Early Sexual Experience Alters Adult Affective Responses and Immune Function

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Graduate School of The Ohio State University

By

John S. Morris, M.A.

Graduate Program in Psychology

The Ohio State University
2013

Professor Randy J. Nelson, Advisor (Chair)

Professor A. Courtney DeVries

Associate Professor Bennett Givens

Associate Professor Derick Lindquist

ABSTRACT

Early life experiences have a lasting imprint on physiology and behavior. In mammals, adolescence is a critical developmental period when various neural structures undergo extensive remodeling, and experiences during this time can have a lasting influence on growth and behavior. The initiation of adolescence occurs around the same time as the onset of reproductive puberty, and the hormonal events that are hallmarks of these developmental periods target the brain creating an intricate link among these processes. Reproductive maturity is reached when steroid hormones present during adolescence adjust and stimulate neural circuits, leading to increases in sensitivity to sexual sensory stimuli, sexual drive, and manifestation of copulatory behaviors in specific social situations. Adolescence can be seen as a gradual phase of transition, rather than a moment of attainment, whereas puberty is merely one of the temporally restricted adolescent points of transition. It is essential to move away from the notion that puberty is a gonadal event and recognize the start of puberty as a brain event with persistent exchanges between steroid hormones and the adolescent nervous system.

The hormonal changes associated with puberty and the timing of environmental events may increase vulnerability to affective and cognitive disorders, as well as modify immune function and alter stress reactivity. Hormones modify neurotransmitter function, and alterations in neurocircuitry provoked during adolescence likely underlie the etiology

of depressive disorder. A positive relationship exists between early sexual activity and adult onset of depression in humans. It is thought that early sexual contact influences depression through remodeling of brain systems not yet equipped or sufficiently mature to handle this type of social interaction, but this hypothesis remains untested. One goal of my thesis is to model depression in animals to provide a means of identifying the development of dysfunctional properties, identifying the neural systems involved, and creating appropriate treatment management strategies in early sex exposed animals as they relate to humans. To that end this dissertation is separated into four chapters. In Chapter 1, I review the previous research on the effects of adolescence on adult cognitive and affective states. Chapter 2 describes an experiment that examines how a salient social interaction, specifically sexual experience, encountered during adolescence, affects adult behavioral responses, immune function, and reproductive development. Chapter 3 describes an experiment that takes a mechanistic approach to examine the possible mediating role of high concentrations of testosterone during adolescence in programming adult brain and behavior. In this experiment testosterone was administered during adolescence in place of a sexual pairing to determine whether similar outcomes resulted as early sexual experience. Finally, Chapter 4 describes an experiment that was designed to assess whether engaging in sex during adolescence influences alcohol consumption in adulthood. Taken together, my dissertation research demonstrates that sex during adolescence leads to increases in anxiety-like depressive-like behaviors, marked concentrations of cortisol in blood serum, a rise in inflammatory markers in the brain, and alterations in neuronal structure in prefrontal brain regions. When T was administered

during adolescence rather than being exposed to sex, T exposed adolescent hamsters showed increases in anxiety- and depressive-like behavior, which recapitulated the changes seen in the hamsters exposed to early sexual experiences. In the final experiment hamsters experiencing sexual interactions during adolescence displayed the highest rates of alcohol intake compared to all other groups. All in all, these results suggest that adolescent experiences can shape the behavioral, psychological and immunological outcome in adulthood.

Dedication

This Dissertation is dedicated to family and my mentor Randy J. Nelson. Without their unwavering support and belief in me I would not be the person or scientist that I am today

Acknowledgments

I am grateful to my adviser, Dr. Randy J. Nelson for his guidance, support, and expertise which made this research possible.

I thank Dr. A. Courtney DeVries, Dr. Zachary Weil and Dr. Gregory J. Norman for their guidance and advice at the beginning of this project.

I thank Dr. Laura Fonken, Dr. James Walton, Dr. Tracy Bedrosian, Jeremy Borniger, Taryn Aubrecht, and Achikam Haim for helpful advice and discussions during the course of this project.

I especially thank Varad Deshmukh and Jobanjot Moti for technical assistance with behavioral testing and tissue collection and processing.

Vita

March 20, 1983 .....Born – Camden, New Jersey, USA

June 2001 .....Dr. Charles E. Brimm Medical Arts H.S.

June 2006 .....B.S. Psychology, The Ohio State University

October 2012.....M.A. Psychology, The Ohio State University

2012 to present .....Graduate Research Associate, Department of Psychology, The Ohio State University

Publications

RESEARCH PUBLICATIONS

1. Carrol, P.J., Arkin, R.M., Seidel, S.D., Morris, J.S. (2009). The relative importance of needs among traumatized and non-traumatized samples. *Motivation Emotion*, 33:373–386.

2. Weil, Z.M., Norman, G.J., Karelina, K., Morris, J.S., Barker, J.M., Su, A.J., Walton, J.C., Bohinic, S., Nelson, R.J., DeVries, A.C. (2009). Sleep deprivation attenuates inflammatory responses and ischemic cell death. *Experimental Neurology*, 218:129.

3. Karelina, K., Norman, G.J., Zhang, N., Morris, J.S., Peng, H., DeVries, A.C. (2009). Social isolation alters neuroinflammatory response to stroke. *Proceedings of the National Academy of Sciences,* 14:5895-900.

4. DeVries, A.C., Norman, G.J., Karelina, K., Morris, J.S., Zhange, N., Cochran, M. (2010). Social interaction prevents the development of depressive-like behavior following nerve injury: A potential role for IL-1β and oxytocin. Brain, Behavior and Immunity, 24(S1), S36

5. Fonken, L.K., Workman, J.L., Walton, J.C., Weil, Z.M., Morris, J.S., Haim, A., and Nelson, R.J. (2010). Light at night increases body mass by shifting the time of food intake. *Proceedings of the National Academy of Science,* 107:18664-18669.

6. Fonken, L.K., Morris, J.S., and Nelson, R.J. (2011). Early life experiences affect adult delayed-type hypersensitivity in short- and long-photoperiods. *Chronobiology International,* 28:101-8.

7. Norman, G. J., Berntson, G.G., Morris, J.S., Karelina, K. Zhang, N., Weil, Z.M., DeVries, A.C. (2010). Social isolation exacerbates autonomic, inflammatory and behavior responses to global cerebral ischemia. Proceedings of the National Academy of Sciences, U S A, 107(37) 16342-16347

8. Norman, G.J., Cacioppo. J.T., Morris, J.S., Karelina, K., Malarkey, W.B., DeVries, A.C., and Berntson, G.G. (2010). Selective influences of oxytocin on the evaluative processing of social stimuli. *Journal of Psychopharmacology,* 1-7.

9. Norman, G.J., Karelina, K., Morris, J.S., Zhang, N., Cochran, M., and DeVries, A.C. (2010). Social interaction prevents the development of depressive-like behavior post

nerve injury: A potential role for oxytocin. *Proceedings of the National Academy of Sciences.* 72:519-26.

10. Norman, G.J., Karelina, K., Zhang, N., Walton, J.C., Morris, J.S., Devries, A.C. (2010). Stress and IL-1beta contribute to the development of depressive-like behavior following peripheral nerve injury. *Molecular Psychiatry*, 4:404-14.

11. Norman, G. J., Cacioppo, J.T., Morris, J.S., Malarkey, W.B., Berntson, G.G., DeVries, A.C. (2011). Oxytocin increases autonomic cardiac control: Moderation by loneliness. Biological Psychology, 86:174-180.

12. Norman, G.J., Morris, J.S., Karelina, K., Weil, Z.M., Zhang, N., Al-Abed, Y., Brothers, H.M., Wenk, G.L., Pavlov, V.A., Tracey, K.J., and DeVries, A.C. (2011). Cardiopulmonary arrest and resuscitation disrupts cholinergic anti-inflammatory processes: A role for cholinergic α7 nicotinic receptors. *Journal of Neuroscience,* 31: 3446-3452.

13. Norman, G. J., Morris, J.S., Karelina, K. Zhang, N., Berntson, G.G., DeVries, A.C. (2012). Heart rate variability predicts cell death and inflammatory responses to global ischemia. Frontiers in Physiology, 3(131), 1-6.

Fields of Study

Major Field: Psychology

Minor Field: Behavioral Neuroscience

Table of Contents

List of Figures

CHAPTER 1

INTRODUCTION

Individuals of all animal species must adequately ascertain and maintain appropriate physiological responses to environmental and homeostatic challenges as a necessary requisite for survival and well being (Ulrich-Lai & Herman, 2009). Challenges or occurrences during an individual's life that prevent or at least impede homeostatic function can have dramatic influences on long-term health and behavior. Instances of adversity during prenatal to late adolescent life can greatly exacerbate the extent of homeostatic dysfunction and susceptibility to psychopathologies later in life. Animal and human studies have revealed that the brain goes through major changes during two time periods in life, early childhood and late adulthood. Current research relates exposure to early-life challenge with increased stress reactivity and cognitive dysfunction later in life, further suggesting that these periods are especially sensitive to stressors and associated hormonal signals (Lupien et al, 2009).

The time between childhood and adulthood that includes maturation of adult social and cognitive behaviors is known as adolescence. Adolescence is a transitional phase when juvenile animals shift into adult typical behavioral and neurological phenotypes (Buwalda, Geerdink, Koolhaas, 2011). Marked plasticity occurs during

adolescence that alters both the structure and function of the brain. Regions of particular interest during this period are the frontal cortical regions of the brain and associated neural circuitry because these areas are highly remodeled over the course of adolescence and early adulthood. Indeed, emotion-related brain regions undergo reorganization following exposure to various forms of chronic and acute stress when neurological tissue is assessed at adult time points. Although the age ranges vary across species, the physiological and behavioral transitions are sufficiently similar among mammalian species to be able to use rodents to model this process in humans. In this dissertation, I chose Siberian hamsters (*Phodopus sungorus*) as the model species because they are less inbred than standard lab animals and are more variable genetically and behaviorally, which more accurately mimics the individual variability seen in humans. Another reason why the Siberian hamster was chosen is because they are a solitary species of animal which allows for the study of social environmental influences on these animals without the added complexities introduced by the presence of cage mates. The dramatic changes in the sensitivity of the reproductive neuroendocrine axis to steroid negative feedback regulation and steroid facilitation of reproductive performance are well documented in these animals (Romeo, Schulz, Nelson, Menard, & Sisk, 2003). Connections among sexual experience, adolescence, and pubertal development on affect and immunity have not been investigated in this species.

Puberty is the developmental period when individuals become capable of sexual reproduction, and this developmental epoch refers to the activation of the hypothalamic-pituitary-gonadal (HPG) axis and ultimately culminates in gonadal maturation. The

transitions from immaturity to the fully developed adult state that occur during adolescence require significant adjustments in behavior and neural circuitry for future reproductive success (Romeo, Richardson, & Sisk, 2002). Akin to humans, altricial rodents (e.g., mice, rats, and hamsters), secrete large amounts of testosterone (T) immediately after birth; T concentrations then gradually decline for the following weeks. Following the perinatal period of development, T concentrations reach nearly undetectable levels and remain low until the onset of puberty at approximately 4 weeks of age. Prior to the onset of puberty, during the juvenile stage, animals are not yet fertile and will not show mating behavior (Sisk & Foster 2004). At approximately 4 weeks of age, T release steadily increases in both the frequency and amplitude of pulsatile release. Increased T concentrations both remodel and trigger activation of neural circuits, which in turn finishes structuring the brain into adult typical behavioral and reproductive patterns (Sisk & Foster 2004). Adolescence is characterized by the neural and behavioral changes of an organism that encompasses more than reproductive function, the primary feature of puberty (Sisk & Zehr, 2005). Although the processes are distinct, the interface between pubertal hormones and the adolescent brain is paramount for the maturation of adult typical behavior and the timing of this interaction has persistent consequences on behavioral and psychological outcome in adulthood

The two stage model of neurodevelopment is a model of development that posits the existence of two sensitive periods for steroid dependent organization of brain and behavior (Phoenix, Goy, Young, 1967; Sisk & Zehr, 2005). The time just before, and immediately after birth, is the time when steroid hormones evoke sexual differentiation of

neural circuits in the brains of rodents. The process of sexual differentiation requires testosterone which is secreted by the fetal testes; testosterone is aromatized to estradiol which inhibits and stimulates specific arrays of gene expression which results in the permanent organization of the developing central nervous system, (CNS), in the direction of the prototypical masculine pattern (Negri-Cesi, Colciago, & Motta, 2004). The hormonal environment at this moment in life is the basis of the sex-related morphological dissimilarity of various brain nuclei, of gender-specific secretion of many hypothalamic and pituitary hormones, and of sexual behaviors (Negri-Cesi, Colciago, & Motta, 2004). Steroid exposure during prenatal and neonatal life has an organizational component because it locks or programs the brain into its sexually-differentiated state, as well as determines the potential for behavioral responses to steroids later in life. Following the determination of gonadal sex, sexual differentiation of the rest of the body takes place, which is a process that is largely driven by the influence of gonadal hormones rather than by the influence of genetic factors (Breedlove, 1994). Particularly in males, testicular hormones are essential elements responsible for masculinization (Breedlove, 1994). During this time during development individuals undergo rapid maturation and any perturbation during this period can have enduring effects later in life.

The traditional perspective posits that exposure to hormones early in life programs the expression of sexual and agonistic behaviors in males by masculinizing (promoting male traits) and defeminizing (removing female traits) behavioral responses to hormones during adulthood; the absence of these hormones leads to demasculinization and feminization of these responses (Schultz & Sisk, 2006). More recently, studies have

shown that the brain retains its capacity for organizational modification well beyond this early critical period because animals that are re-exposed to steroids around the time of puberty appear to display organizational effects on subsequent behavior and development (Romeo et al, 2002; Schultz & Sisk, 2006). Based on research conducted by Sisk and colleagues, the initial proximal goal of my project was to examine whether sexual experiences during adolescence, a hormonally-salient event, had an enduring effect on adult physiology, behavior, and neurodevelopment. Upon completion of the initial study, a follow-up experiment was conducted to uncover a biological mechanism. Lastly, the ultimate goal was to provide a translational framework that could advance the understanding of the long-term effects of adolescent sex in humans. Because of a strong association between the early sexual experience in human males and adult alcohol abuse (Whitebeck, et al., 1999; Yamaguchi & Kandel, 1987), the third aim of this research is focused towards reproducing aspects of the behavioral and environmental components of this process by testing alcohol consumption after peri- or postpubertal sexual experience in Siberian hamsters.

Much is known about the effects of environmental challenges and subsequent release of stress hormones can have on the structure and function of the brain (Kauffmann, Plotsky, Nemeroff, Charney, 2000). However, less is known about how the pubertal brain responds to challenge (Romeo & McEwen, 2006) or how pubertal maturation and experience interact to affect physiological and behavioral outcomes in adulthood. The pubertal period is a recently recognized period of brain plasticity. During puberty the nervous system becomes progressively more responsive to the organizing

effects of steroid hormones (Romeo et al., 2002). Increasing evidence suggests that puberty leads to organization of neural circuits in the brain that initiates maturation signals (Schultz et al., 2009). The enhancement of plasticity during adolescence and puberty may impose increased susceptibility to neurological and behavioral disturbance. Substantial remodeling takes place during this period in brain areas involved in emotion and learning such as the prefrontal cortex (PFC) (Spear, 2000). The PFC is highly sensitive to the effects of environmental pressure, and is associated with socio-emotional disturbances and increased risk for psychopathologies later in life (Spear, 2000; Heim & Nemeroff, 2001; Heim et al., 2004). This same form of enhanced plasticity may, however, offer a unique opportunity to intervene at this point of development to modify the neuro-circuitry in ways that may be neuro-protective (Dahl, 2004).

In addition to the plethora of neurological modifications that early life environmental manipulations can have on the life of individuals, relevant social experiences in adolescence may alter the developmental course of the immune system. A specific example of environmental influences on immune function would be childhood socioeconomic status predicting resistance to infection in adulthood (Miller et al., 2009). Certain types of social experiences are also associated with epigenetic changes in genes associated with immune function and may modulate immune function in adulthood (Uddin et al., 2010). Childhood maltreatment predicts inflammatory bias in adults, possibly due to early life adverse experiences programming stress-responses later in life (Danese et al., 2007). Building an improved understanding of how this debilitating condition emerges is essential to the creation of more effective early intervention

strategies (Morley & Moran, 2011). Depressed patients display increased concentrations of proinflammatory substances in various affective and emotion related brain regions such as: the amygdala, hippocampus, nucleus accumbens, basal ganglia, and frontal cortices (Raison, Capuron, Miller, 2006; Hayley, 2011). Inflammatory mechanisms have the ability to modify neuro-circuitry, as well as peripheral systems (Raison, Capuron, Miller, 2006). A major example of how inflammatory processes influence neuro-circuitry and peripheral systems is illustrated though the body's response to a peripheral infection; pro-inflammatory cytokines act on the brain to cause sickness behavior (Dantzer, 2001; Dantzer et al, 2008). Research into the effects of inflammation on the maturation of the adolescent brain and behavior is essential, because the hormonal changes that individuals experience during puberty and adolescence may be linked to negative outcome in adulthood.

Animal models have provided much insight into the differential phenotypic responses to stress during early development (Champagne, 2008; Meaney, 2001); both rodent and primate models have demonstrated how socially relevant early life experiences produce persistent changes to stress reactivity and immune function in adults (Lewis et al., 2000). One notable method to explore the involvement of brain related gene transcription processes in onset of depression is to assess mRNA levels via rtPCR. Gene expression analysis expands upon the clinical literature by relating it to animal models, thus providing increased understanding of depression and discovering new therapeutic targets for treatment (Kroes et al., 2006).

Recently, some forms of depression have been reported to be associated with inflammatory processes. For instance, patients with major depressive disorder (MDD) display elevated levels of circulating proinflammatory cytokines and other biomarkers of inflammation, which have the capability to access the brain and periphery and interact with most domains pertinent to the pathophysiology of depression (Miller, Maletic and Raison, 2008; Schiepers, Wichers, & Maes, 2005). Interleukin (IL)-1β is detected in the brain of stressed mice, and the presence of this proinflammatory cytokine potentiates depressive-like behavioral responses (Norman et al., 2010). In addition, animal models of depression have established a connection between peripheral measures of depression such as cell mediated immunity and the presence of depressive-like behavior (Maes, 2010). Alterations in immunity following challenge may be a consequence of compensatory investments in immune defense in opposition to other expensive physiological processes such as reproduction (Martin al., 2007). To test whether a relationship of potential clinical relevance exists between adolescent sex experiences and depression, various methodological techniques have been used to measure phenomena that are typically associated with adult onset depression. Immunologic activity, the degree of behavioral modification, and alterations in body mass and reproductive tissue were assessed to determine the impact that early life sex experience has on subsequent development of neuropsychiatric disease.

Much of the research on the effects of social influences on behavior is conducted using adult animals. Early-life social encounters and experiences can establish individuals' developmental trajectory by regulating somatic, immunologic, and

neurologic processes. This dissertation is designed to investigate the potential mechanisms that drive the phenotypic alterations present in adult animals following exposure to sexual encounters during adolescence. One goal is to suggest improvements of therapeutic strategies to attenuate the progression of disease states. I will test whether engaging in sexual behavior during adolescence, a particularly vulnerable developmental period, alters neuronal plasticity leading to lasting effects on individual behavior, health, and mood. Specifically, I tested the hypothesis that hormonal changes associated with puberty and the timing of environmental events may increase vulnerability to affective and cognitive disorders, as well as modify immune function and alter stress reactivity. This hypothesis is based on the previous associations made in humans between adolescent sexual activity and adult affective disorders. What is not known is whether exposure to sexual behavior during adolescence can have persistent influences on mood and incidence of psycho-pathology that extends into adulthood.

