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Effects of Increased Levels of Prenatal Mesotocin on Postnatal Individual Recognition and Stress Responsiveness in Northern bobwhite quail (Colinus Virginianus)

Brittany Yusko
Florida International University, byusk001@fiu.edu

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

EFFECTS OF INCREASED LEVELS OF PRENATAL MESOTOCIN ON POSTNATAL INDIVIDUAL RECOGNITION AND STRESS RESPONSIVENESS IN NORTHERN BOBWHITE QUAIL (*COLINUS VIRGINIANUS*)

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To: Dean Kenneth G. Furton
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This thesis, written by Brittany Yusko, and entitled Effects of Increased Levels of Prenatal Mesotocin on Postnatal Individual Recognition and Stress Responsiveness in Northern Bobwhite Quail *(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.

_____
Anthony Dick

_____
Bennett Schwartz

_____
Robert Lickliter, Major Professor

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The thesis of Brittany Yusko is approved.

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Dean Kenneth G. Furton
College of Arts and Sciences

_____
Dean Lakshmi N. Reddi
University Graduate School

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ABSTRACT OF THE THESIS

EFFECTS OF INCREASED LEVELS OF PRENATAL MESOTOCIN ON POSTNATAL INDIVIDUAL RECOGNITION AND STRESS RESPONSIVENESS IN NORTHERN BOBWHITE QUAIL (*COLINUS VIRGINIANUS*)

by

Brittany Yusko

Florida International University, 2014

Miami, Florida

Professor Robert Lickliter, Major Professor

Oxytocin (OT) plays a key role in the mediation of social and stress behaviors across many species; however, the mechanism is still unclear. The present study investigated the influence of prenatal levels of mesotocin (MT; avian homologue of OT) on postnatal social and stress behavior in Northern bobwhite quail. Experiment one determined endogenous levels of MT during prenatal development using an enzyme-linked immunoassay kit. Experiment two examined the influence of increased MT during prenatal development on chicks' individual recognition ability and stress response to a novel environment. Experiment one showed MT levels increased significantly throughout embryonic development. Experiment two showed significant differences in stress behavior for chicks with increased MT during prenatal development; however, no significant differences were found for social behavior. This study suggests MT serves different functions depending on the stage of embryonic development and that increasing MT levels affects postnatal stress behavior, but not social behavior.

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I. INTRODUCTION

Over the past decade, researchers have focused on the role that the neurotransmitter oxytocin (OT) plays in the mediation of social and stress behaviors across many species; however, how and to what extent this neurotransmitter is involved in these processes is still unclear (Bartz, Zaki, Bolger, & Ochsner, 2011; Ross & Young, 2009). Because of the growing evidence to support the role of OT in several specific disorders such as autism, social anxiety disorders, depression, obsessive-compulsive disorder, and schizophrenia, it is important that we are able to effectively characterize this system (Gimpl & Fahrenholz, 2001). Researchers working with OT Knockout mice have shown that these mice have a significant impairment in social recognition tasks that can be reversed through injection of OT (Ferguson, Aldag, Insel, & Young, 2001). Another key finding came from Stein, Goldin, Sareen, Zorrilla, and Brown (2002) who demonstrated that social avoidance and phobias (i.e., anxiety) increased amygdala activity in humans. Stein and colleagues' (2002) finding is significant because we know the amygdala contains OT receptors, suggesting that OT may be an underlying mechanism reflecting the changes in activity and behavior observed in these disorders. Similar activation of the amygdala was found when participants were shown fearful stimuli and this activation was significantly depressed by intranasal administration of OT (Kirsch, et al., 2005). Additionally, compelling evidence for the role of OT in social cognition comes from Hollander and colleagues, 2006 who found facilitation of the processing and retention of social information, specifically affective speech, in adults diagnosed with Asperger's or Autism after administration of OT. Taken together, these

findings suggest a role for OT in the production of the aberrant behaviors characteristic of these disorders.

Research has also revealed a role of OT in stress responses (Lee, Brady, Shapiro, Dorsa, & Koenig, 2007). Studies examining the effects of OT on stress have found that OT exerts powerful anti-stress effects including decreases in blood pressure, corticosterone/cortisone levels, and increases in insulin, all of which may allow for easier social interactions among conspecifics (Gimpl & Fahrenholz, 2001). Furthermore, stress-induced central release of OT can alleviate stress-induced symptoms of anxiety (Gimpl & Fahrenholz, 2001). These studies suggest that there is overlap between the effects of OT on behaviors related to stress and social interactions. Indeed, in many instances characteristics from both domains are present in a given disorder. For example, anxiety disorder symptoms can include hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis and decreases in social interaction.

In addition, of particular interest for my research, is the substantial body of work demonstrating that changes in the prenatal environment can have effects on subsequent postnatal behaviors. For example, Bertin, Richard-Yris, Möstl, and Lickliter (2009) showed that increasing the amount of prenatal testosterone (T) available to a developing quail embryo significantly increased chicks' postnatal growth rate, stress reactivity, and auditory learning ability.

Mesotocin (MT) is the avian homologue of OT and differs from OT by only one amino acid (Jonaidi, Oloumi, & Denbow, 2003; Gimpl & Fahrenholz, 2001; Bons, 1980) and as we will review below, MT in avian species functions similarly to OT in mammals. In the current study, Northern bobwhite quail were used to investigate the role of

increased levels of MT during prenatal development on postnatal social behavior and stress responsiveness. To investigate this, two experiments were carried out. Experiment one was designed to determine the endogenous levels of MT throughout embryonic development. Experiment two had three aims: (1) to examine the role of increased prenatal MT on postnatal social cognition, specifically examining chicks' ability to discriminate between a familiar and unfamiliar conspecific, (2) to examine the role of increased prenatal MT on chicks postnatal stress responsiveness as measured by an emergence task into a novel environment, and (3) to examine the developmental trajectory of the effects of increased prenatal MT on these postnatal behaviors. To our knowledge, this is the first study to quantify the amount of MT in the brain of the bobwhite quail and to examine the effects of prenatal MT levels on postnatal behaviors.

II. LITERATURE REVIEW

Hormones and Behavior

Hormones are chemicals produced in the body at one location, which project their effects to the activity of another location, referred to as the target tissue. The production locations for hormones are specialized glands known as endocrine glands (*endo* from the Greek root word meaning 'within' and *krinein* meaning 'to release'). Hormones are responsible for coordinating the physiology and behavior of an organism by regulating, integrating, and directing its bodily functions (Nelson, 2010). Hormones are quite similar in function to other chemical transmitters in the body, such as neurotransmitters and cytokines, and the division among these chemical mediators is mostly a reflection of the need to organize the various biological systems into endocrine, nervous, and immune systems (Nelson, 2010). Hormones can be categorized into four

groups: (1) peptides or proteins, (2) steroids, (3) monoamines, and (4) lipid-based hormones. The divisions of the hormones into their respective groups is based on several distinctive characteristics including their mode of release, how they move through the blood, the location of their target tissue receptors, and the manner by which the interaction of the hormone with its receptor results in a biological response (Nelson, 2010). Additionally, the endocrine system itself harbors several general features that distinguishes it from other bodily systems including (1) endocrine glands are ductless, (2) endocrine glands have a rich blood supply, (3) the products of endocrine glands (i.e. hormones) are secreted into the bloodstream, (4) hormones in the blood can circulate to almost every cell in the body and thus have the potential to interact with any cell containing appropriate receptors, (5) hormone receptors are embedded within the membrane or located elsewhere on the cell and these receptors are quite specific binding sites, only interacting with a particular hormone or class of hormones (Nelson, 2010).

Historically, there have been two types of mechanisms that have dominated research aimed at elucidating the physiological mechanisms of animal behavior - neural and hormonal. Until the beginning of the twentieth century, the corresponding systems for these two mechanisms, nervous and endocrine, respectively, were thought to be disparate. However, findings have led to the understanding that the nervous and endocrine systems are significantly integrated (Adkins-Regan & Carter, 2010). For example, nerve cells can synthesize and secret hormones, the behavioral effects of hormones are mediated by their actions on neurons, and the endocrine system is regulated by the brain so that hormone levels associated with behavior respond to both physical and social environments (Adkins-Regan & Carter, 2010). Indeed, it is now known that one of

the most active endocrine organs and one that produces the most diverse array of hormones is the brain (Nelson, 2010). It is also known that many neuro-endocrine mechanisms related to behavior and other complex processes are similar among many vertebrate species.

Some of the first evidence of this concept of neurohormones is credited to Henry Dale in the early 1900's when he showed that secretions from the pituitary gland could be used to induce labor, first in animal models and later in humans (Dale, 1906 as cited in Adkins-Regan & Carter, 2010). Further evidence came from Otto Loewi in 1921 when he demonstrated that secretions from the vagus nerve were capable of affecting heart rate (as cited in Adkins-Regan & Carter, 2010). Loewi's work is said to be the first "modern" evidence for neurohormones (i.e. the idea that hormones in the periphery can, in fact, come from and be regulated by the central nervous system). Together, the work of these and many others during this time generated a powerful interest in the relationship between hormones and their effects on behavior.

Building on the understanding of neuroendocrine mechanisms and their influence on behavior is the work of Ernst Scharrer. In 1928, Scharrer contributed to understanding the role of secretions of the central nervous system in endocrinology by identifying the largest cells in the hypothalamus and subsequently naming them 'magnocellular neurons'. He was also the first to articulate the concept of neurosecretion (Adkins-Regan & Carter, 2010). However, it was not until 1953-1954 that the chemicals secreted by these cells were identified when Vincent du Vigneaud synthesized oxytocin and vasopressin (Adkins-Regan & Carter, 2010). The identification of oxytocin and vassopressin by du Vigneaud set the stage for advancements in the investigations of

hormones and understanding that they were not only produced in peripheral endocrine glands and the pituitary, but in the nervous system as well.

