Early Sexual Experience Alters Adult Affective Responses and Immune Function

MASTER'S THESIS

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By

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ABSTRACT

Early life experiences have a lasting impact 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 impact on growth and behavior (Sachser et al., 2010). 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 (Sisk & Zehr, 2005). 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 particular social situations (Sisk & Foster 2004). Although there is overlap in timing of these two processes, the influence of hormones on reproductive behavior depends to some extent on changes in the adolescent brain that occur separately from gonadal maturation (Sisk & Foster 2004). 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 (Spear, 2000). 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 (Sisk& Zehr, 2005).

The hormonal changes associated with puberty and the timing of environmental events may increase vulnerability to the negative symptoms of depression (Leen-Feldner, Reardon, Hayward, & Smith, 2008). In humans, sexual experience in adolescence can increase susceptibility to mental disorders (Sabia, 2006), modify immune function (Danese et al., 2006) and alter stress reactivity (Danese et al., 2009). Hormones modify the functioning of numerous widespread neurotransmitter systems involved in emotional and cognitive brain functions (Cameron, 2004). The best known adolescence-driven brain modulations occur in prefrontal regions of the brain, stress sensitive dopamine neural circuitry, and the limbic system, which includes the amygdala, hippocampus, hypothalamus, nucleus accumbens, and areas of connection between limbic and prefrontal areas (Crews & Hodge, 2007).

Alterations in neurocircuitry that occur during the critical period of adolescence provide important information that aids in delineating processes that underlie the etiology of depressive disorders. Human studies have revealed that a positive relationship exists between early sexual activity and adult onset of depression (Sabia, 2003). It is thought that early sexual contact influences depression through remodeling of systems not yet equipped or sufficiently mature to handle this type of social interaction. Identifying

mental health problems among adolescent patients who are engaging in sexual intercourse can expand upon current methods of diagnosis in ways that advance the development of new intervention strategies (Anderson & Teicher, 2008). There are benefits of modeling depression in animals as they provide a means of identifying the development of dysfunctional properties, understanding the neural systems involved, and creating appropriate treatment management strategies in early sex exposed animals as they relate to humans (Cryan & Holmes, 2005).

A hamster (*Phodopus sungorus)* model of first sexual encounter in adolescence was used to test the hypothesis that a salient social interaction, specifically sexual experience, affects adult behavioral responses, modifies immunological function in adulthood, and affects reproductive growth. At birth, male hamsters were randomly assigned to one of five groups: (1) experienced first sexual contact with an ovariectomized, estrogen-primed, adult female during puberty at postnatal day 40 (P40) and underwent behavioral testing on day 120, (2) experienced first sexual contact with an ovariectomized, estrogen-primed, adult female during puberty at postnatal day 40 (P40) and tested through behavioral measures at 80 days of life, (3) first sexual bout with an ovariectomized, estrogen-primed, adult female occurred in adulthood at postnatal day 80 (P80) and underwent behavioral testing on day 120, first sexual bout with an ovariectomized, estrogen-primed, adult female occurred in adulthood at postnatal day 80

(P80) and went through behavioral testing on day 160, or (5) received no sexual contact. The group that experienced first sexual contact at 40days and were tested at 80 days (40X40) and the group that experienced first sexual contact at 80days and were tested at 160 days of life (80 X80) where added through a second installment of the study in order to control for age of animal at the time of testing. The addition of additional groups (40X40) and (80X80) accounted for age discrepancies that may have arisen due to differences in the length of time between the first sexual encounter and the beginning of behavioral testing. Hamsters underwent behavioral testing in the form of the elevated plus maze; (EPM), designed to assess anxiety like responses, the Porsolt forced swim test; (FST) and sucrose anhedonia test; (SA), which are measures of behavioral despair and anhedonia, respectively. Following behavioral tests, cell-mediated immune responses were assessed through the use of delayed type hypersensitivity (DTH). Real-time quantitative polymerase chain reaction (PCR) was used to assess the amount of gene expression of specific cDNA levels of inflammatory markers within the emotion and affect related regions of the brain (i.e., PFC, hippocampus, amygdala, striatum). Blood samples were obtained prior to and following sex to determine glucocorticoid concentrations.

Compared to sexually inexperienced hamsters and hamsters that did not experience sexual interactions until 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 cell-mediated immunity 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 brain, specifically in the prefrontal cortex (PFC). The prefrontal cortex is involved in a range of cognitive and executive functions such as: working memory, attentional set shifting, planned behavior, behavioral inhibition, decision making, and integration of voluntary behavior (Dalley, Cardinal, & Robbins, 2004). Recent experimental observations suggest that brief exposure to stress may be sufficient to induce structural neural reorganization within the PFC and that experience driven morphological alterations to the PFC may be accountable for some symptoms of depression (Holmes & Wellman, 2009). The finding that IL-1β is increased in hamsters experiences sex during adolescence suggests that relevant social stimuli during this sensitive time may increase neuroinflammation and subsequent vulnerability to psychopathologies similar to what past research has shown. These animals also reduced overall body mass and accessory reproductive tissue mass, which may be mediated through IL-1β expression.

Taken together, these results suggest that early adolescent sexual experience has long-term effects on affective responses, enduring effects on adult immune function, as

well as lasting effects on reproductive tissue. This work may be useful in understanding the long-term physical and mental health outcomes of adolescent sex in humans.

Dedication

Dedicated to the students at The Ohio State University

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Vita

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CHAPTER 1

INTRODUCTION

Psychological disorders are difficult to diagnose, which often leads to treatment and intervention strategies that are unsuccessful (Laska et al., 2009). Major depression is among the most prevalent, expensive, and severely debilitating psychiatric conditions in the world (Morley & Moran, 2011). Indeed, approximately 18.8 million Americans are currently 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 (Nemeroff, 2000), the precise mechanisms are unknown.

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 a juvenile animal shifts into adult typical behavioral and neurological phenotypes (Buwalda, 2011). Marked plasticity occurs during adolescence that alters both 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 brain tissue is assessed at adult time points. Although the age ranges vary across species, the physiological and behavioral transitions are sufficiently similar between the two species to use hamsters to model this process in humans. Siberian hamsters 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.

Puberty is the period when an individual becomes capable of sexual reproduction. This period 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 take place during adolescence, require drastic adjustments in behavior and neuro-circuitry in order for future reproductive success (Romeo, Richardson, & Sisk, 2002). Akin to humans, altricial rodents (e.g., mice, rats, and hamsters), secrete testosterone (T) immediately after birth, that then gradually declines for the next month. 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, in 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 both its pulse frequency and magnitude of release. These increased T concentrations sum to produce high concentrations of gonadal sex steroid hormones during puberty. Increased T concentrations both remodel and trigger activation of neural circuits, which in turn finishes securing the brain into adult typical behavioral and reproductive patterns (Sisk & Foster 2004).

Early life experiences can alter growth and development (McEwen, 2008). The occurrence of negative signals from the environment during developmentally sensitive periods are predictive of disease onset and risk factors for poor health in adulthood (Johnson, 2005). Past research in humans suggests that certain early social experiences may alter cognitive processes in maladaptive ways increasing vulnerability to the development of depression following adverse events later in life (Morley & Moran, 2011). A region of the brain that is of particular interest is the prefrontal cortex (PFC), which is involved in the emergence of depression as it matures during adolescence. The PFC has a protracted developmental time course, not reaching full maturity until well into adulthood, which may render it vulnerable to environmental challenges faced during

adolescence (Anderson et al., 2008). Other regions of the brain, namely the hippocampus and striatum, undergo alterations due to adolescent stress that may subsequently affect PFC development (Anderson & Teicher, 2008). The environment provides important predictive feedback about future conditions during the developmental process and this type of information can be used as a crucial point of intervention.