Depression. Psychological disorders are difficult to diagnose, which often leads to treatment and intervention strategies that are unsuccessful (Laska et al., 2009). MDD is a highly prevalent, expensive, and severely debilitating psychiatric condition that has devastating effects on the lives of people on a global scale (Morley & Moran, 2011). Indeed, roughly 18.8 million Americans are at present afflicted with a clinical depressive disorder (NIMH, 2011). The traditional classification of depression depicts it as a disorder of the brain that can influence a broad range of psychological processes and behaviors (Sharpley & Agnew, 2011). Although numerous potential neurobiological correlates of depression have been identified, the precise mechanisms remain unknown

(Nemeroff, 2000; Birmaher et al., 1996). To gain an improved understanding of how depression originates it is imperative to carry out studies that are designed to elucidate the mechanisms and interrelationships among the different risk factors, especially those that occur early in life.

Anxiety and Depression. There is a high prevalence of anxiety disorders in North America, with a rate of approximately 13% per year among adults. Anxiety shares a high co-morbidity with other psychopathologies, notably mood disorders (namely depression) and substance abuse. The median age of onset of anxiety disorders is about 15 years compared to a median age of about 26 years for mood disorders, and anxiety disorders are found to be even more persistent and pervasive than are mood disorders. Adolescents are particularly vulnerable to this form of psychopathology, and adverse life experiences in adolescence are important risk factors. Recent evidence in animal models indicates that adolescence may be another opportunity for life experiences to shape brain development either positively or negatively. There is marked development and reorganization of the brain over adolescence in humans and in rats which, in concert with altered HPA function in adolescence, may make adolescents particularly susceptible to the negative consequences of stressors. Nevertheless, there has been limited investigation of the lasting effects of stressors in adolescence in animal models.

Inflammation and Depression. As noted, high levels of inflammation are linked to depression (Capuron & Miller, 2011). The presence of inflammatory factors predicts the onset of depressive disorders (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008). Patients with major depression exhibit increased peripheral blood inflammatory

biomarkers (Miller, Maletic, Raison, 2009). Important inflammatory signaling proteins, known as cytokines, infiltrate the brain and interact with nearly every pathophysiologic domain pertinent to the onset of depression, including the metabolic processes of neurotransmission, the function and communication of neuroendocrine systems, and plasticity in the brain (Raison, Capuron, Miller, 2006). Early life experiences are important modulators of immune function, but the presence of inflammatory mediators has yet to be assessed following exposure to a prominent social event, *viz*., sexual intercourse, during adolescent life. In addition to their role in immune signaling, pro-inflammatory cytokines are potent modulators of behavior and affect (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008). Both exogenous and endogenous proinflammatory cytokines (e.g., IL-1β) induce depressive-like behavioral responses in nonhuman animals (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008). Adult behavioral, hormonal, and immunologic responsivity can be largely modified by early experiences, thus any events that disrupt development early on might lead to a maladaptive stress response system and increased susceptibility to disease (O'Mahony et al., 2009).

Neuroinflammation, Stress, and Depression. Depressed patients display increased quantities of proinflammatory agents in various affective and emotion related brain regions (Raison, Capuron, Miller, 2006). The regulatory capacities of inflammatory mechanisms is well documented and prompted us to explore the possibility that peripheral inflammatory system function undergoes long term modification following sex early in life. In addition, the developmental implications of experience during the sensitive adolescent period prompted us to explore the relationship between adolescent

sex and immunologic and brain structural changes in adulthood. Studies suggest that hormonal changes associated with puberty and the timing of environmental events may increase vulnerability to affective and cognitive disorders, as well as modify immune function and alter stress reactivity (Steinberg, 2005). Hormones modify neurotransmitter function, and alterations in neurocircuitry provoked during adolescence likely underlie the etiology of depressive disorder (Steinberg, 2005). The hormonal fluctuations occurring in adolescence may play a mediating role in this process. The primary goal in my studies was to perform tests to evaluate possible behavioral substrates that undergo change and then transition into testing other possible molecular, physiological, and anatomical changes that may be linked to early exposure to a sexually receptive counterpart or sex steroid hormones.

Drug Use and Depression. Sexual activity, drug use, and depressive symptoms are common among teenagers. There is a widespread notion that adolescents "self-medicate" depression with substance use and sexual behaviors, yet the temporal arrangement of depression and these risk behaviors remains unclear (D.D Hallfors et al., 2005). Associations between risk-taking behaviors and depressive symptoms during adolescence raise the issue of whether the relationship is causal, and if so, the direction of causality. Uncovering a causal link and/or revealing a causal pathway is a critical issue for prevention. For instance, substance abuse may be an inadvertent consequence of self-medicating a mental disorder. If so, then aggressively identifying and treating depression may decrease later substance abuse disorders. Conversely, depression may result from the biological or psychosocial consequences of substance use or from a shared underlying

mechanism that contributes to both. If a causal pathway from risk behavior to depression exists, then intervening to stop or delay the behavior could prevent or lessen subsequent depression. The final experiment in this dissertation was designed to test the hypotheses that adolescent sex influences the intake of alcohol when tested in adulthood.

This type of research can elucidate essential underlying aspects of immune function following exposure to salient social encounters and its possible involvement in the progression of depression. The study of social encounters and how they manipulate the behavior and physiology of animals has been studied in various contexts, but most studies lack a focus on adolescence. Adolescence has recently been accepted as a critical period of development, yet the intricacies of the processes that occur during this time period are largely unknown and under studied. By exploring the questions associated with developmental timing and exposure to steroids or steroid inducing social experiences a better understanding of the mechanisms driving human behavior and vulnerability to psychopathology might be obtained. This work may be useful and clinically relevant in understanding the long-term physical and mental health outcomes of adolescent sexual activity in humans. This is a relatively difficult research question to address in humans without ethical ramifications. Yet, through the use of relevant animal models, such as the one used in this dissertation project, much can be learned regarding the effects of sexual behavior early in life and its implications on adult behavioral and affective outcomes.

CHAPTER 2

EXPERIMENT 1: SEXUAL ACTIVITY EARLY DURING ADOLESCENCE ALTERS ADULT AFFECT, IMMUNITY, AND BEHAVIOR

Early life experiences can have a lasting imprint on mental and emotional well-being; however, precisely how behavior and physiology are influenced remains understudied. Vulnerability to various psychiatric disorders may be linked to the life events that occur during developmentally sensitive windows such as the perinatal and less understood adolescent periods (Nemeroff, 2004). The study of social encounters and how they influence the behavior and physiology of animals has been studied in various contexts, but most studies lack a focus on adolescence. Recently, observations that adverse experiences early in life predispose individuals to the development of affective and anxiety disorders in adulthood have begun to gain a considerable amount of interest within the scientific community. Stressful life events in childhood have been documented to have a predictive and preeminent function in the development of numerous disorders, mainly anxiety and mood disorders in adulthood. Yet, individuals encounter an array of stressors with a wide range of biological and environmental significance and

consequences on the life that an animal lives (Leuner, Glasper, Gould, 2010). The deleterious effects of negative stressors early in life are well documented and generally accepted. Importantly, not all stressors are associated with harmful consequences; there are occasions when stressors have an element of reward and can actually be of benefit to the health and mental function on an individual (e.g., sexual experience and exercise) (Leuner, Glasper, Gould, 2010). In this study, we evaluated the effects of initiation of sexual activity during adolescence on adult behavioral and immunological outcomes in an animal model.

A potential driving force behind these effects may involve the role of differential timing of puberty among boys. In male populations, early maturation has been shown to be by and large advantageous, while late maturation was associated with negative consequences (Duncan et al, 1985). In terms of social-emotional functioning early maturers appear to have an advantage in comparison to those with delayed maturation (Brooks-Gunn, Petersen, & Eichorn, 1985). Research in humans has revealed that early maturers are rated as more well-liked, more relaxed, more pleasant and poised, and viewed as more attractive and popular than late maturers who were rated as being less adequate, less self-assured, and more anxious (Jones and Bayley, 1950). The physical status of an adolescent can have profound and persistent effects on social, emotional, and behavioral development. For instance, it is well known that the best athletes in middle and high school generally go through puberty at an early age. In athletic activities early maturers are in an advantageous position, making it more likely that they will be further personally and socially adjusted than later maturing individuals during adolescence

(Mussen & Jones, 1958). Early maturing boys are seen as more capable of assuming leadership roles and taking on adult male roles in both social and interpersonal contexts (Clausen, 1975; Brooks-Gunn, Petersen, & Eichorn, 1985). Thus age of puberty is highly correlated with time of first sexual encounter in boys; evidence from human studies shows that male and female early maturers reported engaging in more sexual activity and are more susceptible to negative outcomes in adulthood than late maturers (Flannery, Rowe, Gulley, 1993). Based on research that has focused on the study of maturational timing in adolescence it is generally accepted that early maturation can have both positive and detrimental effects on the overall long-term well-being of an individual.

Developmental studies have reported that both early and late pubertal maturation are risk factors for depression (Kaltiala-Heino, Kosunen, & Rimpelä, 2003). Also, there is a positive relationship between early sexual intercourse and development of depression in adult humans. (Rector, Johnson, & Noyes, 2003*)*. The connection between adolescent sexual activity and depression is apparent, yet the direction of that association is not well understood. For example, it may be possible that depressed adolescent individuals turn to sexual activity and deviant behavior (e.g drug use and aggression) in an attempt to alleviate feelings of depression (Rector, Johnson, & Noyes, 2003*)*. Thus, depression might lead to greater sexual activity rather than sexual activity's leading to depression. This experiment removes the age of puberty issue, allowing for the isolated study of sex during adolescence. By using a controlled animal model to study the effects of sex in adolescence we can began to uncover the biological underpinnings of engaging in sexual

activity early in life without the confounding variables that are created through individual differences in the timing of maturation.

The focus of this study was to test the hypothesis that engaging in sexual behavior during adolescence, a particularly vulnerable developmental period, alters neuronal plasticity leading to lasting detrimental effects on individual health and mood. We performed tests to evaluate possible behavioral substrates that undergo change and then transitioned into testing other possible genetic, physiologic, and anatomical changes that may be linked to early developmentally timed exposure to sex. Siberian hamsters (*Phodopus sungorus*) were chosen because they exhibit morphogenic shifts in sensitivity and reactions to steroid hormones. The immense changes in the sensitivity of the reproductive neuroendocrine axis to steroid negative feedback regulation and steroid facilitation of reproductive performance are well documented in these animals (Romeo, Schulz, Nelson, Menard, & Sisk, 2003). Connections among sexual experience, adolescence, and pubertal development on affect and immunity have not been investigated in this species. In Experiment 1, I separated animals into one of five groups: a control group (no sexual experience), two 40 day (adolescent sex) groups that received testing either 40 or 80 days later (40X40 & 40X80, respectively), and two 80 day (adult sex) groups that were assessed either 40 or 80 days later (80X40 & 80X80, respectively). The experimentally manipulated groups were all paired with an ovariectomized (OVX) female to allow for experimental control over levels of circulating sex steroid hormones within female counterparts during the copulatory period. Behavioral testing consisted of performance on the elevated plus maze (EPM), designed to evaluate anxiety-like

responses, and the (Porsolt forced swim test; FST), which is a measure of depressive-like responses. Blood samples were obtained at the conclusion of the study to determine whether glucocorticoid concentrations were involved in this process.

Compared to sexually inexperienced hamsters and those that experienced sex for the first time in adulthood, hamsters with adolescent sexual experience increased anxiety- and depressive-like behavioral responses. Adolescent sexual experience also markedly increased immune responses, suggesting that premature exposure to sexual encounters may increase inflammation, as well as influence affective responses in adulthood. Adolescent sexual experiences increased the presence of the pro-inflammatory cytokine interleukin 1β (IL-1β) in the prefrontal cortex (PFC). The finding that IL-1β is increased in hamsters that experienced sex during adolescence suggests that relevant social stimuli during this sensitive time may increase neuro-inflammation and subsequent vulnerability to psychopathologies as reported previously in studies of humans. These animals also reduced overall body mass and accessory reproductive tissue mass, which may be mediated through IL-1β expression.

Taken together, these results further support the notion that social interactions that occur during adolescence can have significant organizational effects on the brain and behavior. These results also suggest that early adolescent sexual experience has long-term effects on affective responses, adult immune function, as well as reproductive tissue. These findings imply that certain behaviors that have the capability to manipulate sex steroid hormones may have enduring effects if performed early in development.

Materials & Methods

Animals

Siberian hamsters (*Phodopus sungorus*) used in this study were bred in our colony at The Ohio State University from a wild-bred stock obtained from Dr. K. Wynne-Edwards (Kingston, Ontario, Canada). Hamsters were housed in polypropylene cages (28 × 17 × 12 cm) with a nestlet and 1 cm of corncob bedding. Hamsters were weaned at approximately 21 days of age in a long photoperiod (16:8 LD; with lights-off at 1500 Eastern Standard Time [EST] in the room) and housed within this room for the duration of the study. All hamsters had *ad libitum* access to food (Harlan Teklad Rodent Diet 8640, Indianapolis, IN, USA) and filtered tap water, except where experimental protocol dictated otherwise. Animal rooms were maintained at an unvarying temperature and humidity (21 ± 2° C and 50 ± 10%, respectively). All procedures were conducted in accordance with the US National Institute of Health (1986) Guide for the Care and Use of Laboratory Animals, The Ohio State University Institutional Animal Care and Use Committee, and the international ethical standards described previously (Portaluppi et al., 2010).

Experimental Groups

Adult male Siberian hamsters were housed individually from weaning. At 40 or 80 days of age males were paired with an intact female or kept in isolation. The females were removed after 6 h and mating behavior was recorded to ensure that copulation occurred. Behavioral testing was initiated at 120 days of age for all of the experimental groups. Because behavioral testing occurred either 40 or 80 days after sexual contact additional groups were added to account for the elapsed time between sexual experience and experimental testing. Hamsters were still provided sexual experience at 40 or 80 days of age, but were then tested 40 or 80 days later (40 X 40 vs. 40 X 80 vs. 80 X 40 vs. 80 X 80 groups). In the original design all of hamsters were the same age at the time behavioral testing began, yet for adolescent sex group a time period of 80 days passed between the sexual encounter and the initiation of behavioral testing, and for the adult sex group a lesser time period of 40 days passed before behavioral testing was administered following first sexual encounter. The additional groups included in experiment 1B were: the 40 X 40 and 80 X 80 day groups. The 40 X 40 day group corresponds with the 80 X 40 day group from the original study and the 80 X 80 day group matches up with the 40 X 80 day group from the original study, experiment 1A.

All forms of behavioral testing were administered between 15:00 and 18:00 h and hamsters were given 30 min to habituate to the test room before initiation of testing. Tests were performed in the following order: (1) elevated-plus maze, (2) forced swim test, (3)

sucrose anhedonia. Following behavioral testing, the presence of inflammatory markers in the brain was assessed gene expression analysis.

Stimulus Animals

Male hamsters were placed in a novel cage with a sexually receptive female. The stimulus females were introduced to the males and allowed to copulate for a maximum of 6 h. Female ovariectomized (OVX) hamsters were implanted with an estrogen capsule 2 weeks prior to the beginning of experiments. OVX females were brought into heat by subcutaneous injections of progesterone 6 h prior to being sexually paired with a receptive male counterpart. Bouts of mating behavior were conducted in a rectangular box measuring 50 cm × 75 cm × 50 cm (D × W × H), the front wall of which was transparent. Sexual contact was monitored and videotaped in the dark under red light illumination, and tests were conducted between 15:00 and 21:00 h and recorded on VHS-video tape to ensure that copulation had taken place.

Behavioral Tests

Tests of Anxiety-Like Responses

Elevated Plus Maze. At 80, 120, and 160 days of age Siberian hamsters were tested in the elevated plus maze, according to group assignment, which is a method that has been

validated in hamsters through past research in the lab (Prendergast & Nelson, 2005; Weil et al., 2006). The EPM test consists of two open and two closed 6-cm wide arms in a plus-sign configuration 1 m off the floor. The closed arms are enclosed by 15 cm tall black Plexiglas. All arms were covered with contact paper to prevent the hamsters from sliding off, and all surfaces were wiped with 70% alcohol between animals. Each hamster was released into one of the closed arms and allowed to move freely on the maze for a 5-min testing period that was videotaped from above the maze. Hamsters that fell off the maze into compartments below were placed back on the maze for the remainder of the testing period. An observer uninformed about experimental conditions scored the videotapes with The Observer software (Version 5, Noldus Software, Leesburg, VA, USA) for (a) percentage of entries into open arms (b) and total entries into all arms. Hamsters were considered to have entered an arm when all four paws crossed onto an arm of the maze.

Tests of Depressive-like Responses

Forced Swim Test. Hamsters were examined for cessation of attempting to escape water by placing them in 17 cm of room-temperature water (22 ± 1 °C) in a cylindrical tank (24 cm diameter, 53 cm height) with opaque walls. Swimming behavior was videotaped for 7 min and scored by an uninformed observer with The Observer software (Version 5, Noldus Software, Leesburg, VA, USA) to determine (a) swimming (i.e., climbing or scratching directed at the wall of the tank and horizontal movement in the tank), or (b)

floating (i.e., minimal movement required to maintain head above the surface of the water).

Sucrose Anhedonia. The relative consumption of a 3% sucrose solution was measured to quantify anhedonic behavior. All fluid consumption testing took place in the home cage beginning at (15:00 h EST). To acclimate the animals to the novel solution, hamsters were presented with a modified water bottle containing tap water and an empty modified water bottle for 5 h over the course of three consecutive nights (15:00-21:00 h). The normal drinking water was measured each night in order to establish a baseline measurement of overall water consumption. On the fourth day, hamsters were presented with a bottle containing normal drinking water and a bottle containing sucrose solution for 5 h over an additional 6 consecutive nights (15:00-21:00 h). Each night following the 5 h exposure period each bottle was weighed, bottles were replaced in the cage at 15:00 hr the next night, and subsequently weighed again 5 hrs later (21:00 h). To control for possible side preferences, placement of the bottles in the cage was counterbalanced. The modified water bottles were weighed before and after the 5 h sample time to quantify total volume of liquid consumed. Sucrose consumption on both nights was normalized to the average pretesting water consumption.

Gene Expression

Polymerase Chain Reaction. Real-time quantitative polymerase chain reaction (PCR) was used to assess the amount of gene expression of specific mRNA levels of inflammatory

markers within the emotion and affect related regions of the brain (i.e., PFC & hippocampus). Mixed unilateral RNA samples dissected from the PFC and hippocampus were extracted by using a homogenizer (Ultra-Turrax T8, IKA Works) and an RNeasy Mini Kit (Qiagen) according to manufacturer's protocol. Extracted RNA was suspended in 30 µl of RNase-free water, and RNA concentration was determined by a spectrophotometer (NanoDrop ND-1000, Nanodrop Technologies, Wilmington, DE, USA). All RNA samples were stored at −80 °C until analysis. cDNA was created via reverse transcription of 2 µg of RNA from each sample with MMLV Reverse Transcriptase enzyme (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The following inventoried primers and probes (Applied Biosystems) were used: interleukin IL-1β. A TaqMan 18S rRNA primer and probe set, labeled with VIC dye (Applied Biosystems), were used as a control gene for relative quantification. Amplification was performed on an ABI 7000 Sequencing Detection System by using Taqman Universal PCR master mix. The universal 2-step RT-PCR cycling conditions used were: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 sec.

Analysis of Brain Morphology

Histology. Neurons impregnated with the Golgi-Cox solution were chosen within layer III of the PFC and the CA1, CA3, and dentate gyrus (DG) regions of the hippocampus. Only fully impregnated neurons that were not obscured by neighboring neurons and had no obviously truncated dendrites were chosen for analysis. For each animal, six randomly

chosen, representative neurons from different sections were completely traced at 20× (N.A. 0.75) using Neurolucida 8 software (MicroBrightField, Williston, VT, USA) for PC and a Nikon Eclipse E800 brightfield microscope. Dendritic spines were traced in each neuron at 100× (N.A. 1.30) in 4 apical and 4 basilar randomly chosen, representative dendrite segments of at least 20 μm in length, and at least 50 μm distal to the cell body. Morphological characteristics were analyzed using Neurolucida Explorer software (MicroBrightField, Williston, VT, USA) and consisted of: (1) basilar dendritic length, (2) apical dendritic length, (3) basilar dendritic intersections, (4) apical dendritic intersections, (5) dendritic spine density (6) cell body area, (7) and cell body perimeter.