Some of the seminal work examining neuroendocrine mechanisms of behavior involved the measuring of steroid hormones using bioassays. Advances in these hormone measurement techniques have led to an abundance of studies linking the release of hormones, particularly steroid hormones, to specific behaviors. With the surge of interest in measuring hormones and correlating them with specific behaviors questions began to surface as to what evidence would be appropriate in order to establish that a certain hormone was, indeed, responsible for the observed change in behavior or that a behavior was correlated with a change in hormone concentration. It was established that three conditions must be satisfied in order for a causal link to be made between the hormone under investigation and the corresponding behavior: (1) the behavior should disappear when the source of the hormone is removed or the actions of the hormone are blocked, (2) after the behavior is stopped, replacement of the hormone or the hormones source should restore the behavior, and (3) hormone concentrations and the behavior should be covariant (Nelson, 2010). These guidelines are still utilized in the modern investigation of hormones. However, because of the difficulty in maintaining reliable covariant hormone-behavior measures the first two conditions are typically sufficient to establish a link between hormone and behavior (Nelson, 2010). It is likely the complexity of the interactions of the multiple hormones as well as other environmental and social factors that influence behaviors that accounts for the difficulty of obtaining the third criteria of covariant of hormone and behavior. In fact, one of the environmental factors that can alter hormone functioning is the laboratory setting. Because of this, it is of particular

importance to verify hormone-behavior relationships in natural environments. Although there are inherent difficulties associated with examining hormone-behavior relationships in the natural environment it is also useful in distinguishing between artifacts of a laboratory setting and a true biological response (Nelson, 2010).

The early work of researchers interested in the study of hormone-behavior relationships (i.e., ethologists) focused on the non-physiological mechanisms of behavior, such as sensory cues (Adkins-Regan & Carter, 2010). Eventually the underlying mechanisms of behavior began to be investigated by figures such as Erich von Holst and Ursula von St. Paul, when they electrically stimulated the brains of freely moving chickens and observed behaviors such as vocalizations, grooming, feeding, and aggressive attack (Adkins-Regan & Carter, 2010). The work Holst and St. Paul, as well as many others, marked the beginning of a rich interest in the physiological mechanisms of behavior.

Research continues to flourish with novel discoveries on the nature of the hormone-behavior relationship in animals and humans alike. For example, research has demonstrated that adrenal steroid hormones are secreted in different amounts according to the time of day and in doing so, play an important role in coordinating sleep patterns, food seeking behaviors, and processing of information (McEwen, Sakai, & Spencer, 1993). Studies examining effects of sex hormones have found that postnatal increases in testosterone levels are critical for the normal development of the male genitalia and reproductive function (Main, Schmidt, & Skakkebaek, 2005). Alexander, Wilcox, and Farmer (2012) found that hormonal changes in early postnatal life could predict toy preferences in male infants. The 2002 finding of Alexander and colleagues provides a

sensitive time period in which the influence of hormones on sex-specific behaviors may be of particular interest, similar to that of puberty in early adolescence. While the majority of investigations on hormone-behavior relationships have focused on the postnatal period, a number of researchers are also inquiring about the role of hormones in the prenatal environment.

Prenatal Hormones

It has been well established that during prenatal development hormones influence the structure of the brain and determine the basis for behavioral regulation (Worthman, 2011). Some of the earliest work regarding the theory of how hormones function to support organization and regulation of behavior of the organism came from the landmark article published in 1959 by Charles Phoenix and colleagues (Phoenix, Goy, Gerall, & Young, 1959 as cited in McCarthy, Wright, & Schwarz, 2009; Wade, 2006). The results of this article offered two important concepts to the field: (1) It demonstrated that when female guinea pigs were exposed to androgens during the prenatal period masculinization of sexual behavior occurs and (2) it posited the Organizational/Activational theory of the effects of hormones. The theory stated that hormones during the prenatal period have "organizational" effects because they produce permanent changes to brain structure and the corresponding behavior and "activational" effects are those that occur in the postnatal period that produce temporary changes in the brain and behaviors. Since this time, a wealth of evidence has accumulated in support of this notion. One of the most widely known effects of prenatal hormones is regarding sex differentiation of brain structures and the reproductive system. For example, the discovery of sex-specific differences in song control nuclei in birds was one of the earliest discoveries of its kind and led directly

to the discovery of the sexually dimorphic nucleus of the preoptic area in rodents (Gorski, Harlan, Jacobson, Shryne, & Southam, 1980). Following these insights it was soon revealed that sex differences existed in the frequency of dendritic spine versus somatic synapses in the arcuate nucleus (Matsumoto & Arai, 1980) ventromedial nucleus (Matsumoto & Arai, 1986) and amygdala (Nishizuka & Arai, 1981).

In addition to the abundance of work examining the effects of steroid hormones in the prenatal period there has been a growing amount of work investigating glucocorticoids. High levels of maternal stress during pregnancy are associated with increased levels of glucocorticoids that alter the maternal hypothalamic-pituitary-adrenocortical axis and placental axis (Davis & Sandman, 2006). Some key findings have demonstrated that increased glucocorticoids in the prenatal environment can lead to abnormal development of the fetal central nervous system and shorter gestation, both of which increase the risk of infants born with cognitive, emotional, and physical abnormalities (Davis & Sandman, 2006). For example, prenatal exposure of rats to increased glucocorticoids was shown to lead to poorer performance on a spatial memory task as adults (Brabham, Phelka, Zimmer, Nash, Lopez, & Vazquez, 2000). Additional studies using animal models have demonstrated that increased amounts of prenatal glucocorticoids have life-long effects on insulin resistance, blood pressure, hypothalamic-pituitary-adrenal axis functioning, and the central nervous system (Bakker, van Bel, and Heijnen, 2001; Matthews, 2000). These studies not only highlight the importance of prenatal hormones and their effects on multiple aspects of postnatal development, but they also underscore one of the major difficulties of the mammalian model for examining effects of prenatal hormones. When attempting to examine the effects of hormones in the

prenatal period there is the constant influence of the mothers' endocrine state on the fetus. Most research examining hormones has utilized a mammalian model and although there has been a wealth of insight into the effects of hormones on prenatal development, it is difficult to parse apart the effects of the hormone being examined and the many other maternal hormones that are affecting the fetus. Because of this, researchers have employed another model in the study of the effects of prenatal hormones – the avian model. An advantage of using avian species is that all prenatal hormones are deposited prior to incubation (i.e. a one-time event at oogenesis). Thus, the avian paradigm allows for more precise control of the hormone being investigated, providing a clearer picture of what is responsible for the observed alterations in postnatal behavior. Another advantage of the avian model is that in comparison to other non-mammalian species, avian eggs are quite large and therefore allow for easier sampling and manipulation (Groothuis & Engelhardt, 2005). Because of its advantages, the avian model has become an increasingly popular model for the investigation of prenatal hormones and their effects on behavior.

Avian Hormones

The past decade has shown a surge in studies investigating steroid hormones of maternal origin in the avian egg and how these effect offspring development (Groothuis & Engelhardt, 2005). Indeed, much of the research investigating hormones in avian species has focused on hormones of maternal origin, particularly androgens (Müller, Dijkstra, & Groothuis, 2009; Engelhardt, Carere, Dijkstra, & Groothuis, 2006; Müller, et al., 2005; Eising, Müller, Dijkstra, & Groothuis, 2003). Hormones of maternal origin that have been found in the egg prior to incubation are androgens, estrogen, and

corticosterone. These hormones have been shown to have effects on the development of the embryo, the phenotype of the adult bird, and the amount and type of egg hormones can act as a significant source of developmental plasticity beginning in the embryonic stages and continuing throughout postnatal life (Gil, 2003). For example, these hormones have been implicated in the mediation of postnatal attributes of the offspring such as hatching time, muscular growth, growth of body mass and structural size, early begging and competitive behavior, and pre-fledgling survival (Groothuis & Engelhardt, 2005). It has also been found that eggs producing male versus female chicks differ in the content of maternal hormones (Petrie, Schwabl, Brand-Lavridsen, & Burke, 2001). Specifically, eggs hatching males had significantly higher levels of androstenedione, testosterone, dihydrotestosterone, and estradiol compared to female hatching eggs. These findings are important for understanding the effects of maternal hormones on the development and sex differentiation of the embryo as multiple studies have found sexual differentiation of brain structures, phenotypes, and behaviors of offspring.

An advantage for researchers employing the avian model is that there is a large volume of behavioral data collected in birds in a naturalistic setting relative to many other vertebrate taxa (Groothuis & Carere, 2004). For example, ecologists have extensively studied neophobia and exploratory behaviors in birds in relation to ecological plasticity, opportunism, or innovative behavior, as these are all potential driving forces behind the evolution of many species (Groothuis & Carere, 2004). As psychologists, these behaviors are also of particular interest, but for different reasons. For example, examining neophobic behavior can shed light on different aspects of many human psychological disorders (e.g., anxiety, depression, autism, schizophrenia). To better understand the

hormonal mechanisms underlying these concepts researchers have expanded research on hormones to experimental modification. By modifying the endogenous levels of hormones, researchers are attempting to better isolate hormones of interest and gain deeper knowledge regarding their roles in the development of postnatal behaviors and how the relationship between hormones and behavior relate to aspects of human psychological disorders.

