* = .076 (two-tailed)) did not differ significantly between the two conditions.

Comparing measures of exploration between the Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity condition and the Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity condition did not reveal a general difference in level of fearfulness between groups. Although chicks that were postnatally exposed to the novel visual stimulus, then tested in the presence of the novel auditory tone traveled significantly greater distances (*H*(1) = 6.695, *p* = .010 (two-tailed)) and had significantly a greater mean velocity of movement (*H*(1) = 6.771, *p* = .009 (two-tailed)) compared to naïve chicks tested in the presence of the maternal assembly call, both groups did not differ significantly across measures of latency to emerge from the first chamber of the maze (*H*(1) = 2.748, *p* = .097 (two-tailed)), latency to maze completion (*H*(1) = .728, *p* = .393 (two-tailed)), latency to maze completion after emerging from the first chamber of the maze (*H*(1) = .002, *p* = .965 (two-tailed)), percentage of the maze explored (*H*(1) = .453, *p* = .501 (two-tailed)), or immobile duration (*H*(1) = 2.767, *p* = .096 (two-tailed)). These groups also did not differ significantly in duration of time spent in close proximity (*H*(1) = 2.237, *p* = .135 (two-tailed)) or at an extended proximity (*H*(1) = .952, *p* = .329 (two-tailed)) of their respective auditory stimuli during testing.

Comparing measures of exploration between the Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity condition and the Postnatal

Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity condition did not reveal a general difference in level of fearfulness between groups. No significant differences were found among any measures of exploration between the two groups including latency to emerge from the first chamber of the maze ($H(1) = .022$, $p = .882$ (two-tailed)), latency to complete the maze ($H(1) = .004$, $p = .947$ (two-tailed)), latency to complete the maze after emerging from the first chamber of the maze ($H(1) = .423$, $p = .515$ (two-tailed)), percentage of the maze explored ($H(1) = .332$, $p = .564$ (two-tailed)), total distance traveled ($H(1) = 1.470$, $p = .225$ (two-tailed)), immobile duration ($H(1) = 2.099$, $p = .147$ (two-tailed)), and mean velocity of movement ($H(1) = 1.470$, $p = .225$ (two-tailed)). These groups also did not differ significantly in duration of time spent in close proximity ($H(1) = .444$, $p = .505$ (two-tailed)) or at an extended proximity ($H(1) = .238$, $p = .626$ (two-tailed)) of the auditory tone during testing.

Comparing measures of exploration between the Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity condition and the Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity condition did not reveal a general difference in level of fearfulness between groups. No significant differences were found among any measures of exploration between the two groups including latency to emerge from the first chamber of the maze ($H(1) = 3.098$, $p = .078$ (two-tailed)), latency to complete the maze ($H(1) = 1.183$, $p = .277$ (two-tailed)), latency to complete the maze after emerging from the first chamber of the maze ($H(1) = 3.364$, $p = .067$ (two-tailed)), percentage of the maze explored ($H(1) = .031$, $p = .859$ (two-tailed)), total distance traveled ($H(1) = .386$, $p = .535$ (two-tailed)), immobile duration ($H(1) = .154$, $p = .695$ (two-tailed)), and mean velocity of movement ($H(1) = .367$, $p =$

.544 (two-tailed)). These groups also did not differ significantly in duration of time spent in close proximity (*H*(1) = .018, *p* = .894 (two-tailed)) or at an extended proximity (*H*(1) = .547, *p* = .460 (two-tailed)) of the auditory tone during testing.

Comparing measures of exploration between the Postnatal Auditory Tone Exposed – Visual Stimulus Tested and the Naïve Subjects – Visual Stimulus Tested conditions demonstrated that chicks postnatally exposed to the novel auditory tone stimulus, then subsequently tested in the presence of the novel visual stimulus were generally less fearful compared to naïve chicks tested under identical conditions. Compared to naïve chicks, chicks exposed to the novel tone stimulus prior to being tested in the presence of the novel visual stimulus displayed significantly greater distances traveled (*H*(1) = 7.160, *p* = .004 (one-tailed)), shorter latencies to exit the emergence box (*H*(1) = 7.448, *p* = .003 (one-tailed)), greater percentage of the T-maze explored (*H*(1) = 4.190, *p* = .021 (one-tailed)), decreased immobile duration (*H*(1) = 6.467, *p* = .006 (one-tailed)), and greater mean velocity of movement (*H*(1) = 7.082, *p* = .004 (one-tailed)).

Comparing measures of exploration between the Postnatal Auditory Tone Exposed – Visual Stimulus Tested and the Naïve Subjects – No Visual Stimulus Tested conditions did not reveal an overall difference in level of fearfulness between groups. Although exposed chicks tested in the presence of the novel visual stimulus did explore a greater percentage of the T-maze (*H*(1) = 6.459, *p* = .011 (two-tailed)) than naïve chicks tested without any novel stimuli present, they did not differ across measures of total distance traveled (*H*(1) = 1.620, *p* = .204 (two-tailed)), latency to exit the emergence box (*H*(1) = 3.481, *p* = .062 (two-tailed)), immobile duration (*H*(1) = 2.941, *p* = .086 (two-tailed)), or mean velocity of movement (*H*(1) = 1.617, *p* = .204 (two-tailed)).

Comparing measures of exploration between the Postnatal Auditory Tone Exposed – Visual Stimulus Tested and the Postnatal Visual Stimulus Exposed – Visual Stimulus Tested conditions did not reveal an overall difference in level of fearfulness between groups. No significant differences were found among any measures of exploration between the two groups including total distance traveled ($H(1) = 0.000$, $p = .988$ (two-tailed)), latency to exit the emergence box ($H(1) = 2.477$, $p = .116$ (two-tailed)), percentage of the T-maze explored ($H(1) = 0.342$, $p = .559$ (two-tailed)), immobile duration ($H(1) = 0.291$, $p = .589$ (two-tailed)), and mean velocity of movement ($H(1) = 0.000$, $p = 1.000$ (two-tailed)).

Table 21

Experiment 4: Latency to Emerge from First Chamber of Maze

Experimental Conditions	Mean (in seconds)	Standard Deviation
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	207.36	291.93
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	309.33	337.01
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	269.86	307.91
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	293.34	303.35
Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity	296.25	371.43
Naïve Subjects – Auditory Tone Tested – Extended Proximity	451.59	498.27
Naïve Subjects – Auditory Tone Tested – Close Proximity	400.90	417.07
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	263.83	385.71
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	315.93	421.04
Naïve Subjects – No Sound Control Tested – Extended Proximity	277.08	354.51
Naïve Subjects – No Sound Control Tested – Close Proximity	334.59	345.01

Table 22

Experiment 4: Latency to Complete Maze

Experimental Conditions	Mean (in seconds)	Standard Deviation
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	646.91	440.94
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	572.71	382.15
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	698.01	395.31
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	510.99	411.55
Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity	496.25	417.05
Naïve Subjects – Auditory Tone Tested – Extended Proximity	800.93	438.69
Naïve Subjects – Auditory Tone Tested – Close Proximity	794.47	450.79
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	463.17	412.21
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	898.02	408.93
Naïve Subjects – No Sound Control Tested – Extended Proximity	697.43	390.70
Naïve Subjects – No Sound Control Tested – Close Proximity	686.06	407.36

Table 23

Experiment 4: Latency to Complete Maze After Emerging from First Chamber

Experimental Conditions	Mean (in seconds)	Standard Deviation
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	519.45	416.47
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	383.37	388.70
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	484.76	415.31
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	283.09	354.69
Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity	360.00	444.95
Naïve Subjects – Auditory Tone Tested – Extended Proximity	669.27	479.44
Naïve Subjects – Auditory Tone Tested – Close Proximity	593.74	457.18
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	362.01	449.24
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	744.213	434.53
Naïve Subjects – No Sound Control Tested – Extended Proximity	540.35	434.31
Naïve Subjects – No Sound Control Tested – Close Proximity	473.60	411.73

Table 24

Experiment 4: Percentage of Maze Explored

Experimental Conditions	Mean (%)	Standard Deviation
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	86.65	26.40
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	86.60	29.94
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	83.79	27.57
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	89.87	24.46
Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity	85.98	31.70
Naïve Subjects – Auditory Tone Tested – Extended Proximity	70.74	39.64
Naïve Subjects – Auditory Tone Tested – Close Proximity	70.04	37.70
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	85.55	32.85
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	63.92	34.94
Naïve Subjects – No Sound Control Tested – Extended Proximity	85.69	28.67
Naïve Subjects – No Sound Control Tested – Close Proximity	85.98	27.95

Table 25

Experiment 4: Total Distance Traveled

Experimental Conditions	Mean (in centimeters)	Standard Deviation
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	10069.81	5345.40
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	8829.84	6228.17
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	9614.69	5043.90
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	9668.91	5750.42
Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity	11082.30	5816.18
Naïve Subjects – Auditory Tone Tested – Extended Proximity	7557.55	5730.41
Naïve Subjects – Auditory Tone Tested – Close Proximity	5616.92	4693.34
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	8105.50	4324.84
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	8003.94	5485.34
Naïve Subjects – No Sound Control Tested – Extended Proximity	7696.71	4276.52
Naïve Subjects – No Sound Control Tested – Close Proximity	8071.27	4460.56

