Neuroplasticity after Sexual Experience in the Nucleus Accumbens of Syrian hamsters

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Abstract

Research over the past three decades has demonstrated that many neural changes occur in response to rewarding stimuli and behavior. However, most of this research has focused on the changes that occur following drug use and their role in addiction. Less research has investigated the neural changes in response to everyday rewarding behaviors such as eating, exercising, and sexual behavior, and even less has explored whether these changes differ in male and female brains. The goal of this study was to investigate the changes in brain circuitry that occur in Syrian hamsters after exposure to sexual experiences and to identify any possible sex differences involved. Specifically, levels of delta FosB, a transcription factor that is important for long-term neural plasticity following rewarding experiences, was measured in the Nucleus Accumbens (NAc) as a way to quantify these neural changes. This study also aimed at investigating whether the efficiency with which hamsters mate is improved with experience, as measured by the time the hamsters are actively having sex and the amount of sex-related behaviors they perform. It was expected that sexual experiences would lead to an up-regulation of delta FosB in the NAc, that this up-regulation would not differ between the sexes, and that mating efficiency would improve with experience. The results demonstrated that sexual experience led to higher delta FosB levels in the NAc than controls, and that there were no differences in delta FosB levels between males and females of the same group. This study also found that mating efficiency was not improved with experience. The results obtained in this study suggest that the normal rewarding behavior of sexual experience leads to neuroplastic changes in the NAc of Syrian hamsters and that male and female Syrian hamsters likely have similar neuroplastic changes following sexual experiences. This research has the potential to provide a better understanding of

how drugs of abuse take advantage of reward pathways, and eventually lead to better treatments for addiction.

Keywords: Nucleus Accumbens, delta FosB, Syrian hamsters, Neuroplasticity

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The ways in which the brain responds to rewarding stimuli has been heavily researched during the past two decades. Much of this research has been focused on the mesolimbic reward pathway, which is the most studied reward pathway in the brain, and has been shown to be activated in response to most drugs of abuse, as well as gambling and orgasms (Volkow, Fowler, Wang, & Swanson, 2004). Research in this area has been mostly focused in trying to understand the underlying neurological mechanisms behind the addictive behavior tied with drug usage, mainly because of the social and public health implications of these studies. However, less research has been done in trying to understand the brain mechanisms behind normal rewarding experiences such as eating or sexual behavior. More specifically, relatively little research has been done to try to understand the neuroplasticity or the changes in synapses and brain structures that occur in response to naturally-motivated behaviors. Thus, there is a lot of information and evidence regarding the many pathways involved in drug addiction, but there is little information and evidence regarding the neurological effects that normal, everyday rewarding experiences have on the brain and specifically on the reward pathways. Understanding the neurological effects or the neural changes that occur following normal rewarding experiences is important not only because they are for the most part unknown, but also because a better understanding of these changes in response to normal behavior will provide more information about reward pathways and how drugs activate these reward pathways. The main goal of this research will be to provide insights into the neurological changes that occur in response to normal sexual experiences. Specifically, this research will focus on studying the neuroplasticity involved in the Nucleus Accumbens (NAc), a part in the mesolimbic reward pathway, in response to sexual experiences, as well as any neuroplastic differences that might exist between male and female

Syrian hamsters. This research will also focus on investigating whether the efficiency with which hamsters mate is improved with experience.

The Mesolimbic Pathway

The mesolimbic reward pathway was first exposed in 1965 by Fuxe and Dahlstrom (Dahlstrom & Fuxe, 1965). It serves as one of the four major dopaminergic pathways that have been identified in the mammalian brain, alongside the nigrostriatal, mesocortical, and the tuberoinfundibular pathways (Beaulieu & Gainetdinov, 2011). It is also believed to be the primary reward system of the brain and it is highly conserved between different species, as it can be found from mammals to reptiles (Campbell, 1972). The mesolimbic reward pathway activates in response to rewarding behaviors such as gambling, sight of appetizing food, sex, and addictive drugs (Breiter, Aharon, Kahneman, Dale, & Shizgal, 2001; Komisaruk et al., 2004; G. J. Wang et al., 2004; Volkow et al., 2004). This pathway begins in the dopaminergic neurons of the ventral tegmental area (VTA) of the midbrain. The cells in this region then project to the limbic system, specifically the NAc, dorsal striatum, amygdala, and the hippocampus, as well as regions in the prefrontal cortex (Hyman, Malenka, & Nestler, 2006; Kalivas & Volkow, 2005; Koob & Moal, 2005). However, the main projection and terminating area of the dopaminergic neurons in the VTA is the NAc (Adinoff, 2004). The NAc is an essential region within the mesolimbic reward pathway, as well as for drug addiction, as Zito and colleagues demonstrated that lesions to the NAc lead rats to stop engaging in rewarding experiences such as drug usage (Zito, Vickers, & Roberts, 1985). Also, dopaminergic input from the VTA has been shown to regulate the activity of neurons within the NAc and research has shown that all known drugs that exhibit addictive behavior increase dopaminergic transmission from the VTA to the NAc (Hyman et al., 2006; Kalivas & Volkow, 2005; Koob & Moal, 2005; Robison & Nestler, 2011). Apart from addictive