Autopsy

One week following immunological testing between 10:00 and 12:00 h, all hamsters were deeply anesthetized with isoflurane vapors and rapidly decapitated. At this time, reproductive tissues were extracted (testes, epididymal fat pads, seminal vesicles, and epididymides), cleaned of connective tissue, and weighed to the nearest 0.1 mg, for subsequent analysis. Brains were promptly hemi-sectioned (cut in half) and processed for Golgi-Cox staining using a Rapid Golgi Stain Kit (FD NeuroTechnologies). Half brains were submerged in Golgi-Cox solution and stored for 14 days in the dark. Brains were then flash frozen with dry ice and 100 μm coronal sections were sliced on a cryostat and collected onto gelatin-coated glass slides. The stain was developed in NH_4OH for 10 min and sections were counterstained with cresyl violet. Finally, slides were dehydrated

through a series of graded ethanol washes, cleared with xylene, coverslipped with Permount, and dried in the dark for at least 1 week.

Hormone assays

Blood samples were collected from the retro-orbital sinus into microcentrifuge tubes. The samples were centrifuged at 6000 rpm for 30 min at 4 °C; serum was stored at – 80 °C until assayed. Cortisol concentrations were determined by using an I^{125} CORT kit (MP Biomedical, Solon, OH) according to kit instructions. The standard curve was run in duplicate and samples were run in duplicate. All samples in an experiment were run in a single assay, the intra-assay variability was 12.5.

Statistical Analyses

Data analyses were conducted using a one way (ANOVA) with variables being (sex experience x age). Mean differences were considered statistically significant if $p<0.05$. Following a significant F score, multiple comparisons were conducted with Bonferroni's multiple comparison tests.

Results

Several behavioral measures were used to assess anxiety-like and depressive-like responses. Well after sexual contact (i.e., at ages P80, P120, P160), basal levels of anxiety and exploratory behavior of sexually experienced and control animals were studied. The animals underwent three behavioral tests: Elevated plus maze (EPM),

Porsolt forced swim task (FST), and sucrose anhedonia test (SA). No differences were found in measurements of locomotor activity in EPM, as measured through total arm entries in the elevated plus maze (Figure 2.1A). Sexual activity altered behavior in the elevated plus maze [$F(4,37) = 5.901$; $P < 0.05$)]; (Figure 2.1B). For adolescent sex and adult sex hamsters, there was a significant difference in the percentage of time spent in the open arms of the EPM compared to controls (Figure 2.1B). P40, P80, and P80X80 sexual contact hamsters spent significantly less time in the open arms than adult males and none sex experienced male hamsters. Bonferroni's multiple comparison tests revealed that P40, P80, and P80X80 hamsters exhibited increases in anxiety-like behavior when compared to controls, (% time spent in open arm: $t = 4.094$; $t = 3.730$; $t = 3.453$; $P \leq 0.05$, respectively). Neither P40, P80, nor P80X80 hamsters differed significantly in the percentage of time spent in the open arms of the maze. Sexual activity altered behavior in the forced swim test [$F(4,34) = 4.594$; $P < 0.05$)]; (Figure 2.2A). Adolescent sex male hamsters spent significantly more time floating during the forced swim test as compared to the adult sex and the no sex experience groups (Figure 2.2A). For P40 hamsters' sexual experience in adolescent hamsters was coupled to significant increases in time spent floating. Bonferroni's multiple comparison tests revealed that P40 hamsters exhibited increases in depressive-like behavior when compared to P80, P80X80, and controls (% Float time: $t = 3.256$; $t = 3.092$; $t = 3.940$; $P \leq 0.05$, respectively). Neither P80, P80X80, or control hamsters differed significantly in the percentage of time spent floating in the porsolt forced swim test. Following sexual experiences hamsters exhibited decreases in sucrose consumption. Analysis showed that adolescent hamsters with sexual

experience showed the most dramatic decreases in total amount of sucrose consumed (Figure 2.2B). Sexual experience in adolescent hamsters was connected to decreases in total amount of sucrose consumed [$F(4,29) = 6.909$; $P < 0.05$)]. The amount of sucrose consumed for P40 hamsters' was significantly decreased when compared to controls. Bonferroni's multiple comparison tests revealed that P40 hamsters exhibited increases in depressive-like behavior when compared to controls (Amount sucrose intake: $t = 5.157$; $P \leq 0.05$). The P40X40, P80 and P80X80 hamsters did not differ significantly in the amount of sucrose intake in the sucrose anhedonia test. The P40 sex paired hamsters showed the largest decrease in sucrose consumption when compared to controls and adult P80 and P80X80 animals.

During autopsy brains were hemi-sectioned with one hemisphere flash frozen for subsequent gene expression and the other hemisphere placed in golgi solution to later look at morphology. Gene expression analysis of the prefrontal cortex in hamsters that engaged in a first sexual encounter during adolescence revealed increased mRNA expression of IL-1β, a pro-inflammatory cytokine associated with depressive-like behavior following exposure to various forms of environmental challenges, as compared to all other experimental groups [$F(4,27) = 3.073$; $P < 0.05$)]; (Figure 2.3). No differences were found in TNFα gene expression in the PFC. Additionally, no differences were observed in Il-1β or TNFα gene expression in the hippocampus (Figure 2.4).

Golgi impregnated hemi-sectioned brains were observed using a microscope to determine if brain morphology is altered. Scholl analysis of prefrontal cortical regions discovered a decrease in the complexity of basilar dendrites in both P40 and P40X40

male hamsters', as measured through mean intersection number. Adolescent sexual activity altered the number of intersections in basilar dendrites in the PFC [$F(4,22) = 3.514$; ($P < 0.05$)]; (Figure 2.5A). There were no significant differences found in P80, P80X80, and controls. Scholl analysis of mean length of prefrontal basilar dendrites revealed a decrease in dendrite length in both P40 and P40X40 male hamsters when compared to all remaining experimental groups [$F(4,22) = 3.612$; ($P < 0.05$)]; (Figure 2.5B). Similar findings to those shown in basilar dendrites were found when prefrontal apical dendrites where observed. A decrease in the complexity of apical dendrites was observed in both P40 and P40X40 male hamsters', as measured through mean intersection number [$F(4,22) = 4.323$; ($P < 0.05$)]; (Figure 2.6A). Bonferroni's multiple comparison tests revealed that P40X40 sex experienced hamsters exhibited decreases in dendrite intersections when compared to controls (Apical dendrite intersections: $t = 3.475$; $P \leq 0.05$). There were no significant differences found in P80, P80X80, and controls. A decrease in dendrite length in both P40 and P40X40 male hamsters was seen in analyses of mean length of prefrontal apical dendrites when compared to all remaining experimental groups [$F(4,22) = 5.213$; ($P < 0.05$)]; (Figure 2.6B). Bonferroni's multiple comparison tests discovered that P40X40 sex experienced hamsters exhibited decreases in dendrite length when compared to P80 s and control hamster (Apical dendrite length: $t = 3.200$; $t = 3.856$; $P \leq 0.05$, respectively). In terms of apical dendrite length, no significant differences were found among P80, P80X80, and control animals.

Hamsters that underwent sex pairing in adolescence exhibited highest cortisol concentrations [$F(4,31) = 3.105$; ($P < 0.05$)]; (Figure 2.7). In other words, P40 animals

showed the highest concentration of cortisol. The concentration of cortisol was significantly elevated as compared to P80, P80X80 and control males (Figure 2.7). Bonferroni's multiple comparison tests revealed that P40 sex experienced hamsters increases in CORT concentrations when compared to controls hamsters (CORT concentration: $t = 3.340$; $P \leq 0.05$).

Following the end of behavioral and immune testing a number of measurements were taken to calculate somatic and reproductive responses. No significant changes to overall body mass were observed (Figure 2.8A). Paired testis mass was reduced in male hamsters that were sexual paired at P40 as measured up to P40X40, P80, P80X80, and control males [$F(4,37) = 5.972$; ($P < 0.05$)]; (Figure 2.8B). Bonferroni's multiple comparison tests revealed that P40 sex experienced hamsters increased paired testis mass compared to P40X40, P80, P80X80, and control males (Paired testis mass: $t = 3.553$; $t = 3.030$; $t = 4.114$; $t = 4.279$); $P \leq 0.05$, respectively). Accessory reproductive tissue mass was reduced in male hamsters that were sexual paired at P40. The reduction in epididymide tissue mass in P40 animals was significantly decreased as compared to P40X40, P80, P80X80, and control males [$F(4,38) = 3.200$; ($P < 0.05$)]; (Figure 2.8C). Bonferroni's multiple comparison tests revealed that P40 sex experienced hamsters showed decreases in epididymides mass when compared to P80X80 hamsters (epididymides mass: $t = 3.348$; $P \leq 0.05$). Seminal vesicle tissue mass was reduced in P40 hamsters that were sexual paired when match up to P40X40, P80, P80X80, and control males [$F(4,38) = 3.427$; ($P < 0.05$)]; (Figure 2.8D). Bonferroni's multiple comparison tests revealed that P40 sex experienced hamsters showed decreases in seminal vesicle

mass when compared to controls (seminal vesicle mass: $t = 3.219$; $P \leq 0.05$). In all measurements of somatic and reproductive responses no significant differences were observed between P40X40, P80, P80X80, and control males.

Discussion

The first test that was used to assess anxiety-like behavior was the elevated plus maze test, which involved placing the hamster on an elevated platform with two closed chambers and two open arms. This test of spontaneous exploratory behavior was chosen because it is a behaviorally valid test of anxiety in rodents (Pellow, Chopin, File, & Briley, 1985). The number of entries into and time spent on the open arms are interpreted as representing reduced anxiety-like behavior. In terms of anxiety like responsivity, the mere exposure to sex increases anxiety-like behavior. Sexual activity increases anxiety-like behavior independent of the timing of the first sexual encounter. To account for the amount of ambulation done in the testing arena the number of times hamsters crossed a bar, which distinguished between entering a closed or open arm, was observed during a specific time period. Thus, by establishing the rate of bar crossings over time, we were able to confirm that the hamsters were moving at a similar rate, but spending more time in a given arm of the maze. Because there were no significant differences in locomotor activity, I can state that not only were the hamsters all moving at the same rate, but also that they spent their time in different arms. Thus, by moving around in the closed arms rather than the open ones, I can conclude that sexual activity is likely playing a role in mediating these anxiety-like responses.

The next step was to look at depressive-like behavioral responses because an increase in depressive symptoms has also been linked to sexual contact (Kaltiala-Heino, Kosunen, Rimpela, 2003). Depressive-like behavior was evaluated through the use of two different tests of rodent depression. Both the FST and SAT tests were used to measure depressive-like behavior because they have high face and predictive validity. Notably, the administration of antidepressants reverses depressive symptomatology in these tests. The Porsolt forced swim test was used because it directly measures how much time is allocated to immobility during the most stressful of all the tests. The reasoning behind this test is because much like symptoms of anxiety, depressive symptoms have been linked to sexual contact (Kaltiala-Heino, Kosunen, Rimpela, 2003). Symptoms including decreased energy and effort have been identified in animal studies in the past (Anisman & Matheson, 2005). Reduced active escape behaviors and far more time remaining immobile reflect not only depressed symptomatology, but also a learned helpless response. The hamsters that had early sexual experiences during adolescence spent less time displaying active escape behaviors and more time remaining immobile, suggesting a learned helplessness response. Furthermore, the adolescent sexually active hamsters stopped displaying active escape behavior more quickly than did the controls or the adults. Another different measure of depressive like behavior the SAT was used to evaluate anhedonic behavior. Anhedonia is a core feature of depression that refers to the expression of indifference to a previously rewarding stimulus. When given a choice between tap water and sucrose rodents typically prefer to drink the sweetened solution because it is highly palatable to them. But a lack of preference in this test would indicate

an anhedonic response. Adolescent sexually active hamsters displayed the highest levels of anxiety-like behavior in this test.

The results of the behavioral testing reported here suggest that behavioral responses are in part shaped by experiences that take place early during individuals' lives. Exposure to sexual contact within the adolescent sensitive period alters functioning of affective systems involved in depression. The findings are of particular interest because sexual activity is typically interpreted as a positive and pleasurable experience that has neuro-protective properties (Leuner, Glasper, Gould, 2010). In rodents sexual experience is a powerful motivator with the potential to impose organizational change in neuro-circuitry. For instance, chronic sexual experience enhances cell propagation, adult neurogenesis, and dendritic spine numbers in emotion and memory related areas in the brains of adult male rats (Leuner, Glasper, Gould, 2010). The findings in this study are more in line with research that indicates that stressful life events lead to anxiogenic and depressive behavioral manifestations, as well as impairments in immune function (Pryce et al., 2005). The exposure to a salient social interaction, such as sex during adolescence, may be interpreted similarly to a stressor in an individual that is not yet fully equipped to deal with the nuances of this type of occurrence.

Human studies suggest that adverse early life experiences affect mood and immunity possibly through inflammatory pathways. Depressed patients have been shown to exhibit elevated levels of inflammation (Miller, Maletic, Raison, 2009). Research has suggested that the immune system operates similarly to a sensory organ notifying the brain when antigens are present. The types of neurochemical changes elicited by

inflammatory factors are akin to those brought forth by environmental stressors, so it has been proposed that immune activation might be translated as a stressor by the central nervous system and may play a role in the onset of mood and anxiety disturbances (Anisman & Merali, 2003; Anisman, 2009). Activation of inflammatory pathways could be one of the mechanisms through which early life adverse experiences alter long-term health outcomes (Danese et al, 2006). Alterations in immunity following challenge may be a consequence of compensatory investments in immune defense in opposition to other expensive physiological processes such as reproduction (Martin al., 2007). It was important to verify if early sex experience impacted immune function like what's seen in clinically depressed individuals. We assessed the gene expression of various known pro-inflammatory cytokines (IL1β and TNFα) in mood related regions of the brain (Hippo and PFC). Regions of the PFC and Hippo were dissected from the brain and mRNA was extracted from this tissue and reverse transcribed into cDNA so that rtPCR could be undergone to measure levels of cytokine gene expression. The adolescent sex experienced hamsters had marked increases in the pro-inflammatory cytokine IL1β, specifically in the prefrontal cortex. No differences were found in TNFα gene expression in the PFC or in IL1β and TNFα in the hippocampus. The results of the immune tests suggest that immunologic changes occur in response to developmentally relevant social interactions. The immune consequences of stressors have been shown in other studies, but the present investigation is unique because it demonstrates these effects in adults after exposure to reproductive activities during adolescence. An enhanced inflammatory response is often indicative of better immune function. But too strong of an immune

response can be detrimental leading to autoimmunity and host tissue damage. Taken together, these results suggest that early adolescent sexual experience has profound effects on the neurocircuitry that governs affective responses, as well as long-term effects on one assay of adult immune function.

Several papers have recently reported abnormal structural, maturational changes in the prefrontal regions following early life and adolescent stress. For, instance, human studies indicate that the hippocampus, cerebellum, prefrontal cortex, and corpus callosum, regions of the brain that are linked to emotion regulation and stress reactivity, share characteristically elongated postnatal maturational windows and a predominant vulnerability to stress (Spinelli, et al., 2009; Andersen & Teicher, 2008). The prefrontal cortex is an area of the brain involved in executive functions that has a protracted maturational trajectory, with this particular brain region not reaching full maturity until well into adulthood. Disruptions in the morphological characteristics of this brain region are in line with the notion that sex can alter brain regions that are not fully mature at the time of sexual pairing. It was important to investigate whether there are changes in the structural integrity of neurons; therefore brains were collected following behavioral testing and stained using the golgi-Cox method. This technique darkly stains a subset of neurons so that different structural characteristics can be observed under a microscope. Neurons in the PFC and hippo were traced and analyzed for different dendritic complexities like intersections and length, using Scholl analysis. Adolescent sex experienced individuals showed a reduction in intersections and length in both Basilar and apical dendrites in the PFC. Neurons in the CA1 and CA3 regions of the hippo were

measured but no differences were observed. The brain morphology results indicate that there is neural organization occurring following adolescent sexual experience. A reduction in these features could reflect altered communication between neurons and could possibly have some involvement in the behavioral changes seen.

Depressed patients exhibit elevations in cytokine expression, heightened glucocorticoid concentrations, and enhanced inflammation. Psychosocial stress has been recently recognized to activate the inflammatory response both peripherally and in the brain (Miller, Maletic, Raison, 2009). One potential pathway of activating inflammatory processes is through the presence of cytokines. Cytokines may influence HPA axis function by impairing negative feedback regulation of HPA axis and by decreased responsiveness to glucocorticoids, which is a hallmark of major depression (Miller, Maletic, Raison, 2009). Blood samples were obtained to determine if changes in glucocorticoid concentrations were involved in this process. The adolescent sex experienced hamsters were revealed to have the highest concentrations of cortisol in blood serum. These results indicate that glucocorticoids may play a role in the affective and behavioral responses seen in adolescent sex exposed animals.

Somatic and reproductive tissues were removed and weighed at the conclusion of this experiment. Hamsters that engaged in sexual activity in adolescence showed decreases in overall weight of reproductive and accessory reproductive tissue. Adolescent sexual experience appears to creating maladaptive reproductive responding. This suggests that sex early in life may prompt the body and brain to boost inflammation. This is in line with research that proposes that a trade-off exists between reproductive function and

immunity; better immune function commonly comes at a cost to reproductive function (Lochmiller, 2003).

My general conclusion is that early life events are crucial for creating and manipulating the foundation for emotional, affective, immunological, and physiological health and well-being. Early life experiences reveal an important aspect of developmental plasticity and how environmental factors can promote both normal and abnormal maturation of the brain. Alternative explanations may become known but currently all of the pathways and processes that could account for the observed group differences have yet to be delineated. This type of research can elucidate essential underlying aspects of immune function following exposure to salient social encounters and its possible involvement in the progression of depression. My research further supports the concept of a two stage model of neuro-development and may be useful and clinically relevant in understanding the long-term physical and mental health outcomes of adolescent sexual activity in humans.

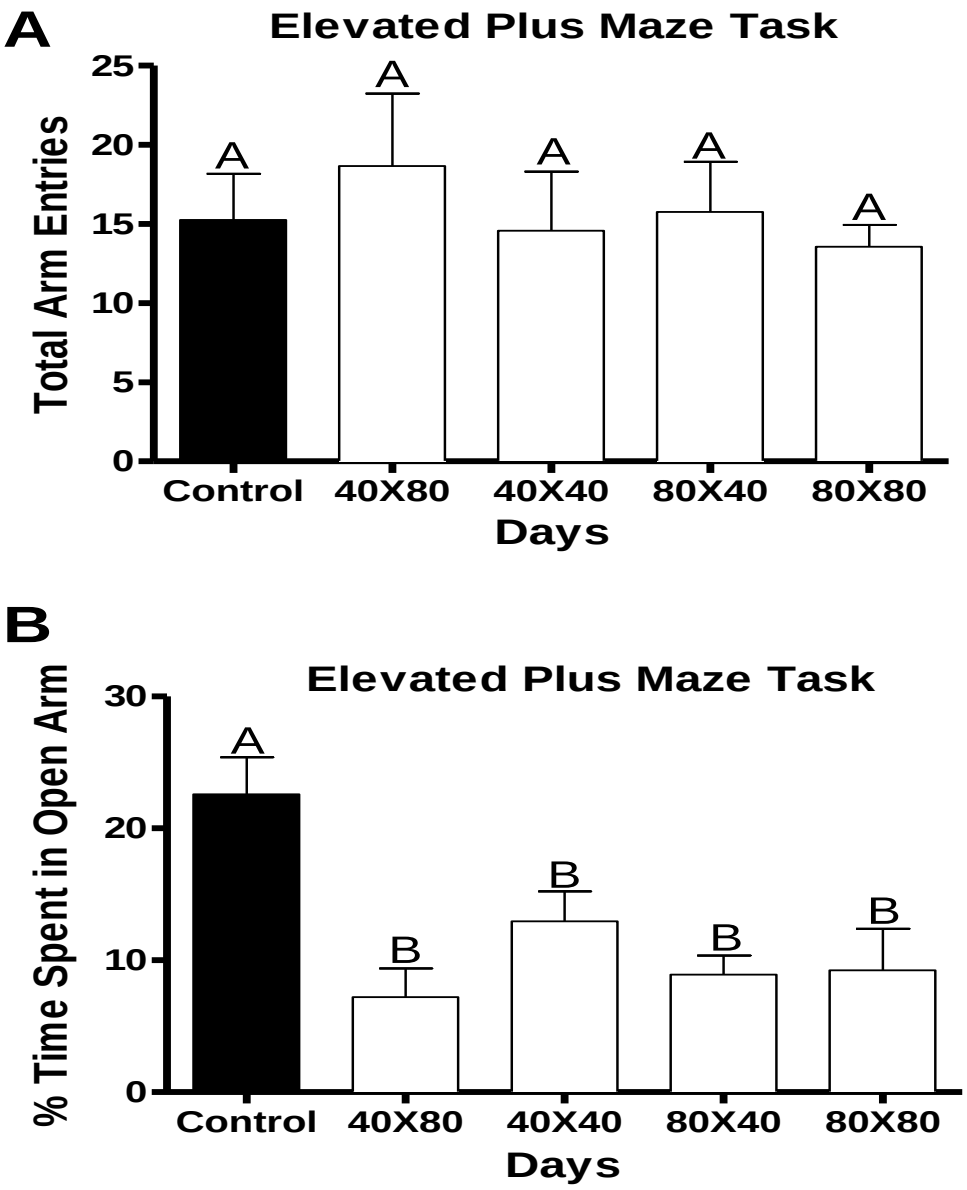

Figure 2.1. Adolescent sexual did not alter locomotor activity in the elevated plus maze

Effects of adolescent sexual activity on behavioral responses (mean ±SEM) in the elevated plus maze task. A) Total number of arm entries in the testing arena. B) Percentage of time spent in the open arm of the testing arena. *Bars sharing the same letters* are not statistically different from each other.