To make progress in understanding the brain mechanisms underlying depression, rodent models are often used. Several notable behavioral techniques have been utilized to measure anxiety-like and depressive-like behavioral responses. One test of anxiety-like behavior in rodents is the elevated plus maze test. The elevated plus maze test is the most common test used to assess anxiety and consists of placing a rodent on an elevated platform with two closed chambers and two open ends (shaped like a plus sign) (Hogg, 1996). Rodents are generally prey species and tend to avoid open spaces. Thus, the number of entries made onto the open arms and the time spent on these arms indicates low anxiety-like responses (Hogg, 1996). Treatment with anti-anxiety drugs tends to reduce the avoidance of the open arms. This test of spontaneous behavior was chosen because it is a behaviorally, physiologically, and pharmacologically valid test of anxiety in rodents (Pellow, Chopin, File, & Briley, 1985) and because both the physiological and behavioral responses shown are similar in humans and animals, providing face and construct validity, the animal model can serve a distinct function of investigating

pathogenic aspects of anxiety disorders (Ohl, 2005). A commonly used measure of depressive-like behavior is the so-called Porsolt or forced swim task which consists of placing a rodent into a cylindrical tank filled with water (Porsolt, Le Pichon & Jalfre, 1977). Rodents tend to swim vigorously as they circle the container presumably searching for a way out of the water. At some point, individuals tend to stop actively swimming and float. Reductions in swim duration and immobility are recognized as signs of behavioral despair as animals abandon their search for an escape mechanism (Porsolt et al., 1977). Immobility is interpreted to be a sign that the animal has learned that any attempt of escape is futile and hope is discarded (Castagne, Moser, Roux, Porsolt, 2001). This passive form of behavior, i.e., immobility and non-vigorous swimming represents an animal's disengagement in escape in an attempt to conserve energy for events that may provide an opportunity of escape (Cryan, Valentino & Lucki, 2005). Treatment with antidepressants tends to prolong swim duration. Other animal models of depression employ tests to quantify anhedonia through measurements of consumption of desirable foods. Anhedonia is acknowledged as a core symptom of major depression and is defined as a distinctly diminished reactivity to pleasurable stimuli and general reduction in sensitivity to reward (American Psychiatric and Association, 1994). For example, measurement of sucrose consumption is regularly used to measure anhedonia following chronic, unpredictable, and mild forms of stress (Mathews, Forbes, & Reid, 1994). Decreased sucrose consumption is seen as desensitization of brain reward

mechanisms (Rygula et al., 2005). Because anhedonia a central indicator of depression this social stress paradigm may be reliably evaluating depressive-like responses in humans (Rygula et al., 2005). Again, treatment with antidepressants tends to reverse the anhedonic responses.

Early life events may alter the developmental course of the immune system. A specific example of environmental reorganization of 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). Studies show that 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). Animal models have provided much insight into the differential phenotypic responses to stress in 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 (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 clinical literature

by relating it to animal models thus providing increased understanding of depression and discovering new therapeutic targets for treatment (Kroes, 2005). Recently, some forms of depression have been reported to be associated with inflammatory processes. For instance, patients with major depression 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). Research in rodents has found the presence of IL-1β in the brain of stressed mice, and the presence of this proinflammatory cytokine potentiates depressive-like behavior (Norman, Karelina, Walton, Morris, & Devries, 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). A technique that is regularly employed to assess changes in immune activation and inflammation is the delayed hypersensitivity (DTH) test, which is a measure of an antigen specific cell mediated immune response. 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 were used to measure phenomena that are typically associated with adult onset depression. The amount of

immunologic activity, the degree of behavioral modification, and alterations in body mass and reproductive tissue were collected and measured in order to assess the impact that early life sex experience has on subsequent development of neuropsychiatric disease. These various forms of behavioral and physiological examination are insightful, since the effects of early life events sexual contact on immune function have not been assessed in this particular context.

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 then initiates signals that evoke maturation of an adult behavioral profile (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 PFC (Spear, 2000). This particular brain region is highly sensitive to the effects of environmental pressure, as

well as associated with later onset of socio-emotional disturbances (Spear, 2000), and increased risk for psychopathologies later in life (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 growth to modify the neuro-circuitry in ways that may be neuro-protective (Dahl, 2004).

In the experiments described below, I aim to investigate the impact that early sexual experience may have on adult behavior, immunity, and reproduction in hopes of elucidating some of the possible mechanisms at work in the etiology and progression of pathologies such as depression. The focus of this study was to address the question of whether engaging in sex during adolescence, a particularly vulnerable developmental period, alters neuronal plasticity leading to lasting detrimental effects on individual health and mood. We initially wanted to perform tests to evaluate possible behavioral substrates that undergo change and then transition into testing other possible genetic, physiologic, and anatomical changes that may be linked to early developmentally timed exposure to sex. In this study we separated animals into one of three groups: a control, 40 day (adolescent sex), 80 day (adult sex) groups. 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. At 120 days of age experimental testing began for all hamsters. Behavioral testing consisted of performance on the elevated plus maze (EPM), designed

to assess anxiety-like responses, and the (Porsolt forced swim test; FST), which is a measure of depressive-like responses. Following behavioral tests cell-mediated immune responses were assessed through the use of (delayed type hypersensitivity; DTH). Blood samples were obtained prior to sex, following sex, and during immune testing in order to determine whether glucocorticoid concentrations were involved in this process.

Chapter 2

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. 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).

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 isolation. The females were removed after 6 hours and mating behavior was recorded to insure that copulation took place. Behavioral testing was initiated at 120 days of age for all of the experimental groups. Since the behavioral analyses tend to be the most variable additional groups were created to provide more control. In regards to the behavioral measures of anxiety-like and depressive-like behavior an additional study was carried out which included a 40X40 day and an 80X80 day groups to control for the age at which behavioral testing began in adult hamsters. Adding additional groups is justified because the original set up failed to account for age discrepancies of animals at the time of testing. In the original design the 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 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.Both the 40X40 and 80X80 day groups were added for somatic and immune measures but further analysis is required and is currently underway.

All forms of behavioral testing were administered between 15:00 and 18:00 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, immune function was assessed through

the use of DTH testing. Measurements for DTH tests were obtained between 07:30 and 09:00 h; this was well in advance of the lights being turned off, which occurred at 15:00 h, in the animal rooms.

Copulatory behavior

The test arena was a rectangular box measuring 50 cm × 75 cm × 50 cm (D × W × H). The tests were conducted between 15:00 and 21:00 h and recorded on VHS-video tape for subsequent quantification. The stimulus females were introduced to the males and allowed to copulate for a maximum of 6hrs. Copulatory behavioral parameters quantified included: mount latency (latency from introduction of female to first mount), intromission latency (latency from introduction of female to first intromission), and ejaculation latency (latency from first intromission to ejaculation). From these data, the post-ejaculatory interval (time from first ejaculation to first intromission in the second copulatory series) was derived. Animals that did not complete the copulatory stages within the 6hr time limit were terminated from the study and excluded from statistical analysis if no mount was observed, if no intromission was observed, if no ejaculation occurred, if ejaculation was not followed by intromission throughout the testing time period.

Behavioral Tests

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 recognized to work in hamsters through past research in the lab. 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 are 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) total entries into all arms, (b) latency to enter open arms, (c) number of entries into open arms, (d) percentage of entries into open arms, and (f) the number of fecal boli expelled (an indication of an anxiety-like state; Blizard & Bailey, 1979). Hamsters were considered to have entered an arm when all four paws crossed onto an arm of the maze.

Forced Swim Test. Hamsters were examined for cessation of attempting to escape water (i.e., floating instead of active swimming) by placing them in 17 cm of room-temperature water 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) latency to first floating bout, (b) the total number of floating bouts, (c) the total time spent floating, and (d) the number of fecal boli expelled.

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). Prior to presentation of the sucrose solution, tap water was administered to hamsters in modified water bottles for three consecutive nights to control for novelty of the modified water being placed in the home cage. Sucrose consumption on both nights was normalized to the average pretesting water consumption. Over the next 6 days consumption of a 3% sucrose solution over 5 h of the dark phase (1500–2000 h EST) were recorded in all hamsters to measure sucrose anhedonic responses (Willner et al., 1992). The modified water bottles were weighed before and after the 5-h sample time to quantify total volume of liquid consumed.

Immune Tests

DTH. Hamsters were separately brought into a procedure room, anesthetized with isoflurane vapors, weighed, and a 1 x 2 cm shaved patch of the dorsum on each hamster was sensitized to 2,4, dinitro-1-flurobenzene (DNFB; Sigma, St. Louis, Missouri, USA;

25 µl of 0.5% DNFB solution (wt/vol)in 4:1 acetone/olive oil). The DNFB solution was prepared fresh daily and applied to the dorsal skin in the same location on two consecutive days (Dhabhar and McEwen 1997). Baseline measurements of both the left and right pinna (ear) thickness were measured during sensitization with a constant loading dial micrometer (Mitutoyo, America Corp., Aurora, IL, USA). Hamsters were then left undisturbed for 7 days, after which they were again anesthetized, measurements of pinna thickness were obtained, and hamsters were challenged with 20 µl of 0.2% (wt/vol) DNFB in 4:1 acetone to olive oil on the surface of the right pinna. Left pinna was treated with equivalent volumes of vehicle solution. Over the following week both pinnae were measured every 24 h. Pinnae thickness values obtained on each day were expressed as a percentage of baseline thickness. Vehicle treated (left) pinnae showed no change in swelling or thickness. All measurements occurred between 7:30 and 9:00 h EST. Values of maximum swelling across all daily measurements and swelling over the entire measurement period were observed from these data.