drugs, research has also shown that natural reinforcers, such as food, water, or a sexual partner, stimulate the release of dopamine into the NAc (Malenka, Nestler & Hyman, 2009). The NAc is thus an integral brain region of the mesolimbic reward pathway and its activation is tied to the addictive effects of drugs as well as to the rewarding experiences of natural or normal reinforcers.

Nucleus Accumbens

The NAc, which is also known as the accumbens septi or the accumbens nucleus, is a brain structure that is part of the ventral striatum, which in turn is part of the basal ganglia (Carlson & Neil, 2013). It is known to be involved in reward, motivation, motor function, and learning (Carlson & Neil, 2013; Shirayama & Chaki, 2006). The major brain regions that input to the NAc are the basolateral amygdala, prefrontal cortex, and the VTA, while the primary brain regions that the NAc outputs to or sends axons to are the ventral pallidum, substantia nigra, reticular formation, and the basal ganglia (Barrot et al., 2012; Nestler, 2013; Robison & Nestler, 2011).

The neurons in the NAc are mostly inhibitory GABAergic medium spiny neurons that express dopamine receptors (Self, 2010). Specifically, they express D1-type dopamine receptors, D2-type dopamine receptors, or both D1-type and D2-type dopamine receptors. It is believed that around 40% of neurons in the NAc contain both types of receptors (Ferre et al., 2010). D1-type dopamine receptors are the most abundant type of dopamine receptor in the central nervous system and they function by activating cyclic AMP-dependent protein kinases (National Center for Biotechnology Information [NCBI] Reference Sequence, 2015a). They have been shown to mediate behavioral responses and regulate neuronal growth (NCBI Reference Sequence, 2015a). On the other hand, D2-type dopamine receptors are G-protein coupled receptors that function by

inhibiting adenylyl cyclase activity (NCBI Reference Sequence, 2015b). D2-type receptors have been demonstrated to play a primary role in the control of locomotion activity and mutations in these receptors have been associated with myoclonus dystonia and schizophrenia (Beaulieu & Gainetdinov, 2011; NCBI Reference Sequence, 2015b). However, both D1-type and D2-type dopamine receptors have been shown to be necessary for learning and memory mechanisms (Goldman-Rakic, Castner, Svensson, Siever, & Williams, 2004; Wang, Buck, Yang, Macey, & Neve, 2005). Finally, research has suggested that concurrent activation of both types of dopamine receptors is required for the reward processes that occur in the NAc as well as for the neural and behavior sensitization that occurs in response to drug addiction (Capper-Loup, Canales, Kadaba, & Graybiel, 2002; Dziedzicka-Wasylewska, 2004; White, Bednarz, Wachtel, Hjorth, & Brooderson, 1988).

The NAc has two main anatomical subdivisions: the core, which forms the inside part of the NAc, and the shell, which covers the outside of the core (Hyman et al., 2006). Both the shell and the core contain medium spiny neurons that express mostly D1-type or D2-type dopamine receptors (Ferre et al., 2010). In terms of function, research has determined that the shell is involved in the processing of motivational salience, while the core is involved in the processing of motor action related to future reward attainment (Ito, Robbins, & Everitt, 2004; Malenka, et al., 2009). In other words, the core of the NAc is involved in the regulation of goal-directed behavior. Another difference between the NAc core and shell is that addictive drug usage seems to be related to greater release of dopamine in the shell compared to the core, leading to the hypothesis that drugs of abuse have a greater effect in the shell than in the core (Malenka, et al., 2009). Similarly, research has suggested that the NAc shell plays a relatively important role in the psychostimulant effects of addictive drugs (Ito et al., 2004). In terms of neuroanatomy, it is

known that neurons in the core have around 50% more surface area than neurons in the shell. This suggests that neurons in the core of the NAc have greater potential for accumulating synaptic information than neurons in the shell (Meredith, Agolia, Arts, Groenewegen, & Zahm, 1992). Because of the multiple neuroanatomical, behavioral, and functional differences that exist between the NAc core and shell, as well as both of their involvement in addictive behavior, the present research will focus on analyzing both sub-regions of the NAc.

Why Syrian hamsters?