Table 26

Experiment 4: Immobile Duration

Experimental Conditions	Mean (in seconds)	Standard Deviation
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	726.90	240.59
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	798.16	253.35
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	764.23	216.21
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	782.45	232.43
Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity	707.80	245.48
Naïve Subjects – Auditory Tone Tested – Extended Proximity	836.98	268.82
Naïve Subjects – Auditory Tone Tested – Close Proximity	893.12	235.66
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	792.17	216.39
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	817.72	232.64
Naïve Subjects – No Sound Control Tested – Extended Proximity	814.42	215.15
Naïve Subjects – No Sound Control Tested – Close Proximity	800.48	219.34

Table 27

Experiment 4: Mean Velocity of Movement

Experimental Conditions	Mean (in centimeters / second)	Standard Deviation
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	8.46	4.43
Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	7.38	5.17
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity	8.46	4.22
Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity	8.02	4.79
Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity	9.25	4.83
Naïve Subjects – Auditory Tone Tested – Extended Proximity	6.30	4.78
Naïve Subjects – Auditory Tone Tested – Close Proximity	4.79	3.95
Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity	6.75	3.60
Naïve Subjects – Maternal Assembly Call Tested – Close Proximity	6.69	4.58
Naïve Subjects – No Sound Control Tested – Extended Proximity	6.42	3.57

Table 28

Experiment 4: Latency to Exit from Emergence Box into T-Maze

Experimental Conditions	Mean (in seconds)	Standard Deviation
Naïve Subjects – No Visual Stimulus Tested –	179.05	114.62
Naïve Subjects – Visual Stimulus Tested –	207.93	114.85
Postnatal Visual Stimulus Exposed – Visual Stimulus Tested –	163.00	123.99
Postnatal Auditory Tone Exposed – Visual Stimulus Tested –	124.55	115.25

Table 29

Experiment 4: Total Distance Traveled

Experimental Conditions	Mean (in centimeters)	Standard Deviation
Naïve Subjects – No Visual Stimulus Tested –	1615.74	1338.63
Naïve Subjects – Visual Stimulus Tested –	1132.31	1111.19
Postnatal Visual Stimulus Exposed – Visual Stimulus Tested –	1982.42	1481.27
Postnatal Auditory Tone Exposed – Visual Stimulus Tested –	2040.41	1298.52

Table 30

Experiment 4: Percentage of T-Maze Explored

Experimental Conditions	Mean (%)	Standard Deviation
Naïve Subjects – No Visual Stimulus Tested –	35.71	44.91
Naïve Subjects – Visual Stimulus Tested –	42.24	48.22
Postnatal Visual Stimulus Exposed – Visual Stimulus Tested –	57.88	48.16
Postnatal Auditory Tone Exposed – Visual Stimulus Tested –	74.35	38.85

Table 31

Experiment 4: Immobile Duration

Experimental Conditions	Mean (in seconds)	Standard Deviation
Naïve Subjects – No Visual Stimulus Tested –	135.20	96.06
Naïve Subjects – Visual Stimulus Tested –	163.15	107.06
Postnatal Visual Stimulus Exposed – Visual Stimulus Tested –	116.65	103.02
Postnatal Auditory Tone Exposed – Visual Stimulus Tested –	101.10	38.26

Table 32

Experiment 4: Mean Velocity of Movement

Experimental Conditions	Mean (in centimeters / second)	Standard Deviation
Naïve Subjects – No Visual Stimulus Tested –	5.43	4.47
Naïve Subjects – Visual Stimulus Tested –	3.82	3.74
Postnatal Visual Stimulus Exposed – Visual Stimulus Tested –	6.66	4.97
Postnatal Auditory Tone Exposed – Visual Stimulus Tested –	6.89	4.41

Consistent with the hypothesis that fearfulness of a novel stimulus could be decreased through the early postnatal exposure of a markedly different novel stimulus, I predicted that chicks tested in the presence of a novel auditory stimulus after having been exposed to a novel visual stimulus for 24 hours immediately following hatching would demonstrate decreased fearfulness compared to naive chicks. Results revealed that chicks postnatally exposed to the novel visual stimulus prior to being tested in the presence of the novel auditory tone were generally less fearful during testing than naïve chicks tested under identical conditions (five measures indicating increased exploration motivation).

Results also revealed that visually exposed chicks tested in the presence of the novel auditory tone stimulus did not differ in overall level of fearfulness compared to chicks that had received prenatal or postnatal exposure to the auditory tone itself prior to testing. These results demonstrate that early-life experience with a novel auditory stimulus can generalize and effectively serve to decrease fearfulness of a different novel visual stimulus. Additionally, visually exposed chicks tested in the presence of the novel auditory tone did not differ in overall level of fearfulness compared to naïve chicks tested in the presence of the maternal assembly call suggesting that this type of postnatal exposure effectively worked to shift the aversive and attractive properties of the novel auditory tone to be equivalent to those of the maternal assembly call.

Similarly, I predicted that chicks tested in the presence of the novel visual stimulus after having been exposed to the novel auditory stimulus for 24 hours following hatching would also demonstrate decreased fearfulness compared to naïve chicks. Results revealed that chicks postnatally exposed to the novel auditory tone stimulus prior to being tested in the presence of the novel visual stimulus were generally less fearful during testing than naïve chicks tested under identical conditions (all five measures indicating increased exploration motivation). In addition, chicks exposed to the novel auditory tone then tested in the presence of the novel visual stimulus did not differ in level of fearfulness compared to chicks that had received postnatal exposure to the visual stimulus itself prior to testing. These findings expand previous results by demonstrating that chicks are able to generalize their early-life experiences with novel visual stimuli to subsequent novel auditory stimuli, and vice-versa.

Generally, these results suggest that exposure to a specific novel stimulus is not required to decrease fearfulness of that novel stimulus, instead demonstrating that exposure to one type of novel stimulus can have far reaching fear-reducing effects on markedly different novel stimuli. The ability of this type of exposure to affect such disparate stimuli (even across sense modality) suggests that chicks not only generalized their experiences from one stimulus to another, but may have fundamentally changed how they would subsequently react in the presence of novelty in a more profound manner.

CHAPTER XI

GENERAL DISCUSSION

Previous research has shown that bobwhite quail chicks exhibit an increase in fearful behaviors when tested within a maze in the presence of a novel auditory stimulus at 24 hours of age; however, the role of novelty on this phenomenon, whether this occurs across sensory modalities, and the extent to which these results can be attenuated or generalized have remained relatively unexplored (Suarez, 2012). In Experiment 1 of the present study, chicks were exposed *postnatally* to the novel auditory tone stimulus for 24 hours following hatching and subsequently tested within a novel maze in the presence of the previously exposed auditory tone. Chicks receiving postnatal exposure to the auditory stimulus demonstrated decreased fearfulness compared to naïve chicks tested under identical conditions, and behaved more similarly to chicks tested in the presence of a bobwhite maternal assembly call vocalization (a known attractive stimulus). These findings demonstrate that novelty is an important factor in determining the extent to which an auditory stimulus will elicit fearful responses in chicks. These findings also reveal that 24 hours of early postnatal exposure is sufficient to significantly attenuate the aversive properties of a novel auditory stimulus, thereby allowing chicks to exhibit increased exploratory behaviors in its presence (Buss & Plonin, 1984; Einon & Morgan, 1976; Cavigelli & McClintock, 2003).

Experiment 2 of the present study expanded on the first by examining the extent to which the aversive properties of a novel auditory stimulus could be reduced through *prenatal* exposure to that stimulus. Chicks were exposed to the novel auditory tone stimulus for 24 hours immediately prior to hatching, then subsequently tested within a novel maze in the presence of the previously exposed auditory tone. Chicks having received prenatal exposure to the tone expressed mixed results with regards to how they compared to naïve chicks tested under identical parameters. Although exposed chicks demonstrated decreased fearfulness when tasked with avoiding the auditory stimulus (starting within close proximity of the tone), they did not differ significantly when tasked with approaching the tone (starting at an extended proximity). This suggests that prenatal exposure to the auditory tone may have played a role in decreasing fearfulness of the tone, but did not necessarily increase the attractiveness of the tone so as to motivate chicks to move toward its direction during testing (Elliot & Covington, 2001; Elliot, 2006). Additionally, these findings expand on previous research by demonstrating that prenatal auditory learning is not limited to reinforcing or neutral stimuli, instead revealing that aversive auditory stimuli can also be learned through prenatal exposure (Lickliter, 1989; Lickliter & Hellewell, 1992).

Comparisons made between chicks tested in Experiment 1 and Experiment 2 revealed evidence suggesting that although both forms of auditory exposure decreased fearfulness by means of reducing the aversive properties of the auditory tone, postnatal exposure may have additionally increased the attractive properties of the auditory tone during testing. Comparisons between prenatal and postnatal exposure conditions with corresponding starting positions revealed no difference in level of fearfulness between both groups. Further comparisons revealed that postnatally exposed chicks displayed similar motivation to stay at a close proximity of the auditory tone during testing as chicks displaying motivation to remain at a close proximity of the maternal assembly call auditory stimulus; this similarity was not found with prenatally exposed chicks who showed less motivation to stay in close proximity of the auditory tone. This is further demonstrated by results indicating that postnatally exposed chicks displayed increased motivation to explore toward the direction of the auditory tone rather than in the opposite direction during testing, whereas prenatally exposed chicks displayed no such difference, instead exhibiting similar exploratory behavior both toward the direction of the auditory tone and away from the tone during testing. In general, postnatally exposed chicks demonstrated increased motivation to approach the auditory tone during testing demonstrating that the tone had increased in level of attractiveness and had shifted from a negative (punishing) stimulus to a more positive (reinforcing) stimulus; a shift that was not found in prenatally exposed chicks (Elliot & Covington, 2001; Elliot, 2006).