Modifying Avian Hormones

The ability to manipulate hormones of interest has afforded researchers the opportunity to gain more direct insights into hormonal mechanisms involved in behavior. Following the work in mammals, recent research with avian species has focused a considerable amount of attention on modifying hormones in the prenatal environment. For example, Müller and colleagues (2005) found that increases in prenatal levels of testosterone and androstenedione produced negative effects on cell-mediated immunity and humoral (i.e. antibody-mediated) immunity in gull chicks. Other research examining prenatal exposure to increased testosterone in bobwhite quail embryos found that this influenced postnatal growth rates, increased chicks' emotional reactivity, and facilitated auditory learning ability (Bertin, et al., 2009). Taken together these studies suggest that elevated prenatal levels of androgens can have significant effects on postnatal development and learning. Nordgreen, Janczak, and Bakken (2006) investigated the effects of increased corticosterone in the prenatal period on postnatal filial imprinting in the domestic chicken. It was found that chicks that were exposed to elevated corticosterone did not show a preference for the imprinting stimulus during a discrimination task compared to the control group that did show a preference. These

studies have provided evidence for the influence of hormones of maternal origin in multiple avian species on postnatal behaviors. Because studies investigating prenatal hormones in avian species have focused on hormones of maternal origin, almost exclusively, it is imperative that this research be extended to other classes of hormones. Extending this type of research to other hormones will provide novel insight into mechanisms driving multiple aspects of development, including behavior.

Oxytocin, Social Cognition, and Stress Responsiveness

Oxytocin. Oxytocin (OT) and OT-like peptides are neurohypophysial hormones that have been shown to be a key modulator in social behavior across a wide variety of species. It was also the first peptide hormone to have its structure determined and the first to be chemically synthesized in a biologically active form (Ross & Young, 2009). Virtually all vertebrate species possess an OT-like peptide (Gimpl & Fahrenholz, 2001). OT, like all neurohypophysial hormones, is a nonapeptide that is constituted with a six amino acid cyclic part and a COOH-terminal alpha-amidated three-residue tail. OT is synthesized in the magnocellular neurosecretory cells of the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the hypothalamus. It is released from the hypothalamus and stored in the Herring Bodies of the axon terminals in the posterior pituitary. It is here that OT is bound to its' transporter protein neurophysin I and released into the blood stream when stimulated. Similarly, MT which differs from OT by only one amino acid (Jonaidi, Oloumi, & Denbow, 2003; Gimpl & Fahrenholz, 2001; Bons, 1980) has been shown to be produced and stored in the same relative brain structures as OT in humans and other mammal species (Ross & Young 2009; Tennyson, Nilaver, Hou-Yu, Valiquette, & Zimmerman, 1986). Research has also provided support for MT serving

similar functional purposes as those of OT (Jonaidi et al., 2003; Milewski, Ivell, Grossman, & Ellendorff, 1989; Tennyson et al., 1986; Blähser & Heinrichs, 1982; discussed in further detail below).

In humans, OT has been found in equivalent amounts in the posterior pituitary and plasma of both sexes, providing evidence for the notion that OT has other physiological functions in addition to its well-known role in reproductive functioning in females (Gimpl & Fahrenholz, 2001). For example, stress has been shown to stimulate the release of OT from the posterior pituitary into circulation. Studies investigating the functional properties of the posterior pituitary have found that stimulating this area only causes release of OT into the blood stream, whereas stimulation of the PVN releases OT into the blood and the CSF in rats, which is in accord with the finding that the PVN contains OT producing projections to the spinal cord (Gimpl & Fahrenholz, 2001). Further support comes from studies in which hypophysectomy resulted in the disappearance of OT in the blood, but increased OT concentrations in the CSF in rats. It is important to note that most of what we know about OT has come from rat studies and there is also evidence of species-specific differences.

Social Cognition. Social cognition can be defined as the encoding, storage, retrieval and processing of information in the brain related to conspecifics. Social cognition is comprised of many different aspects including attachment, joint attention, face recognition, theory of mind, and individual recognition. For example, the capacity to discriminate between ones own conspecifics is an important skill for navigating the social environment. Indeed, the ability to discriminate between conspecifics is an essential component to the survival and reproductive success of any social species, including

humans. Having the ability to assess and remember others to determine who is an appropriate social partner or a potential enemy is beneficial to all social species. It is easy to understand why the abilities comprising social cognition are essential to being successful in this domain. The capacity to think about what another is thinking, recognize and discriminate between familiar and unfamiliar conspecifics, or being able to coordinate ones attention with that of a social partner, such as following eye gaze, to share an experience related to an object or event can be pivotal to the survival of an organism (Mundy, Gwaltney, & Henderson, 2010).

Researchers have integrated social cognition with research from biology and neuroscience. This integration has linked specific biology to specific behaviors in the organism by supporting the notion that these behaviors are occurring concurrently with neurohormonal systems and brain circuits. The influence of this paradigm shift on the investigation of social affiliation remains widely evident today. Thus, there has been a surge in the study of physiological mechanisms underlying overt behaviors specifically related to social affiliation or cognition (Gweon, Dodell-Feder, Bedny, & Saxe, 2012; Haan, Pascalis, & Johnson, 2002). For example, Haan and colleagues (2002) were able to show using electroencephalogram (EEG) that when adults were shown pictures of human or nonhuman primate faces, and upright or inverted faces, the adults' face-responsive N-170 event-related potential (ERP) component showed specificity to the upright human faces only. Recently, Gweon and colleagues (2012) used functional magnetic resonance imaging (fMRI) to demonstrate that children and adults showed increased activation of the right temporo-parietal junction while listening to descriptions of characters' mental states compared to descriptions of physical states. Taken together, these studies provide

evidence that certain areas of the brain respond preferentially to species-specific social features. A study conducted in human participants in 2007, using eye tracking, found that participants administered OT showed an increased number of fixations and total gaze time toward the eye region when compared with controls (Guastella, Mitchel, & Dadds, 2007). This provides evidence that OT increases gaze specifically towards the eye region of the human face and suggests the possibility that eye gaze may be a mechanism by which emotion recognition, interpersonal communication, and social approach behavior is mediated.

Work with animals has provided insight into other possible neural mechanisms supporting social cognition. Much of this animal research has focused on neurohormonal mechanisms, particularly the OT system; in fact, some of the pioneering studies investigating the role of OT were in relation to social recognition in rats. These studies revealed that, in male rats, central injections of low doses of OT enhanced the amount of time (i.e., duration) a conspecific was remembered (Benelli, et al., 1995; Popik, Vetulani, & van Ree, 1992; Dantzer, Bluthe, Koob, & Le Moal, 1987). Specifically, Benelli and colleagues (1995) used a simultaneous choice task in relation to social recognition using familiar versus novel conspecifics. Preference (i.e., recognition) of the familiar was indicated by more time spent investigating the novel compared to the familiar conspecific. Furthermore, studies using OT knockout mice have shown that on social recognition tasks, like the one described above, show impairments, indicating that this peptide is essential for familiarity recognition (Choleris, Ogawa, Kavaliers, Gustafsson, Korach, Muglia, & Pfaff, 2006; Clipperton, Cragg, Wood, Langmo, & Choleris, 2006; Ferguson, Young, Hearn, Matzuk, Insel, & Winslow, 2000; and reviewed in Choleris et

al., 2009). In addition to the work on underlying mechanisms of social cognition, there is a substantial body of work demonstrating that early experiences within the environment can have substantial effects on social behaviors. For example, a study examining visual imprinting of species-specific maternal preferences in ducklings found that if exposed to a stuffed hen for 30 minutes, ducklings at 24 hours of age would develop a visual preference for the familiar mallard over an unfamiliar stuffed hen in a simultaneous choice task at 48 and 72 hours, but only if the ducklings were reared under unrestricted social interactions with siblings. If ducklings were reared in social isolation, the visual preference for the familiar stuffed hen would not occur. This study underscores the importance of normal early social experiences on visual imprinting of filial behavior (Lickliter & Gottlieb, 1985)

The importance of the early environment in the development of social cognition extends beyond the perinatal period and into the prenatal environment. Studies have examined behaviors such as perceptual learning and emotional reactivity in quail and found quite interesting results (Lickliter, 2005). For example, Lickliter (1989) found that exposing bobwhite quail embryos to patterned visual stimulation in the period immediately prior to hatching interfered with species typical auditory preferences in the post-hatch period. In contrast, another study examining the effects of prenatal auditory stimulation on bobwhite quail embryos showed enhanced postnatal responsiveness. Specifically, enhanced prenatal auditory stimulation produced an accelerated pattern of species typical visual responsiveness by 24 hours of age (Lickliter & Stoumbos, 1991). In addition, studies examining the effects of prenatal steroid hormones on postnatal ontogeny of behaviors found that increasing the amount of prenatal testosterone (T)

available to a developing quail embryo significantly increased chicks' postnatal growth rate, stress reactivity, and auditory learning ability (Bertin, et al., 2009).

Stress. Studies in male and female rats have shown that OT exerts anti-stress effects such as decreases in blood pressure and corticosterone levels (Gimpl & Fahrenholtz, 2001). For example, immobilization stress has been shown to increase the amount of OT mRNA in rats (Jezova, Skultetyova, Tokarev, Bakos, & Vigas, 1995). Thus, it is thought that perhaps stimulated release of OT serves to activate the hypothalamic-pituitary-adrenal (HPA) axis to increase glucocorticoid release. Corticotropin releasing factor (CRF) is produced in the hypothalamus, which serves as a precursor to adrenocorticotropic hormone (ACTH). Adrenocorticotropic hormone is produced in and secreted by the anterior pituitary and is responsible for stimulating the release of glucocorticoids, such as cortisol, from the adrenal gland. The effects of ACTH can have both long-term and short-term physiological effects. The release of glucocorticoids by the adrenal glands acts as an inhibitory negative feedback mechanism to inhibit the release of CRF from the hypothalamus. The connections among the elements of this cascade of hormone release are known as the HPA axis. Copious amounts of research have shown that the HPA axis is often activated in response to biological stress. Cortisol is one of the main stress hormones involved in the HPA axis and glucocorticoids, such as cortisol, are known for their anti-inflammatory and immunosuppressant effects. Almost every cell contains receptors for glucocorticoids and a diurnal cycle (for cortisol in particular) has been observed across several mammalian species with levels typically being highest in the morning and lowest at night (Gimpl & Fahrenholz, 2001).