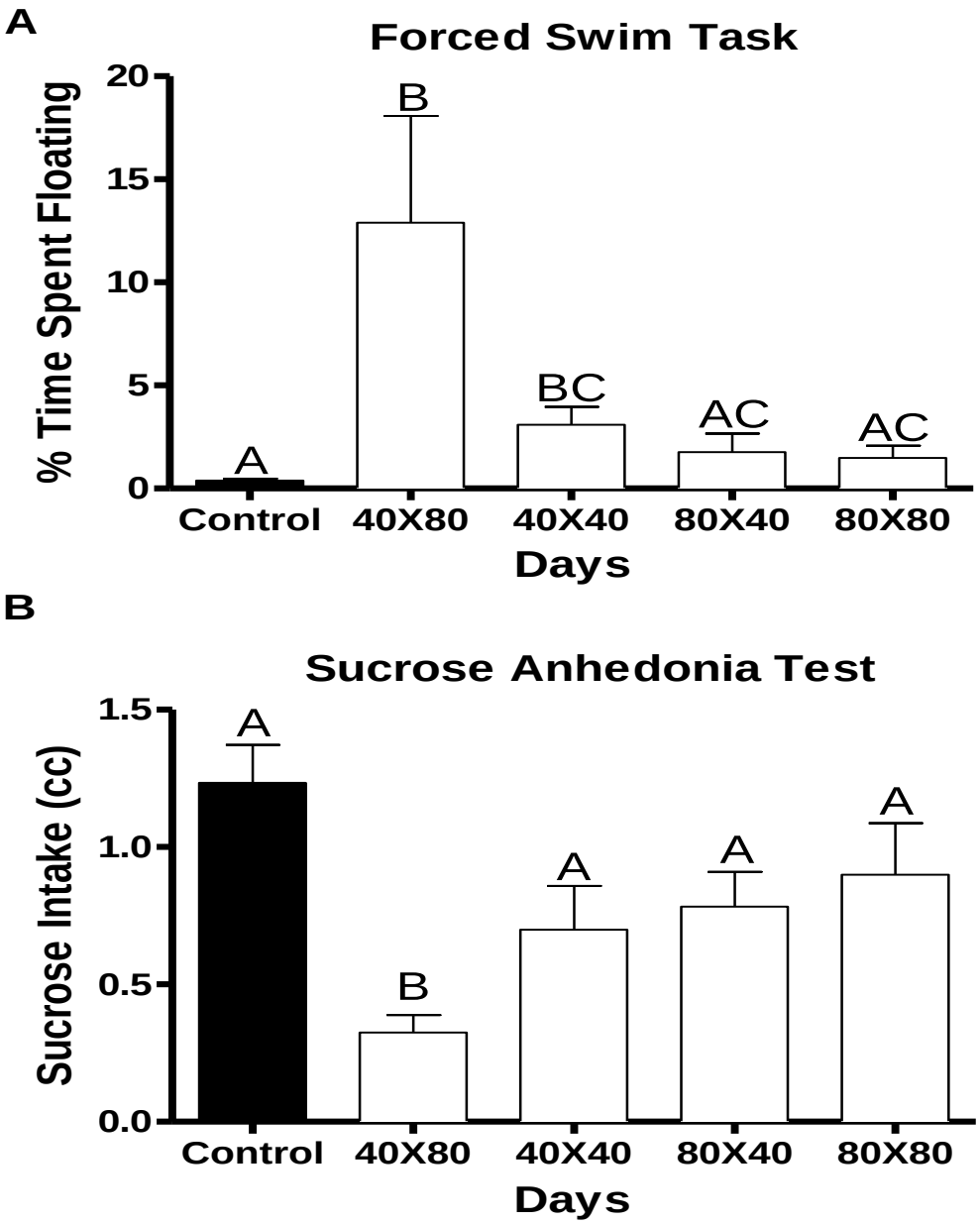

Figure 2.2. Adolescent sexual activity alters behavioral parameters.

Effects of adolescent sexual activity on behavioral responses (mean ±SEM) in the forced swim task. A) Percentage of time spent floating in the testing arena. B) Amount of sucrose (cc) consumed over the duration of the sucrose anhedonia test. *Bars sharing the same letters* are not statistically different from each other

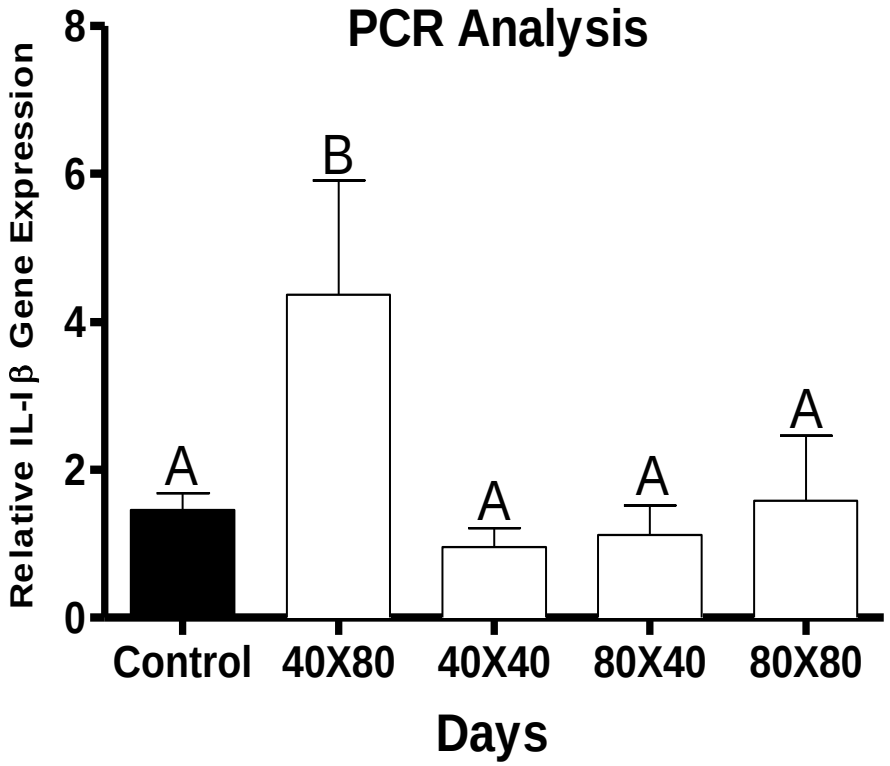

Figure 2.3. Adolescent sexual modulates expression of inflammatory markers in the brain.

Effects of adolescent sexual activity on gene expression (mean ±SEM) relative to 18s rRNA. A) Adolescent sexual experience exacerbates interleukin-1β in the prefrontal cortex (PFC). *Bars sharing the same letters* are not statistically different from each other.

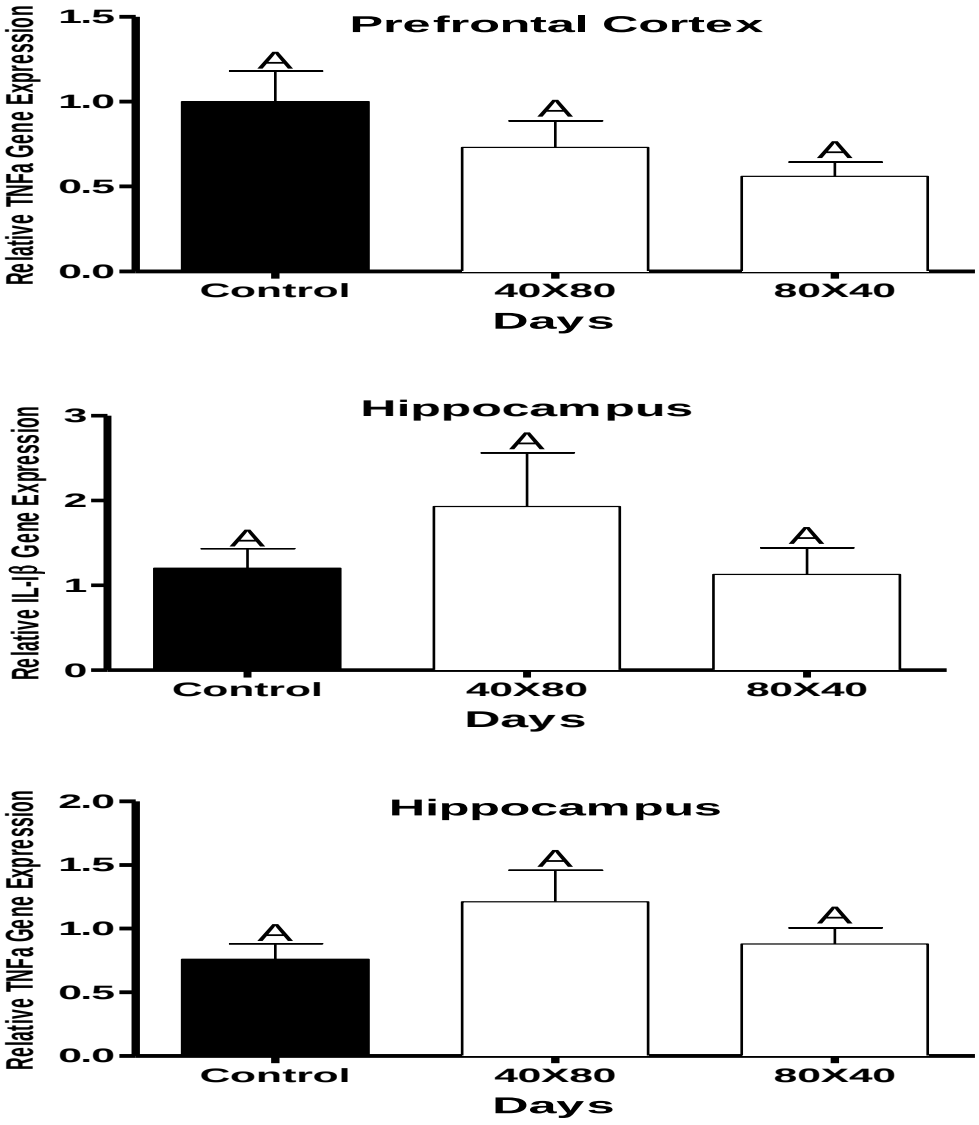

Figure 2.4. Adolescent sexual did not alter gene expression of (TNFα) in the PFC or (IL-1β or TNFα) in the brain.

Effects of adolescent sexual activity on gene expression (mean ±SEM) relative to 18s rRNA. A) No differences in TNFα gene expression in the prefrontal cortex (PFC). B) No differences in IL-1 β gene expression in the Hippocampus (Hippo). C) No differences in IL-1 β gene expression in the Hippocampus (Hippo). *Bars sharing the same letters* are not statistically different from each other.

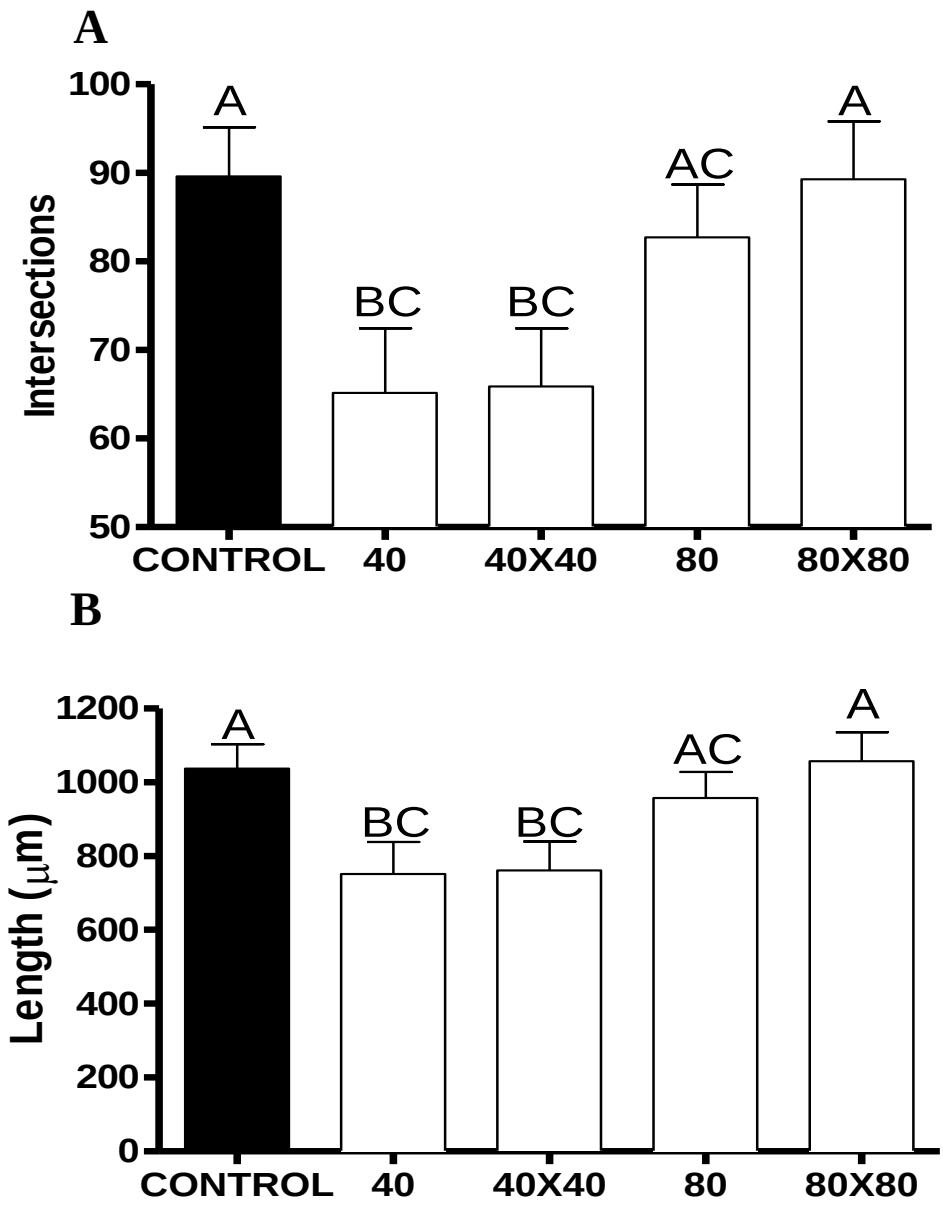

Figure 2.5. Adolescent sexual activity reduces the complexity and length of basilar dendrites in the prefrontal cortex.

Effects of adolescent sexual activity on dendritic morphology (mean ±SEM) in the prefrontal cortex. A) Mean number of intersections of Basilar Dendrites, (BD). B) Mean length of (BDs). *Bars sharing the same letters* are not statistically different from each other.

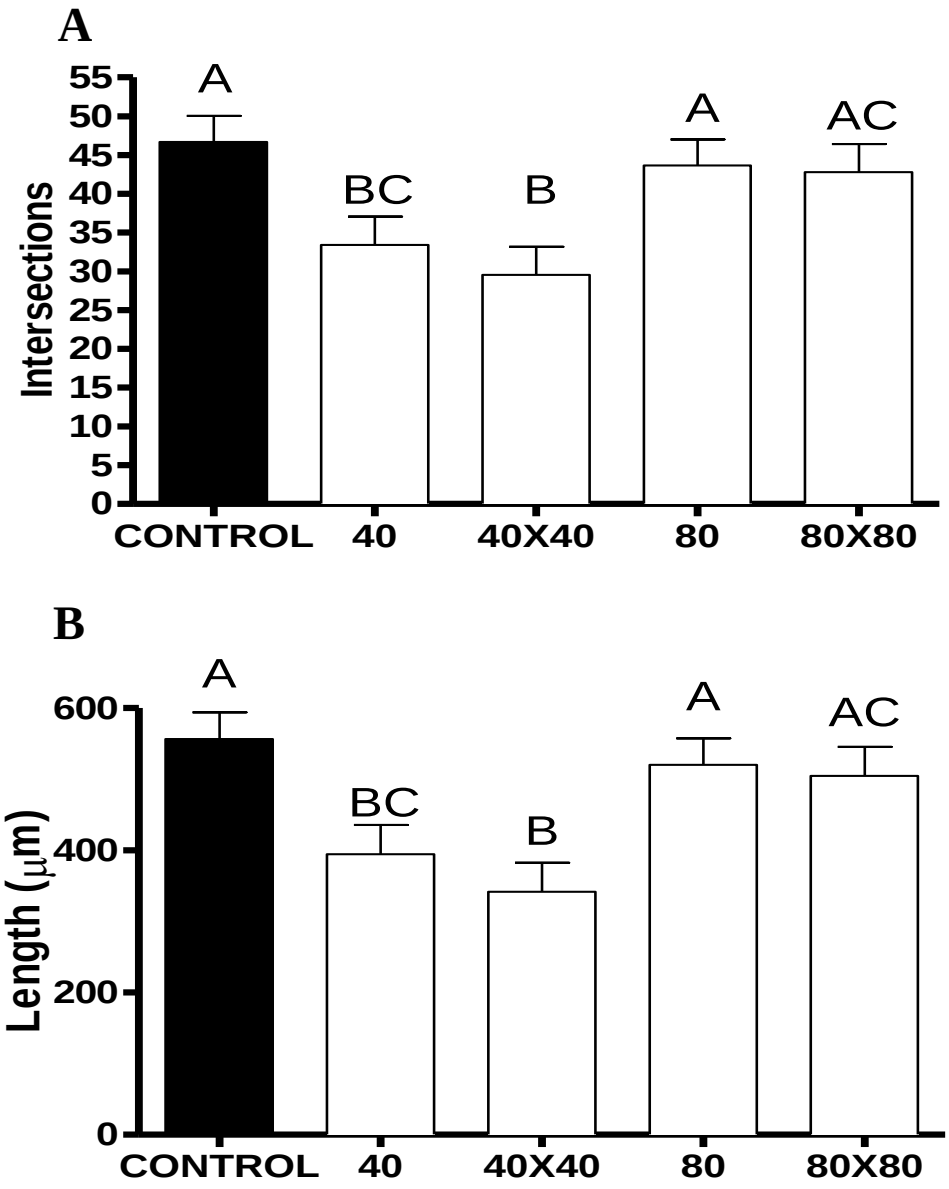

Figure 2.6. Adolescent sexual activity reduces the complexity and length of apical dendrites in the prefrontal cortex.