Experiment 3 examined whether the previous findings were unique to auditory stimuli, or if they would generalize across sense modalities. Rather than expose chicks to a novel auditory stimulus, this experiment exposed chicks to a novel visual stimulus postnatally for 24 hours, then tested them in the presence of that stimulus within an emergence box / T-maze apparatus. In line with previous findings, chicks receiving postnatal exposure demonstrated a reduction in fearfulness when subsequently tested in the presence of the previously exposed visual stimulus compared to naïve chicks tested under identical conditions. This suggests that early life exposure could be used to reduce fearfulness of not only auditory stimuli, but also visual stimuli and potentially across other sense modalities as well.

Lastly, Experiment 4 examined how early postnatal exposure to one novel stimulus could potentially generalize and serve to decrease fear of different novel stimuli during testing. To accomplish this, some methods from Experiments 1 and 3 were combined to compare how exposure to the auditory tone could subsequently influence fearfulness in the presence of the novel visual stimulus, and vice-versa.

Chicks that received postnatal exposure to the auditory tone, and were subsequently tested in the presence of the novel visual stimulus demonstrated a decrease in fearfulness compared to naïve chicks tested under identical conditions. This difference in fearfulness was surprisingly stronger than that found when chicks were postnatally exposed to the visual stimulus itself (significant differences found in 5 measures as opposed to 3). Similarly, chicks that received postnatal exposure to the visual stimulus, and were subsequently tested in the presence of the novel auditory tone demonstrated a decrease in fearfulness compared to naïve chicks tested under identical conditions. Surprisingly, this difference in fearfulness was also stronger than that found when chicks were postnatally exposed to the auditory tone and tasked with approaching the tone during testing (significant differences found in 5 measures as opposed to 3). Although previous research has demonstrated that a high degree of generalization can be expected for approach responses (Brown, 1942), the extent to which early life exposure to one type of stimulus was able to generalize across sense modalities to markedly different stimuli has not previously been shown.

When comparing the types of exposure outlined in this experiment to the more direct forms outlined in Experiments 1-3, no significant differences were found between exposure type and subsequent fearfulness during testing. Chicks postnally exposed to the auditory tone, then subsequently tested in the presence of the visual stimulus displayed similar levels of fearfulness to those having been previously exposed to the visual stimulus itself. Similarly, chicks postnatally exposed to the visual stimulus, then subsequently tested in the presence of the auditory tone displayed similar levels of fearfulness to those having been previously prenatally or postnatally exposed to the auditory tone itself. These findings suggest that early life exposure to one type of novel stimulus can reduce the expression of fearfulness in the presence of markedly different novel stimuli. The ability of this type of exposure to generalize across such disparate stimuli suggests that chicks may be able to form associations based not only on the physical factors of stimuli, but rather based on underlying factors that link very different novel stimuli to one another (Philip, 1947).

This generalization between specific stimuli and novelty in a more broad sense is of importance for the practicality and utility of this type of early-life intervention for neophobia. My results show that exposure to each different type of novel stimulus is not necessary for reducing neophobia in chicks; instead results suggest that exposure to one type of novel stimulus may generalize to reduce fearfulness in the presence of several different novel stimuli. Unlike exposure therapy which focuses on the reduction of one type of phobia at a time for an extended amount of time, early life exposure to novelty may provide a relatively simple method for targeting an extensive set of commonly feared stimuli (Marks, 1979, Myers & Davis, 2006; Rothbaum & Schwartz, 2002).

Limitations and Future Directions

The present study, although having attained several notable results, is not without its limitations. Firstly, most significant results were attained by comparing experimental conditions against one another, rather than by comparing experimental conditions with their respective control conditions. Although some significant differences exist between experimental conditions and no auditory stimulus or no visual stimulus control conditions, most measures compared between these conditions were not shown to differ significantly. Chicks tested within these control conditions reliably performed at levels between those of experimental conditions in which chicks did not receive any type of exposure to the novel auditory or visual stimuli prior to testing (high levels of fearfulness), and those of experimental conditions in which chicks did receive prior exposure to the novel stimuli prior to testing, or those in which chicks were tested in the presence of the maternal assembly call auditory stimulus (low levels of fearfulness). Although these results were to be expected, the control conditions could have had increased utility had they differed to a greater extent from their respective experimental conditions.

The present study controlled for developmental age by maintaining the time of testing constant across groups (24 hours after hatch). This presents a limitation between conditions that received prenatal exposure to the novel tone stimulus and those that received postnatal exposure. Chicks that received 24 hours of prenatal exposure to the auditory tone stimulus inherently had a 24 period immediately after hatching in which they did not receive exposure to the auditory stimulus, whereas chicks receiving postnatal exposure did not. Although few differences were found between prenatal and postnatal exposure groups, this brings into question the long-term effects of early life exposure on the reduction of neophobia. Further studies should further explore the extent to which this 24 hour gap in exposure may have affected the results of the present study, and more importantly explore the long-term effects early life exposure to novelty may have across development.

Perhaps the most important limitation of the present study is the extent to which measures of exploration represent increased fearfulness. Although willingness to explore novel environments is often indicative of fearfulness in animal subjects, decreased willingness to explore can also be a result of lack of motivation to explore (Buss & Plonin, 1984; Einon & Morgan, 1976; Cavigelli & McClintock, 2003; Miller et al., 2005). This presented a particular issue within experimental conditions starting at a close proximity of different auditory stimuli. Because organisms are usually motivated to approach or stay in close proximity of attractive stimuli, it is difficult to discern whether chicks that started in close proximity of any particular auditory stimulus may have demonstrated decreased explorative tendencies because of increased levels of fearfulness, or as a result of the attractive properties of the stimulus (Schneirla, 1959, 1965; Elliot & Covington, 2001). Although the present study is able to look to experimental conditions in which chicks started at an extended proximity of the auditory stimuli to ascertain whether explorative tendencies were due to level of fearfulness or decreased motivation to explore in the opposite direction of an attractive stimulus, future studies should employ different measures to more clearly differentiate these two factors.

Some additional future directions for this research would involve testing the extent to which this type of exposure may serve to decrease neophobia across sense modalities beyond auditory and visual, as well as observing the extent to which the results found in the present study may generalize to more naturalistic environments. Although the present study tested the effects of exposure to both auditory and visual stimuli on neophobia, it is not clear whether similar results would be found across different sense modalities (olfactory, somatosensory, gustatory, etc.). These results could also be expanded by observing the effects of early life exposure on neophobia within a more naturalistic setting than that of the standard laboratory conditions utilized within the present study. This may provide insight into the extent to which this type of manipulation may persist within more complex environments as well as providing information as to how it may affect exploratory behavior and reactivity to predators within the natural environment.

Human Significance

Although results attained from studies utilizing animal models cannot be directly applied to human subjects, they do allow us to make better informed decisions on future directions for human research. Neophobia can emerge early in life in humans, sometimes negatively affecting individuals across development. Not unlike quail, neophobic humans often experience heightened anxiety, behavioral inhibition, and decreased willingness to explore when in the presence of novelty. If the results of the present study are expanded demonstrating the utility of early life exposure across development, these methods may be adaptable to human populations, potentially providing a means of preventing neophobia in children. Although a great deal of future research would be necessary to realize this ultimate goal, the present study should serve as a first step in this process.

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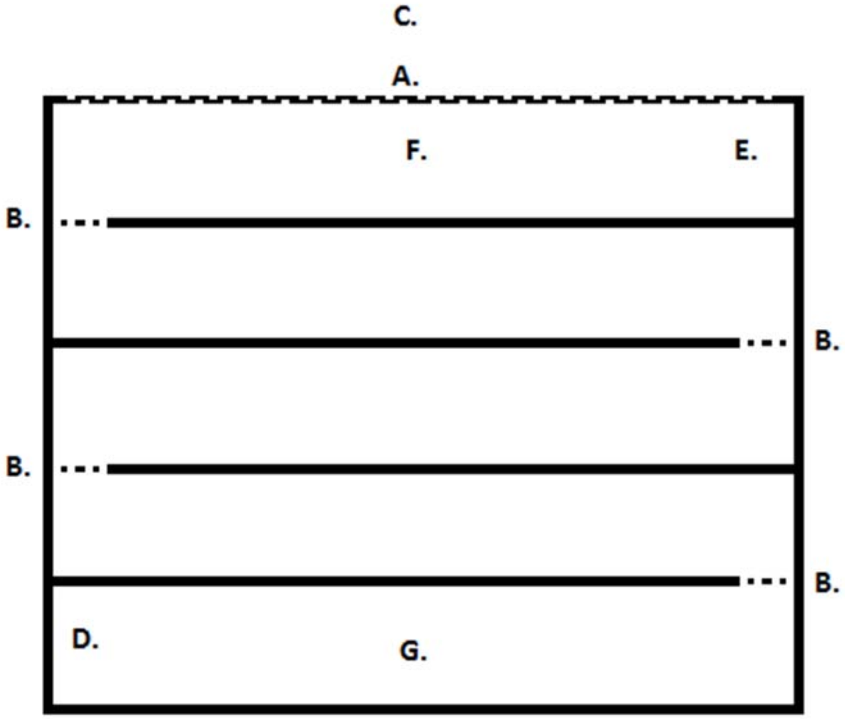
APPENDICES