Other research has shown that stress-induced central release of OT can ameliorate stress-associated symptoms, such as anxiety, in rats and mice (Gimpl & Fahrenholz, 2001). Interestingly, OT has also been shown to act as an anti-depressant in two different animal models of depression (Arletti & Bertolini, 1987) and it is possible that these effects may be mediated by an influence of OT on the dopaminergic neurotransmission in the limbic brain regions from projections of OT neurosecretory neurons from the PVN of the hypothalamus (McCarthy, 1995). Some research suggests that these OT-induced effects may be explained by the anxiolytic actions of OT (e.g., by reducing the inhibition inherent in social encounters; Gimpl & Fahrenholz, 2001). It has also been posited that OT may act as an anxiety reducer when animals are experiencing a stress-response to a novel environment or unfamiliar conspecific (McCarthy, 1995).

Mesotocin and The Avian Model

As was briefly reviewed above, MT is a homologue of OT. Mesotocin is found in birds, lungfish, reptiles, amphibians, and some marsupials (Goodson, Kelly, & Kinsbury, 2012). Like OT in mammals, MT is synthesized in the parvocellular cells of the paraventricular nucleus (PVN) of the hypothalamus (Mikami, Tokado, & Farner, 1978) and can also be found in the magnocellular cells of the PVN (Aste, Muhlbauer, & Grossmann, 1996). Goodson and colleagues (2012) reported that, similar to mammals, MT-immunoreactive fibers in birds have also been found in the nucleus accumbens, medial and lateral Bed Nucleus of the Stria terminalis (BNST), medial amygdala, lateral septum, habenula, periaqueductal gray, and ventral tegmental area. One of the only studies to actually quantify MT in an avian model showed that in chicken embryos peak levels of MT in the infundibulum and neural lobe occur between days 17 and 18 and

decrease thereafter to amounts similar to those found on the first postnatal day (Nouwen, Decuypere, Michels, & Kühn, 1982). In fact, the most widely used avian model has been the domestic chicken, however, other avian models are becoming more abundant in the study of hormones and behavior including the zebra finch, Japanese quail, and the bobwhite quail. Relevant to our research, Charvet and Striedter (2010) compared brain development during the prenatal period of domestic chickens with that of bobwhite quail. They found that when compared in absolute days of incubation (21 days for chickens vs. 23 days for quail) neural events occur later in quail than in the chicken; However, when compared in percentage of incubation period the timing of neural events overlap between the two species.

While the study of the MT system in birds has been investigated for quite some time, it is only recently that the effects of MT on avian behavior have been examined. For example, when examining the effects of an MT antagonist on group size preferences in male and female zebra finches, findings revealed that both peripheral and intraventricular administration produced a decrease in the percent of time spent near the larger group of conspecifics and a concomitant increase in the time spent near the smaller group (Goodson, Schrock, Klatt, Kabelik, & Kingsbury, 2009). This effect could then be reversed by central administration of MT, indicating that MT plays an important role in modulating avian social behavior in relation to group size preference. Additionally, using a modified version of the above paradigm where the choices were instead a familiar or novel same-sex conspecific, the same investigators examined the effects of peripheral and central administration of an MT antagonist and found that both administration routes reduced the preference of subjects for the familiar conspecific. As reviewed above,

similar results have been shown with rats and mice using this same task (reviewed in Choleris, Clipperton-Allen, Phan, & Kavaliers, 2009).

While some work on the topic of MT and avian behavior is emerging, the most common being social and affiliative behavior, to our knowledge, there has yet to be any work examining the relationship between MT and stress reactivity in avian species; However, several studies are investigating the relationship between MT and stress using a mammalian model (i.e., rats and mice) and have found evidence that MT modulates at least some aspects of stress reactivity (see the section titled "Stress"; Lee, Brady, Shapiro, Dorsa, & Koenig, 2007; Jezova, et al., 1995; McCarthy, 1995; Arletti & Bertolini, 1987). Additionally, there is no research on the relationship between MT during the prenatal period and how manipulations of MT during this stage of development may affect postnatal behavior. Thus, my study was designed to determine the effects of elevated prenatal levels of MT on postnatal social cognition and stress responsiveness in Northern bobwhite quail. We intended to (1) Build upon the knowledge of hormones and behavior, particularly during the prenatal period, while utilizing the advantages of employing an avian model and (2) Extend upon the hormone research within the avian paradigm by examining the influence of a hormone that is not of maternal origin (i.e., mesotocin).

III. HYPOTHESES AND PREDICTIONS

The first hypothesis of the proposed experiment regards the characterization of the endogenous levels of MT throughout the four embryonic developmental time points. On the basis of previous research of Charvet and Striedter (2010), who analyzed the embryonic developmental trajectory of brain maturation in the domestic chicken and bobwhite quail as well as the research from Nouwen, and colleagues (1982) who

quantified MT levels in the embryonic brains of domestic chickens, it was hypothesized that the lowest levels of MT would be observed on embryonic day 8 and the highest would be observed on embryonic day 16. The second hypothesis concerns the effect of prenatally elevated MT on social cognition. Chicks were administered an individual recognition task in which a familiar and a novel chick of the same age were placed on opposite sides of an open arena. On the basis of the social recognition tasks reviewed above, I predicted that chicks with increased levels of MT in the prenatal period would show a preference for a familiar conspecific over a novel chick when compared to both control groups (Choleris, Clipperton-Allen, Phan, & Kavaliers, 2009; Goodson et al., 2009; McCarthy, 1995). The third hypothesis concerns chicks' stress response to a novel environment. On the basis of the literature reviewed above examining the effects of OT on stress responsiveness I predicted that elevated levels of MT in the prenatal period would decrease chicks stress response to a novel environment (McCarthy, 1995).

IV. EXPERIMENT ONE

Method

Subjects. The brains of 40 quail embryos were collected to determine the average endogenous levels of mesotocin present throughout the 23 days of prenatal development in Northern bobwhite quail eggs. Eggs were weighed prior to incubation and brain tissue was collected and weighed on embryonic days 8, 12, 16, and 20 (Charvet & Striedter, 2010; Milewski et al., 1989; Tennyson et al., 1986; Blähser & Heinrichs, 1982). An additional 13 embryos were used to verify the efficacy of the injection procedure once the baseline levels of MT were determined. All eggs were subjected to the same tissue collection, tissue preparation, and hormonal assay procedure as outlined here (see the

section titled "injections" in the methods section of experiment two for a description of the injection procedure). All animal care and work was carried out in accordance with the recommended institutional guidelines for animal care and use (Florida International University IACUC approval #13-015).

Procedure

Tissue Collection. Embryos were euthanized via rapid decapitation (Ubuka, Ueno, Ukena, & Tsutsui, 2003; Milewski et al., 1989) and the whole brain was removed from the skull with the aid of standard micro-dissection tools and a dissection microscope. Tissue was weighed, flash frozen in liquid nitrogen, and stored at -80ºC until the time of analysis.

Tissue Preparation. MT was extracted from the cells following the extraction procedure outlined in the oxytocin enzyme-linked immunoassay kit (ELISA; Abcam) with some modifications. An approximate equal volume of 0.1% triflouroacetic acid in water (0.1% TFA-H_2O) was added to each sample. Tissue was homogenized using manual micro tissue homogenizers and centrifuged for 15 minutes at 17,000 x g at 4ºC. Supernatant was collected. Five hundred mg C18 SEP-PAK columns were used to perform solid phase extraction for each sample. Each column was equilibrated using 2ml of acetonitrile followed by 15 ml of 0.1% TFA-H_2O. The supernatant was applied to the column and washed with 15 ml of 0.1% TFA-H2O. Finally, the column was eluted with 4 ml of a solution comprised of 40% acetonitrile and 0.1% TFA-H2O. Eluants were collected and evaporated to dryness overnight using a centrifugal concentrator under vacuum in cold temperature. Dried samples were stored at -20ºC until reconstitution with assay buffer at time of analysis.

Hormonal Assays. A commercial oxytocin enzyme-linked immunoassay kit was used to quantify levels of MT present in the brains of the quail embryos. Cross-reactivity between the oxytocin ELISA kit and MT was determined to be 131%. Plates were read at 405nm. Calculations were performed using the Gen5 software (version 2.00). Briefly, a standard curve was made for each plate with OT concentrations of 1000, 500, 250, 125, 62.5, 31.2, and 15.6 pg/ml. Percent bound versus concentration of OT (pg/ml) for the standard curve was plotted and used to determine the concentration of MT in the samples. The average amount of MT present for each of the time points was calculated and used to determine the amount of MT to inject for experiment two.

Data Analysis

Data were analyzed using the statistical software program Statistical Package for the Social Sciences (SPSS). The N is 10 for all four time points in the endogenous group. In the injected group Ns for each time point are as follows: day 8, $N = 4$, day 12, $N = 4$, day 16, $N = 3$, and day 20, $N = 2$ (see General Discussion section). The accepted significance level was $p < 0.05$.