Analysis of brain morphology

All hamsters were deeply anesthetized with isoflurane vapor and rapidly decapitated on the same day between 10:00 and 12:00 h. Brains were promptly hemi-sectioned 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,

followed by a 30% sucrose solution for 4 days. 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.

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) dendritic length, (2) cell body area, (3) cell body perimeter, and (4) dendritic spine density.

Cortisol radioimmunoassay

Statistical Analyses

Data analyses were conducted using a 3 x 2 analysis of variance (ANOVA) with variables being (sex experience x age). Responses to DTH were analyzed using a 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 analysis was conducted through the use of SPSS software, version 16.0 (SPSS, Chicago, IL). In all cases, mean differences were considered to be statistically significant when $p \leq .05$.

CHAPTER 3

RESULTS

Somatic and Reproductive Responses.

Body Mass. Exposure to sex in adolescence significantly decreased overall body mass compared to adult and non sex hamsters ($p<0.05$; Fig. 1). When measured in adulthood only male hamsters that had sex in adolescence showed fluctuations in body mass. *Accessory reproductive tissue*. Hamsters exposed to sex in either adolescence or adulthood did not display significant differences in testis volume when compared to control animals ($p>0.05$ in each comparison). However, accessory reproductive tissue mass was reduced in male hamsters that were sexual paired in adolescence.

Anxiety-like Behavior and Depressive-Like.

Elevated Plus Maze. Following the conclusion of the sexual initiation period, much later in adulthood (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 two behavioral tests: Elevated plus maze (EPM), Porsolt forced swim task (FST), and sucrose anhedonia. 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. Hamsters that experienced sex spent more time in the closed arms than non experienced males (effect not found for the control group). The percentage of time hamsters spent in the open: no sex males 21.10 ± 2.87; versus P40 adolescent sex males 8.88 ± 1.97 ($P^* < 0.01$); versus 40X40 adolescent sex males 12.73 ± 5.86; versus P80 adult sex males 8.92 ± 1.46 ($P^* < 0.01$); versus 80X80 adult sex males 7.39 ± 1.80 ($P^* < 0.01$); Figure 3).

Forced swim task. Adolescent sex male hamsters spent significantly more time swimming in a non-vigorous manner during the forced swim test as compared to the adult sex and the no sex experience groups. The percentage of time hamsters spent swimming non-vigorously: no sex males 0.51 ± .18; versus P40 adolescent sex males 10.19 ± 3.97 ($P < 0.01$); versus 40X40 adolescent sex males 3.17 ± 1.46 ($P < 0.01$); versus adult sex males 1.76 ± 0.90; versus 80X80 adult sex males 1.55 ± 0.84 (Figure 4).

Sucrose Anhedonia Test. Sex decreased sucrose consumption ($p<0.05$, Figure 5). Analysis showed that adolescent sex experienced hamsters showed the most profound decreases in total amount of sucrose consumed 0.38 ± 0.08 ($p < 0.01$); followed by the adult sex hamsters who also exhibited reductions in sucrose consumption 0.78 ± 0.13 ($p < 0.01$); both sex manipulation groups were significantly different than the non sex group of hamsters.

Delayed Type Hypersensitivity. Vehicle treated (left) pinna showed no swelling as compared to the baseline measurement during the experiment (data not shown). In contrast, DNFB elicited a strong swelling response on the right pinna in both groups of hamsters that were sexually experienced. Both the adolescent sex animals and the adult sex hamsters groups, showed significant enhancement of swelling during DTH testing. The adolescent sex males exhibited significant elevations in the percentage swelling compared to both the adult sex and no sex males. The percentage of swelling in the non sex experienced male hamsters 63.09 ± 6.54 ($P < 0.05$), versus adolescent sex males 68.65 ± 5.79 ($P < 0.05$; Figure 3), and versus adult sex males 40.80 ± 10.62 ($P < 0.05$; Figure 6).

Brain morphology

Prefrontal cortex IL-1β mRNA levels. 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 ($p < 0.01$; Figure 4). PFC IL-1β mRNA expression was similar among the adult sex group and the no sex group ($p > 0.05$; Figure 7).

Golgi Analysis. Scholl analysis of prefrontal cortical regions discovered a decrease in the complexity of basilar dendrites located between 150 to 210 concentric circle radii from

the center of the soma (10 micron increments), in adolescent sex hamsters compared to non sex hamsters (($p < 0.05$(150), ($p < 0.05$(160), ($p < 0.05$(170), ($p < 0.05$(180), ($p < 0.05$(190), ($p < 0.05$(200), ($p < 0.05$(210); Figure 8). Scholl analysis of CA3 hippocampal brain regions revealed a decrease in the complexity of apical dendrites located between 60 to 100 concentric circle radii from the center of the soma (10 micron increments), in adolescent sex hamsters compared to non sex hamsters ((($p < 0.05$(60), ($p^* < 0.01$(70), ($p < 0.05$(80), ($p < 0.05$(90), ($p < 0.05$(100); Figure 11). Also, Scholl analysis of apical dentritic length showed a decrease in length between 40 and 100 concentric circle radii from the center of the soma (10 micron increments), in adolescent sex hamsters compared to non sex hamsters (($p < 0.05$(40), ($p < 0.05$(50), ($p < 0.05$(70), $p < 0.05$(80), ($p < 0.05$(90), ($p < 0.05$(100); Figure 12.

CHAPTER 4

DISCUSSION

In this study, we evaluated the influence of early sexual exposure on behavioral, physiological, and neurological development in an animal model. As predicted, exposing adolescent hamsters to sex led to marked increases in anxiety- and depression-like behaviors in adulthood. Interestingly, we also observed dramatic alterations in immunological functioning which may illuminate a potential pathway by which early sexual exposure influences behavior and health.

In regards to anxiety, numerous studies have demonstrated that exposure to sex is a sufficient, but not necessary, condition for persistent changes in anxious-like behaviors. This effect appears to be consistent across development as both adolescent and adult hamsters significantly decreased exploratory behaviors following sex compared to control animals. Moreover, in the present study general locomotor activity, a common confounding factor in the exploration paradigm, was assessed and was comparable across all experimental groups.

Sexual contact in adolescence has also been linked to increased depression (Kaltiala-Heino, Kosunen, Rimpela, 2003). Depressive symptoms such as decreased energy or effort, impaired appetite, depressed mood (i.e., anhedonia) and despair have

been repeatedly identified in animal studies (Anisman & Matheson). Specifically, sexual contact during adolescence is associated with both decreased activity and appetite. For example, studies using the forced swim test, in which animals are placed in an inescapable bucket of water, have reported (i.e., non-vigorous swim bouts, and float time) in animals exposed to sexual contact during adolescence. In addition, researchers have suggested that sucrose consumption is an indicator of anhedonia. This has been supported by findings that animals that experienced the first sexual encounter in adolescence decreased sucrose consumption (Jamieson & Wade, 2011). In this study, adult sex animals also consumed less sucrose compared to controls, but not when compared to hamsters exposed to sexual behavior during adolescence. However, the hamsters that experienced early sexual exposure exhibited the largest decrease overall. Further, when considering sucrose consumption as a marker of depressive behavior it is also important to consider other factors such as measurements of body mass which may influence motivation to engage in appetitive behaviors. Body mass was recorded throughout the testing period to confirm that metabolic factors were not affecting food sucrose intake.

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). Early life experiences are important regulators 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. To determine whether peripheral inflammatory system function undergoes long term modification following sex early in life, cell mediated immune responses were examined. In order to assess cell mediated immune functioning DTH tests were conducted. Adolescent sexual activity is linked to the highest elevations in DTH swelling response when compared to both adult sex and non sex experienced hamsters. Animals that experienced sex during adolescence showed elevated levels of swelling in relation to control hamsters examined in DTH tests.

Depressive patients display increased quantities of proinflammatory substances in various affective and emotion related brain regions (Raison, Capuron, Miller, 2006). The PFC was of interest because it plays a role in mediating depressive behaviors. Following behavioral testing and analysis, tissue was collected from the prefrontal cortex and relevant limbic regions (i.e., amygdala and hippocampus). Hamsters that experienced early sexual interactions increased levels of IL-1β mRNA expression within the prefrontal cortex in adulthood. Elevated levels of the proinflammatory cytokine IL-1β within the prefrontal cortex in combination with elevated levels of inflammation in the periphery, as measured through DTH testing, provide particularly interesting information given the connection between inflammatory cytokines and induction of depression. These data provide evidence that sexual interaction modulates the development of depressive-

like behavior and possibly through the activation of inflammatory pathways, which could be one of the mechanisms through which early life adverse experiences alter long-term health outcomes (Danese et al., 2006).