Effects of adolescent sexual activity on dendritic morphology (mean ±SEM) in the prefrontal cortex. A) Mean number of intersections of Apical Dendrites, (AD). B) Mean length of (ADs). *Bars sharing the same letters* are not statistically different from each other.

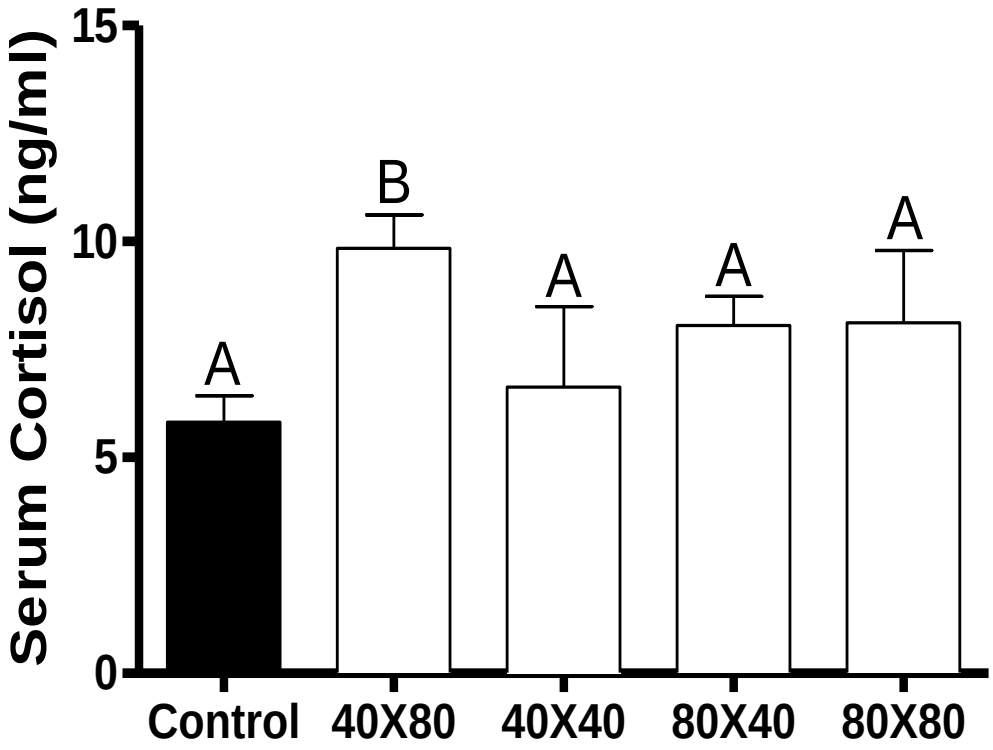

Figure 2.7. Adolescent sexual activity alters circulating steroid concentrations.

Effects of adolescent sexual activity on serum cortisol (ng/ml; mean ±SEM) in males. A) Cortisol (ng/ml) *Bars sharing the same letters* are not statistically different from each other.

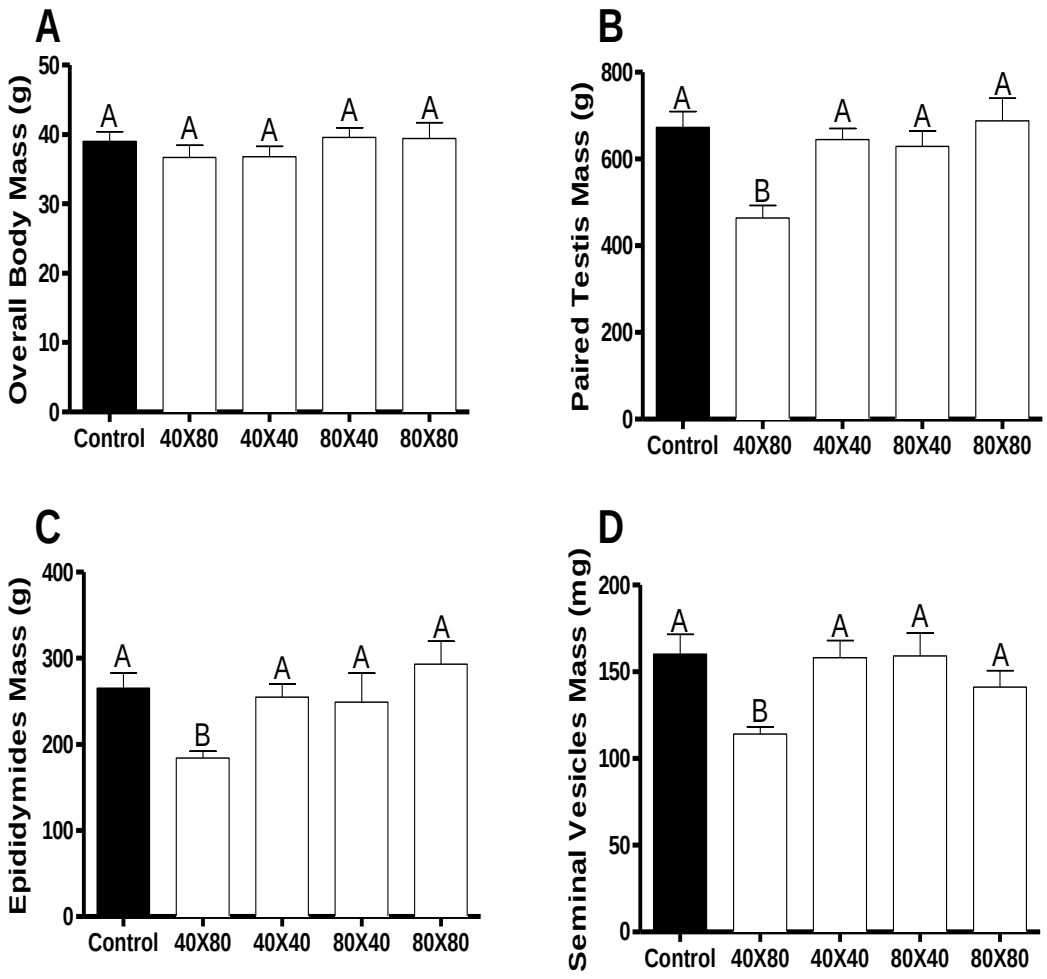

Figure 2.8. Adolescent sexual activity alters somatic and reproductive responses.

Effects of adolescent sexual activity on mass (mean ±SEM) of somatic and reproductive tissues. A) Overall body mass B) Paired testis mass C) Epididymides mass D) Seminal vesicles mass. *Bars sharing the same letters* are not statistically different from each other.

CHAPTER 3

EXPERIMENT 2: EXOGENOUS TESTOSTERONE TREATMENT EARLY DURING ADOLESCENCE ALTERS ADULT BEHAVIOR

As noted, puberty and adolescence are two fundamental processes that take place as mammalian animals' transition from childhood to adulthood. There is overlap in the timing of these two occurrences; puberty is associated with the initiation of adolescence, but it is vital to understand that these states are two distinct processes. The beginning of puberty occurs when both internal and external environmental cues trigger the release of GnRH, which culminates in steroid hormones production, particularly testosterone. Puberty can be thought of as a subset of fairly synchronous maturational changes that are contained within the elongated developmental process of adolescence (Dahl, 2006). Conversely, the prolonged developmental stage that includes the maturation of adult typical emotional, social, cognitive behaviors, and the ability to make judgments and decisions, is known as adolescence (Sisk & Foster, 2004). Elevated testosterone concentrations during adolescence are crucial to establish a proper maturational trajectory and for the full repertoire of reproductive behavior to emerge. This developmentally

timed testosterone secretion plays a role in adolescence by remodeling neural circuits (Sisk & Foster, 2004). Until recently, it was thought that organization (the permanent sculpting or programming of the nervous system by steroid hormones) occurred primarily during a narrow temporal window during perinatal development (Sisk & Zehr, 2005). It has recently been demonstrated that steroid dependent organization of behavior continues into adolescence, extending beyond the classically described perinatal phase (Sisk & Zehr, 2005). These types of events have the potential to bring about lasting behavioral effects in adulthood because of testosterones ability to make permanent changes to brain structure. Because adolescence is a phase in which the brain is highly sensitive, any permanent neural changes have a chance of making a long-term, undesirable impact on both the structure and function of the brain (Romeo & McEwen, 2006). The resulting effects of this sensitivity to testosterone include changes in long-term behavioral phenotype and adult mental health.

The significance of the early adolescent maturation for depression has been explored in terms of biological, mental and social factors (Kaltiala-Heino & Rimpelä, 2003). The process of adjusting to the hormonal changes associated with puberty could be the challenge that increases the risk of depressive affect (Kaltiala-Heino & Rimpelä, 2003). Maturing early may alter the trajectory of developmental processes that are programmed to be completed in mid-adolescence (Tschann et al, 1994). In experiment 2, we attempted to mimic the occurrence of early maturation through pharmacological administration of exogenous T to adolescent male hamsters. This form of experimentation allows for the isolated observation of the influence of a pulse of

testosterone in adolescence on outcome in adulthood. This experiment was intended to test the hypothesis that exposure to testosterone during adolescence has an enduring effect on behavior, specifically, behavior related to anxiety and depression. Based on my hypothesis and results from previous studies I predicted that one of two outcomes will occur: (1) either adolescent hamsters that were exposed to testosterone will display similar results (i.e., increased anxiety- and depressive-like responses) as adolescents in the previous sexual experience studies or (2) they will spend more time displaying such behaviors than hamsters from the sexual experience study. The effects that testosterone can have on the developmental process is widely studied, however, uncovering the role of testosterone early in the developmental process and its possible link to the occurrence of premature and accelerated maturation receives less attention. In addition, the presence of testosterone early on in life may influence susceptibilities to anxiety and depression related conditions, but this postulation has yet to be firmly established. In Experiment 2, I separated animals into one of five groups: a control group (no T administered), two 40 day (adolescent T administered) groups that received testing either 40 or 80 days later (40X40 & 40X80, respectively), and two 80 day (adult T administered) groups that were assessed either 40 or 80 days later (80X40 & 80X80, respectively). Behavioral testing consisted of performance on the elevated plus maze (EPM), designed to evaluate anxiety-like responses, and the (Porsolt forced swim test; FST), which is a measure of depressive-like responses.

Materials and Methods

Animals

Siberian hamsters *(Phodopus sungorus)* used in this project were bred in a colony at Ohio State University and were derived from progenitors obtained from Dr. K. Wynne-Edwards (Kingston, Ontario, Canada). Hamsters dwelled in polypropylene cages (28 x 17 x 12 cm) with a nestlet and corncob bedding (1 cm). Hamsters were weaned at approximately 21 days of age in a long photoperiod (16:8 L: D; lights-off at 15:00 EST in the room) and housed within this room for the duration of the project. All subjects had unrestricted access to food (Harlan Teklad Rodent Diet 8640, Indianapolis, IN, USA) and filtered tap water. Rooms were held at constant temperature and humidity (21 ± 2° C and 50 ± 10%, respectively). All procedures were conducted in accordance with the US National Institute of Health (1986) Guide for the Care and Use of Laboratory Animals, The Ohio State University Institutional Animal Care and Use Committee, and the international ethical standards (Portaluppi et al., 2010).

Experimental Groups

At either 40 or 80 days of age, the experimental hamsters were subcutaneously injected with testosterone at a concentration of 250 μg/kg in olive oil, whereas the control animals received the oil vehicle. Depending on the group assignment, behavioral testing was then conducted on either Day 80, 120, or 160 of the experiment. In the beginning of the study,

behavioral testing was initiated at 120 days of age for all of the experimental groups. However, this created a situation in which 80 days lapsed between tests for the 40-day group and 40 days lapsed for the 80-day group. To mitigate this variable, additional groups were added. Thus, hamsters were still provided sexual experience-like testosterone concentration at either 40 or 80 days of age, but now they were also being tested 40 or 80 days later (i.e., there were now four experimental groups plus a control, *viz.*, P40 X 40, P40 X 80, P80 X 40, P80 X 80). All forms of behavioral testing were administered between 15:00 and 18:00 h and hamsters were given 30 min to get used to the test room before initiation of testing. Tests were performed in the following order: (1) open field test, (2) elevated-Plus maze/bar cross, and (3) Porsolt forced swim test. This specific order was chosen because it presents the hamsters with a gradual increase in stress, so that stress from previous tests was not acting as a confounding variable in the next test.

Behavioral Tests

Tests of Anxiety-Like Responses

Open Field Test. Hamsters were examined for exploratory behavior in the center of an open field relative to zones near the walls. The zones were established by putting colored adhesive tape on the floor in the shape of a small rectangle. A larger terrarium was placed so that the glass walls surrounded the tape rectangle and made a 4 cm border.

This border was the walled zone, whereas the interior side of the rectangle was the open region. This test was carried out under red light and videotaped for 7 min and scored by an uninformed observer with The Observer software (Noldus Corp., Leesburg, VA, USA) to determine (a) time spent in the center of the open field, (b) time spent by the wall.

Elevated Plus Maze/Bar Cross. At 80, 120, or 160 days of age, the hamsters were tested in the elevated plus maze, according to group assignment. The EPM test consists of two open and two closed 6-cm wide arms in a plus-shaped configuration 1 m high (Hogg, 1996). The closed arms are surrounded by 15 cm tall opaque Plexiglas. Each hamster was released into one of the closed arms and allowed to freely explore the apparatus for 5-min testing period that was videotaped from above the maze. Hamsters that fell off were placed back on the apparatus for the remainder of the testing period. All surfaces were wiped with 70% alcohol between animals. An observer uninformed about experimental conditions scored the videotapes with The Observer software (Version 5, Noldus Software, Leesburg, VA) for (a) percentage of entries into open arms (b) and total entries into all arms. Hamsters were considered to have entered an arm when all four paws crossed onto an arm.

Tests of Depressive-like Responses

Porsolt Forced Swim Test. Hamsters were examined for their ending of an attempted escape of water by placing them in 17 cm of tepid tap water (22 ± 1 °C) in a cylindrical chamber (24 cm diameter, 53 cm height) with opaque walls (Castagne et al, 2003). This test was conducted under red light and videotaped for 7 min and scored by an uninformed observer with The Observer software (Noldus Corp., Leesburg, VA, USA) to determine (a) active escape behavior (i.e., climbing or scratching directed at the wall of the tank and horizontal movement in the tank), (b) passive escape behavior (i.e., minimal movement required to maintain head above the surface of the water).

Data Analyses

Data analyses were conducted using a 5 x 2 analysis of variance (ANOVA) with variables being (testosterone vs. age). All data analyses were conducted through the use of SPSS software, version 16.0 (SPSS, Chicago, IL). Mean differences were considered statistically significant if $p<0.05$. Following a significant F score, multiple comparisons were conducted with Bonferroni's multiple comparison tests.

Results

Animals that were exposed to T during adolescence displayed increased anxiety- and depressive-like behavioral responses, compared to the non-T and adult-T injected hamsters. Measurements of total activity were taken while hamsters performed the

elevated plus maze test, each experimental group moved at a comparable rate with the others; no significant differences were observed (Figure 3.1A). In the elevated plus maze, the P40 and P40X40 hamsters spent significantly less time in the open arms [$F(4,29) = 4.273$; ($P < 0.05$)]; (Figure 3.1B). Bonferroni's multiple comparison tests revealed that P40 T administered hamsters showed increases in anxiety-like to controls (% time spent in open arm: $t = 3.457$; $P \leq 0.05$). In the forced swim test, the P40 and P40X40 adolescent exposure to testosterone significantly increased the percentage of time spent floating [$F(4,29) = 5.310$; ($P < 0.05$)]; (Figure 3.1C). Bonferroni's multiple comparison tests revealed that P40 T administered hamsters showed increases in depressive-like to controls (% time spent floating: $t = 4.422$; $P \leq 0.05$).

Discussion

The objective of the present experiment was to test the hypothesis that elevated testosterone during adolescence could recapitulate the effects of early sexual experience on anxiety- and depressive-like behaviors in adulthood. The major aim of this experiment was to evaluate the effects of exposure to elevated concentrations of testosterone during adolescence and the potential effects it could have on behavioral development and adult phenotype. When administered during the early part of puberty, T treatment led to more profound adult sex typical behavior such as: mounts, intromissions, and ejaculations in Syrian hamsters, (Sisk & Zehr, 2005). Furthermore, a correlation exists between testosterone and anxiety and depressive-related behavior in this species (Sisk & Zehr, 2005).

The elevated plus test was used to assess anxiety-like responses. In this test T had differing effects on the adolescent the adult animals. Indeed, the hamsters exposed to T during adolescence spent less than half the time on the open arm than the vehicle-treated and adult-exposed hamsters, suggesting that adolescent testosterone treatment programmed the brain in such a way to increase anxiety-like behavior. Similar to experiment 1 the amount of ambulation in the testing arena was measured by the number of times hamsters crossed a bar, which distinguished between entering a closed or open arm. It was, again, confirmed that there were no significant differences in locomotor activity. These results imply that T is likely playing an underlying role in mediating these anxiety-like responses. The administration of T partially recapitulates what was shown in adolescent sexually active hamsters because it did not induce anxiety in adult animals. These findings make sense because the adult sex group T is administered outside of any critical developmental windows so there likely less of an opportunity for organizational change to occur in adults.

The FST was used to measure depressive-like behavior. The hamsters that had early sexual experiences and testosterone administration during adolescence spent less time displaying active escape behaviors and more time remaining immobile, suggesting a learned helplessness response. Furthermore, the adolescent hamsters exposed to T stopped displaying active escape behavior more quickly than did the controls or the adults. These results are consistent with what was seen in adolescent sex hamsters. These results lend further support for the involvement of sex steroid hormones in the manifestation of depressive-like behavior in adulthood following adolescent sex and

possibly early maturation.

The behavioral analyses of both sexual experience and testosterone-treated hamsters mirrored each other in a way that suggests that testosterone plays a crucial role in mediating this process. By administering testosterone, it allowed us to confirm that the presence of adult typical concentrations of testosterone presented during mid-adolescence, when the finishing of neurocircuitry is still occurring, is sufficient to alter adult affective behaviors. What remains to be determined is whether the presence of elevated concentrations of testosterone is necessary to produce similar effects or not. Research that would uncover the biological mechanism associated with this is required and is underway.

This also leads me to conclude that sexual experience-like concentrations of testosterone are yielding organizational effects, which mediate these behaviors in adulthood. If this were not the case, then the P40X80 group would not have deviated so much from the adults and controls in the elevated plus maze as well as the forced swim test. This is a probable explanation because adolescent development is not yet complete, leaving the hamsters still in a critical maturational phase. I suspect that if this is the case, then testosterone is acting on a more sensitive brain, and in turn organizing the nervous system in a way that yields such behavior. This is why the adults (whose critical phase has passed) in the forced swim test exhibited little depressive-like behavior compared to the adolescents.

Taking into account testosterone actions in the brain, it is a potential candidate for having a mechanistic role in the alterations in the physiology, behavior, and brain of

mammals. Most of the masculinizing actions that testosterone has on the brain are via estrogen receptors following aromatization (Romeo, Richardson, Sisk, 2002). Differential exposure of male and female brains to steroids, during the period just before and after birth, leads to sexual differentiation of neural circuits and determines the potential for behavioral responses to steroids in adulthood (Romeo, Richardson, Sisk, 2002). Importantly the brain evidently retains a considerable capacity for organizational modifications well outside of this early critical period, and puberty is another window of development when the nervous system undergoes additional and striking change to sustain the expression of adult behaviors (Arnold & Breedlove, 1985). Also there are data that acute testosterone alters anxiety like behavior and the relationship between those data and these is significant because it suggests that testosterone may have the ability to alter the brain and other aspects of physiology during adolescence when individuals have a substantial amount of development to undergo.