Results

The requirements for data on the brain weights (weights in milligrams) and MT levels (pg/ml) in the brains of chicks during embryonic development were not obtained; therefore, Kruskall-Wallis and post-hoc Mann-Whitney U-tests were used for between group comparisons with embryonic age (8,12,16, 20) and condition (endogenous vs. injected) as the between subjects factors. To examine the effect of MT on brain growth during the prenatal period, brain weights were recorded for each subject at the time of tissue collection. In the embryos used to determine endogenous amounts of MT, brain

weights across the four developmental time points ranged from 109.5 mg to 396.9 mg. Average brain weights for each time point were 157.9 mg (*SD* = 2.43 mg), 120.1 mg (*SD* = 0.59 mg), 232.9 mg (*SD* = 0.94 mg), and 348.7 mg (*SD* = 2.13 mg) for 8-, 12-, 16-, and 20-day-old embryos, respectively. Between group comparisons revealed a significant effects of Age on brain weight (Kruskall-Wallis, $H = 36.482$, $p = .000$) for these embryos. Post hoc Mann-Whitney U-tests revealed significant differences between days 8 and 12 (U = -3.704, $p = .000$), 12 and 16 (U = 3.780, p = .000), and 16 and 20 (U = 3.797, p = .000). In the embryos used to analyze the effects of the injection procedure brain weights across the four stages ranged from 100mg to 340mg. Average brain weights for each time point were 152.5 mg (*SD* = 2.99 mg), 115.00 mg (*SD* = 1.29 mg), 220.00 mg (*SD* = 1.00 mg), and 335 mg (*SD* = .71 mg) for 8-, 12-, 16-, and 20-day-old embryos, respectively. Between group comparisons revealed a significant main effect of Age ($H = 10.119$, $p = .018$); However, post hoc Mann-Whitney U-Tests did not reveal significant differences between any age. No significant effect of Condition was found for brain weight.

To address the primary research question of determining the profile of levels of MT throughout embryonic development Kruskall-Wallis and Mann-Whitney U-tests were conducted. The endogenous levels of MT throughout the four time points were lowest at the beginning of embryonic development and increased through embryonic day 20. The average amount of MT present in the brains of the quail embryo at day 8 was 43pg/ml (*SD* = 5.05). Day 12 revealed levels nearly 5 times this amount at 210pg/ml (*SD* = 42.36). From day 12 to day 16 levels of MT increased approximately 10-fold to 1,961pg/ml (*SD* = 392.79) and on day 20 levels were measured at another

approximate10-fold increase of 3,047pg/ml (*SD* = 650.19). Between group comparisons revealed a main effect of Age (H = 35.700, p = .000). Post-hoc Mann-Whitney U-tests revealed significant differences between days 8 and 12 (U = 3.808, p = .000), 12 and 16 (U = 3.780, p = .000), and 16 and 20 (U = 3.250, p = .000). After injection of synthetic MT, levels of MT for the days examined were found to be 42.24pg/ml (*SD* = 2.02), 150.22pg/ml (*SD* = 58.87), 1,414.02pg/ml (*SD* = 336.90), and 3,192.55pg/ml (*SD* = 1,057.03), respectively. Between group comparisons revealed a significant effect of Age on MT levels (Kruskall-Wallis, H = 11.207, p = .011). Post hoc Mann-Whitney U-tests revealed a significant difference in MT levels between days 8 and 12 (U = 2.323, p = .029). No significant effect of Condition was found (See *Figures 1* and *2*).

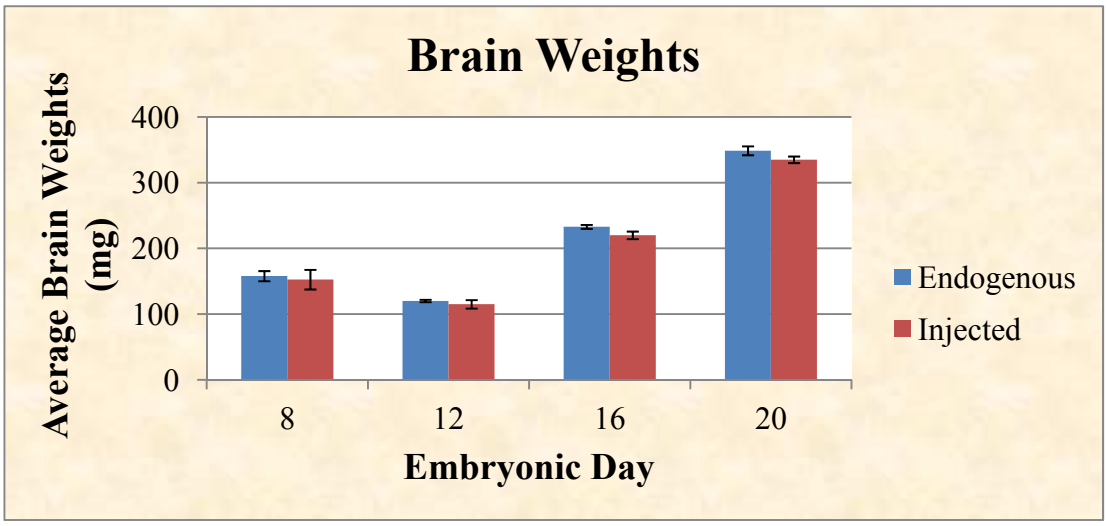

Figure 1. Average brain weights of chicks in the Endogenous and Injected conditions for the four prenatal development time points. Standard error bars represent the standard error of the mean.

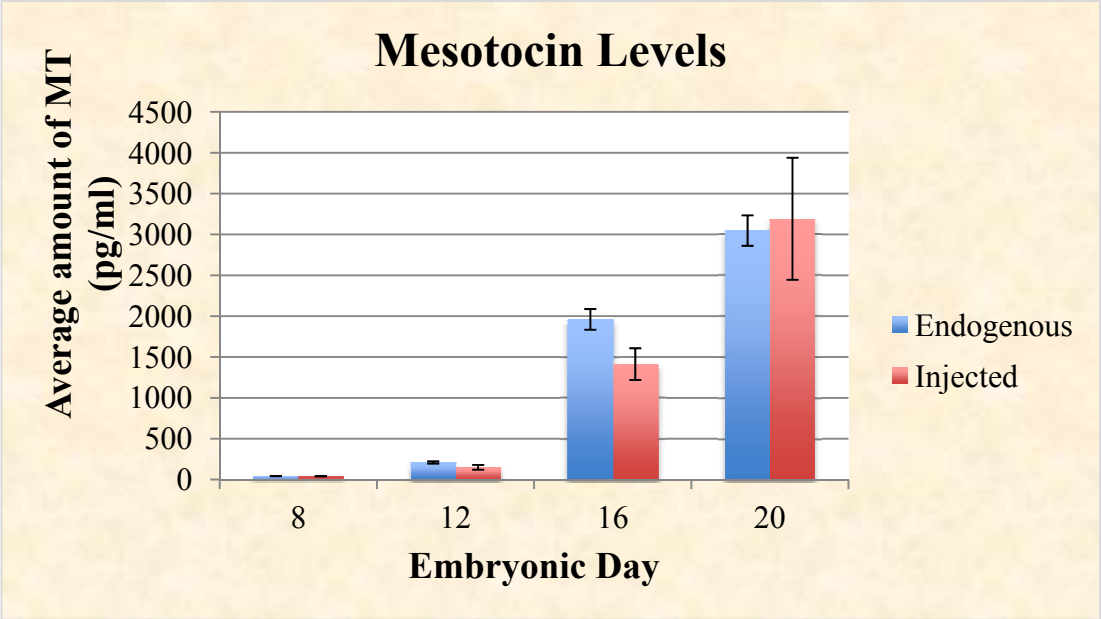

Figure 2. Average level of mesotocin of chicks in the Endogenous and Injected conditions for the four prenatal development time points. Standard error bars represent the standard error of the mean.

Discussion

We are the first to quantify levels of MT in the brain of the quail embryo. The dramatic changes in the level of MT across developmental stages suggest that MT may play an important role in at least some aspects of prenatal development and that its role changes depending on the age of the embryo. For example, between embryonic days 16 and 20 in the quail we observed a near 10-fold increase in the amount of MT. During the equivalent developmental stage in the chicken embryo (time in percent of incubation; Charvet & Striedter, 2010) the visual and auditory systems are at peak development and further maturation of neurotransmitter systems is occurring (Rogers, 1995). Given the data provided by Charvet and Striedter (2010) it is likely that peak development of the visual and auditory systems, along with maturation of neurotransmitter systems is

occurring between days 16-20 in the bobwhite quail; Thus, our findings may provide insight into the role that MT plays in the development of these two key sensory systems. This finding is of interest to our work as research has shown abnormal processing of auditory and visual stimuli in certain disorders, such as children diagnosed with autism spectrum disorders (ASD; see Bahrick and Todd, 2012 for a review). Furthermore, abnormal levels of OT have been shown in children with ASD suggesting a possible correlation between OT and at least some of the symptoms of ASD associated with the auditory and visual systems (Modhal, Green, Fein, Morris, Waterhouse, Feinstein, & Levin, 1998).

Additionally, I showed that there were no significant differences in the levels of MT between conditions (i.e., MT injected vs. no injection). MT levels remaining the same between conditions could indicate that increasing the levels of MT pre-incubation causes down-regulation of the MT system in the embryo. If the injection did, in fact, cause a down-regulation of the endogenous MT system, it is likely that increasing the amount of MT available to the developing embryo early in development allowed for a compensatory mechanism to adjust for the added amount of the hormone. It would be interesting to explore whether or not this would also be the case if the embryos were injected later in development. However, one issue with exploring this is the development of the blood-brain barrier, which in the chicken, occurs at approximately embryonic day 14 (Ribatti, Nico, & Bertossi, 1993).

Future investigations could utilize a radioactive tracer along with the injected hormone which would afford the opportunity to examine where the hormone is distributed after injection and for how long it remains there in order to develop better

techniques for injecting hormones that are not of maternal origin to more accurately mimic the natural introduction of the hormone in the embryo. Another possible line of inquiry is to examine the effects of an antagonist for MT at different embryonic stages. Further investigation is needed to better understand how the bobwhite quail embryo adapts to altered levels of hormones during embryonic development.

In addition to experiment 1, we were interested in examining the behavioral effects of administering exogenous MT during embryonic development. If MT is affecting the visual and auditory systems during prenatal development are we able to measure any associated changes in postnatal behavior? Because of the known deficits in social cognition and aberrant reactivity of stress systems associated with many developmental and psychiatric disorders we explored chicks ability to recognize a familiar conspecific in a simultaneous forced choice test between a familiar and novel chick and chicks' stress response to a novel environment, at two postnatal ages.