The presence of inflammatory markers in the brain and elevated immune responses in the periphery following sex early in life are in line with the notion that signaling pathways of proinflammatory cytokines represent important points of intervention in the treatment of depression. Several studies provide evidence supporting this contention. For example, experiments in both humans and animals show that exposure to cytokines induce depressive-like responses and altered behavior. Animal studies revealed that brain cytokines produced behavioral changes in anhedonic responses, suppressed libido, weight loss or anorexia, exploration, and altered social behavior (Pollack & Yirmiya, 2002). High levels of IL-1β in human patients with major depressive disorder are predictive of current depressive severity (Thomas et al., 2005). The observations from this study and others suggest that inhibition of proinflammatory cytokines may a feasible way to treat patients with depressive disorder.

A potential explanation for the inflammation related findings could involve the trajectory of maturation in the brain, more specifically the prefrontal cortex, which is a region of the brain that has a protracted maturational window continuing well into adulthood. Studies have reported structural and functional modification of prefrontal

circuitry following environmental challenge in adolescence. This could be one of the mechanisms of change in immunity and behavior following various forms of early life experience. In the present study, initiation of copulatory behavior in adolescence markedly elevated the level of IL-1β, specifically in the prefrontal cortex. This suggests that the adolescent brain is not fully developed or organized to cope with the pressures associated with sexual reproduction. This form of social interaction could alter development and neurocircuitry, ultimately increasing susceptibility to depression in adulthood. Other emotion related limbic structures, particularly the hippocampus and amygdala, are involved in integrating threat responses and social interactions associated with behavioral depression. Morphological alterations within the hippocampus and amygdala are intricately linked to the onset of depression in both clinical and animal research. This is intriguing; however, additional studies will have to be conducted in order to pinpoint whether such regions of the brain are associated with the findings of the present study.

In sum, behavioral responses are largely shaped by experiences that take place early during individuals' lives. These results suggest that exposure to sexual contact within the adolescent sensitive period alters functioning of affective systems involved in depression. Exposure to sex increases susceptibility to anxiety and depression, but with exposure during the critical window of adolescence leading to more negative responses.

Human studies suggest that adverse early life experiences affect mood and immunity possibly through inflammatory pathways. The results of this study suggest that immunologic changes occur in response to developmentally relevant social interactions. More inflammation is usually in indication of better immune function. But too strong of an immune response can be detrimental leading to autoimmunity. 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 adult immune function.

The implications for this type of research are extensive and intriguing. 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. This work may be useful and clinically relevant in understanding the long-term physical and mental health outcomes of adolescent sexual activity in humans.

APPENDIX

FIGURES

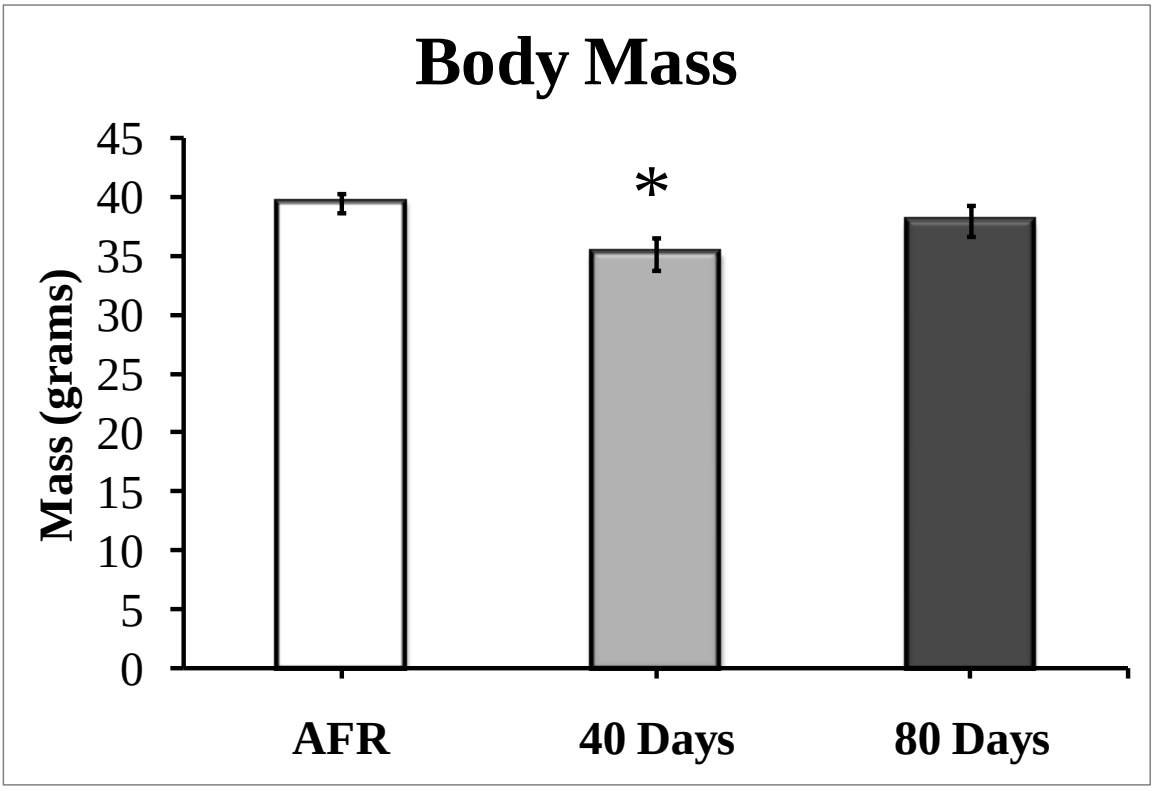

Figure 1. **Adolescent sex decreases overall body mass.** Mean body mass (g) ± SEM. Overall body mass was reduced in male hamsters that were sexual paired at P40. The percentage of swelling in P40 animals was also significantly enhanced as compared to both P80 and control males. The percentage of swelling in control animals 63.09 ± 6.54 versus stressed males 68.65 ± 5.79 (n s.) control females 69.47 ± 6.21 versus stressed females 40.80 ± 10.62 ($P < 0.05$).