The present research will utilize Syrian hamsters (*Mesocricetus auratus)* as the model organism to study the neuroplasticity involved in the NAc in response to regular sexual experiences, as well as any neuroplastic differences that might exist between males and females. Syrian hamsters are greatly utilized as a model organism in research; it is believed that around 90% of all hamsters used for research purposes in the U.S. are Syrian Hamsters ("Hamsters: biology, care, diseases & models," n.d.). Also, a PubMed search for studies that have utilized Syrian Hamsters revealed over 21,500 results. This popularity of the use of Syrian Hamsters for research purposes originates from the fact that they are easily bred in captivity, develop rapidly, are relatively free from diseases, and have many features that resemble the physiology and metabolism of humans (Adler, 1948; Boschert, 2015; Gao et al., 2014; "Hamsters: biology, care, diseases & models," n.d.). More recently, Syrian hamsters have been studied extensively regarding motivated behavior such as addiction, sexual behavior, maternal behavior, and aggression (L. Been, 2015). Apart from this, there is also a long history of the use of Syrian Hamsters for research purposes, as U.S research institutions started using them as early as the 1940’s (Murphy, 1985). As a result, there exists a large amount of research regarding this animal

and thus there is a lot of data available for use in the present research. There is also significant research regarding the procedures used in the current study.

The second reason for the use of Syrian Hamsters to test the hypothesis of the current research was that female Syrian hamsters, in contrast to other rodents such as rats, display minimal motor activity during a sexual experience (L. Been, 2015). During a sexual experience, female Syrian hamsters assume the lordosis position, which is the position of sexual receptivity for female Syrian hamsters characterized by the arching of the spine. Notably, female hamsters stay in that position for the majority of the sexual experience. This lack of motor activity during a sexual experience thus allows for the possibility to associate perceived changes in the brain with the sexual experience and not with other motor behaviors (L. Been, 2015). Therefore, the use of female Syrian hamsters will limit the potential confounds that could arise from other motor behaviors since they display minimal motor activity during a sexual experience.

Another reason for using Syrian hamsters as the animal model to test the hypothesis of the current research was that expression of sexual behavior in Syrian hamsters is very tightly coupled to blood levels of gonadal hormones, which are steroid hormones, such as testosterone, progesterone, and estradiol, that are released into the bloodstream by the gonads (Faruzzi, Solomon, Demas, & Huhman, 2005; L. Been, 2015; Meisel, Sterner, & Diekman, 1988; Nature, 2015). For Syrian hamsters to be receptive to sexual behavior, they must have specific blood levels of these gonadal hormones. This fact provides researchers with a relatively simple way to induce sexual receptivity in Syrian hamsters by providing the hamsters with the specific combination of gonadal hormones via injections. Thus, sexual receptivity in Syrian hamsters can be easily manipulated and precisely controlled. This fact is significant for the aims of this study,

since the procedure will involve multiple sexual experiences and quantifying the effect these experiences have on neuroplasticity in the NAc.

What is Neuroplasticity?

Neuroplasticity or brain plasticity can be broadly defined as the process by which the neural pathways and synapses of the brain are altered in response to behavioral, environmental, and neural changes (Jensen, 2013). Before the 1970's, most researchers and neuroscientists believed that neuroplasticity in humans only occurred during the developmental stages and once adulthood was reached, the brain remained fixed and neuroplasticity was not possible (O'Rourke, n.d.). Today, it is widely believed that neuroplasticity is constantly occurring throughout the nervous system of most mammals including adult human beings, whether at the cellular level, molecular level, or at the large-scale brain networks level (Pascual-Leone et al., 2011). Recently, research has shown that neuroplasticity is largely retained throughout life, even during old age (Park & Reuter-Lorenz, 2009). Research has also recognized that neuroplasticity is essential for the establishment and maintenance of brain circuitry and brain connectivity, as well as for the acquisition of a new skill and the adaptation after an injury (Pascual-Leone et al., 2011). Neuroplasticity can thus be viewed as a powerful evolutionary mechanism that allows the brain to respond to unforeseen changes in the environment. Pascual-Leone and colleagues summarize this idea as: "the brain does not remain static but, instead, continues to change as the obligatory consequence of each sensory input, motor act, association, reward signal, action plan, and awareness" (Pascual-Leone et al., 2011, p. 303).

Neuroplasticity can be more specifically defined as any lasting change in the nervous system that occurs at the level of synapses (synaptic plasticity) and cells (non-synaptic plasticity and neurogenesis) (Citri & Malenka, 2007). Synaptic plasticity refers to the activity-dependent

adaptation of the proficiency or strength of synaptic transmission between two neurons (Citri & Malenka, 2007). Changes in the activity of synaptic transmission, which can occur in response to alterations in levels of released neurotransmitters, levels of neurotransmitter receptors, and the efficiency with which receptors respond to their respective neurotransmitters, can result in either a decrease or increase in the strength of the synapse (Gaiarsa, Caillard, & Ben-Ari, 2002). Persistent changes in neurotransmitter and receptor levels can lead to long lasting effects on synaptic strength and activity in the form of Long Term Potentiation (LTP). LTP is a well studied mechanism involved in the formation of stable memories in the brain of mammals and is defined as a long-lasting increase in synaptic strength following stimulation (Shors & Matzel, 1997). These long term changes are important for learning, memory formation, and brain development (Gaiarsa et al., 2002).