Presumably, the next step in the process is to determine whether it is androgens or estrogen products of T that drives these alterations to adult behavior. Today, it is regarded as an axiom that the sexual differentiation of the rodent brain may be accomplished due in large part to the involvement of estrodial (E2), which is a form of estrogen created locally from the androgen testosterone (Roselli, Liu, Hurn, 2009). Numerous experimental standpoints have been utilized to examine the mechanism whereby testosterone acts to organize and sexually differentiate brain architecture and chemical profile. The evidence that the brain was capable of converting androgen to estrogen, albeit at very low levels, was the confirmation used in the formulation and substantiation

of the aromatization hypothesis (Hutchison et al., 1991). According to the aromatization hypothesis testicular androgens synthesized by the fetal testis diffuses into the male brain where it is locally aromatized to E2; these locally synthesized estrogens in the brain act to amplify and diversify the action of circulating T (Roselli, Liu, Hurn, 2009). The estrogen formed through the aromatization of T has classically been associated with the regulation of sexual differentiation and masculinization of the male brain. However, new data indicate that the aromatization of testosterone to E2 is involved in the activation of sexual behavior and control of the neuroendocrine system in the male hamster (Hutchison et al., 1991).

A pivotal step in understanding the underlying biological aspects of adult outcome following a pulse of T in early adolescence is determining if these effects are mediated by androgen receptors or estrogen receptors or if there mediated by both. Experiments can be conducted to examine the role of estrogen as a possible mediator of the behavioral alterations seen in adolescent T administered hamsters when tested in adulthood. One possible experimental design to test the role of estrogen could take advantage of pharmacological techniques that employ the administration of exogenous E2 at developmentally relevant time points. If E2 administration results in expression of similar behavioral profiles as those shown following adolescent T exposure then estrogen can be retained as a potential mediator of this process. If the results of T administration can be recapitulated through the administration of E2 it would suggest that estrogen plays a role in this phenomenon because testosterone is converted into estrogen through the process of aromatization. Research can be conducted to study the role of androgen as a

prospective mediator of the behavioral changes exhibited by adolescent T administered hamsters when tested in adulthood. One feasible experimental design to assess the role of androgen could make use of pharmacological techniques that use the administration of exogenous 5α-Dihydrotestosterone (DHT) at adolescent time points. In mammals T is the primary steroid product synthesized in the testes, and this testis derived T is under control of luteinizing hormone (LH) that originates from the pituitary (Mcdonald et al., 1970). After T reaches the target cells in the urogenital sinus, the enzyme 5α-Reductase found in the target tissue itself metabolizes T into 5α-Dihydrotestosterone (Shima et al., 1990). The biological action of androgens is mediated through DHT as it binds to the androgen receptor (AR) and regulates gene transcription, which can result in the growth of the prostate, the development of external genitalia, and the production of several secondary male characteristics in puberty (Mainwaring, W. I. P., 1975; Heinlein & Chang, 2002). Administration of DHT would help to determine if the behavioral alterations seen in adolescent T administered hamsters is an androgen mediated phenomenon. If the administration of DHT can produce similar behavioral outcomes in adults as those of adolescent T administered hamsters it would imply that androgen may be involved in the occurrence of this phenomenon because testosterone is metabolized into DHT by the enzyme 5α-Reductase type 2. To determine if the behavioral alterations associated with T administration in adolescence was mediated by a combination of both estrogen and androgen receptors could be investigated through the use of pharmacological manipulation. A plausible experiment that institutes the simultaneous administration of both exogenous E2 and DHT, followed by administration of a selective agonist for either

E2 or DHT would adequately address the question of whether both forms of receptors are involved in this process. If an agonist for either E2 or DHT were to block the effects seen in adults then we can establish whether or not there is a combinatorial influence of E2 and DHT.

A next logical step would be to perhaps discover where in the brain these T mediated changes are occurring. In a male the developing testis produces testosterone, which subsequently causes the brain to become masculinized (Roselli, Liu, Hurn, 2009). During a critical period, like perinatal life, testosterone produced in the testes is believed to shift the male brain from the inherent feminization program into a program that masculinizes and defeminizes the male brain and induces male-typical behaviors in adulthood (Sato et al., 2004). Endocrinological research indicates that during adolescence there is further organization and masculinization of behavior, which supports the notion that adolescence is an additional critical period when T can have organizational effects on the brain (Schultz et al., 2004). The modulational power of exposure to androgens and estrogens during developmentally sensitive periods in development is demonstrated by research carried out by Romeo and colleagues that demonstrated that a week of testosterone propionate, dihydrotestosterone, or estradiol benzoate treatment increased mounts, intromissions, and ejaculations in adult but not prepubertal males, further substantiating the importance of developmental timing in the organization of the mammalian brain (Romeo et al., 2001;Romeo et al., 2002). Conceivably, the brain could be further altered in a maladaptive way when the processes that occur during another critical point, adolescence, are disrupted by the presence of

uncharacteristically elevated T. It would be reasonable to set up experiments that are aimed at elucidating the various brain regions that may have some involvement in the progression of behavioral modification following adolescent T administration. One way to investigate the locations in the brain were these T mediated changes are happening would be to perform experiments that are set up to observe the localization of androgen or estrogen receptors in brains regions that are densely populated with these types of receptors, such as hypothalamus, regions of the telencephalon, medial preoptic and ventromedial nuclei, lateral septal nucleus, the medial and cortical nuclei of the amygdala, the amygdalohippocampal area, the bed nucleus of the stria terminalis, and the medial prefrontal cortex (Simerly et al., 2004).

The last and most ambitious step would be to ultimately study this phenomenon in humans. Even though animal models are representatives of what could occur in humans, there are social and environmental factors that are still unique to humans. Testosterone exposure early during adolescent development could uncover mechanisms that explain the biological underpinnings of adolescence in humans and physiological and psychological outcome in adulthood and shed light into the pathophysiology of depressive disorders. Could these effects be directly due to the presence of steroid hormones? Or could my effects represent T withdrawal-like symptoms, similar to those experienced by androgen anabolic steroid users (Uzych, 1992)? What I did was a pharmacological test in a very controlled setting. Although it does establish that testosterone is sufficient to evoke maladaptive responses, it leaves open the question as to whether those responses will play a role in human interactions, or whether social and

environmental factors could mitigate them altogether.

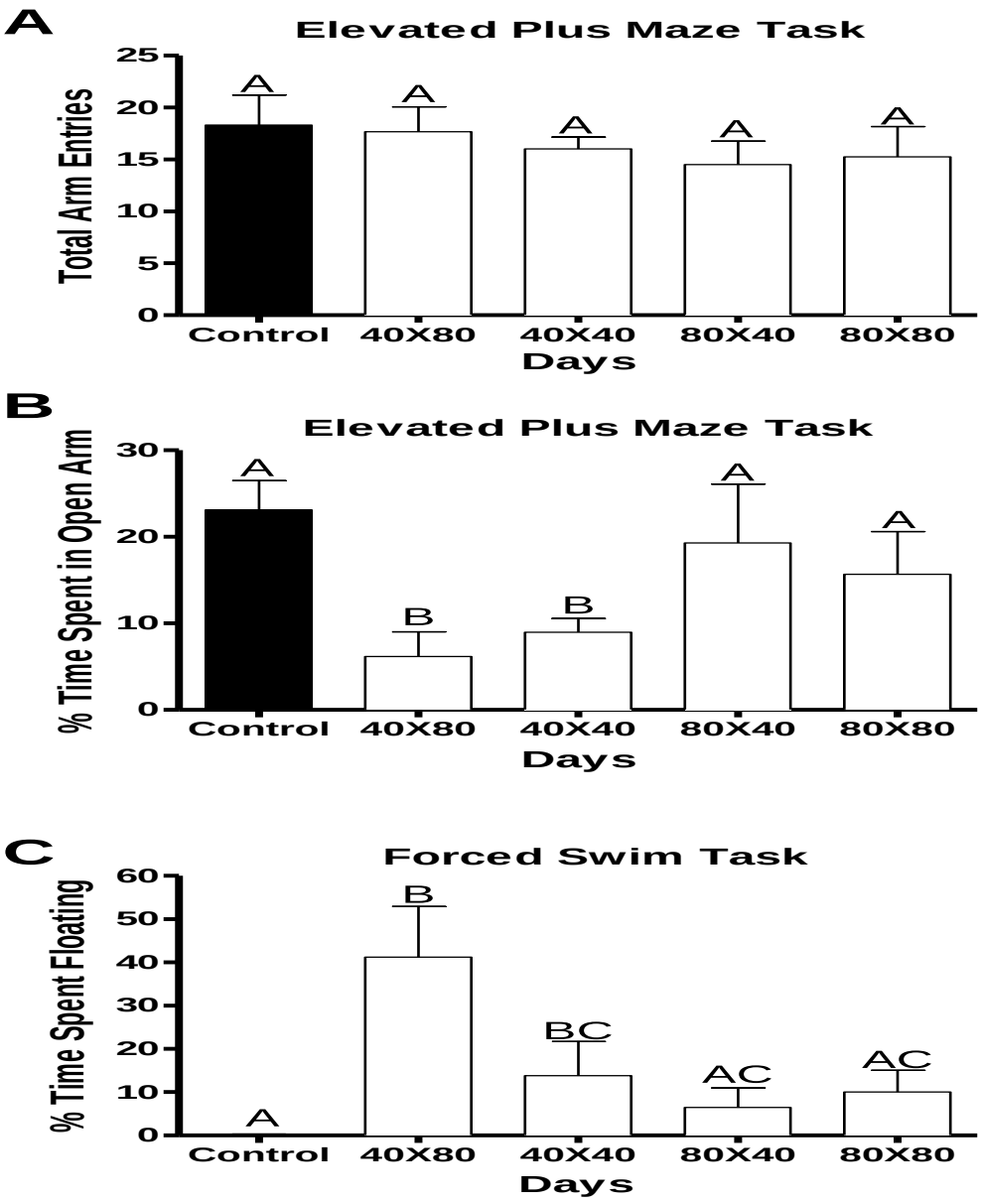

Figure 3.1. Adolescent testosterone administration alters behavioral parameters.

Effects of adolescent T administration on behavioral responses (mean ±SEM) in the elevated plus maze task. A) Total bar crossings in the open arm of the testing arena. B) Percentage of time spent in the open arm of the testing arena. C) Percentage of time spent floating in the testing arena. *Bars sharing the same letters* are not statistically different from each other.

CHAPTER 4

EXPERIMENT 3: SEX DURING ADOLESCENCE ALTERS ADULT ALCOHOL INTAKE

Human adolescence has been described as a time of turmoil and turbulence, characterized by an immense and overwhelming quantity of developmental processes occurring at once (Hall, 1904). This notion of adolescence imposing added emotional, physiological, and behavioral disruptions due to a bottleneck of resources has faced much scrutiny (Higgins & Parsons, 1983;Eccles, et al., 1993;Eccles & Lord, 1996). Contemporary scientists rebuff the merit of this traditional view of adolescence because it is not a universal and well-definined phenomenon; not all adolescent individuals react to the adjustment that adolescence entails in a maladaptive manner (Eccles, et al., 1993). Thus, the traditional dogma requires amending to account for the discrepancies of individual differences in adolescent transitional phases (Arnett, 1999). Although adolescence may not be a time of turmoil for everyone, studies have revealed that it is a time when individuals are increasingly susceptible to the effects of stress, neural reorganization, and drug abuse and addiction (Eccles, et al., 1993).

Among the issues faced during adolescence is an increased incidence of pathological disorders such as depression and anxiety. In addition, the abuse of drugs and

alcohol is quite prevalent at this time in life. This may reflect poor choices indicating impaired cognitive processes, or may reflect an attempt to self-medicate anxiety and depressive-like symptoms (Mehrabian, 2001;McCarthy et al., 2005). Approximately half of the US population will meet the criteria for a *DSM-IV* disorder at some point in their life, usually with the first case in childhood or adolescence (Kessler et al., 2005). In the United States alcohol and drug use by adolescents is highly prevalent (Mitchell et al., 2013). Research in humans indicates that by the time they have reached 12th grade, a large proportion of adolescents have used alcohol or another drug (Johnston et al., 2011). Alcohol is the most frequently used drug among adolescents, and it is the leading cause of mortality and morbidity in this age group; more than all other drugs collectively (Muramoto & Leshan, 1993). In this study, I decided to investigate a model of the effects of early sexual experience on adult alcohol consumption using Siberian hamsters. This work is important because the use of alcohol is a pervasive issue in western society and more research is required in order to develop better screening and intervention strategies.

As previously mentioned, early pubertal maturation is a risk factor for engaging in sexual and delinquent behaviors, as well as the onset of depression (Kaltiala-Heino, Kosunen, & Rimpelä, 2003). An interface between developmental changes at both the individual and social environmental levels influences the transitional nature of early adolescence (Eccles, et al., 1993). It has been posited that maturing early may lead to disruptions in developmental tasks that are programmed to be completed in mid-adolescence (Tschann et al, 1994). Maturing early may expose individuals to increased environmental and social pressures (e.g., conformity, sexual activities, drug use) because

they are perceived as being more mature (Brooks-Gunn, Petersen, & Eichorn, 1985; Tschann et al, 1994). Research conducted on humans' suggests that males that mature early are more likely to engage in sexual activity and delinquent behaviors (Flannery, Rowe, Gulley, 1993). A connection between early maturation, adolescent sexual activity, and drug use has been well documented, but the underlying mechanisms and direction of this relationship remain undefined. Experiment 3, as with experiment 1, attempts to remove the age of puberty issue by using a controlled animal model that permits the isolated examination of sex during adolescence and its potential influence on adult alcohol intake. Although there are strong associations, what is not known is whether exposure to early sexual interactions during adolescence increases the odds of alcohol use and abuse and whether this type of sexual experience can play a role in the maladaptive phenotypes shown by many during their adolescent years of development? The goal of this experiment is to evaluate whether sexual experience during adolescence leads to alterations in the expression of other potentially rewarding behaviors such as alcohol consumption. In this study, I evaluated the influence of sexual experience during adolescence on adult reactivity to the presence of alcohol. I hypothesize that the mechanisms implicated in adult outcome, following sexual experience early in life, may also regulate self-administration of ethanol. This aim is designed to test whether exposure to sex during adolescence affects the amount of alcohol consumed in adulthood. I predict that the adolescent sex experience animals will drink more alcohol then control and adult sex paired animals; this effect may be associated with the depressive and anxiety-like behaviors expressed by this group. Previous data from other labs have shown that

depressed and anxious animals tend to consume high quantities of alcohol compared to non-anxious and non-depressed conspecifics.

Materials and Methods

Animals

Siberian hamsters (*Phodopus sungorus*) used in this study were bred in our colony at The Ohio State University from a wild-bred stock obtained from Dr. K. Wynne-Edwards (Kingston, Ontario, Canada). Hamsters were housed in polypropylene cages (28 × 17 × 12 cm) with a nestlet and 1 cm of corncob bedding. Hamsters were weaned at approximately 21 days in a long photoperiod (16:8 LD; with lights-off at 1500 Eastern Standard Time [EST]) and housed within this room for the duration of the study. All hamsters had *ad libitum* access to food (Harlan Teklad Rodent Diet 8640, Indianapolis, IN, USA) and filtered tap water, except where experimental protocol dictated otherwise. Animal rooms were held at constant temperature and humidity (21 ± 2° C and 50 ± 10%, respectively). All procedures were conducted in accordance with the US National Institute of Health (1986) Guide for the Care and Use of Laboratory Animals, The Ohio State University Institutional Animal Care and Use Committee, and the international ethical standards described previously (Portaluppi et al., 2010).

Stimulus Animals

Male hamsters were placed in a novel cage with a sexually receptive female. The stimulus females were introduced to the males and allowed to copulate for a maximum of 6hrs. Sexual behavior was induced between intact males and ovariectomized females. Female OVX hamsters were implanted with a 5cm long estrogen capsule 2 weeks prior to the beginning of experiments. OVX females were brought into heat by subcutaneous injections of progesterone 6 h prior to being sexually paired with a male. Bouts of mating behavior were conducted in a rectangular box measuring 50 cm × 75 cm × 50 cm (D × W × H), the front wall of which was transparent. Sexual contact will be monitored and videotaped in the dark under red light illumination, and tests will be conducted between 15:00 and 21:00 h and recorded on VHS-video tape to ensure that copulation had taken place.

Experimental Groups

In experiment 3, adult male Siberian hamsters were housed individually from weaning. At 40 or 80 days of age males were paired with an intact female or kept isolation. The females were removed after 6 h and mating behavior was recorded to insure that copulation took place. Hamsters were provided with a sexual experience at 40 or 80 days of age, and then were tested 40 or 80 days later (40 X 40 vs. 40 X 80 vs. 80 X 40 vs. 80 X 80 groups). Additional groups were created because the original design failed to

account for the elapsed time between sexual experience and testing. In the original design all of hamsters were the same age at the time behavioral testing begins, yet for adolescent sex group a time period of 80 days passed between the sexual encounter and the initiation of behavioral testing, and for the adult sex group a lesser time period of 40 days passed before behavioral testing was administered following first sexual encounter. The 40 X 40 day group corresponds with the 80 X 40 day group from the original study and the 80 X 80 day group matches up with the 40 X 80 day group from the original study.

All behavioral testing was conducted between 15:00 and 18:00 h and hamsters were each given 30 min to habituate to the test room before initiation of testing. Tests were performed in the following order: (1) open field test, (2) elevated-plus maze, (3) forced swim test, (4) ethanol intake test.

Behavioral Tests

Tests of Anxiety-Like Responses

Elevated Plus Maze. At 80, 120, and 160 days of age Siberian hamsters were tested in the elevated plus maze, according to group assignment, a method that has been recognized to work in hamsters through past research in the lab. The EPM test consisted of two open and two closed 6-cm wide arms in a plus-sign configuration 1 m off the floor. The closed arms were enclosed by 15 cm tall black Plexiglas. All arms were covered with contact paper to prevent the hamsters from sliding off, and all surfaces were wiped with 70%

alcohol between animals. Each hamster was released into one of the closed arms and allowed to move freely on the maze for a 5-min testing period that was videotaped from above the maze. Hamsters that fell off the maze into compartments below were placed back on the maze for the remainder of the testing period. An observer uninformed about experimental conditions scored the videotapes with The Observer software (Version 5, Noldus Software, Setauket, NY) for (a) percentage of entries into open arms (b) and total entries into all arms. Hamsters were considered to have entered an arm when all four paws crossed onto an arm of the maze.

Tests of Depressive-like Responses

Forced Swim Test. Hamsters were examined for cessation of attempting to escape water by placing them in 17 cm of room-temperature water (22 ± 1 °C) in a cylindrical tank (24 cm diameter, 53 cm height) with opaque walls. Swimming behavior was videotaped for 7 min and scored by an uninformed observer with The Observer software (Noldus Corp., Leesburg, VA, USA) to determine (a) vigorous swimming (i.e., climbing or scratching directed at the wall of the tank and horizontal movement in the tank), (b) non-vigorous swimming (i.e., minimal movement required to maintain head above the surface of the water).

Consummatory Behavior

Alcohol Consumption. Hamsters self-administered ethanol using a sucrose-substitution procedure that provides ad lib access to a sipper tube containing ethanol. Initially, two

sipper tubes were introduced into the home cage, one tube containing ethanol solution in 2% sucrose in water and the other tube containing water. Daily measurements were made in the middle of the light period by reading fluid volume to the nearest 0.2 mL. The positions of the tubes was switched every 24 h to control for side preferences. Ethanol solutions were tested for 8 days: during the first 4 days, mice received 3% ethanol/2% sucrose, and during the next 4 d they received 10%/2% sucrose ethanol. Consumption of ethanol and water was measured daily at the same time. Body masses were recorded daily. The tests were designed to avoid any possible "carry-over" effects from taste solutions on ethanol intake.

Statistical Analyses

Data analyses were conducted using a 3 x 2 analysis of variance (ANOVA) with variables being (sex experience x age). Intake of alcohol was analyzed with repeated-measures ANOVA, with the between-subjects factors being sexual experience and age of sex initiation and day as the within-subjects factor. All data analyses will be conducted through the use of SPSS software, version 16.0 (SPSS, Chicago, IL). Mean differences were considered statistically significant if $p<0.05$. Following a significant F score, multiple comparisons were conducted with Bonferroni's multiple comparison tests.