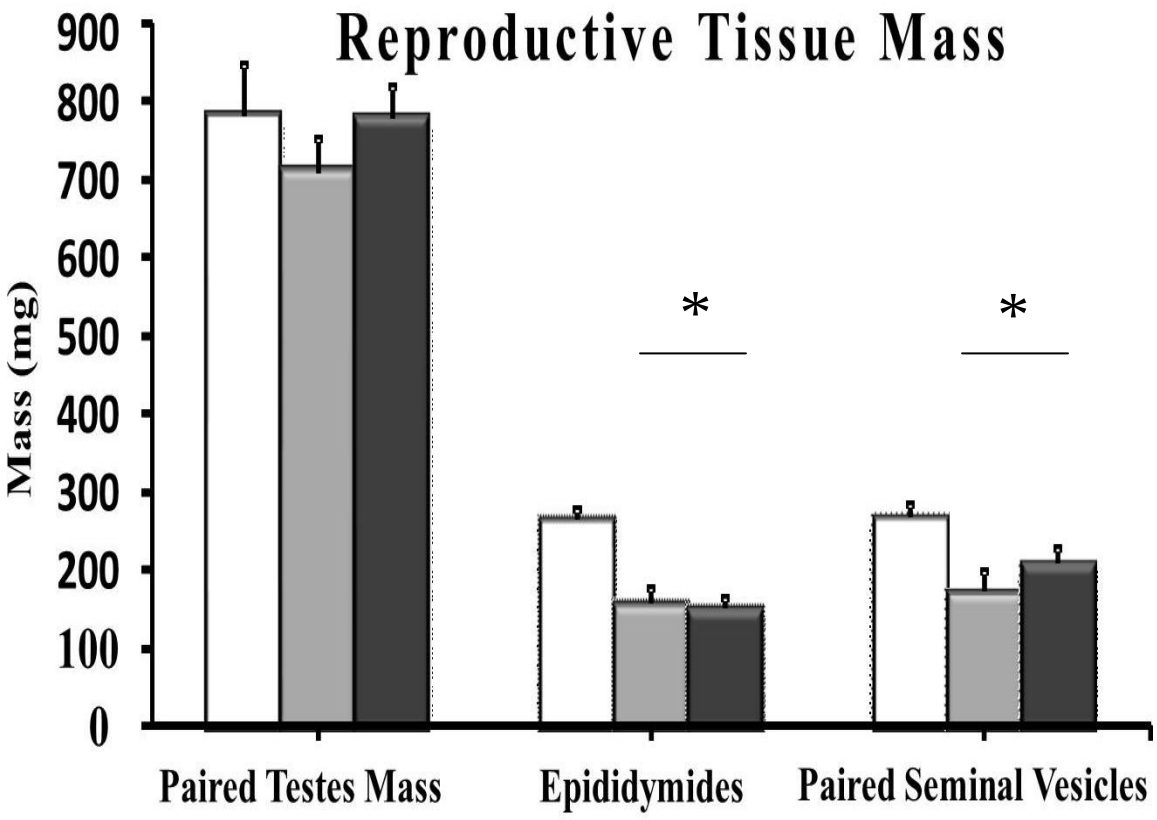

Figure 2. **Adolescent and adult sex decreases the size of accessory reproductive tissue.** Mean accessory reproductive tissue mass (g) ± SEM. Overall accessory reproductive tissue mass were reduced in male hamsters that were sexual paired at P40 & P80. The reduction in accessory tissue mass in P40 animals was also significantly decreased as compared to both P80 and control males. The tissue mass in control animals 63.09 ± 6.54 versus adult sex males 68.65 ± 5.79 (n s.) adolescent sex males 69.47 ± 6.21 ($P < 0.05$).

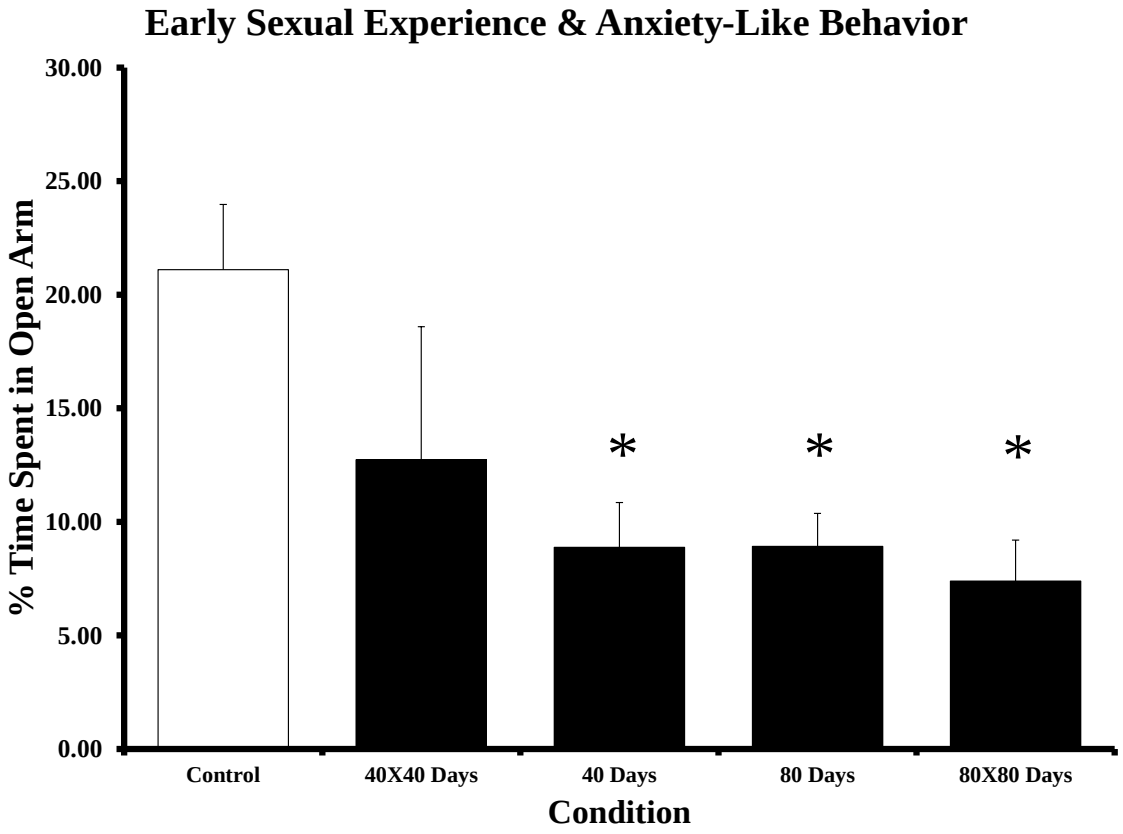

Figure 3. **Adolescent and adult sex increases anxiety-like responses in the elevated plus maze.** Percentage of time (s) spent in the open arms (mean ± SEM) of the elevated plus maze. P40 and P80 sexual contact hamsters spent significantly less time in the open arms than adult males and none sex experienced male hamsters. Percentage of time adolescent sex hamsters (P40) spent in the open arms of the EPM 0.07 ± .02 ($P < 0.01$); versus P80 males 0.09 ± 0.01 ($P < 0.02$) versus control males 0.21 ± 0.04 ($P < 0.02$). Neither P40 nor P80 differed significantly in the percentage of time spent in the open arms of the maze.

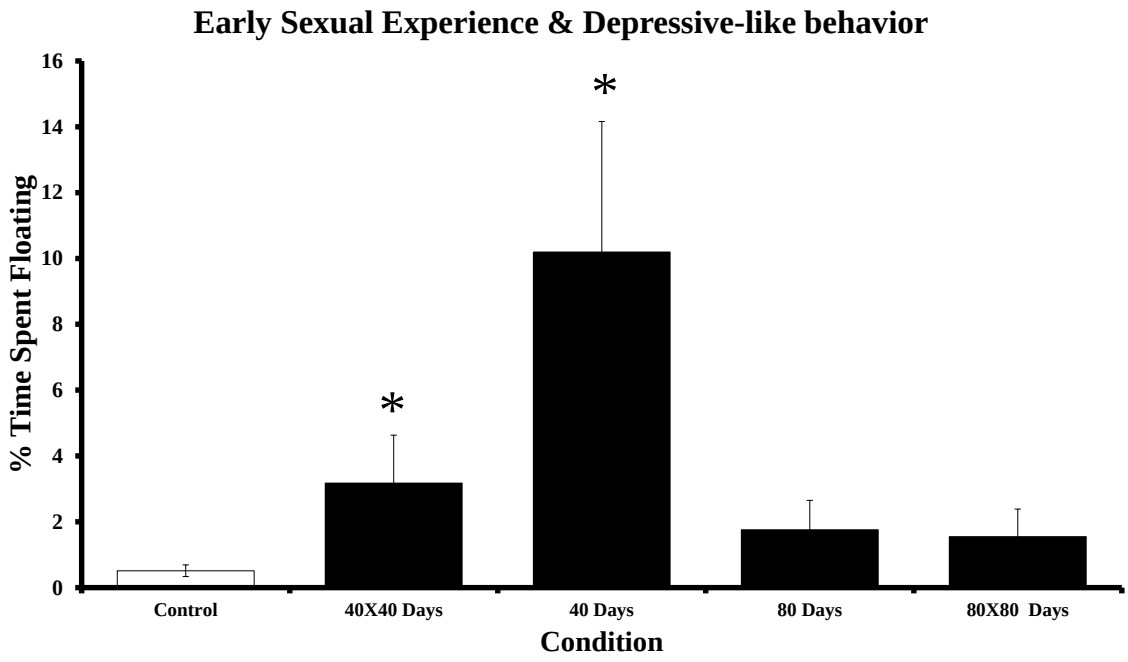

Figure 4. **Adolescent and adult sex increases depressive-like responses in the forced swim task.** Percentage of time (sec) consumed (mean ± SEM) in the Porsolt forced swim test. For P40 animals sexual experience in adolescent hamsters was coupled to significant increases in time spent swimming in a non-vigorous way total amount of sucrose consumed 0.129 ± 0.052 ($P < 0.01$). Neither P80 nor control hamsters differed significantly in the percentage of time spent in the open arms of the maze.