Non-synaptic plasticity refers to changes that occur in any part of the neuron except at synapses. Thus, non-synaptic plasticity involves changes in the axon, dendrites, and the cell body of a neuron (Mozzachiodi & Byrne, 2010). Although this is a relatively newly discovered mechanism or form of plasticity, recent research has suggested that this type of plasticity, specifically in the axon, occurs through an alteration of membrane constituents such as voltage dependent ion channels (Mozzachiodi & Byrne, 2010). This in turn results in modifications in the excitability of the neuron. It is believed that the mechanism behind this type of neuroplasticity involves constant changes of patterns of neuronal activity (Mozzachiodi & Byrne, 2010). Non-synaptic neuroplasticity is believed to play an important part in classical conditioning and the formation of new memories (Brons & Woody, 1980; Woody & Black-Cleworth, 1973).

Neurogenesis is considered the birth, growth, and development of new neurons. In mammals, the process of neurogenesis is most active during the developmental years and it begins early in gestation (Mandal, 2014). Although the level of neurogenesis in the brain significantly decreases once adulthood is reached, it continues to occur in the brain regions of the hippocampus, the dentate gyrus, lateral ventricle walls, and olfactory bulb (Eriksson et al., 1998; Mandal, 2014). Recent research has revealed that neuroplasticity might occur in other parts of the brain, such as the cerebellum (Ponti, Peretto, & Bonfanti, 2008). As with other forms of neuroplasticity, neurogenesis is believed to be involved in the formation of new memories and learning (Tashiro, Makino, & Gage, 2007).

Dendritic Spines: A form of neuroplasticity

A specific form of non-synaptic neuroplasticity involves the formation, rearrangement, movement, and maturation of dendritic spines. Dendritic spines are membrane protrusions that extend outwards of the neuronal surface of dendrites (Tashiro et al., 2007). They consist of a head and a spine neck, which connects the head to the membrane of the dendrite (Tashiro et al., 2007). Dendritic spines were first discovered by Ramon y Cajal in the late 19th century and are found in all vertebrates and even some invertebrates (Cajal, 1888; Coss, Brandon, & Globus, 1980; Nassel & Strausfeld, 1982; Sarnat & Netsky, 1985). Although dendritic spines vary greatly in terms of their morphology, there are three main categories that are commonly used to describe them. Mushroom spines are characterized by a constricted spine neck, stubby spines are characterized by the absence of constriction between the head and the dendrite, and thin spines are characterized by a narrow neck and a small head (Tashiro et al., 2007). Irrespective of the type and category of dendritic spine, all of them are involved in the formation and regulation of excitatory synapses (Bourne & Harris, 2008). Research has also shown that increased levels of

excitatory synaptic activity can induce dendritic spine formation (Tashiro et al., 2007). Although it is under much debate and controversy, scientists believe that the specific function of dendritic spines is to provide a structural skeleton that acts as biochemical and electrical compartments (K. F. Lee, Soares, & Beique, 2012). In terms of their role as biochemical compartments, it is believed that dendritic spines function to compartmentalize signaling molecules such as calcium and other proteins involved in signaling cascades that are major components for the regulation of synaptic plasticity (Harvey, Yasuda, Zhong, & Svoboda, 2008; Noguchi, Matsuzaki, Ellis-Davies, & Kasai, 2005; Lee et al., 2012; Lee, Escobedo-Lozoya, Szatmari, & Yasuda, 2009; Yuste, Majewska, & Holthoff, 2000). In terms of the role of dendritic spines as electrical compartments, it is believed that they are capable of regulating the kinetics and integration of synaptic potentials (Lee et al., 2012). More specifically, research suggests that dendritic spines have a significant degree of charge accumulation (Lee et al., 2012). It is also believed that the differences in morphology of dendritic spines may influence the degree of biochemical and electrical compartmentalization (Lee et al., 2012; Tsay & Yuste, 2004). Dendritic spines have been recently implicated in several neurological disorders. Research involving neuropathology has demonstrated that a number of diseases, ranging from schizophrenia to autism spectrum disorder, display abnormal dendritic spine morphology and density (Srivastava, Woolfrey, & Penzes, 2011).