V. EXPERIMENT TWO

Method

Egg Injections. Fertile, unincubated eggs were selected each week and divided into one of the three experimental conditions. Injections were performed prior to incubation as described by Bertin et al. (2009). All eggs receiving injections were disinfected with 70% ethanol and a hole was bored in the eggshell above the air sac using a sterile 25 1/2 -G needle. The solution (20 µl vehicle (Sodium Chloride (NaCl); Sigma and MT ((Ile^{8})-Oxytocin; Bachem, No. H-2505) for M-treated or vehicle only for V-controls) was delivered using a 100 µl Hamilton syringe mounting 25 1/2 -G sterile needle. The injection hole was sealed using the procedure described by Rubolini,

Romano, Martinelli, Leoni, and Saino (2006) by gluing a tiny piece of cleaned and disinfected eggshell over the hole immediately after injection. After hatching chicks were transported to their home-cage where they were housed for the remainder of the study.

Subjects. Subjects consisted of 147 incubator-reared Northern bobwhite quail chicks. There were three groups of embryos: a mesotocin treated group (M; N = 27), a vehicle treated group (V-controls; N = 60), and an untreated group (controls; N = 60). Chicks were housed in clear plastic bins (25cm wide x 15 cm high x 45 cm long) placed on shelves in a Nuaire Model NU-605-500 Animal Isolator in groups of 12-15 chicks to mimic typical brood conditions for the bobwhite quail. Food and water were available *ad libitum*. All animal care and work was carried out in accordance with the recommended institutional guidelines for animal care and use (Florida International University IACUC approval #13-015).

Apparatus. The individual recognition task was conducted in a circular, open field arena (diameter = 130 cm, height = 24 cm) located in a sound-attenuated room. Two semi-circular areas each corresponding to 5% of the total area of the arena were demarcated on opposite sides of the arena. A clear, plexi-glass box (17cm x 15cm x 15cm) was placed in each area. For testing, a familiar chick (reared with the subject chick) and a novel chick (not reared with the subject chick, but of the same age) were placed in the plexi-glass boxes. Immediately following, the subject chick was placed in the center of the arena to begin testing. Time (seconds) spent investigating the novel chick and the familiar chick was recorded.

For the stress responsiveness task, a small box (25.4 cm x 17.78 cm) was placed on the outer edge of the arena. There is a small opening in the front of the box covered by

a door that allowed the chick to exit the box. A one-minute acclimation period preceded the start of the test before the experimenter opened the door to the box, allowing the chick to exit into the arena. Latency to exit the box and time spent in the open arena were measured.

Chicks were tested at 72 and 96 hours post-hatching. After chicks completed the test at 72 hours, they were returned to the home cage for the intervening 24-hour period. At 96 hours post-hatching chicks were subsequently tested on the same task. Counterbalancing for side-bias took place across all conditions and between age of testing (72- versus 96-hours post-hatch). All testing trials lasted 5 minutes and were recorded via a video camera that is mounted directly above the testing arena. The chicks were tracked using the software program Ethovision. All data were stored within the Ethovision software until the data were exported for analysis.

Data Analysis

To achieve adequate power for our parametric and non-parametric statistical analyses, we have found that an N of approximately 30-40 chicks per experimental condition is usually required. Data were analyzed using the statistical software program Statistical Package for the Social Sciences (SPSS). The requirements for parametric tests were not obtained for these data; therefore, Kruskall-Wallis and post-hoc Mann-Whitney U-tests were used for between group comparisons. Due to the non-independence of the duration scores for the time spent investigating the novel versus the familiar chick in the individual recognition task, duration scores were converted into proportion of total duration scores.

Between group comparisons were performed on the differences between these scores (i.e. familiar minus novel). For the emergence task, latency to emerge into the open arena was measured.

Results

Individual Recognition Task. To address the primary research question of whether MT injected chicks showed a preference for the novel over the familiar chick Kruskall-Wallis tests were performed for the proportion of time spent investigating the novel versus the familiar chick. Within group comparisons (Wilcoxon- signed rank tests) were conducted to examine the effect of Age on the duration of time chicks spent investigating the novel versus the familiar chick and revealed a marginally non-significant effect of Age ($Z = 1.677$, $p = .093$). The effect of Condition on time spent investigating the novel versus familiar chick was not significant (*Figure 3*).

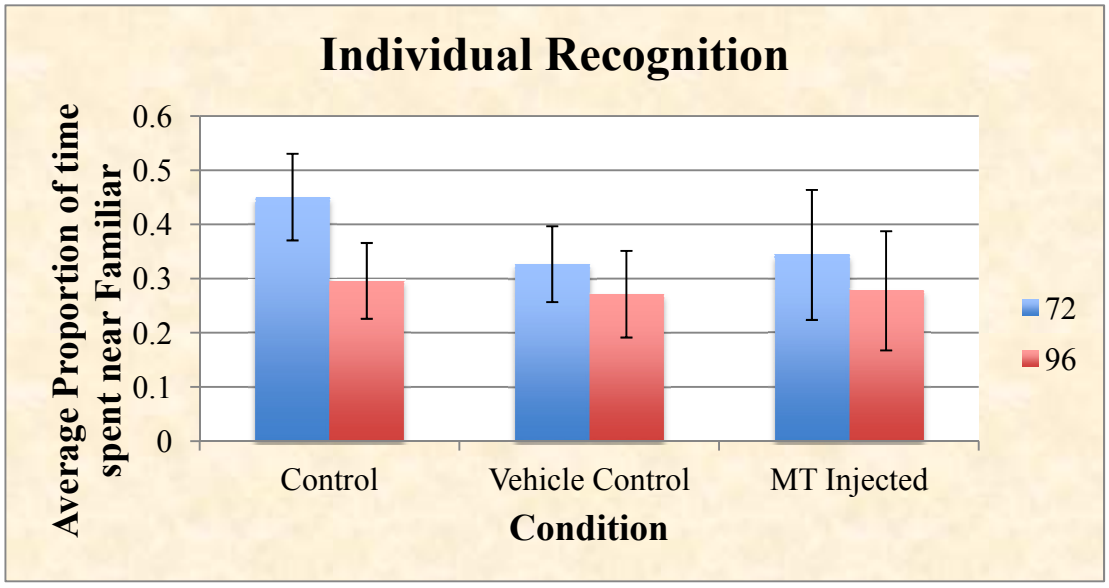

Figure 3. Mean proportion of time spent near the familiar chick. Standard error bars represent the standard error of the mean.

Emergence Task. To address the primary research question of whether MT injected chicks showed a reduced stress response to a novel environment we performed analyses on the latency to exit the box into the open arena. Results revealed a significant main effect of Condition (Control, Vehicle Control, MT injected) for latency to exit into the open arena at 72 hr, $H = 10.546$, $p = .005$ and a marginally significant effect at 96 hr, $H = 5.229$, $p = .073$. Post-hoc Mann-Whitney U-tests revealed significant differences between control chicks and MT injected chicks and between vehicle injected chicks and MT injected chicks for latency to exit in to the open arena at 72 hr, $U = 126$, $p = .007$ and $U = 98$, $p = .001$, respectively. No differences were found between the control and vehicle control conditions on latency to exit into the open arena at 72 hr. There was no significant effect of age (*Figure 4;* note that in the mesotocin condition at 72 hours the average time to emerge is zero, which, in this case is indicating that the chicks in this group never emerged).

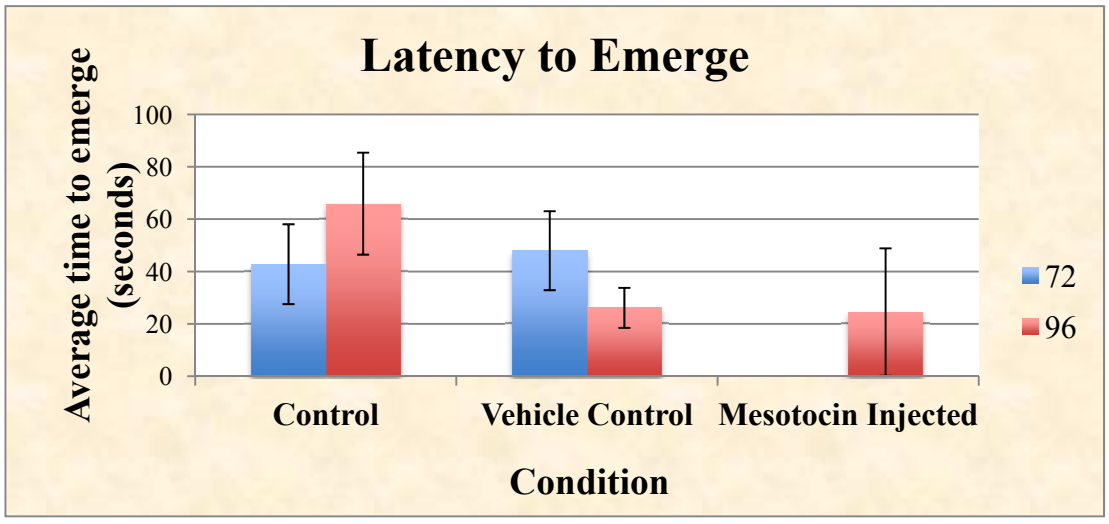

Figure 4. Mean of the difference scores for latency to emerge into the open arena. Standard error bars represent the standard error of the mean.