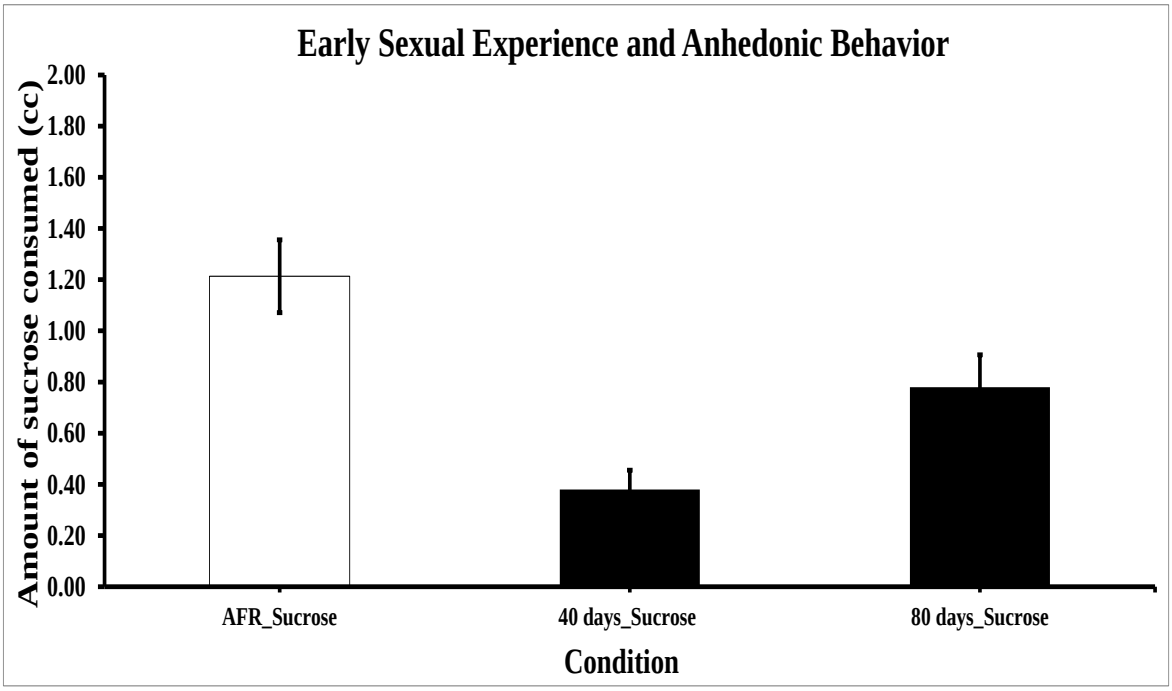

Figure 5. **Adolescent and adult sex increases depressive-like responses in measures of anhedonia.** Amount of sucrose (cc) consumed/amount of water consumed (mean ± SEM) in the sucrose anhedonia test. For P40 animals sexual experience in adolescent hamsters was connected to decreases in total amount of sucrose consumed/water consumed 0.38 ± 0.08 ($P < 0.01$) P80 hamsters also exhibited reductions in sucrose consumption/water consumed 0.78 ± 0.13 ($P < 0.01$). Both P40 & P80 sex experienced consumed less sucrose than control animals.

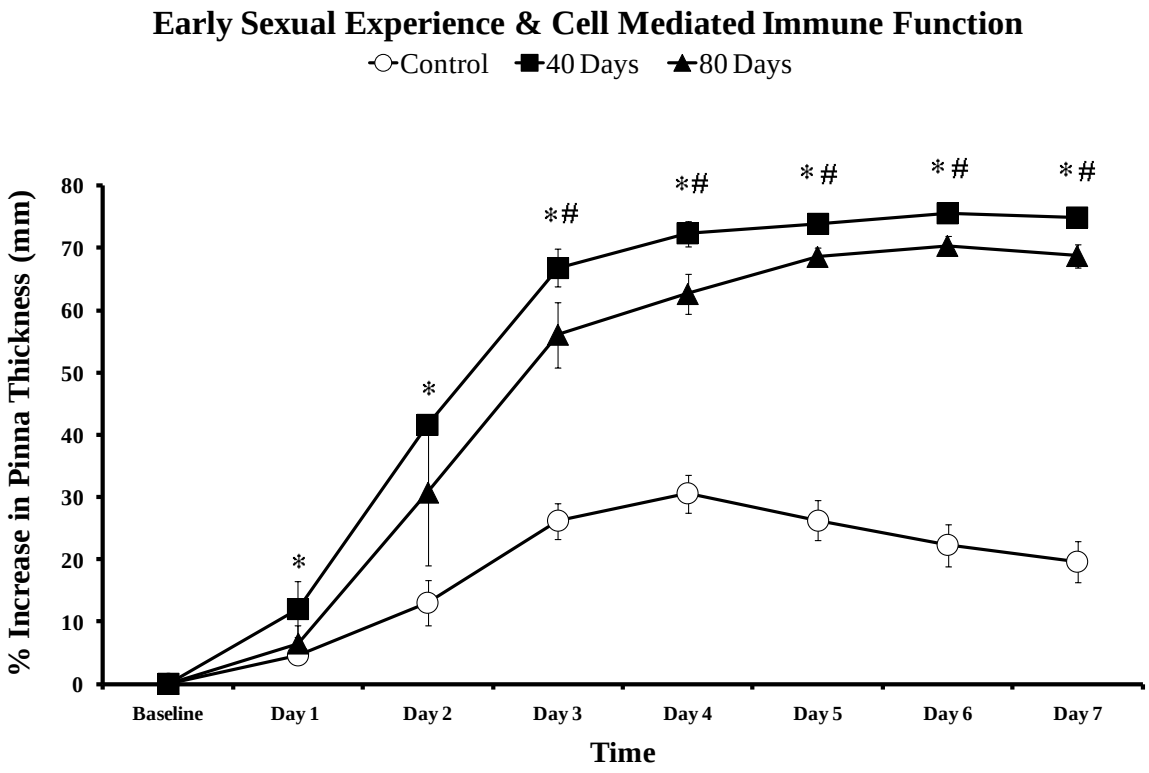

Figure 6. **Adolescent and adult sex increases swelling response in measures of immune function.** Mean delayed-type hypersensitivity responses in non sex experienced males, adolescent sex experienced male, and adult sex experienced male Siberian hamsters (±SEM). *P < 0.05 between P40 and AFR hamsters; #P < 0.05 between P80 and AFR hamsters. Male hamsters that underwent sexual pairing at P40 exhibited increased swelling compared to AFR and P80 hamsters of a 7 day period.

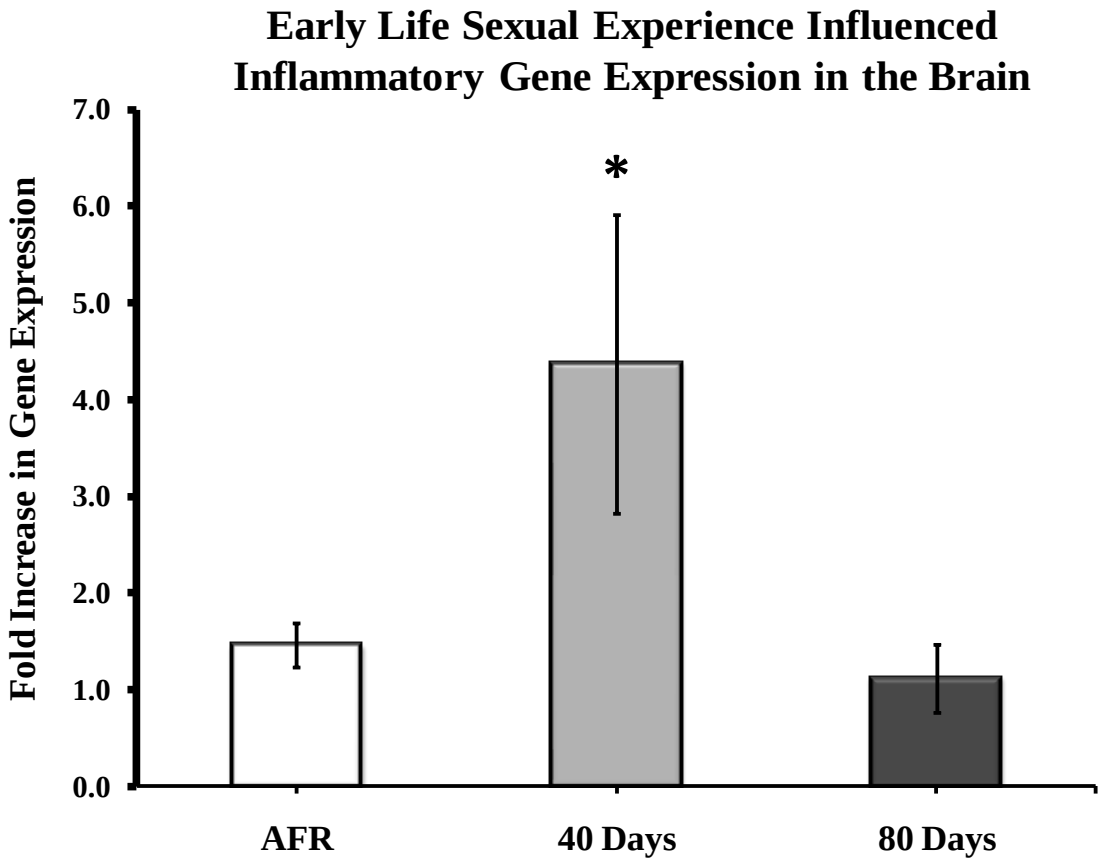

Figure 7. **Adolescent sex increases expression of the pro-inflammatory cytokine IL-1β in the brain**. Only 40 day sex hamsters showed increased levels of the pro-inflammatory cytokine (IL-1β) in the prefrontal cortex. The expression of IL-1β in P40 animals was also significantly enhanced as compared to both P80 and control males.