Appendix A

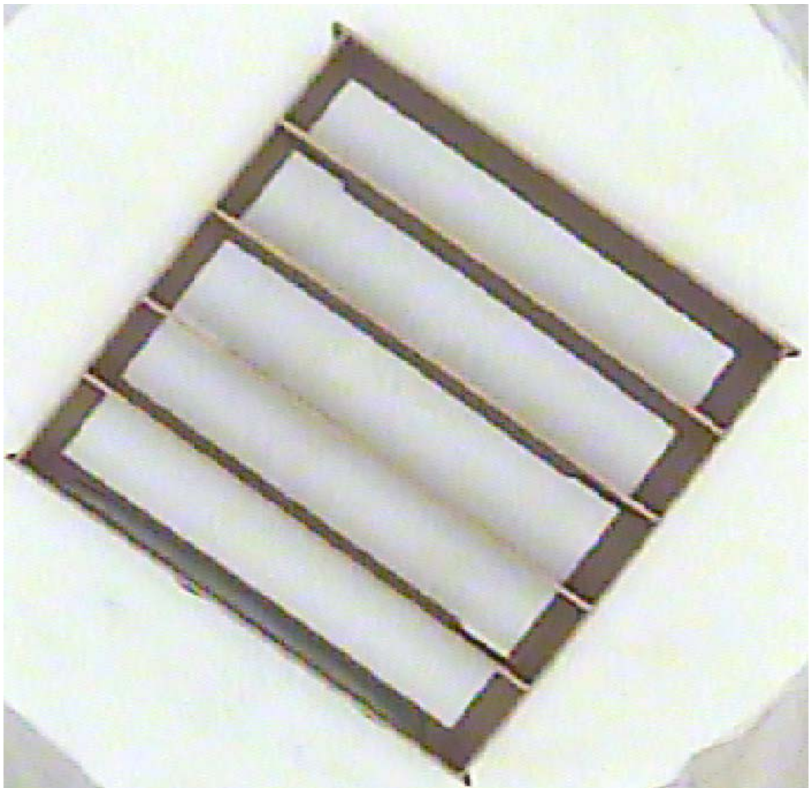
Square Maze Layout and Relevant Details



Legend:	A. Wire Screen
	B. 8cm by 9.5cm Openings
	C. Speaker Location
	D. Extended Proximity Starting Location
	E. Close Proximity Starting Location
	F. Extended Proximity Maze Completion Area
	G. Close Proximity Maze Completion Area

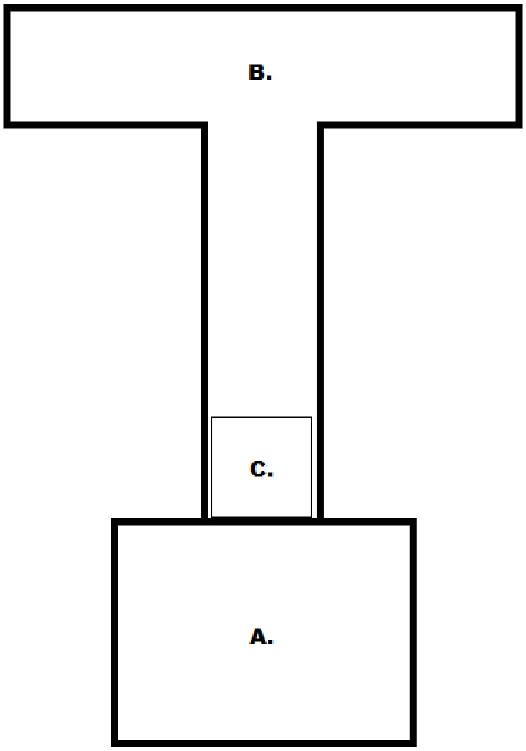
Picture of Square Maze with Chick Proxy at Extended Proximity Starting Location

Ethovision XT View of Square Maze



Appendix B

Emergence Box / T-Maze Layout and Relevant Details

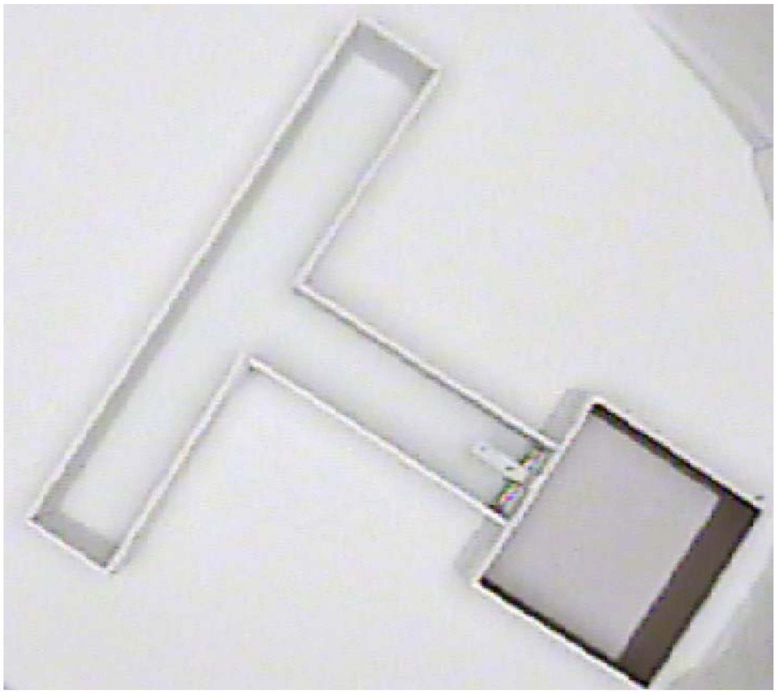


Legend: A. Emergence Box / Subject Starting Location

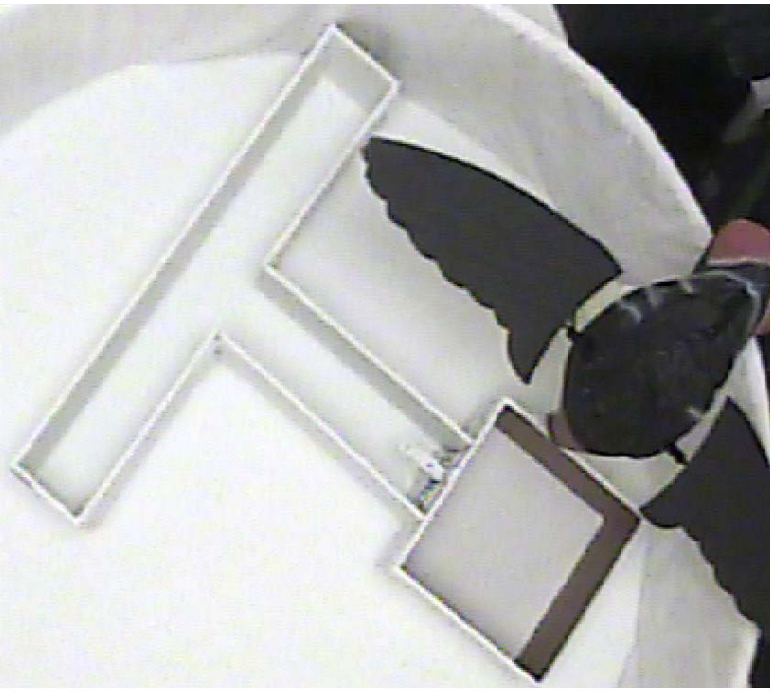
B. T-Maze Extension

C. Remotely Controlled Door

Ethovision XT View of Emergence Box / T-Maze (Without Hawk)

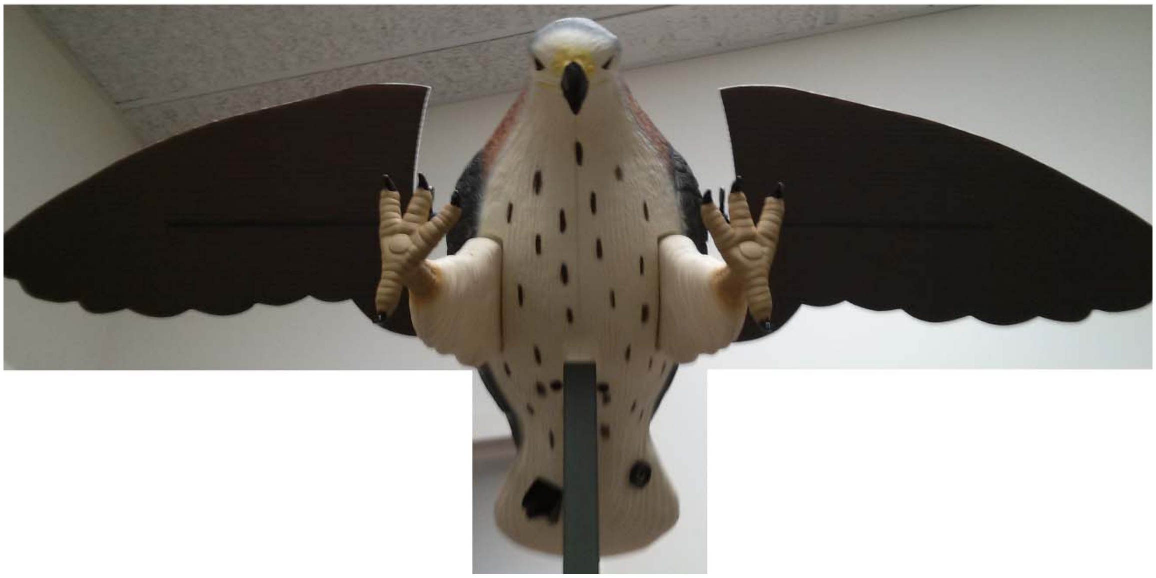


Ethovision XT View of Emergence Box / T-Maze (With Hawk)