Results

In this section behavior analysis was conducted with the goal of recapitulating the findings from the initial sex experience study. Measurements of total activity were taken while hamsters performed the elevated plus maze test, each experimental group moved at a comparable rate with the others; no significant differences were observed (Figure 4.1A). As expected hamsters that were exposed to sex exhibited increased anxiety- and depressive-like behavioral responses, independent of timing of first sexual encounter For adolescent sex animals and adult sex hamsters, there was a significant difference in the percentage of time spent in the open arms of the EPM compared to controls [$F(4,26) = 5.365$; ($P < 0.05$)]; (Figure 4.1B). P40, P40X40, and P80 sexual contact hamsters spent significantly less time in the open arms than none sex experienced male hamsters. Bonferroni's multiple comparison tests revealed that P40, P40X40, and P80 sex experienced hamsters showed increases in anxiety-like behavior when compared to controls (% time spent in the open arm: $t = 3.807$; $t = 3.253$; $t = 3.8969$; $P \leq 0.05$, respectively). In the forced swim test, the P40 and P40X40 adolescent sex hamsters' significantly increased the percentage of time spent floating [$F(4,23) = 10.59$; ($P < 0.05$)]; (Figure 4.1C). Bonferroni's multiple comparison tests revealed that P40 and P40X40 sex experienced hamsters showed increases in depressive-like behavior when compared to P80, P80X80, and control hamsters (% time spent floating: $t = 4.163$; $t = 4.350$; $t = 3.719$; $t = 3.861$; $t = 4.528$; $t = 4.723$; $P \leq 0.05$, respectively). Measurements of alcohol intake were analyzed. Hamsters that were exposed to sex in adolescence demonstrated an increase in the amount of 3% alcohol in 2% sucrose solution consumed

over 4 days. There was a significant difference in the amount of alcohol intake in adolescent sexual experienced males when compared to adult and control males [$F(4,26) = 5.062$; ($P < 0.05$)]; (Figure 4.2A). Bonferroni's multiple comparison tests revealed that P40 sex experienced hamsters showed increases intake of a 3% alcohol in 2% sucrose solution when compared to P80X80, and control hamsters (Amount of alcohol intake: $t = 3.085$; $t = 4.245$; $P \leq 0.05$, respectively). When the percentage of the alcohol in sucrose solution was increased from a 3% alcohol in 2% sucrose solution to a 10% alcohol in 2% sucrose solution, again hamsters that were exposed to sex in adolescence demonstrated an increase in the amount of alcohol consumed. There was a significant difference in the amount of this higher percentage alcohol intake in adolescent sexual experienced males when compared to adult and control males [$F(4,26) = 3.828$; ($P < 0.05$)]; (Figure 4.2B). Bonferroni's multiple comparison tests revealed that P40 sex experienced hamsters showed increases intake of a 10% alcohol in 2% sucrose solution when compared to P80X80, and control hamsters (Amount of alcohol intake: $t = 3.392$; $t = 3.457$; $P \leq 0.05$, respectively).

Discussion

The purpose of conducting this experiment was to determine if being exposed to sex during adolescence would influence the intake of alcohol that is presented for the first time in adulthood. In this study my aim was to assess the effects that early sexual experience has on adult intake of alcohol. A correlation has been shown between boys who undergo puberty and reach sexual maturity at early ages and the likelihood of

engaging in risky behaviors such as: the use of drugs and alcohol, violent and aggressive activities, and sexual intercourse (Bratberg et al., 2005; Celio, Karnik, & Steiner, 2006). Early physical maturity, in conjunction with antisocial/delinquent behavior and substance use, predicted early onset of sexual intercourse (Capaldi, Crosby, & Stoolmiller, 1996). It has yet to be determined whether sexual intercourse can induce premature expression of physical maturity or if it can drive the consumption of drugs like what's seen in early maturing individuals.

A hypothesis of early maturation attempts to explain the dissimilarity that's seen between children that mature earlier vs. their peers by suggesting that those who mature earlier have developed physically before their social resources have fully developed, leaving them inadequately prepared to cope with the challenges associated with physical maturation (Westling, Andrews, & Peterson, 2012). The results reported previously, in (Chapters 2 & 3) of this dissertation, support the notion that early physical development may be initiated through social interactions and/or biologically relevant hormonal signals, which may disrupt the maturation of less developed social processes. It is important to recognize if the social environment that an animal encounters during early development can have a modulatory influence over developmental trajectory, initiation of puberty, or onset of psychiatric conditions later in life.

Alcohol was used as a test of drug use because it is the most abused substance in the world and therefore it has clinical relevance. To ensure that the alcohol was palatable to the hamsters a modified sucrose substitution procedure was used. Over successive sessions, the concentration of sucrose was reduced and the ethanol concentration

increased, until 10% ethanol in sucrose was the solution presented (Samson, Sharpe, Denning, 1999). The elevated alcohol intake results imply that exposure to sex early in adolescence is positively associated with the intake of alcohol when presented for the first time in adulthood. In terms of behavior analysis findings recapitulated what was discovered in the original sexual experience study, described in Chapter 2 of this dissertation. As expected, sex-exposed animals showed higher levels of anxiety-like and depressive-like behavior in the elevated plus maze and Porsolt forced swim task, respectively.

Taken together, salient social experiences, especially sex, may mediate the expression of risky behaviors in adolescent animals. To determine if environmental or biological mechanisms have more significance or if this is a combinatorial process, further research is required. This type of research possesses high clinical significance, because it supports the idea that the social environment of an animal is of critical importance as an animal transitions from an immature to mature state. The pathophysiology and etiology of psychiatric disorders could be intricately tied to this type of process so more research is required in this area. In terms of treatment and intervention, identifying potential mechanisms of disruption early in the developmental process may allow researchers and clinicians to uncover ways to circumvent the negative outcomes seen in many adults. As noted earlier adolescent and adult psychiatric disorders are highly prevalent and devastating and the study of early development may be a key area of research that could expand the knowledge on this topic.

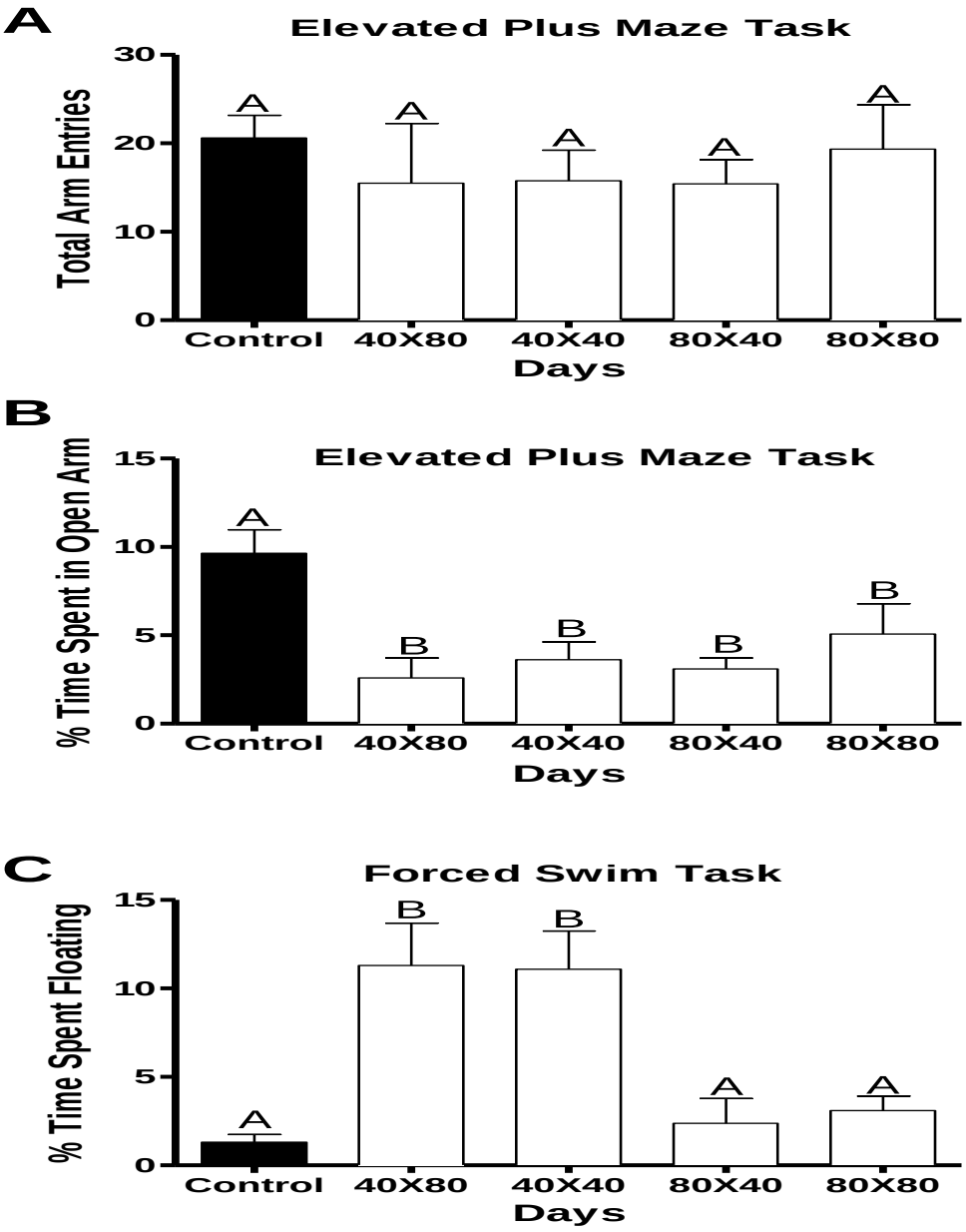

Figure 4.1. Adolescent sexual activity alters behavioral parameters (recapitulated).

Effects of adolescent sexual activity on behavioral responses (mean ±SEM) in the behavioral measure. A) Total number of arm entries in the testing arena. B) Percentage of time spent in the open arm of the elevated plus maze. C) Percentage of time spent floating in the forced swim test. *Bars sharing the same letters* are not statistically different from each other.

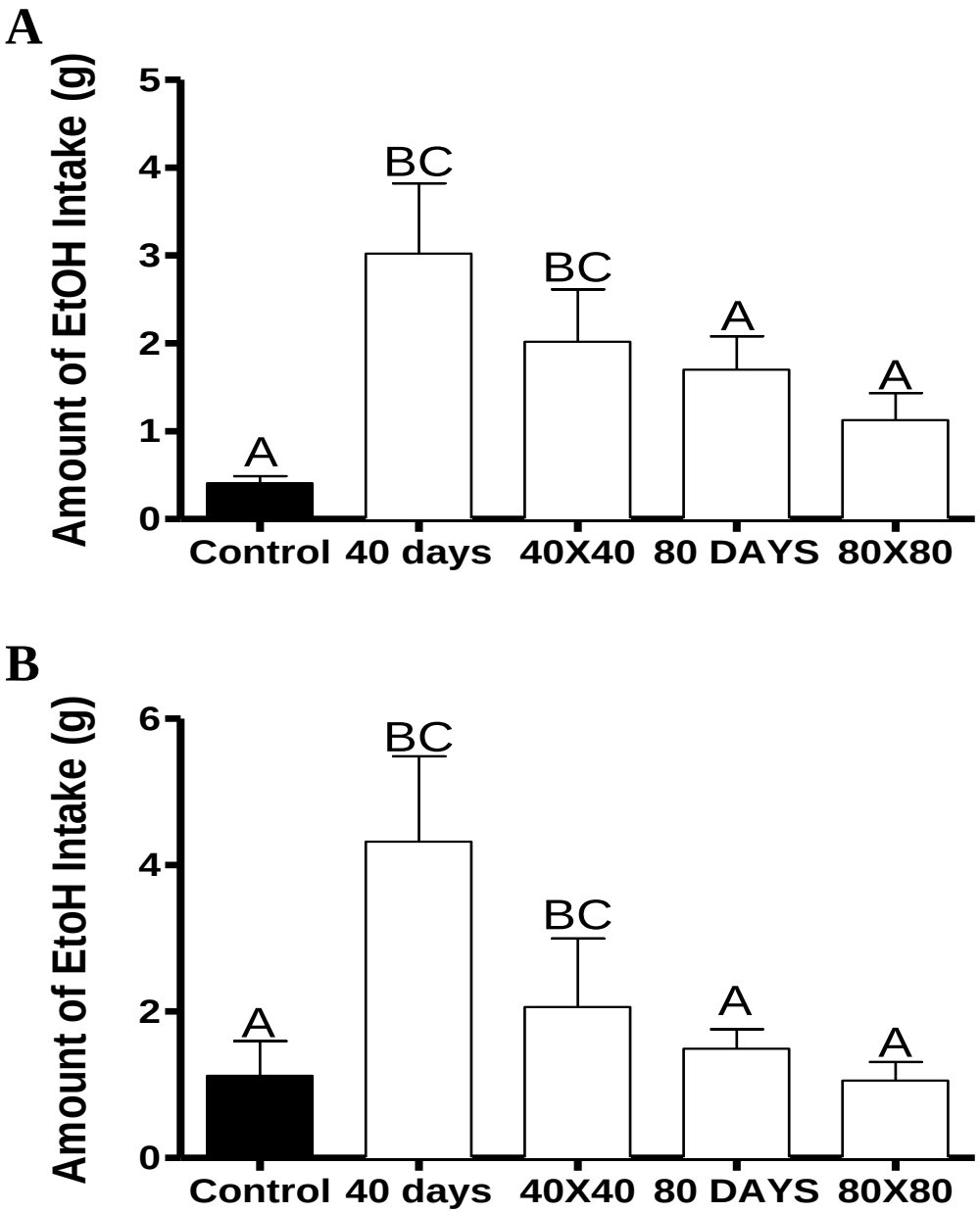

Figure 4.2. Adolescent sexual activity alters influences alcohol intake.

Effects of adolescent sexual activity on intake (mean ±SEM) in tests of alcohol consumption. A) Amount alcohol 3% ethanol/2% sucrose solution (g) consumed over 4 days. B) Amount alcohol 10% ethanol/2% sucrose solution (g) consumed over 4 days. *Bars sharing the same letters* are not statistically different from each other.

CONCLUSION

The objective of this dissertation was to identify the biological consequences of adaptation to the experience of social environmental variables early in life and to investigate the proximate mediators of the behavior adjustments that are present in adulthood. Specifically, this dissertation addressed the influence of sexual experience or sex steroid hormone exposure early in the maturational process and its modulatory control of inflammatory responses, neural development and functioning, and behavior in adult animals. These experiments are novel because the influence of early sex experience on adult immune function has yet to be investigated. The findings of these studies suggest that hormones likely underlie the alterations in affect and behavior that were observed in adolescent sexually active males by altering adult brain structure and function including inflammatory responses.

The first section of this dissertation addressed the relationship among the experience of sex for the first time during adolescence on physiology, immune function, and behavior responses when tested during adulthood. It is generally accepted that early developmental stress can lead to persistent and damaging neurobiological alterations in adult animals and probably represents an important biological basis and major predisposing factor for increased susceptibility to the onset of psychopathology (Heim &

Nemeroff, 2002). Although elevated levels of stress and stress related hormones have been associated with deleterious health effects, there are instances when stressors that contain a component of reward, namely sexual experience and exercise, result in benefits to the health and mental function of individuals (Leuner, Glasper, Gould, 2010). In general, certain aspects of positive stressors may have the ability to act as a buffer from the potentially harmful consequences associated with adverse stressors (Leuner, Glasper, Gould, 2010). The exposure to positive stressors has not been assessed in adolescence, a time of rapid development, when an individual may be ill equipped to interpret the positive valence of sexual experiences in an adaptive way. To determine whether sex during adolescence affects the developmental process, sexually naïve adolescent male hamsters were exposed to a sexually receptive female counterpart and subsequently evaluated for alterations in behavior, neuro-circuitry, and immune function. The adolescent sex exposed animals displayed the highest levels of anxiety and depressive-like behaviors, pronounced concentrations of cortisol in blood serum, largest elevations in inflammatory markers in the brain, and significant alterations in neuronal structure in prefrontal brain regions. The results of Experiment 1, strongly suggest that the physical and emotional state of adult individuals may be modified by the social environment early in development. Furthermore, the results of this aim of the study imply that sexual experiences during adolescence may be interpreted as an adverse stressor in less mature hamsters and may be a possible route for the onset of maladaptive brain, inflammatory, emotional, and behavioral responses.

In Chapter 3, I asked two questions. First, I wanted to determine whether adolescent exposure to testosterone shifts the maturational process forward. Second I wanted to determine whether the presence of adult typical levels of testosterone functions similar to the experience of sex at the same developmentally critical time point altered adult neuronal morphology and behavior. There is a body of literature that suggests that testosterone levels rise substantially following exposure to a receptive female (e.g., Macrides et al., 1974). It appears that this boost in neuroendocrine activity may be sufficient to mediate effects similar to early sex exposure, but further research is necessary to gain a better understanding of this phenomenon. Research on pubertal timing proposes that boys and girls who undergo early puberty engage in more sexual activity and delinquent behavior than later maturing cohorts (Flannery, Rowe, Gulley, 1993).To further elucidate the underlying features of this process, it was important to determine whether engaging in sexual activity in adolescence influenced the maturational timing and trajectory of young animals. To fully understand the mechanistic features of early adolescent maturation, sexual activity, and adult outcome it is essential to establish if the affective salience (assessed by stress hormone concentrations) of the sexual experience or the biological significance of premature sex steroid hormone exposure (testosterone exposure) modulates the increased vulnerability to anxiety- and depressive-like responses. In this section of the dissertation, hamsters were injected with testosterone during either adolescence or adulthood and evaluated for alterations in behavior. The hamsters exposed to T during adolescence display the most dramatic increases in anxiety- and depressive-like behavior, which recapitulated the changes seen

in the hamsters exposed to early sexual experiences in Chapter 2. All in all, the results of this section imply that the spike in T during an early sexual experience may at least, in part, drive the changes seen in adult animals.

The experiment described in Chapter 4 was designed to investigate the consequences that sexual activities early in development have on later alcohol use. Research shows that early maturing individuals are more likely to engage in sex and drug use (Flannery, Rowe, Gulley, 1993). Studies also show that adolescents that abuse drugs and alcohol are more likely to engage in sex at earlier ages (Rosenbaum & Kandel, 1990). Increasingly, studies are beginning to focus on the impact of various risk factors in adolescence such as: socioeconomic status, peer and familial support, biological maturity, and history of drug use (Rosenbaum & Kandel, 1990). There is less research that is devoted to the experience of sex early in life and the role this experience may play in maturation and adult mental health. As mentioned previously, male testosterone levels increase in the presence of a receptive female counterpart (Macrides et al., 1974). An important question was posed in Chapter 4 regarding sexual activity and the role it may play in the maturation time course. Do sexual activity and the surge in T observed in males shift the phase of maturation, particularly when the sex occurs at a developmentally critical time point when T levels are comparatively low? It was hypothesized that adolescent sexually active animals would exhibit similar patterns of alcohol abuse that is commonly seen in early maturing individuals. As expected, the hamsters experiencing sexual interactions during adolescence displayed the highest rates of alcohol intake compared to all other groups. Taken together, these results suggest that

social experiences early in life are important mediators of adult behavior and decision making.

The implications for this type of research are extensive and intriguing. The experiments presented here provide evidence that the type of environmental variables are processed by the nervous, neuroendocrine, and immune systems and can have potent modulatory effects on these systems. This work may be useful and clinically relevant in understanding the long-term physical and mental health outcomes of adolescent sexual activity in humans. Consideration of this type of environmental regulation is likely to yield valuable information to researchers interested in understanding the underlying features of pubertal and adolescent development as well as to scientists and clinicians interested in uncovering the etiology of the various pathological conditions that an abundance of people face around the world.

REFERENCES

Anderson, S. L., & Teicher, M. H. (2008). Stress, sensitive periods and maturational events in adolescent depression. *Trends in Neurosciences*, 31, 183-191.

Anderson, S.L. et al. (2008). Preliminary evidence for sensitive periods in the effect of childhood sexual abuse on regional brain development. *J. Neuropsychiatry Clinical Neuroscience.* 20, 292-301.