Discussion

The results of experiment two provide evidence that injection of MT during prenatal development has lasting effects on at least some postnatal behaviors. At 72 hr of age chicks injected with MT during the prenatal period took significantly longer to exit the box into the open arena as measured in the emergence task compared to the two control groups. Because the emergence task is designed to test stress reactivity to a novel environment, we assert that chicks that were injected with MT during prenatal development exhibited a greater stress response to a novel environment compared to chicks that did not receive MT in the prenatal period. Because reduced levels of MT have been shown to increase fear and stress responses it may be possible that the effects of the injection procedure continued to have effects on MT levels postnatally, causing a down-regulation of this system. When chicks were tested again at 96 hr of age, this effect disappeared; suggesting that either 1) the system is beginning to compensate for the effects of increased levels of MT prenatally, or 2) it may also indicate reduced novelty of the environment. Future studies could examine this by testing two separate groups of chicks at each age to parse apart possible effects of compensatory mechanism versus reduced novelty to further understand the developmental plasticity of the chick MT system.

No significant results were found for either condition or age for our measure on the individual recognition task. This suggests that either 1) our procedure of increasing MT does not affect this set of social behaviors in the bobwhite quail or 2) that some component of this measure is simply not useful for measuring this type of social behavior in bobwhite quail. To address the first possibility, it is possible that the potential down-

regulation of the MT system caused by the injection is sufficient to impair chicks' ability to discriminate between conspecifics, as we know that decreased levels of MT can have effects on social recognition tasks (Goodson et al., 2009). If the injected MT is not stable enough to last in the system once injected, it may be that the MT is no longer present at a critical time when it could have effects on areas of the brain that are involved in social behavior. For example, we know that centrally released MT from the PVN to areas of the amygdala and forebrain are main pathway by which social behavior is affected (Goodson et al., 2012). We also know that approximately embryonic day 8 in chicks is the peak time of neuron formation in the forebrain (Rogers, 1995). If MT is no longer available to have an effect during this time then we may not see any differences in social behavior. It is also possible that at this age the brain systems necessary for this type of social discrimination are not developed enough to perform such discriminations between conspecifics.

To address the second possibility that some component of our task may not be useful for measuring this type of social behavior in the quail it is important to consider other variables such as age or experience of the animal. For example, we assume that because chicks are raised in postnatal groups in separate rearing bins and do not have visual stimulation from chicks of other groups that these chicks are novel to one another; however this may not be true. We know that chicks rely heavily on their auditory sense so it is possible that being exposed to the calls of other chicks in the postnatal rearing environment is enough to make a chick familiar. Chicks are able to discriminate between familiar and unfamiliar calls as early as 24 hrs post-hatching, which suggests that this sense is very important for determining who is familiar and who is not. To elucidating the

role of the auditory sense in individual discrimination of familiar and novel chicks it would be necessary to raise groups of chicks truly separate from one another. Furthermore, because we know that the prenatal environment can have effects on postnatal behavior, it would be important to incubate groups of chicks separately as well.

VI. SUMMARY AND IMPLICATIONS OF THE FINDINGS

This was the first study to quantify levels of MT in the developing Northern bobwhite quail brain. We found that from embryonic day 8 to embryonic day 20 levels of MT increase significantly from an average of 43 pg/ml to an average of 3047 pg/ml, respectively. Our results suggest that MT in the brain plays an important role in at least some aspects of prenatal development in the bobwhite quail. However, our results showed no differences in MT levels between our injected group and control group, possibly suggesting a compensatory mechanism of the MT system in early prenatal development. Experiment 1 also indicated that changes observed in the levels of MT during prenatal development occur around the same time that major developmental changes are occurring in the visual and auditory systems. Because research has shown abnormal processing of visual and auditory information in disorders such as ASD (see Bahrick and Todd, 2012 for a review) and abnormal levels of OT have been shown to be associated with the aberrant social and stress reactivity observed in ASD and other disorders (Modhal, Green, Fein, Morris, Waterhouse, Feinstein, & Levin, 1998), experiment 2 was designed to address the relationship between abnormal levels of MT during prenatal development and postnatal social and stress behaviors.

Experiment 2 showed that chicks that had been injected with MT prenatally exhibited an increase in stress reactivity to a novel environment. We would have

predicted this result in stress behavior had we observed a decrease in MT levels after the injection of MT prenatally, however, the levels remain the same at each of the embryonic time points examined. Given these results, it is possible that levels of MT decrease to below normal levels later in prenatal development or in early postnatal stages. We did not find any significant results on our individual recognition task. This could suggest that MT is not affecting this behavior due to its' lack of presence during a critical time for development of pathways in the brain associated with social behavior (Goodson et al., 2012). Further studies should work to elucidate the role of MT in prenatal development on postnatal behaviors associated with developmental and psychiatric disorders by developing new techniques to inject hormones not of maternal origin and by implementing behavioral paradigms to assess the behavioral components associated with these disorders.

VII. LIMITATIONS

An important point to address when considering the results for this study is that chicks receiving MT injections exhibited an approximate 50% mortality rate. This is the main reason for our lower N values for the MT injected birds. Because we do not see any difference in brain weight/growth nor in MT levels after injection yet a disruption in early development is evident it is possible that having elevated levels of a hormone that is not of maternal origin and therefore not typically present pre-incubation may cause too great of a disruption for at least half of the chicks to survive. In order to better understand the role of MT in the prenatal environment and attempt to correct the 50% mortality rate it will be necessary to develop methods to inject later in prenatal development to mimic the endogenous rise in MT levels (Viglietti-Panzica, Mura, & Panzica, 2007).

REFERENCES

Adkins-Regan, E. and Carter, C. (2010). Neurobiology, endocrinology, and behavior. *Elsevier*, 549-556.

Alexander, G., Wilcox, T., and Farmer, E. (2009). Hormone-behavior associations in early infancy. *Hormones and Behavior, 56*, 498-502.

Arletti, R., and Bertolini, A. (1987). Oxytocin acts as an antidepressant in two animal models of depression. *Life Sciences, 41,* 1725–1730.

Aste, N., Muhlbauer, E., and Grossmann, R. (1996). Distribution of AVT gene expressing neurons in the prosencephalon of Japanese quail and chicken. *Cell and Tissue Research, 286,* 365-373.

Bahrick, L. and Todd, J. (2012). Multisensory processing in autism spectrum disorders: Intersensory processing disturbance as a basis for atypical development. In B.E. Stein (Ed.), *The new handbook of multisensory processes* (p.657-674), Cambridge, MA: MIT Press.

Bakker, J., van Bel, E., and Heijnen. C. (2001). Neonatal glucocorticoids and the developing brain: short term-treatment with life long consequences? *Trends in Neuroscience, 24,* 649-653.

Bartz, J., Zaki, J., Bolger, N., and Ochsner, K. (2011). Social effects of oxytocin in humans: context and person matter. *Trends in Cognitive Sciences, 15,* 301-309.

Benelli, A., Bertolini, A., Poggioli, R., Menozzi, B., Basaglia, R., and Arletti, R. (1995). Polymodal dose-response curve for oxytocin in the social recognition test. *Neuropeptides, 28,* 251-255.

Bertin, A., Richard-Yris, M., Möstl, E., and Lickliter, R. (2009). Increased yolk testosterone facilitates prenatal perceptual learning in Northern bobwhite quail (*colinus virginianus*). *Hormones and Behavior, 56,* 416-422.

Blähser, S., and Heinrichs, M. (1982) Immunoreactive neuropeptide systems in avian embryos (domestic mallard, domestic fowl, Japanese quail). *Cell and Tissue Research, 223,* 287-303.

Bons, N. (1980). The topography of mesotocin and vasotocin systems in the brain of domestic mallard and Japanese quail: immunohistochemical Identification. *Cell and Tissue Research, 213,* 37-51.

Brabham, T., Phelka, A., Zimmer, C., Nash, A., Lopez, J., and Vazquez, D. (2000). Effects of prenatal dexamethasone on spatial learning and response to stress is influenced by maternal factors. *American Journal of Physiology: Regulatory, Integrative, Comparative, and Physiology, 279,* R1899-R1909.

Charvet, C., and Striedter, G. (2010). Bigger brains cycle faster before neurogenesis begins: a comparison of brain development between chickens and bobwhite quail. *Proceedings of the Royal Society, 277,* 3469-3475.

Choleris, E., Clipperton-Allen, A., Phan, A., and Kavaliers, M., (2009). Neuroendocrinology of social information processing in rats and mice. *Frontiers in Neuroendocrinology, 30,* 442-459.

Choleris, E., Ogawa, S., Kavaliers, M., Gustafsson, J., Korach, K., Muglia, L., and Pfaff, D. (2006). Involvement of estrogen receptor alpha, beta, and oxytocin in social discrimination: A detailed behavioral analysis with knockout female mice. *Genes, Brain, and Behavior, 5,* 528-539.

Clipperton, A., Cragg, C., Wood, A., Langmo, A., and Choleris, E. (2006). Influence of gonadal hormones on social behavior in male and female mice. *Abstract at the Society for Behavioral Neuroendocrinology.*

Dale, H. (1906). On some physiological actions of ergot. *Journal of Physiology, 34,* 163-206.

Dantzer, R., Bluthe, R., Koob, G., and Le Moal, M. (1987). Modulation of social memory in male rats by neurohypophyseal peptides. *Psychopharmacology, 91,* 363-368.

Davis, E. and Sandman, C. (2006). Prenatal exposure to stress and stress hormones influences child development. *Infants and Young Children, 19,* 246-259.

Eising, C., Muller, W., Dijkstra, C., and Groothuis, T. (2003). Maternal androgens in egg yolks: relation with sex, incubation time and embryonic growth. *General and Comparative Endocrinology, 132,* 241-247.

Engelhardt, N., Carere, C., Dijkstra, C., and Groothuis, T. (2006). Sex-specific effects of yolk testosterone on survival, begging, and growth of zebra finches. *Proceedings of the Royal Society: Biological Sciences, 273,* 65-70.