Appendix C

Picture of Hawk Visual Stimulus



Appendix D

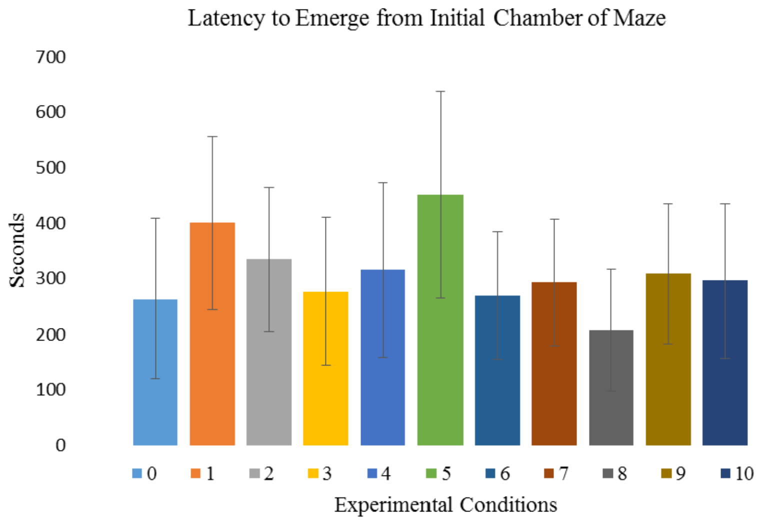
Latency to Emerge from Initial Chamber of Maze

	0	1	2	3	4	5	6	7	8	9	10
0		0.039*	0.010**	0.352**	0.323*	0.063*	0.214**	0.062**	0.859**	0.081**	0.097**
1	0.039*		0.982**	0.149*	0.219**	1.000**	0.170*	0.329*	0.032*	0.364*	0.234*
2	0.010**	0.982**		0.036**	0.056**	0.524**	0.211**	0.544**	0.004**	0.510**	0.219**
3	0.352**	0.149*	0.036**		0.824**	0.432**	0.620**	0.183**	0.329**	0.371**	0.300**
4	0.323*	0.219**	0.056**	0.824**		0.299**	0.230*	0.198**	0.292*	0.242**	0.154*
5	0.063*	1.000**	0.524**	0.432**	0.299**		0.287*	0.953**	0.058*	0.953**	0.477*
6	0.214**	0.170*	0.211**	0.620**	0.230*	0.287*		0.263*	0.193**	0.473**	0.882**
7	0.062**	0.329*	0.544**	0.183**	0.198**	0.953**	0.263*		0.038**	0.976**	0.564**
8	0.859**	0.032*	0.004**	0.329**	0.292*	0.058*	0.193**	0.038**		0.034**	0.078
9	0.081**	0.364*	0.510**	0.371**	0.242**	0.953**	0.473**	0.976**	0.034**		0.861**
10	0.097**	0.234*	0.219**	0.300**	0.154*	0.477*	0.882**	0.564**	0.078**	0.861**	

Grey results outlined in paper

* One-tailed *p* value

** Two-tailed *p* value



Latency to Emerge from Initial Chamber of Maze

LEGEND

0 = Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity
1 = Naïve Subjects – Auditory Tone Tested – Close Proximity
2 = Naïve Subjects – No Sound Control Tested – Extended Proximity
3 = Naïve Subjects – No Sound Control Tested – Close Proximity
4 = Naïve Subjects – Maternal Assembly Call Tested – Close Proximity
5 = Naïve Subjects – Auditory Tone Tested – Extended Proximity
6 = Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity
7 = Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity
8 = Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity
9 = Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity
10 = Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity

Appendix E

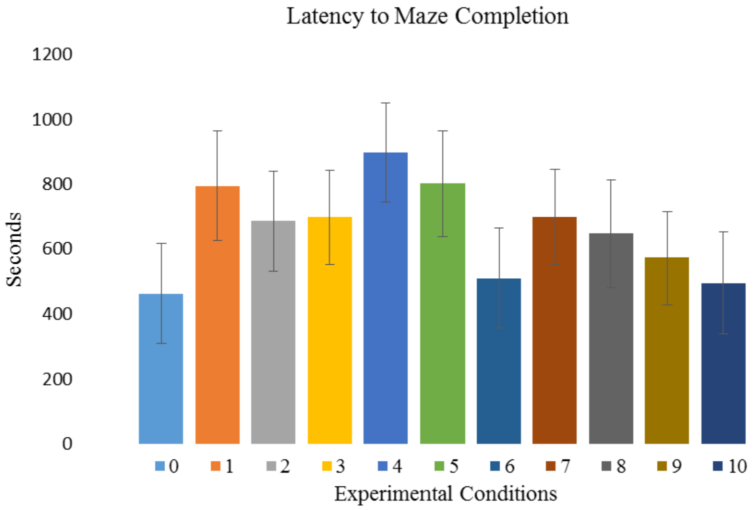
Latency to Maze Completion

	0	1	2	3	4	5	6	7	8	9	10
0		0.005*	0.012**	0.008**	0.000*	0.002*	0.558**	0.006**	0.115**	0.195**	0.393**
1	0.005*		0.365**	0.195*	0.279**	0.920**	0.011*	0.241*	0.105*	0.030*	0.016*
2	0.012**	0.365**		0.788**	0.041**	0.297**	0.50**	0.731**	0.550**	0.265**	0.046**
3	0.008**	0.195*	0.788**		0.055**	0.381**	0.037**	0.988**	0.329**	0.196**	0.021**
4	0.000*	0.279**	0.041**	0.055**		0.279**	0.019*	0.057**	0.009*	0.004**	0.001*
5	0.002*	0.920**	0.297**	0.381**	0.279**		0.006*	0.453**	0.078*	0.054**	0.008*
6	0.558**	0.011*	0.50**	0.037**	0.019*	0.006*		0.013*	0.278**	0.454**	0.947**
7	0.006**	0.241*	0.731**	0.988**	0.057**	0.453**	0.013*		0.455**	0.243**	0.021**
8	0.115**	0.105*	0.550**	0.329**	0.009*	0.078*	0.278**	0.455**		0.307**	0.277
9	0.195**	0.030*	0.265**	0.196**	0.004**	0.054**	0.454**	0.243**	0.307**		0.354**
10	0.393**	0.016*	0.046**	0.021**	0.001*	0.008*	0.947**	0.021**	0.277**	0.354**	

Grey results outlined in paper

* One-tailed *p* value

** Two-tailed *p* value



LEGEND

0 = Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity
1 = Naïve Subjects – Auditory Tone Tested – Close Proximity
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8 = Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity
9 = Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity
10 = Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity

Appendix F

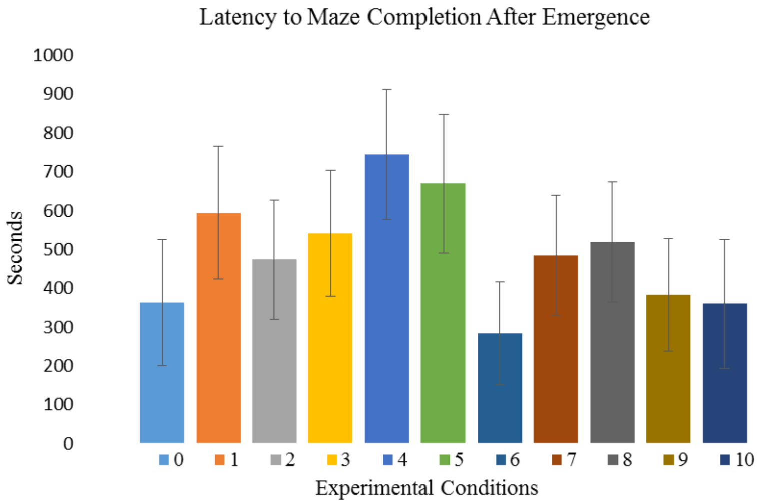
Latency to Maze Completion After Emergence

	0	1	2	3	4	5	6	7	8	9	10
0		0.039*	0.238**	0.056**	0.002*	0.011*	0.525**	0.139**	0.078**	0.574**	0.965**
1	0.039*		0.371**	0.387*	0.183**	0.504**	0.006*	0.244*	0.407*	0.540*	0.037*
2	0.238**	0.371**		0.501**	0.019**	0.134**	0.035**	0.906**	0.460**	0.437**	0.203**
3	0.056**	0.387*	0.501**		0.131**	0.292**	0.006**	0.554**	0.894**	0.141**	0.032**
4	0.002*	0.183**	0.019**	0.131**		0.835**	0.000*	0.036**	0.042*	0.004**	0.001*
5	0.011*	0.504**	0.134**	0.292**	0.835**		0.001*	0.131**	0.172*	0.038**	0.007*
6	0.525**	0.006*	0.035**	0.006**	0.000*	0.001*		0.015*	0.007**	0.181**	0.515**
7	0.139**	0.244*	0.906**	0.554**	0.036**	0.131**	0.015*		0.605**	0.308**	0.113**
8	0.078**	0.407*	0.460**	0.894**	0.042*	0.172*	0.007**	0.605**		0.082**	0.053
9	0.574**	0.540*	0.437**	0.141**	0.004**	0.038**	0.181**	0.308**	0.082**		0.407**
10	0.965**	0.037*	0.203**	0.032**	0.001*	0.007*	0.515**	0.113**	0.053**	0.407**	