Anisman, H. (2009). Cascading effects of stressors and inflammatory immune system activation: implications for major depressive disorder. *Journal of psychiatry and neuroscience.* 34(1), 4-20.

Anisman, H., & Matheson, K. (2005). Stress, depression, and anhedonia: caveats concerning animal models. *Neuroscience & Biological Reviews*. 29, 525-546.

Anisman, H., & Merali, Z. (2003). Cytokines, stress and depressive illness: brain-immune interactions. *Ann Med*. 35, 2-11.

Arnett, J. J. (1999). Adolescent Storm and Stress, Reconsidered. *American Psychologist*. 54(5), 317-326.

Arnold, A. P., & Breedlove, S. M. (1985). Organizational and activational effects of sex steroids on brain behavior: a reanalysis. *Horm Behav.* 19, 469-498.

Bilbo, S. D., & Nelson, R. J (2000). Sex steroid hormones enhance immune function in male and female Siberian hamsters. *Am J Physiol Regulatory Integrative Comp Physiol*, 280, 207-213.

Birmaher, B., Ryan, N. D., Williamson, S. E., Brent, D. A., Kaufman, J., Dahl, R. E., Perel, J., & Nelson, B. (1996). Childhood and adolescent depression: A review of the past 10 years. *Part 1. J. Am. Acad. Child. Adolesc. Psychiatry*. 35(11), 1427-1439.

Bratberg, G. H., Nilsen, T. I., Holmen, T. L., & Vatten, L. J. (2005). Sexual maturation in early adolescence and alcohol drinking and cigarette smoking in late adolescence: a prospective study of 2,129 Norwegian girls and boys. *European Journal of Pediatry*. 164(10), 621-625.

Breedlove, S. M. (1994). Sexual differentiation of the human nervous system. *Annu. Rev. Psychol*. 45, 389-418.

Brooks-Gunn, J., Petersen, A. C., & Eichorn, D. (1985). The study of maturational timing effects in adolescence. *Journal of Youth and Adolescence*. 14(3), 149-161.

Bulwada, B., Geerdink, M., Koolhaas, J. M. (2010). Social behavior and social stress in adolescence: A focus on animal models. *Neuroscience and Behavioral Reviews*. 35, 1713-1721.

Cameron, J. (2004). Interrelationships between hormones, behavior, and affect during adolescence. *Ann. N.Y. Acad. Sci.* 1021, 110-123.

Capaldi, D. M., Crosby, L., & Stoolmiller, M. (1996). Predicting the timing of first sexual intercourse for at-risk adolescent males. *Child Development*. 67, 344-359.

Capuron, L., & Miller, A. H. (2006). Immune system to brain signaling: neuropsychopharmacological implications. *Pharmacology & Therapeutics.* 130, 226-238.

Castagne, V., Moser, P., Roux, S., Porsolt, R. D. (2001). Rodent models of depression: forced swimming and tail suspension behavioral despair tests in rats and mice. *Current Protocols in Neuroscience.* 14, 1-8.

Celio, M., Karnik, N. S., Steiner, H. (2006). Early Maturation as a risk factor for aggression and delinquency in adolescent girls: a review. *International Journal of Clinical Practice*. 60(10), 1254-1262.

Clausen, J. A. (1975). The social meaning of differential physical and sexual maturation. In Dragastin, S. E., & Elder, G. H. JR. (eds.), *Adolescence in the Life Cycle: Psychological change and the Social Context.* Halsted, New York.

Crews, F., He, J., & Hodge, C. (2007). Adolescent cortical development: A critical period of vulnerability for addiction. *J. Pharm Bio Behav.* 86, 189-199.

Cryan, J. F. & Holmes, A., (2005). Model organisms: The ascent of mouse: advances in modeling human depression and anxiety. *Nature Reviews Drug Discovery*. 4, 775-790.

Cryan, J. F., Valentino, R. J., Lucki, I., (2005). Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. *J. Neu Bio Rev*. 29, 547-569.

Dalley, J. W., Cardinal, R. N., Robbins, T. W. (2004). Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *J. Neu Bio Rev*. 28, 771-784.

Dantzer, R. (2001). Cytokine-induced sickness behavior: Where do we stand? *Brain, Behavior, and Immunity*. 15(1), 7-24.

Dantzer, R., O'Connor. J. C., Freund, G. G., Johnson, R. W., & Kelley, K. W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nature Review Neuroscience*. 9, 46-56.

Demas, G. E., Johnson, C., Polacek, K. M. (2004). Social interactions differentially affect reproductive and immune responses of Siberian hamsters. *Physiology & behavior*, 83, 73-79.

Duncan, P. D., Ritter, P. L., Dornbusch, A. M., Gross, R. T., and Carlsmith, J. M. (1985). *Journal of Youth and Adolescence*. 14(3), 227-235.

Dunman, R. S., Malberg, J., Nakagawa, S., D'Sa, C. (2000). Neuronal plasticity and survival in mood disorders. *Biological Psychiatry*. 48, 732-739.

Eccles, J. S., & Lord, S. (1996). School transitions in early adolescence: What are we doing to our young people? *Transitions through adolescence: Interpersonal domains and context. 251-284.*

Eccles, J. S., Midgley, C., Wigfeild, A., Buchanan, C. M., Reuman, D., Flanagan, C., & Iver, D. M. (1993). Development during adolescence: The impact of stage-environment fit on young adolescents' experiences in schools and in families. *American Psychologist*. 48(2), 90-101.

Flannery, D. J., Rowe, D. C., & Gulley, B. L. (1993). Impact of pubertal status, timing, and age on adolescent sexual experience and delinquency. *Journal of Adolescent Research*. 8(1), 21-40.

Hall, G. S. (1904). Adolescence: its Psychology and its Relations to Physiology. *Anthropology, Sociology, Sex, Crime, Religion, and Education (D. Appleton & Co. 1904).*

Hayley, S. (2011). Toward an anti-inflammatory strategy for depression. Frontiers in *Behavioral Neuroscience*. 5(19), 1-7.

Heim. C., & Nemeroff, C. B. (2002). Neurobiology of early life stress: Clinical studies. *Seminars in Clinical Neuropsychiatry*. 7(2), 147-159.

Heinlein, C. A., & Chang, C. (2002). Androgen receptor (AR) coregulators: An overview. *Endocrine Reviews*. 23(2), 175-200.

Higgins, E. T., & Parsons, J. E. (1983). Social cognition and the social life of the child: Stages as subcultures. *Social Cognition and Social Behavior: Developmental Issues*. 15-62.

Holmes, A & Wellman, C. L. (2009). Stress-induced prefrontal reorganization and executive dysfunction in rodents. *J. Neu Bio Rev*. 33, 773-783.

Hutchison, R. E., Hutchison, J. B., Steimer, T., Steel, E., Powers, J. B., Walker, A. P., Herbert, J., & Hastings, M. H. (1991). Brain aromatization of testosterone in the male Syrian hamster: effects of androgen and photoperiod. *Neuroendocrinology*. 53, 194-203.

Jacobs, B. L., Praag, H., Gage, F. H., (2000). Adult brain neurogenesis and psychiatry: a novel theory of depression. *Molecular Psychiatry*. 5, 262-269.

Johnston, L. D., O'Malley, P. M., Bachman, J. G., & Schulenburg, J. E. (2011). Monitoring the future. National results on adolescent drug use: *Overview of key findings 2010. Ann Arbor: Institute for Social Research, University of Michigan*.

Jones, M. C., & Bayley, N. (1950). Physical maturing among boys as related to behavior. *Journal of Educational Psychology*. 41, 129-148.

Kaltiala-Heino, R., Kosunen, E., Rimpelä, Matti. (2003). Pubertal timing, sexual behavior and self reported depression in middle adolescence. *Journal of Adolescence.* 26, 531-545.

Kaufmann, J., Plotsky, P. M., Nemeroff, C. B., Charney, D. S. (2000). Effects of early adverse experiences on brain structure and function: clinical implications. *Biological Psychiatry*. 48, 778-790.

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime prevelance and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication. *Arch Gen Psychiatry.* 62(6), 593-602.

Konsman, J. P., Parnet, P., & Dantzer, R. (2002). Cytokine-induced sickness behavior: mechanisms and implications. *Trends in Neuroscience.* 25, 154-159.

Kroes, R. A., Panksepp, J., Burgdorf, J., Otto, N. J., Moskal, J. R. (2006). Modeling depression: social dominance-submission gene expression patterns in rat neocortex. *Neuroscience*. 137(1), 37-49.

Laska, E. M., Mallinkrodt, C. H., Mundt, J. C., Leber, P., Vaccarino, A. L., Kalali, A. H., Greist, J. H. (2009). Assessing onset of treatment benefit in depression and anxiety: conceptual considerations. *J Clin Psychiatry*. 70, 1138-1145.

Leuner, B., Glasper, E. R., & Gould, E. (2010). Sexual experience promotes adult neurogenesis in the hippocampus despite an initial elevation in stress hormones. *PLoS ONE*. 5(7).

Leussis, M. P., & Andersen, S. L. (2008). Is adolescence a sensitive period for depression? Behavioral and neuroanatomical findings from a social stress model. *Synapse*. 62, 22-30.

Lieberburg, I., Maclusky, N. J., Roy, E. J., & McEwen, B. S. (1978). Sex steroids in the perinatal rat brain. *Amer. Zool.* 18(3), 539-544.

Lockmiller, R. L. (2003). Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos*. 88(1), 87-98.

Lupien, S.J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behavior and cognition. *Nature Reviews Neuroscience*. (10), 434-445.

Macrides, F., Bartke, A., Fernandez, F., D'Angelo, W. (1974). Effects of exposure to vaginal odor and receptive females on plasma testosterone in male hamster. *Neuroendocrinology*. 15(6), 355-364.

Mainwaring, W. I. P. (1975). A review of the formation and binding of 5α-Dihydrotestosterone in the mechanism of action of androgens in the prostate of the rat and other species. *Journal of the Society for Reproduction and Fertility*. 44, 377-393.

McCarthy, D. M., Tomlinson, K. L., Anderson, K. G., Marlatt, G. A., & Brown, S. A. (2005). Relapse in alcohol- and drug-disordered adolescents with comorbid psychopathology: Changes in psychiatric symptoms. *Psychology of Addictive Behaviors.* 19(1), 28-34.

McCormick, C. M., Mathews, I. Z., Thomas, C., Waters, P. (2010). Investigations of HPA function and the enduring consequences of stressors in adolescence in animal models. *Brain and Cognition,* 72, 73-85.

McCormick, C.M., Smith, C., Mathews, I.Z. (2008). Effects of chronic social stress in adolescence on anxiety and neuroendocrine response to mild stress in male and female rats. *Behavioural Brain Research*, 187, 228–238.

McDonald, P., Beyer, C., Newton, F., Brien, B., Baker, R., Tan, H. S., Sampson, C., Kitching, P., Greenhill, R., & Pritchard, D. (1970). Failure of 5α-Dihydrotestosterone to initiate sexual behavior in the castrated male rat. *Nature*. 227, 964-965.

Mehrabian, A. (2001). General relations among drug use, alcohol use, and majoe indexes of psychopathology. *The Journal of Psychology*. 135(1), 71.

Mitchell, S. G., Gryczynski, J., O'Grady, K. E., & Schwartz, R. P. (2013). SBIRT for adolescent drug and alcohol use: Current status and future directions. Journal of Substance abuse Treatment. 1-10.

Miller, A. H., Maletic, V., & Raison, C. L. (2008). Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *J. Biological psychiatry.* 65, 732-741.

Mizoguchi, K., Shoji, H., Ikeda, R., Tanaka, Y., & Tabira, T. (2008). Persistent depressive state after chronic stress in rats is accompanied by hpa axis dysregulation and reduced prefrontal dopaminergic neurotransmission. *J. Pharm Bio Behav.* 91, 170-175.

Morley, T. E., & Moran, G. (2011). The Origins of cognitive vulnerability in early childhood: mechanisms linking early attachment to later depression. *Clinical Psychology Review*. 31, 1071-1082.

Muramoto, M.L., & Leshan L. (1993). Adolescent substance abuse: recognition and early intervention. Prim Care, 20, 141–154.

Mussen, P. H., & Jones, M. C. (1958). The behavior-inferred motivations of late- and early-maturing boys. *Child development.* 29(1), 61-67.

Negri-Cesi, P., Colciago, A., Celotti, F., & Motta, M. (2004). Sexual differentiation of the brain: role of testosterone and its active metabolites. Journal of Endocrinological Investigation. 27(6), 120.

Nestler, E. J., Barrot, M., DiLeone, R. J., Eisch, A. J., Gold, J. S., Monteggia, L. M. (2002). Neurobiology of depression. *Neuron*. 34, 13-25.

Nemeroff, C. B. (2004). Neurobiological consequences of childhood trauma. *Journal of Clinical Psychiatry*. 65, 18-28.

Norman G. J., Karelina, K., Zhang, N., Walton, J. C., Morris, J. S., & Devries A. C. (2010). Stress and IL-1beta contribute to the development of depressive-like behavior following peripheral nerve injury. *Molecular Psychiatry*. 4, 404-14.

Ohl, F. (2005) Animal models of anxiety. *Anxiety and Anxiolytic Drugs*. 169, 35-69.

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A., Quigley, E. M., Cryan, J. F., Dinan, T. G. (2009). Early stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. *J. Biological psychiatry*. 65(3), 263-267.

Pellow, S., Chopin, P., File, A. E., & Briley, M. (1985) Validation of open : closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neuroscience Methods*. 14, 149-167.

Phoenix, C. H., Goy, R. W., & Young, W. C. (1967). Sexual behavior: General aspects. *Neuroendocrinology*. 2.

Prendergast, B. J., & Nelson, R. J. (2005). Affective responses to changes in day length in Siberian hamsters (Phodopus sungorus). *Psychoneuroendocrinology*. 30(5), 438-452.

Raison, C. L., Capuron, L., Miller, A. H. (2006). Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends in Immunology*. 27, 24-31.

Rector, R., Johnson, K. A., & Noyes, L. R. (2003). Sexually active teenagers are more likely to be depressed and attempt suicide. *Center for Data Analysis Report.* 3(4).

Romeo, R. D., Cook-Wiens, E., Richardson, H. N., & Sisk, C. L. (2001). Dihydrotestosterone activates sexual behavior in adult male hamsters but not in juveniles. *Physiology and Behavior*. 73, 579-584.

Romeo, R. D., & McEwen, B. S. (2006). Stress and the Adolescent Brain. *Ann. N.Y. Acad. Sci.,* 1094, 202–214.

Romeo, R. D., Richardson, H. N., & Sisk, C. L. (2002). Puberty and the maturation on the male brain and sexual behavior: recasting a behavioral potential. *Neuroscience & Biobehavioral Reviews*. 26(3), 381-391.

Romeo, R. D., Schulz, K. M., Nelson, A. L., Menard, T. A., Sisk, C .L. (2003). Testosterone, puberty, and the pattern of male aggression in syrian hamsters. *Developmental Psychobiology*, 43, 102-108.

Romeo, R. D., Wagner, C. K., Jansen, H. T., Diedrich, S. L., & Sisk, C. L. (2002). Estradiol induces hypothalamic progesterone receptors but does not activate mating behavior in male hamsters (Mesocricetus auratus) before puberty. *Behavioral Neuroscience*. 116, 198-205.

Roselli, C. E., Liu, M., & Hurn, P. (2009). Brain aromatization: Classical roles and new perspectives. Seminars in Reproductive Medicine. 27(3), 207-217.

Rosenbaum, E., & Kandel, D. B. (1990). Early onset of adolescent sexual behavior and drug involvement. *Journal of Marriage and Family. 52(3), 783-798.*

Rygula, R., Abumaria, N., Flugge, G., Fuchs, E., Ruther, E., & Havemann-Reinecke, U. (2005). Anhedonia and motivational deficits in rats: impact of chronic social stress. *Brain Behavioral Research*. 162, 127-134.

Samson, H. H., Sharpe, A. L., & Denning, C. (1999). Initiation of ethanol self-administration in the rat using sucrose substitution in a sipper-tube procedure. *Psychopharmacology*. 147(3), 274-279.

Sato, T., Matsumoto, T., Kawano, H., Watanabe, T., Uematsu, Y., Sekine, K., Fukuda, T., Aihara, K., Krust, A., Yamada, T., Nakamichi, Y., Yamamoto, Y., Nakumura, T., Yoshimura, K., Yoshizawa, T., Metzger, D., Chambon, P., & Kato, S. (2004). Brain masculinization requires androgen receptor function. *Proceedings of the National Academy of Sciences of the United States of America.* 101(6), 1673-1678.

Schiepers, O. J. G., Wichers, M. C., & Maes, M. (2005). Cytokines and major depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 29(2). 201-217.

Schulz, K. M., Molenda-Figueira, H. A., Sisk, C. L. (2009). Back to the future: The organizational-activational hypothesis adapted to puberty and adolescence. *Hormones and Behavior*, 55, 597-604.

Schulz, K. M., Richardson, H. N., Zehr, J. L., Osetek, A. J., Menard, T. A., & Sisk, C. L. (2004). Gonadal hormones masculinize and defeminize reproductive behavior during puberty in the male Syrian hamster. *Hormone and Behavior*. 45, 242-249.

Shima, H., Tsuji, M., Young, P., & Cunha, G. R. (1990). Postnatal growth of mouse seminal vesicle is dependent on 5α-Dihydrotestosterone. *Endocrinology*. 127, 3222-3233.

Simerly, R. B., Swanson, L. W., Chang, C., Muramatsu, M. (2004). Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: An in situ hybridization study. *The Journal of Comparative Neurology*. 294(1), 76-95.

Sisk, C. L., & Foster, D. L. (2004). The neural basis of puberty and adolescence. *Nature Neuroscience*. 7, 1040-1047.

Sisk, C. L., & Zehr, J. L. (2005). Pubertal hormones organize the adolescent brain and behavior. *Frontiers in Neuroendocrinology*. 26, 163-174.

Sisk, C. L., Schulz, K. M., Zehr, J. L. (2003). Puberty: A finishing school for male social behavior. *Ann N.Y Acad Sci.* 1007, 189-198.

Solomon, G. F., Levine, S., & Kraft, J. K. (1968). Early experience and immunity. *Nature*. 220, 821-822.

Spear, L. P. (2000). Neurobehavioral changes in adolescence. *Current Directions in Psych Science*. 9, 111-114.

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience & Biobehavioral Reviews*. 24, 417-463.

Spinelli, S., Chefer, S., Suomi, S. J., Higley, J. D., Barr, C. S., & Stein, E. (2009). Early-life stress induces long-term morphologic changes in primate brain. *Arch Gen Psychiatry*. 66(6), 658-665.

Toledo-Rodriguez, M., & Sandi, C. (2007). Stress before puberty exerts a sex- and age-related impact on auditory and contextual fear conditioning in the rat. *Neural Plasticity*. 2007, 1-12.

Tschann, J. M., Adler, N. E., Irwin, C. E., Millstein, S. G., Turner, R. A., & Kegeles, S. M. (1994). Initiation of substance use in early adolescence: The roles of pubertal timing and emotional distress. *Health Psychology*. 13(4), 326-333.

Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature Reviews Neuroscience*. (10), 397-409.

Weil, Z. M., Hotchkiss, A, K., Gatien, M. L., Pieke-Dahl, S., & Nelson, R. J. (2006). Melatonin receptor (MT1) knockout mice display depression-like behaviors and deficits in sensorimotor gating. *Brain Research Bulletin*. 68(6), 425-429.

Westling, E., Andrews, J. A., & Peterson, M. (2012). Gender differences in pubertal timing, social competence, and cigarette use: a test of early maturation hypothesis. *Adolescent Health*. 51(2), 150-155.

Whitbeck, L. B., Yoder, K. A., Hoyt, D. R., & Conger, R. D. (1999). Early adolescent sexual activity: A developmental study. *Journal of Marriage and the Family*. 61(4), 934-946.

Yamaguchi, K., & Kandel, D. (1987). Drug use and other determinants of premarital pregnancy and its outcome: A dynamic analysis of competing life events. *Journal of Marriage and the Family*. 49(2), 257-270.