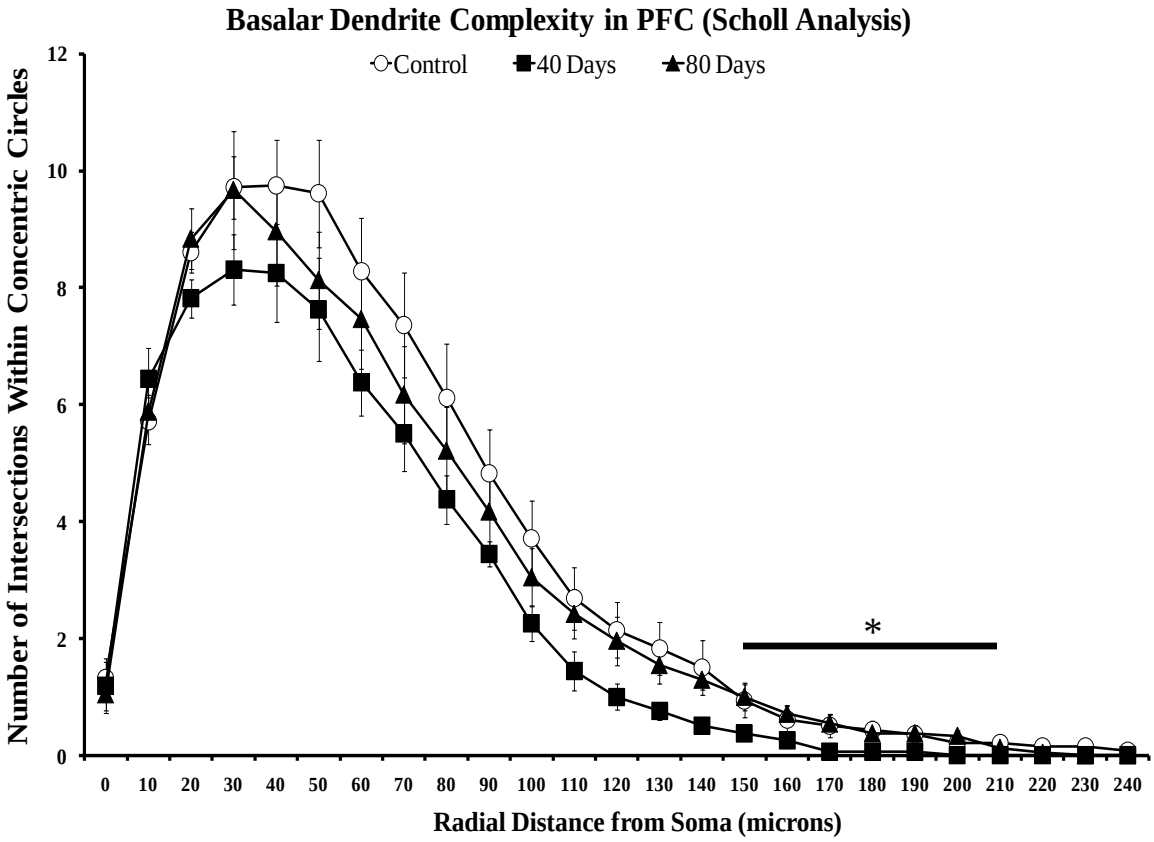

Figure 8. Experiencing first sexual contact in adolescence alters complexity of basilar dendrites. Number of intersections within concentric circle area (μ) within prefrontal cortical regions (mean ± SEM) of intersections. P40 sexual contact hamsters had significantly fewer radius intersections than none sex experienced male hamsters.

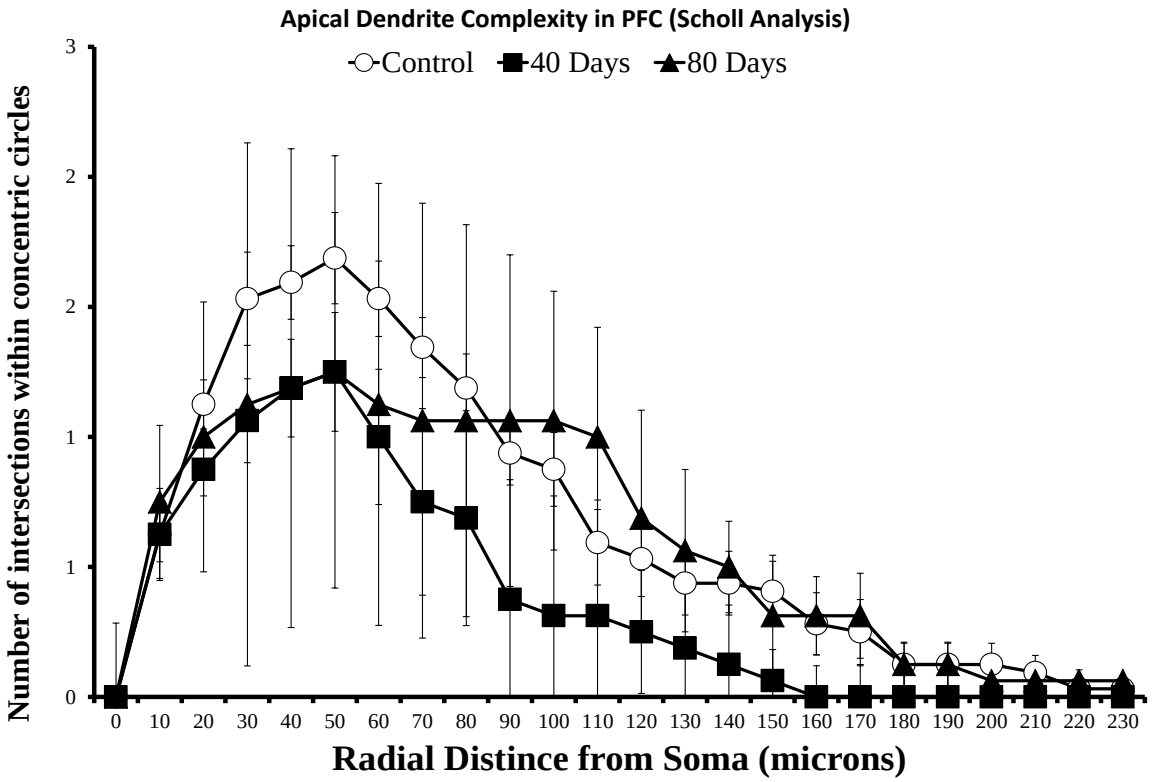

Figure 9. Experiencing first sexual contact in adolescence did not significantly alter the complexity of apical dendrites in the prefrontal cortex. Number of intersections within concentric circle area (μ) within the PFC region of the brain (mean ± SEM) of intersections. No significant differences were found in radius intersections between sex experienced and none sex experienced male hamsters.