Grey results outlined in paper

* One-tailed *p* value

** Two-tailed *p* value



LEGEND

0 = Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity
1 = Naïve Subjects – Auditory Tone Tested – Close Proximity
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7 = Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity
8 = Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity
9 = Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity
10 = Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity

Appendix G

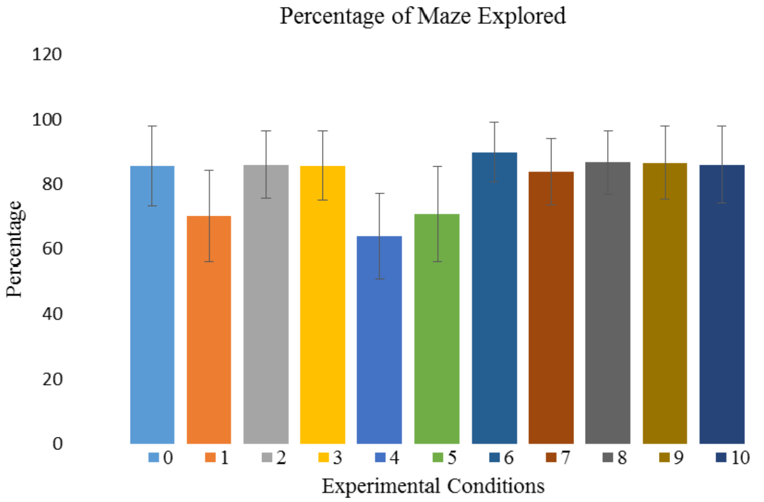
Percentage of Maze Explored

	0	1	2	3	4	5	6	7	8	9	10
0		0.051*	0.144**	0.288**	0.001*	0.116*	0.204**	0.001**	0.515**	0.174**	0.501**
1	0.051*		0.318**	0.102*	0.322**	0.762**	0.005*	0.486*	0.022*	0.144*	0.026*
2	0.144**	0.318**		0.589**	0.024**	0.652**	0.038**	0.193**	0.261**	0.679**	0.114**
3	0.288**	0.102*	0.589**		0.009**	0.478**	0.019**	0.032**	0.174**	0.882**	0.139**
4	0.001*	0.322**	0.024**	0.009**		0.160**	0.000*	0.139**	0.001*	0.011**	0.001*
5	0.116*	0.762**	0.652**	0.478**	0.160**		0.021*	0.784**	0.089*	0.438**	0.061*
6	0.204**	0.005*	0.038**	0.019**	0.000*	0.021*		0.000*	0.657**	0.034**	0.564**
7	0.001**	0.486*	0.193**	0.032**	0.139**	0.784**	0.000*		0.013**	0.033**	0.001**
8	0.515**	0.022*	0.261**	0.174**	0.001*	0.089*	0.657**	0.013**		0.102**	0.859
9	0.174**	0.144*	0.679**	0.882**	0.011**	0.438**	0.034**	0.033**	0.102**		0.076**
10	0.501**	0.026*	0.114**	0.139**	0.001*	0.061*	0.564**	0.001**	0.859**	0.076**	

Grey results outlined in paper

* One-tailed *p* value

** Two-tailed *p* value



LEGEND

0 = Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity
1 = Naïve Subjects – Auditory Tone Tested – Close Proximity
2 = Naïve Subjects – No Sound Control Tested – Extended Proximity
3 = Naïve Subjects – No Sound Control Tested – Close Proximity
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6 = Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity
7 = Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity
8 = Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity
9 = Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity
10 = Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity

Appendix H

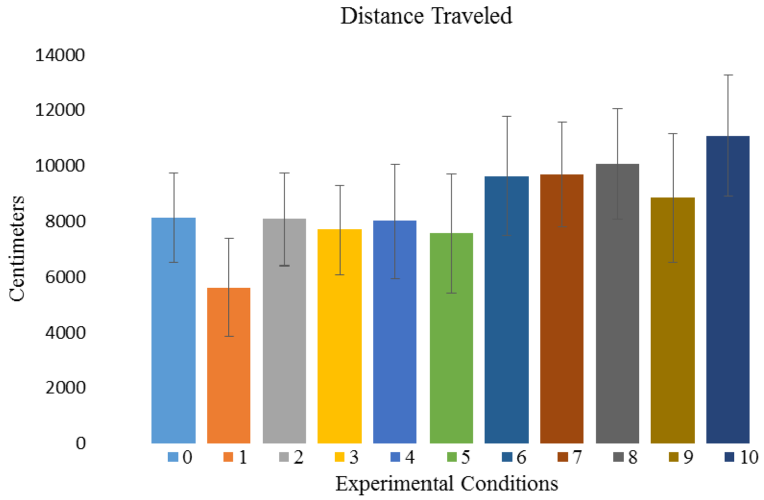
Distance Traveled

	0	1	2	3	4	5	6	7	8	9	10
0		0.028*	0.972**	0.814**	0.308*	0.480*	0.308**	0.223**	0.110**	0.701**	0.010**
1	0.028*		0.069**	0.045*	0.104**	0.220**	0.006*	0.002*	0.001*	0.037*	0.001*
2	0.972**	0.069**		0.790**	0.668**	0.842**	0.308**	0.217**	0.107**	0.871**	0.012**
3	0.814**	0.045*	0.790**		0.745**	0.836**	0.174**	0.135**	0.058**	0.965**	0.005**
4	0.308*	0.104**	0.668**	0.745**		0.906**	0.151*	0.193**	0.046*	0.941**	0.014*
5	0.480*	0.220**	0.842**	0.836**	0.906**		0.087*	0.181**	0.049*	0.941**	0.008*
6	0.308**	0.006*	0.308**	0.174**	0.151*	0.087*		0.436*	0.615**	0.544**	0.225**
7	0.223**	0.002*	0.217**	0.135**	0.193**	0.181**	0.436*		0.657**	0.391**	0.243**
8	0.110**	0.001*	0.107**	0.058**	0.046*	0.049*	0.615**	0.657**		0.116**	0.535
9	0.701**	0.037*	0.871**	0.965**	0.941**	0.941**	0.544**	0.391**	0.116**		0.084**
10	0.010**	0.001*	0.012**	0.005**	0.014*	0.008*	0.225**	0.243**	0.535	0.084**	

Grey results outlined in paper

* One-tailed *p* value

** Two-tailed *p* value



LEGEND

0 = Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity
1 = Naïve Subjects – Auditory Tone Tested – Close Proximity
2 = Naïve Subjects – No Sound Control Tested – Extended Proximity
3 = Naïve Subjects – No Sound Control Tested – Close Proximity
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6 = Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity
7 = Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity
8 = Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity
9 = Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity
10 = Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity

Appendix I

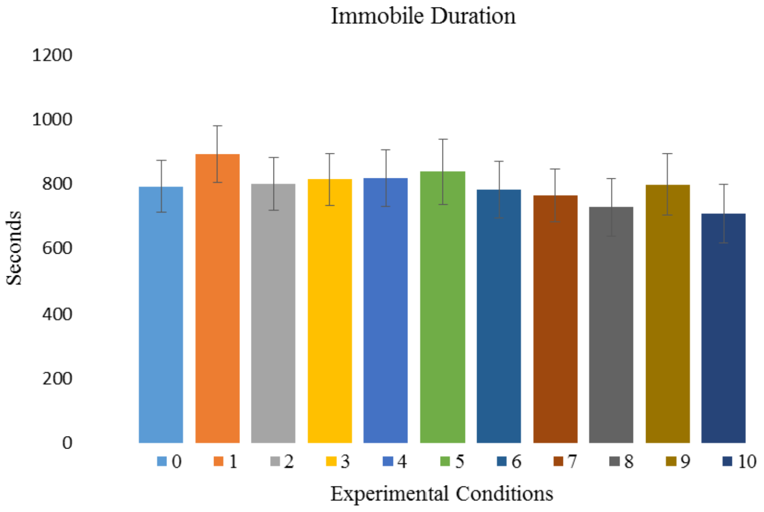
Immobile Duration

	0	1	2	3	4	5	6	7	8	9	10
0		0.039*	0.860**	0.824**	0.275*	0.297*	0.906**	0.684**	0.284**	0.600**	0.096**
1	0.039*		0.117**	0.070*	0.209**	0.391**	0.038*	0.028*	0.004*	0.113*	0.002*
2	0.860**	0.117**		0.871**	0.701**	0.734**	0.767**	0.544**	0.198**	0.836**	0.076**
3	0.824**	0.070*	0.871**		0.734**	0.790**	0.668**	0.442**	0.135**	0.871**	0.048**
4	0.275*	0.209**	0.701**	0.734**		0.929**	0.595*	0.379**	0.056*	0.923**	0.030*
5	0.297*	0.391**	0.734**	0.790**	0.929**		0.253*	0.294**	0.072*	0.836**	0.038*
6	0.906**	0.038*	0.767**	0.668**	0.595*	0.253*		0.340*	0.294**	0.723**	0.147**
7	0.684**	0.028*	0.544**	0.442**	0.379**	0.294**	0.340*		0.520**	0.559**	0.271**
8	0.284**	0.004*	0.198**	0.135**	0.056*	0.072*	0.294**	0.520**		0.081**	0.695
9	0.600**	0.113*	0.836**	0.871**	0.923**	0.836**	0.723**	0.559**	0.081**		0.096**
10	0.096**	0.002*	0.076**	0.048**	0.030*	0.038*	0.147**	0.271**	0.695**	0.096**	