Dendritic spines have been shown to occur in virtually any part of the nervous system that contains excitatory connections within synapses. They are highly dynamic and can change shape as well as quantity (Srivastava et al., 2011). They have been shown to be critical for the processing and storage of information within the brain (Srivastava et al., 2011). Research conducted in animal models suggests that learning a new behavior is correlated with increased

dendritic spines in many areas of the brain (Lowndes & Stewart, 1994; O'Malley, O'Connell, Murphy, & Regan, 2000). Specifically, Lowndes and Stewart found that the brain of chicks that had successfully learned and retained a pecking task, had significantly higher numbers of dendritic spines in the majority of the brain areas examined (Lowndes & Stewart, 1994). Similarly, O'Malley and colleagues trained adult rats to successfully complete a water maze task and then analyzed several brain regions for increased dendritic spine density (O'Malley et al., 2000). They found that the rats had a significantly higher density of dendritic spines in many brain areas involved in spatial awareness. A similar result was obtained by Moser and colleagues (Moser, Trommald, & Andersen, 1994). Specifically, they found that spatially-trained rats had a significantly greater density of dendritic spines in the hippocampus as compared to spatially-untrained rats.

Research in rats has also shown that exposure to rewarding experiences increased dendritic spine density in multiple brain areas (Glasper et al., 2015; Stranahan, Khalil, & Gould, 2007). Glasper and colleagues demonstrated that exposing adult male rats to sexually receptive females once a day for an entire week lead to increased dendritic spine density in neurons of the prefrontal cortex and the dentate gyrus (Glasper et al., 2015). Similarly, Stranahan and colleagues demonstrated that long-term voluntary running, a rewarding experience, increases the density of dendritic spines in multiple areas of the brain (Stranahan et al., 2007). They showed that allowing rats to run on a running wheel for a period of two months lead to increased density of dendritic spines as well as changes in the morphology of the dendritic spines in the dentate gyrus and the medial temporal lobe of the adult rats. This research is significant to the current study, as it provides evidence of rewarding behaviors altering dendritic spine density, which the current research will be studying in Syrian hamsters.

Relatedly, recent research has demonstrated that exposure to drugs of abuse causes changes in the density and morphology of dendritic spines in multiple brain areas (Miller et al., 2012). Studies have shown that repeated and prolonged exposure to cocaine increases the density of dendritic spines in the medium spiny neurons of the NAc and the medial prefrontal cortex (Li, Acerbo, & Robinson, 2004). Shen and colleagues showed that rats injected with daily doses of cocaine had not only increased spine density but also increased spine diameter in the NAc compared to rats receiving daily saline injections (Shen et al., 2009). Similarly, Robinson and colleagues demonstrated that rats that were allowed to self administer cocaine for one hour a day for one month had significantly increased dendritic spine density in the medium spiny neurons of the shell of the NAc and in neurons of the prefrontal and parietal cortex when compared to rats that were not allowed to self administer cocaine (Robinson, Gorny, Mitton, & Kolb, 2001). Apart from cocaine, research has demonstrated that sustained use of other stimulants such as amphetamines also leads to increased spine density in the medium spiny neurons of the NAc and neurons in the medial prefrontal cortex (Robinson & Kolb, 1997; Russo et al., 2010). In contrast to most stimulants, research has demonstrated that prolonged use of opiates decreases the number and complexity of dendritic spines in the medium spiny neurons of the NAc, medial prefrontal cortex, and hippocampus (Robinson & Kolb, 2004; Russo et al., 2010). The neuroplastic effects of dendritic spines in structures of the brain such as the NAc that occur in response to drugs of abuse are believed to play a central role in the addictive effects of these drugs (Robinson et al., 2001). Dendritic spines thus serve an important role in learning new tasks and are involved in rewarding experiences such as running, sexual behavior, and drug usage.

Delta FosB: A molecular marker for changes in dendritic spines

Molecular mechanisms underlie all of the different forms of neuroplasticity. These molecular mechanisms are mostly comprised of proteins that interact with genes and other proteins that are involved in signaling cascades (Breedlove, 2013). One such protein that is part of the molecular mechanism behind dendritic spine neuroplasticity is delta FosB. Delta FosB is a member of the Fos family of transcription factors that combine with Jun family proteins to form an activated complex called Activator protein 1 (AP-1) that, in turn, controls the transcription of specific genes (Nestler, Barrot, & Self, 2001). It is believed that delta FosB becomes active in neurons of the NAc in response to increased dopaminergic transmission to the neurons of the NAc (Hilton, 2013; Nace & Tinsley, 2007). As stated earlier, this increase in dopaminergic transmission to neurons in the NAc occurs in response to rewarding experiences. Thus, increases in delta FosB activation should be correlated to rewarding experiences. Recent research has demonstrated that expression of delta FosB modified dendritic spine morphology and density in the NAc (Grueter, Robison, Neve, Nestler, & Malenka, 2013). Specifically, elevated levels of delta FosB lead to an increase in the number of dendritic spines in the NAc (Nestler et al., 2001). The hypothesized mechanism by which delta FosB increases the density of dendritic spines is: the activated version of delta FosB (AP-1) activates specific genes that in turn express proteins which account for the changes of dendritic spines (Nace & Tinsley, 2007).