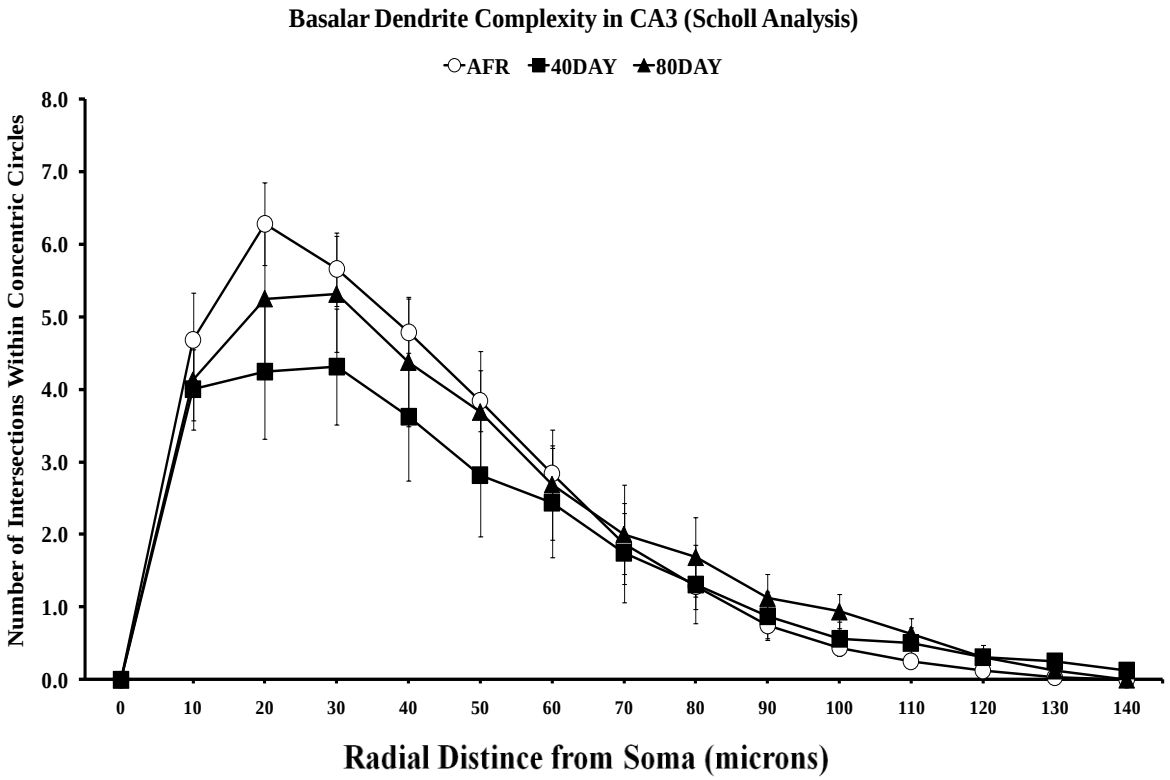

Figure 10. Experiencing first sexual contact in adolescence did not significantly alter the complexity basilar dendrites in the hippocampus. Number of intersections within concentric circle area (μ) within the CA3 region of the brain (mean ± SEM) of intersections. No significant differences were found in radius intersections between sex experienced and none sex experienced male hamsters.

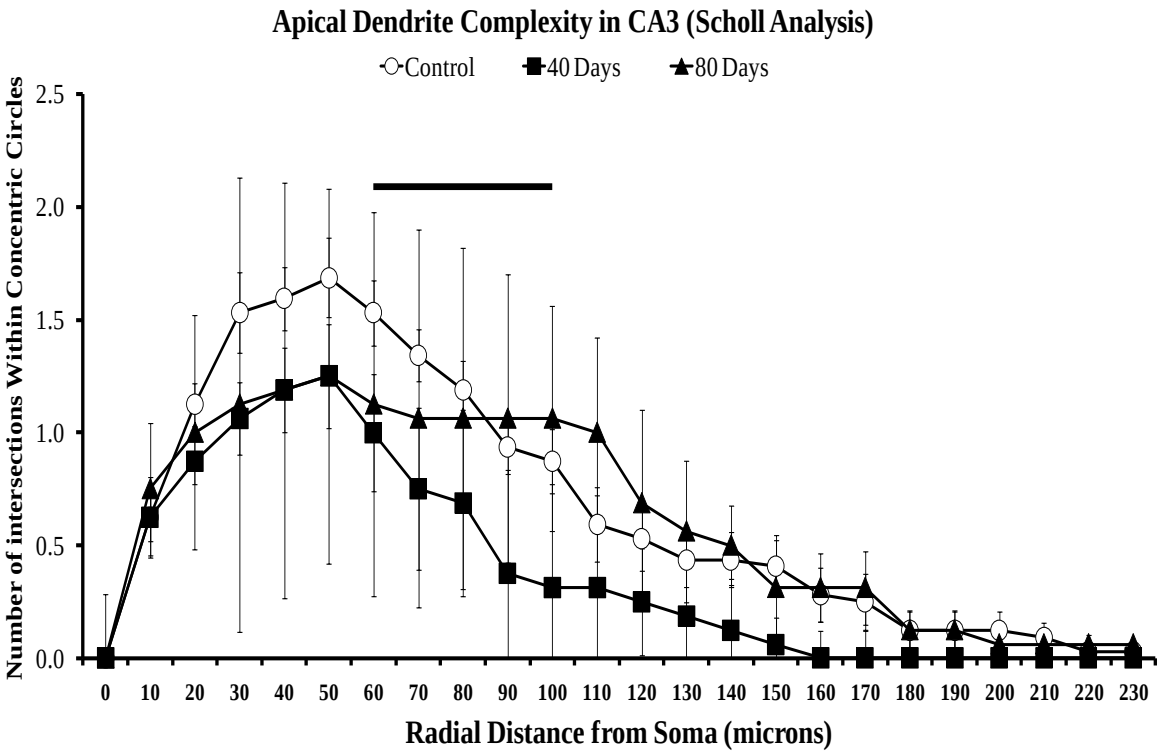

Figure 11. Experiencing first sexual contact in adolescence alters complexity of apical dendrites. Number of intersections within concentric circle area (μ) within CA3 cortical regions (mean ± SEM) of intersections. P40 sexual contact hamsters had significantly fewer radius intersections than non sex experienced male hamsters.

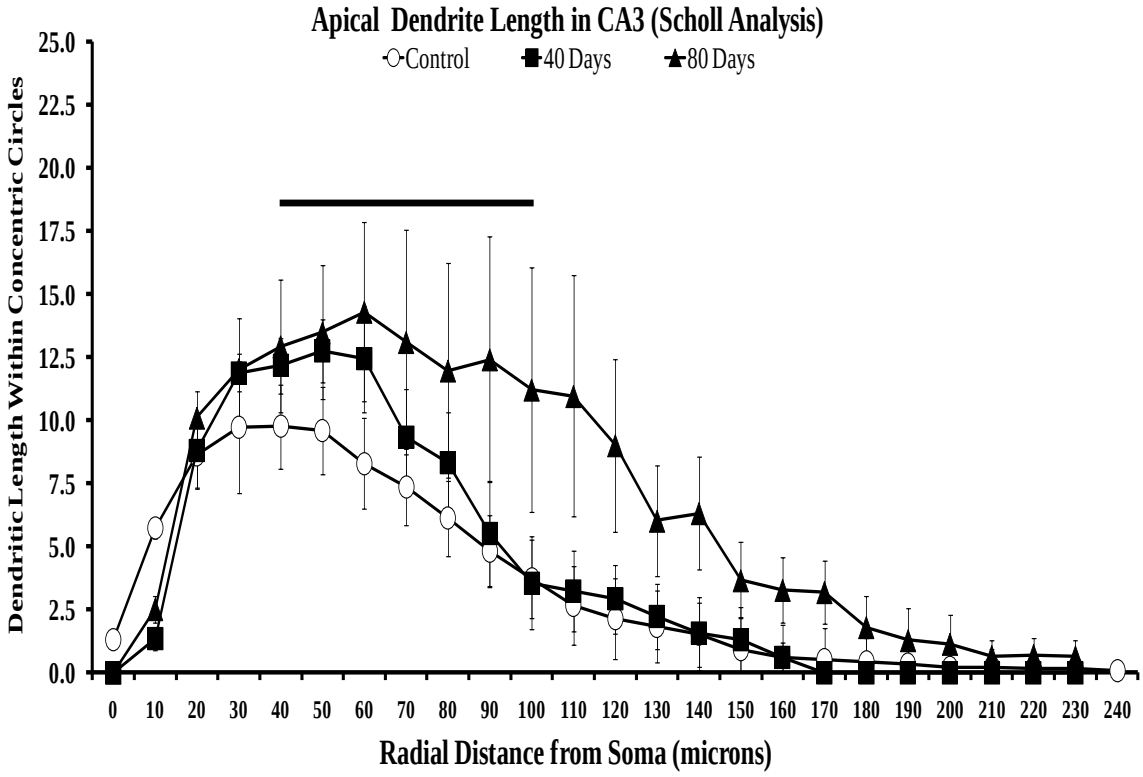

Figure 12. Experiencing first sexual contact in adolescence changes the length of apical dendrites. Number of intersections within concentric circle area (μ) within the CA3 region of the hippocampus (mean ± SEM) of length of apical dendrite. P40 sexual contact hamsters had significantly shorter lengths than non sex experienced male hamsters.

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