Grey results outlined in paper

* One-tailed *p* value

** Two-tailed *p* value


Immobile Duration

LEGEND

0 = Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity
1 = Naïve Subjects – Auditory Tone Tested – Close Proximity
2 = Naïve Subjects – No Sound Control Tested – Extended Proximity
3 = Naïve Subjects – No Sound Control Tested – Close Proximity
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6 = Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity
7 = Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity
8 = Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity
9 = Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity
10 = Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity

Appendix J

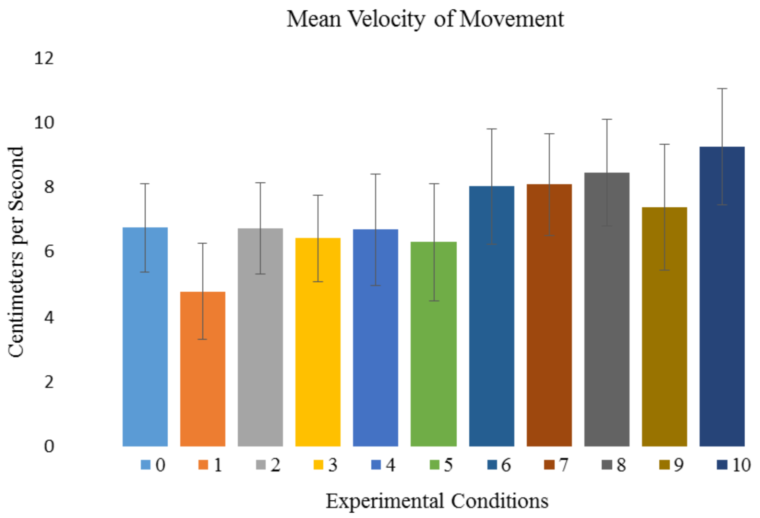
Mean Velocity of Movement

	0	1	2	3	4	5	6	7	8	9	10
0		0.034*	0.982**	0.814**	0.318*	0.480*	0.301**	0.217**	0.104**	0.701**	0.009**
1	0.034*		0.081**	0.054*	0.128**	0.243**	0.007*	0.003*	0.001*	0.049*	0.001*
2	0.982**	0.081**		0.790**	0.668**	0.830**	0.308**	0.217**	0.092**	0.871**	0.011**
3	0.814**	0.054*	0.790**		0.745**	0.824**	0.179**	0.143**	0.056**	0.953**	0.004**
4	0.318*	0.128**	0.668**	0.745**		0.894**	0.298*	0.193**	0.042*	0.906**	0.015*
5	0.480*	0.243**	0.830**	0.824**	0.894**		0.090*	0.171**	0.043*	0.953**	0.008*
6	0.301**	0.007*	0.308**	0.179**	0.298*	0.090*		0.436*	0.595**	0.644**	0.225**
7	0.217**	0.003*	0.217**	0.143**	0.193**	0.171**	0.436*		0.647**	0.399**	0.237**
8	0.104**	0.001*	0.092**	0.056**	0.042*	0.043*	0.595**	0.647**		0.105**	0.544
9	0.701**	0.049*	0.871**	0.953**	0.906**	0.953**	0.644**	0.399**	0.105**		0.081**
10	0.009**	0.001*	0.011**	0.004**	0.015*	0.008*	0.225**	0.237**	0.544**	0.081**	

Grey results outlined in paper

* One-tailed *p* value

** Two-tailed *p* value



LEGEND

0 = Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity
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3 = Naïve Subjects – No Sound Control Tested – Close Proximity
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5 = Naïve Subjects – Auditory Tone Tested – Extended Proximity
6 = Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity
7 = Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity
8 = Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity
9 = Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity
10 = Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity

Appendix K

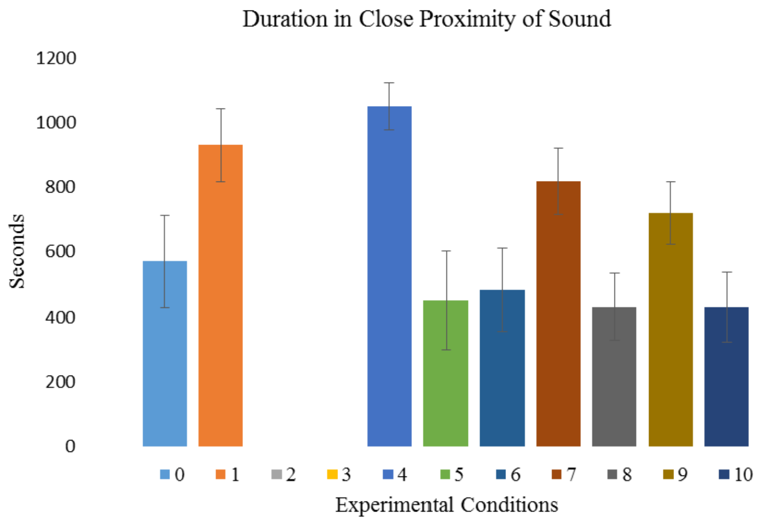
Duration in Close Proximity to Sound

	0	1	2	3	4	5	6	7	8	9	10
0		0.000*			0.000*	0.133*	0.293**	0.013**	0.133**	0.101**	0.135**
1	0.000*				0.258**	0.000*	0.000*	0.019*	0.000*	0.001*	0.000*
2											
3											
4	0.000*	0.258**				0.000*	0.000*	0.000**	0.000*	0.000**	0.000*
5	0.133*	0.000*			0.000*		0.323*	0.001*	0.491*	0.002*	0.483*
6	0.293**	0.000*			0.000*	0.323*		0.000*	0.544**	0.006*	0.505**
7	0.013**	0.019*			0.000**	0.001*	0.000*		0.000*	0.217**	0.000**
8	0.133**	0.000*			0.000*	0.491*	0.544**	0.000*		0.000**	0.894
9	0.101**	0.001*			0.000**	0.002*	0.006*	0.217**	0.000**		0.000**
10	0.135**	0.000*			0.000*	0.483*	0.505**	0.000**	0.894**	0.000**	

Grey results outlined in paper

* One-tailed *p* value

** Two-tailed *p* value



LEGEND

0 = Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity
1 = Naïve Subjects – Auditory Tone Tested – Close Proximity
2 = Naïve Subjects – No Sound Control Tested – Extended Proximity
3 = Naïve Subjects – No Sound Control Tested – Close Proximity
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9 = Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity
10 = Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity

Appendix L

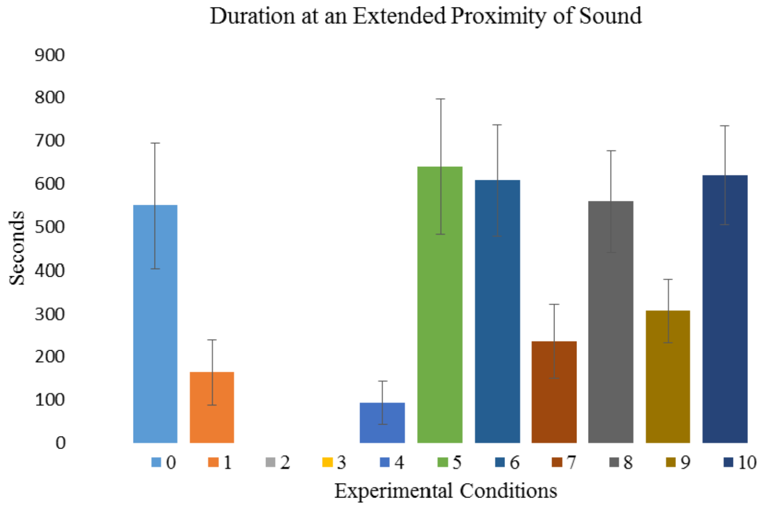
Duration at an Extended Proximity from Sound

	0	1	2	3	4	5	6	7	8	9	10
0		0.000*			0.000*	0.244*	0.469**	0.000**	0.668**	0.018**	0.329**
1	0.000*				0.185**	0.000*	0.000*	0.062*	0.000*	0.006*	0.000*
2											
3											
4	0.000*	0.185**				0.000*	0.000*	0.003**	0.002*	0.003**	0.002*
5	0.244*	0.000*			0.000*		0.298*	0.001*	0.228*	0.001*	0.412*
6	0.469**	0.000*			0.000*	0.298*		0.000*	0.739**	0.001*	0.626**
7	0.000**	0.062*			0.003**	0.001*	0.000*		0.000*	0.144**	0.000**
8	0.668**	0.000*			0.002*	0.228*	0.739**	0.000*		0.001**	0.460
9	0.018**	0.006*			0.003**	0.001*	0.001*	0.144**	0.001**		0.000**
10	0.329**	0.000*			0.002*	0.412*	0.626**	0.000**	0.460**	0.000**	

Grey results outlined in paper

* One-tailed *p* value

** Two-tailed *p* value



LEGEND

0 = Naïve Subjects – Maternal Assembly Call Tested – Extended Proximity
1 = Naïve Subjects – Auditory Tone Tested – Close Proximity
2 = Naïve Subjects – No Sound Control Tested – Extended Proximity
3 = Naïve Subjects – No Sound Control Tested – Close Proximity
4 = Naïve Subjects – Maternal Assembly Call Tested – Close Proximity
5 = Naïve Subjects – Auditory Tone Tested – Extended Proximity
6 = Postnatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity
7 = Postnatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity
8 = Prenatal Auditory Tone Exposed – Auditory Tone Tested – Extended Proximity
9 = Prenatal Auditory Tone Exposed – Auditory Tone Tested – Close Proximity
10 = Postnatal Visual Stimulus Exposed – Auditory Tone Tested – Extended Proximity