Research has demonstrated that acute and prolonged use of virtually every drug of abuse induces delta FosB expression in multiple brain areas but most prominently in the NAc and the dorsal striatum (Hope, Kosofsky, Hyman, & Nestler, 1992; Nestler, 2008; Graybiel, Moratalla, & Robertson, 1990). Hope and colleagues demonstrated that the NAc and the striatum of rats showed a long lasting increase in delta FosB and the activated form of delta FosB (AP-1) after

exposure to a chronic cocaine treatment (Hope et al., 1994). Similarly, Pich and colleagues found that rats that were allowed to self administer intravenous cocaine and nicotine had greater levels of delta FosB expression in the shell and the core of the NAc than rats that were not exposed to the drugs (Pich et al., 1997). Also, McDaid and colleagues demonstrated that injecting rats 5 times a day with methamphetamine resulted in increased levels of delta FosB in the NAc of the rats (McDaid, Graham, & Napier, 2006). Research has demonstrated that this increase in delta FosB expression in response to drugs of abuse is one of the main contributors to the addictive phenotype or behavior that characterizes these drugs (Nestler et al., 2001; Whisler, Kelz, Chen, Nestler, & Self, 1999). Recent data has indicated that delta FosB increases an animal's sensitivity to the majority of drugs of abuse (Nestler et al., 2001). Studies in rats and mice have also shown that delta FosB produces changes in qualitative behavior that in turn promote drug-seeking behavior (Nestler et al., 2001; Whisler et al., 1999).

Apart from its central role with drugs of abuse, expression of delta FosB has also been shown to increase in response to natural rewarding behaviors. Studies involving rats have demonstrated that exposure to a diet high in fat increased the expression of delta FosB in the NAc (Teegarden & Bale, 2007). Also, Wallace and colleagues showed that giving rats a diet high in sucrose for ten days led to an increase in expression of delta FosB in the NAc (Wallace et al., 2008). The same researchers also demonstrated that sexual behavior, defined by allowing rats to mate for 14 sessions, led to greater levels of delta FosB in the NAc (Wallace et al., 2008). Similarly, Pitchers and colleagues showed that repeated sexual experiences caused increased delta FosB expression in the NAc and other limbic brain regions of male rats (Pitchers et al., 2010). Wallace and colleagues also determined that the greater the levels of delta FosB in the NAc of rats, the greater the sucrose intake of the rats. The researchers also showed that rats with

greater levels of delta FosB in the NAc displayed enhanced sexual behavior, defined by a significant decrease in the time required to reach ejaculation, than rats with lower levels of delta FosB in the NAc (Wallace et al., 2008). This enhanced sexual behavior was shown to be attenuated when expression of delta JunD, a negative binding partner of delta FosB which prevents delta FosB from becoming active, in the NAc increased (Pitchers et al., 2010). These results, along with the results implicated earlier with drugs of addiction, suggest the possibility that delta FosB plays an important role in not only the reinforcements effects of rewarding experiences, but also in mediating and promoting behavior, specifically reward inducing behavior (Pitchers et al., 2010).

One of the main goals of the current research is to investigate if repeated exposure to sexual experiences leads to neuroplasticity in the NAc. One of the ways neuroplasticity can be measured is by changes in dendritic spine density in the medium spiny neurons of the NAc. However, quantifying dendritic spines and identifying differences in dendritic spine density is a very complicated and tedious procedure that requires technology that will not be available to the researchers of the current study. On the other hand, quantifying and identifying differences in levels of delta FosB require a relatively simple procedure. Because increased levels of delta FosB is associated with increased dendritic spine density, the authors of the current study can use this simple procedure as a baseline way of identifying neuroplastic changes in the NAc of these rodents. In addition to giving the researchers an easier way to quantify dendritic spine density changes, quantifying changes in delta FosB levels will also allow the researchers to begin identifying the molecular signaling components underlying this structural change in the NAc.

Sex differences?