Ferguson, J., Aldag, J., Insel, T., and Young, L. (2001). Oxytocin in the medial amygdala is essential for social recognition in the mouse. *The Journal of Neuroscience, 21(20),* 8278-8285.

Ferguson, J., Young, L., Hearn, E., Matzuk, M., Insel, T., and Winslow, J. (2000). Social amnesia in mice the oxytocin gene. *Nature Genetics, 25,* 284-288.

Gil, D. (2003). Golden eggs: Maternal manipulation of offspring phenotype by egg androgen in birds. *Ardeola, 50,* 281-294.

Gimpl, G. and Fahrenholz, F. (2001). The oxytocin receptor system: structure, function, and regulation. *Psychological Reviews, 81(2),* 629-683.

Goodson, J., Kelly, A., and Kingsbury, M. (2012). Evolving nonapeptide mechanisms of gregariousness and social diversity in birds. *Hormones and Behavior, 61,* 239-250.

Goodson, J., Schrock, S., Klatt, J., Kabelik, D., and Kingsbury, M. (2009). Mesotocin and nonapeptide receptors promote estrildid flocking behavior. *Science, 325,* 862-866.

Gorski, R., Harlan, R., Jacobson, C., Shryne, J., and Southam, A. (1980). Evidence for the existence of a sexually dimorphic nucleus in the preoptic area of the rat. *Journal of Comparative Neurology, 193,* 529–539.

Groothuis, T. and Carere, C. (2005). Avian personalities: characterization and epigenesis. *Neuroscience and Biobehavioral Reviews*, *29*, 137-150.

Groothuis, T. and Engelhardt, N. (2005). Investigating maternal hormones in avian eggs: measurement, manipulation, and interpretation. *Annals of the New York Academy of Sciences, 1046,* 168-180.

Guastella, A., Mitchel, P., and Dadds, M. (2007). Oxytocin increases gaze to the eye region in human faces. *Biological Psychiatry, 63,* 3-5.

Gweon, H., Dodell-Feder, D., Bedny, M., and Saxe, R. (2012). Theory of mind performance in children correlates with functional specialization for a brain region for thinking about thoughts. *Child Development, 00,* 1-16.

Haan, M., Pascalis, O., and Johnson, M. (2002). Specialization of neural mechanisms underlying face recognition in human infants. *Journal of Cognitive Neuroscience, 14,* 199-209.

Jezova, D., Skultetyova, I., Tokarev, D., Bakos, P., and Vigas, M. (1995). Vasopressin and oxytocin in stress. *Annals of the New York Academy of Sciences, 771,* 192-203.

Jonaidi, H., Oloumi, M., and Denbow, D. (2003). Behavioral effects of intracerebroventricular injection of oxytocin in birds. *Physiology and Behavior, 79,* 725-729.

Kirsch, P., Esslinger, C., Chen, Q., Mier, D., Lis, S., Siddhanti, S., Gruppe, H., Mattay, V., Galhofer, B., and Meyer-Lindenberg, A. (2005). Oxytocin modulates neural circuitry for social cognition and fear in humans. *Journal of Neuroscience, 25,* 11489-11493.

Lee, P., Brady, D., Shapiro, R., Dorsa, D., and Koenig, J. (2007). Prenatal stress generates deficits in rat social behavior: reversal by oxytocin. *Brain Research, 1156,* 152-167.

Lickliter, R. (1989). Premature visual stimulation accelerates intersensory functioning in bobwhite quail neonates. *Developmental Psychobiology, 23,* 15-27.

Lickliter, R. (2005). Prenatal sensory ecology and experience: implications for perceptual and behavioral development in precocial birds. *Advances in the Study of Behavior, 35,* 235-274. Academic Press, New York.

Lickliter, R and Gottlieb, G. (1985). Social interactions with siblings is necessary for visual imprinting of species-specific maternal preferences in ducklings (*Anas platyrhynchos*). *Journal of Comparative Psychology*, *99,* 371-379.

Lickliter, R., and Stoumbos, J. (1991). Enhanced prenatal auditory experience facilitates species-specific responsiveness in Bobwhite quail chicks (*Colinus virginianus). Journal of Comparative Psychology, 105,* 89-94.

Main, K., Schmidt, I., and Skakkebaek, N., (2005). A possible role for reproductive hormones in newborn boys: progressive hypogonadism with the postnatal testosterone peak. *Journal of Clinical Endocrinology Metabolism, 85,* 4905–4907.

Matsumoto, A. and Arai, Y. (1980). Sexual dimorphism in 'wiring pattern' in the hypothalamic arcuate nucleus and its modification by neonatal hormonal environment. *Brain Research, 19,* 238–242.

Matsumoto, A., and Arai, Y. (1986). Male–female differences in synaptic organization of the ventromedial nucleus of the hypothalamus in the rat. *Neuroendocrinolgy, 42,* 232–236.

Matthews, S. (2000). Antenatal glucocorticoids and programming of the developing CNS. *Pediatric Research, 47*, 291-300.

McCarthy, M. (1995). Estrogen modulates oxytocin and its relation to behavior. *Advances in Experimental Medicine and Biology, 395,* 235-245.

McCarthy, M., Wright, C., and Schwarz, J. (2009). New tricks by an old dogma: mechanisms of the organizational/activational hypothesis of steroid-mediated sexual differentiation of brain and behavior. *Hormones and Behavior, 55,* 655-665.

McEwen, B., Sakai, R., and Spencer, R. (1993). Adrenal steroid effects on the brain: versatile hormones with good and bad effects. In J. Schulkin (Ed.), Hormonally-Induced Changes in Mind and Brain. San Diego, CA: Academic Press.

Mikami, S., Tokado, H., and Farner, D. (1978). The hypothalamic neurosecretory systems of the Japanese quail as revealed by retrograde transport of horseradish peroxidase. *Cell and Tissue Research, 194,* 1-15.

Milewski, N., Ivell, R., Grossman, R., and Ellendorff, F. (1989). Embryonal development of arginine vasotocin/mesotocin gene expression in the chicken brain. *Journal of Neuroendocrinology, 1,* 473-484.

Muller, W., Groothuis, T., Kasprzik, A., Dijkstra, C., Alatalo, R., and Siitari, H. (2005). Prenatal androgen exposure modulates cellular and humoral immune function in black-headed gull chicks. *Proceedings of the Royal Society: Biological Sciences, 272,* 1971-1977.

Mundy, P., Gwaltney, M., and Henderson, H. (2010). Self-referenced processing, neurodevelopment and joint attention in autism. *Autism, 14,* 408-429.

Nelson, R. (2010). Hormones and behavior: basic concepts. In *Encyclopedia of Animal Behavior*. (Vol. 2, pp. 97-105). Oxford, UK: Academic Press.

Nishizuka, M. and Arai, Y. (1981). Sexual dimorphism in synaptic organization in the amygdala and its dependence on neonatal hormone environment. *Brain Research, 211,* 31–38.

Nordgreen, J., Janczak, A., and Bakken, M. (2006). Effects of prenatal exposure of corticosterone on filial imprinting in the domestic chicken (*gallus domesticus*). *Animal Behaviour, 72,* 1217-1228.

Nouwen, E., Decuypere, E., Michels, H., and Kühn, E. (1982). The presence of vasotocin and mesotocin in serum and the hypothalamo-neurohypophysial axis of chick embryos before hatching and in posthatch chicks. *General and Comparative Endocrinology, 50,* 445-451.

Petrie, M., Schwabl, H., Brand-Lavridsen, N., and Burke, T. (2001). Sex differences in avian yolk hormone levels. *Nature, 412,* 498-499.

Phoenix, C., Goy, R., Gerall, A., and Young, W. (1959). Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology, 65,* 369-382.

Popik, P., Vetulani, J., and van Ree, J. (1992). Low doses of oxytocin facilitate social recognition in male rats. *Psychopharmacology, 106,* 71-74.

Ribatti, D., Nico, B., and Bertossi, M. (1993). The development of the blood brain barrier in the chick. Studies with evans blue and horseradish peroxidase. *Annals of Anatomy, 175,* 85-88.

Rogers, L. (1995). *The development of brain and behaviour in the chicken*. Sydney: CAB International.

Ross, H. and Young, L. (2009). Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. *Frontiers in Neuroendocrinology, 30,* 534-547.

Rubolini, D., Romano, M., Martinelli, R., Leoni, B., and Saino, N., (2006). Effects of prenatal yolk androgens on armaments and ornaments of the ring-necked pheasant. *Behavioral Ecology and Sociobiology, 59,* 549-560.

Stein, M., Goldin, P., Sareen, J., Zorrilla, L., and Brown, G. (2002). Increased amygdala activation to angry and contemptuous faces in generalized social phobia. *Archives of General Psychiatry, 59,* 1027-1034.

Tennyson, V., Nilaver, G., Hou-Yu, A., Valiquette, G., and Zimmerman, E. (1986). Immunocytochemical study of the development of vasotocin/mesotocin in the hypothalamo-hypophysial system of the chick embryo. *Cell and Tissue Research, 243,* 15-31.

Ubuka, T., Ueno, M., Ukena, K., and Tsutsui, K. (2003). Developmental changes in gonadotropin-inhibitory hormone in the Japanese quail (*coturnix japonica*) hypothalamo-hypophysial system. *Journal of Endocrinology, 178*, 311-318.

Viglietti-Panzica, C., Mura, E., and Panzica, G. (2007). Effects of early embryonic exposure to genistein on male copulatoy behavior and vsotocin system of Japanese quail. *Hormones and Behavior, 51,* 355-363.

Wade, J. (2006). Relationships among hormones, brain, and behavior: Exceptions in search of a rule. *Hormones and Behavior, 49,* 577-579.

Worthman, C. (2011). Hormones and Behavior. In *Encyclopedia of Adolescence*. (vol. 1, pp. 177-186). Atlanta, GA: Academic Press.