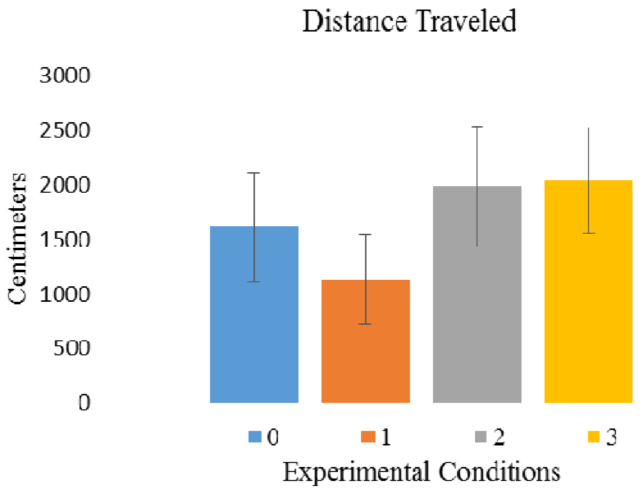
Appendix M

Latency to Exit Emergence Box

	0	1	2	3
0		0.223**	0.660**	0.062**
1	0.223**		0.098*	0.003*
2	0.660**	0.098*		0.058*
3	0.062**	0.003*	0.058*	

* One-tailed *p* values

** Two-tailed *p* values



LEGEND

0 = Naïve Subjects - No Visual Stimulus Tested

1 = Naïve Subjects - Visual Stimulus Tested

2 = Postnatal Visual Stimulus Exposed - Visual Stimulus Tested

3 = Postnatal Auditory Tone Exposed - Visual Stimulus Tested

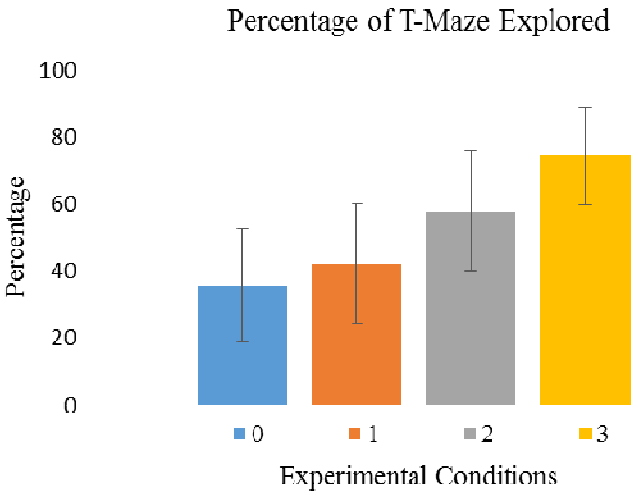
Appendix N

Percentage of T-Maze Explored

	0	1	2	3
0		0.771**	0.158**	0.011**
1	0.771**		0.077*	0.022*
2	0.158**	0.077*		0.280*
3	0.011**	0.021*	0.280*	

* One-tailed *p* values

** Two-tailed *p* values



LEGEND
0 = Naïve Subjects - No Visual Stimulus Tested
1 = Naïve Subjects - Visual Stimulus Tested
2 = Postnatal Visual Stimulus Exposed - Visual Stimulus Tested
3 = Postnatal Auditory Tone Exposed - Visual Stimulus Tested

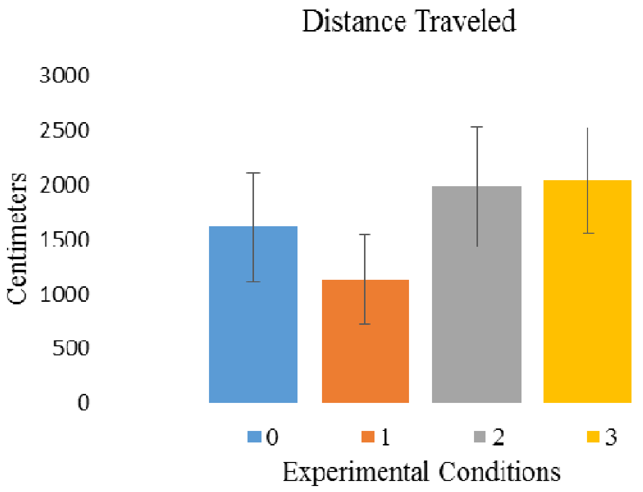
Appendix O

Distance Traveled

	0	1	2	3
0		0.152**	0.338**	0.204**
1	0.152**		0.01*	0.004*
2	0.338**	0.01*		0.494*
3	0.204**	0.004*	0.494*	

* One-tailed *p* values

** Two-tailed *p* values



LEGEND
0 = Naïve Subjects - No Visual Stimulus Tested
1 = Naïve Subjects - Visual Stimulus Tested
2 = Postnatal Visual Stimulus Exposed - Visual Stimulus Tested
3 = Postnatal Auditory Tone Exposed - Visual Stimulus Tested

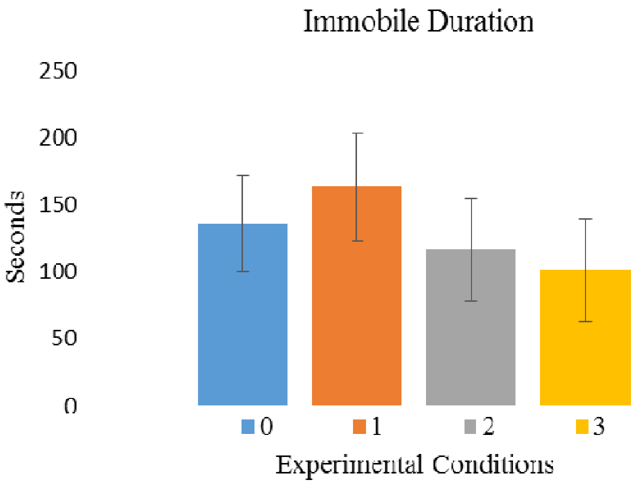
Appendix P

Immobile Duration

	0	1	2	3
0		0.344**	0.287**	0.086**
1	0.344**		0.027*	0.006*
2	0.287**	0.027*		0.295*
3	0.086**	0.006*	0.295*	

* One-tailed *p* values

** Two-tailed *p* values



LEGEND
0 = Naïve Subjects - No Visual Stimulus Tested
1 = Naïve Subjects - Visual Stimulus Tested
2 = Postnatal Visual Stimulus Exposed - Visual Stimulus Tested
3 = Postnatal Auditory Tone Exposed - Visual Stimulus Tested

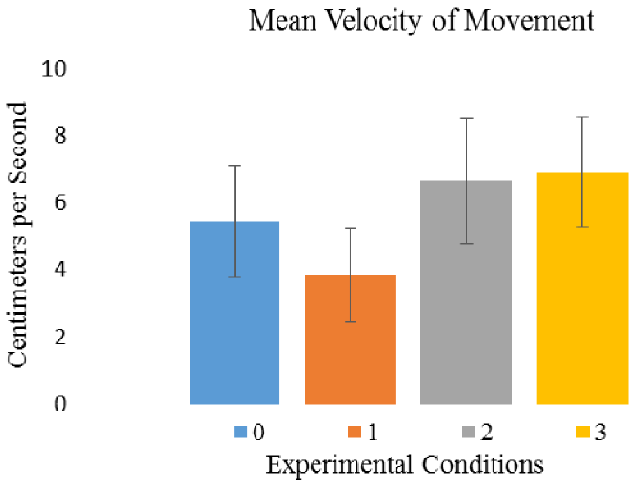
Appendix Q

Mean Velocity of Movement

	0	1	2	3
0		0.160**	0.329**	0.204**
1	0.160**		0.010*	0.004*
2	0.329**	0.010*		0.500*
3	0.204**	0.004*	0.500*	

* One-tailed *p* values

** Two-tailed *p* values



LEGEND
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PUBLICATIONS AND PRESENTATIONS

Suarez, M. (2012, October). *Predicting explorative behavior by level of emotional reactivity in bobwhite quail neonates (Colinus virginianus).* Research presented at the FIU MBRS Mini-Symposium, Miami, FL.

Suarez, M. & Lickliter, R. (2012, October). *Predicting explorative behavior by level of emotional reactivity in bobwhite quail neonates (Colinus virginianus).* Poster presented at the International Society for Developmental Psychobiology, New Orleans, LA.

Suarez, M. (2012). Predicting explorative behavior by level of emotional reactivity in bobwhite quail neonates (Colinus virginianus). *FIU Electronic Theses and Dissertations.* Paper 810.

Suarez, M. (2013, October). *Studying the effects of early life experience on fearfulness and exploration in bobwhite quail neonates (Colinus virginianus).* Research presented at the FIU MBRS Mini-Symposium, Miami, FL.

Suarez, M. & Lickliter, R. (2013, November). *Decreasing fearfulness through prenatal and postnatal exposure to fear-inducing auditory stimuli*. Poster presented at the International Society for Developmental Psychobiology, San Diego, CA.

Suarez, M,. Logan, M. A., Ceballos, N., & Lickliter, R. (2014, October). *Studying generalization of fear inducing stimuli and its implications for the treatment of neophobia during early development.* Research presented at the FIU MBRS Mini-Symposium, Miami, FL.

Suarez, M,. Logan, M. A., Ceballos, N., & Lickliter, R. (2014, November). *Fear of novelty is reduced by early postnatal exposure to novel stimuli in bobwhite quail.* Poster presented at the International Society for Developmental Psychobiology, Washington DC.