Most of the research involving neuroplasticity and brain differences in animal models rarely use both sexes to test their hypotheses; most studies use either male or female animal subjects, but rarely use and compare both sexes. Thus, there is very little data regarding sex differences involved in the neuroplasticity in response to drugs of abuse and rewarding experiences in general. For example, in terms of neuroplasticity, there is only one study that specifically examines sex differences. The study, led by Shors, found that stress had opposite effects on dendritic spines in the hippocampus of male and female rats (Shors, Falduto, & Leuner, 2004). The current research aims to shed light on differences in neuroplasticity between sexes. This could lead to a better understanding of differences in the reward pathway between sexes and therefore possible sex-dependent differences in addiction. If differences are found, it could suggest that male and female brains react differently in response to rewarding behavior. It could also suggest that addiction is different between men and women, which could lead to the development of different treatments for addiction between men and women. On the other hand, if no differences are found, it would indicate that male and female brains react and change in the same ways to rewarding behaviors. This would disprove any doubt whether men or women are more susceptible to addiction due to how their brains react to rewarding behaviors.

Improvements in mating behavior

The process of mating in most species of animals is a complex behavior that relies on environmental and physiological factors (Saleem, Ruggles, Abbott, & Carney, 2014). In Syrian hamsters for example, successful mating involves detecting and interpreting chemosensory cues such as pheromones of the opposite sex, as well as being hormonally primed for sexual behavior (Wood & Newman, 1995). As with most complex behaviors, practice or prior experience is

expected to lead to an improvement in mating behavior. This, along with the evolutionary pressures that exist, including the need to pass on your genes effectively to continue your genetic line, leading to competition among mating suitors, leads researchers to expect that prior sexual experience or multiple exposures to mating experiences would lead to improvements in mating behaviors. However, research into this topic has obtained mostly mixed and inconsistent results. For example, both Hedges and colleagues and Bradley found that prior sexual experience led to increased mating efficiency in female Syrian hamsters (Bradley, 2003; Hedges et al., 2009). In others studies, however, the same group of researchers found that prior sexual experience did not lead to improvements in mating behavior in female Syrian hamsters (Bradley & Meisel, 2001; Bradley et al., 2005). Therefore, a secondary goal of the current research is to potentially clarify our understanding of whether male and female Syrian hamsters improve their mating behavior by calculating and monitoring the efficiency by which male and female hamsters mate as they gain sex experience.

Hypothesis and predicted results

The present research has the primary goals of understanding the changes in brain circuitry that occur after exposure to sexual experiences, a naturally motivating behavior, and identifying the possible sex differences involved. It is important to study the changes in brain circuitry or the neuroplasticity involved in response to naturally rewarding experiences for two main reasons. The first is that there is relatively little research regarding the changes that occur in the brain in response to normal rewarding experiences. The second is that understanding the changes that occur in the brain in response to normal rewarding experiences will lead to a better understanding of how drugs of abuse take advantage of these brain regions and reward pathways, and could eventually lead to better treatments for addiction. Identifying the possible sex

differences involved in how the brain changes in response to rewarding experiences is paramount mainly because there is very little data regarding the subject. The main hypothesis of the current research is that delta FosB influences neural plasticity in the NAc following sex experience. Two major predictions arise from this main hypothesis. The first is that if delta FosB plays a critical part of the molecular pathway underlying neuroplasticity in the NAc following sexual experiences, then sexual experiences should lead to an up-regulation of delta FosB in the NAc. The second is that if the reward pathway in male and female brains react similarly to sexual experiences, then the predicted up-regulation of delta FosB should not be significantly different between the sexes.

The secondary goal of the current research is to understand if male and female Syrian hamsters improve their mating behavior following sexual experience. This is important to investigate because there is contradicting evidence in the literature regarding whether Syrian hamsters improve their mating efficiency following sex experiences. The secondary hypothesis of the current research is that male and female Syrian hamsters improve their mating behavior after exposure to sexual experiences. The researchers predict that if hamsters are allowed to have prior sexual experiences, then male and female hamsters will increase their mating efficiency by spending more time actively having sex and performing more sex-related behaviors.

Methods

Subjects

A total of 32 Syrian Hamsters (*Mesocricetus auratus)* that were acquired from Charles River Laboratories (Wilmington, Massachusetts) were used throughout the experimental procedure. Half of the total subjects, or 16 hamsters, were males, while the other half were females. All of the hamsters were housed individually in plastic rat cages, which are larger than

typical hamster cages, and contained aspen bedding. The hamsters had constant availability of food and water, and were kept in the Haverford College vivarium under a reverse light/dark circadian schedule. Housing the hamsters individually in cages likely did not lead to emotional or psychological strain on the animals because Syrian hamsters, unlike mice and rats, are strictly solitary animals (Meisel, Hays, Del Paine, & Luttrell, 1990; Zimmer & Gattermann, 1996). A reverse light/dark circadian schedule meant that the hamsters were placed under light during nighttime and under darkness during the daytime. The reason behind this reversed schedule is that Syrian hamsters are nocturnal animals, and thus are most active during the night when it is dark. Having them on a reverse schedule allowed for procedures to be conducted during the day when the hamsters were most active. The vivarium had a constant temperature ranging from 68-72 degrees Fahrenheit. This temperature falls under the recommended housing temperature range for Syrian hamsters ("Hamsters: biology, care, diseases & models," n.d.; L. Been, 2015). All animal procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Many of the procedures were adapted from the studies: *Metabotropic glutamate receptor and fragile X signaling in a female model of escalated aggression* and *Cell-type specific increases in female hamster nucleus accumbens spine density following female sexual experience* (L. E. Been, Moore, Kennedy, & Meisel, 2015; Staffend, Hedges, Chemel, Watts, & Meisel, 2014).

Design

The different procedures that were conducted in order to test the hypothesis include: an ovariectomy surgery where the ovaries of female hamsters were removed, injections of gonadal hormones to prime the female hamsters for sexual behavior, several weeks of sexual experiences, subject sacrifice via anesthetized rapid decapitation, obtaining brain samples via tissue punches,

processing of tissue samples for analysis, and analyzing tissue samples via Western Blotting technique. The procedures were conducted in the order presented above. The reason behind Ovariectomy was to prevent female hamsters from becoming pregnant, thus allowing them to be exposed to multiple weeks of repeated sexual experiences. However, because the ovaries are the main source of gonadal hormones in female Syrian hamsters, and these hormones are largely responsible for sexual behavior in the hamsters, ovariectomy would have resulted in decreased sexual behavior (Been, 2015). Therefore, in order to allow for multiple sexual experiences to occur, it was necessary to inject the female hamsters with gonadal hormones before the experiences. These sexual experiences entailed hormone-primed females undergoing one ten-minute sexual encounter with a male hamster every week for six weeks, as research has demonstrated that having hamsters undergo exactly six weeks of sexual experiences is sufficient to induce prolonged increases in dopamine, expression of transcription factors such as c-Fos and delta FosB, and spine density in the NAc of Syrian Hamsters (Bradley & Meisel, 2001; Kohlert & Meisel, 1999; Staffend et al., 2014)**.**

Anesthetized rapid decapitation allowed for a quick procedure that provided relatively unrestricted access to the NAc and other control brain regions. Tissue samples from the NAc and the caudate nucleus were taken using the tissue punches method, which allowed for the extraction of diminutive amounts of brain tissue from specific locations in the brain (Leica, 2015). After the obtained tissue samples were processed, they were then analyzed using Western Blotting. Western Blot is a technique used to detect, separate, and quantify specific proteins in a sample. In the context of the current research, the technique was used to detect and quantify the amount of delta FosB found in the tissue samples. The reason for utilizing Western blotting instead of other protein detection and quantifying methods, such as immunohistochemistry, was

that Western blotting is a more quantitative and a more sensitive technique than most other protein quantifying methods ("Antibodies, Proteins, Kits and Reagents for Life Science", 2016; L. Been, 2015; Ricks, 2009).

Throughout the entire experimental procedure, there were 4 different groups of hamsters, comprised of male control, male experimental, female control, and female experimental groups. All female hamsters underwent the ovariectomy surgery as well as received all of the gonadal hormonal injections. Both male and female experimental groups underwent 6 sexual experiences, while both male and female control groups had no sexual experiences. Similarly, although both of the male groups did not undergo the ovariectomy surgery as well as receive the gonadal hormonal injections, the difference between the male control group and the male experimental group was that the control group did not undergo any sexual experiences while the experimental group underwent all of the sexual experiences. The reason for controlling for sexual experiences goes back to our hypothesis that sexual behavior will induce neural changes. Therefore, it is important that any perceived neural changes can be attributed to the sexual experience, instead of some other behavior or experience. Thus, we gave each rodent in the experimental condition exactly 6 sexual experiences, and each rodent in the control experience no sexual experiences. The hamsters in all of the 4 groups were then sacrificed and samples of their brains were collected, processed, and analyzed. The results obtained from the different groups were then compared against each other. Each group contained 8 Syrian hamsters. Having 8 hamsters per group provided the necessary statistical power to detect significant differences between groups while still allowing for variability among the groups. Although power analysis was not run in the current research, the specific number of hamsters per group was based on previous studies that

have demonstrated its statistical power as well as on the past experiences of Professor Been (L. E. Been et al., 2015; L. Been, 2015).

Apart from controlling for sexual experiences, the current research also controlled for brain anatomical specificity. It was important to assure that any observed changes in delta FosB levels were specific to areas of the brain that are associated with the reward pathways, such as the NAc. If these changes would have been found in unrelated areas, it would have suggested that the differences occurred throughout the brain and were likely not based on the rewarding sexual experiences. Anatomical specificity was measured by collecting and analyzing tissue punches from both the target structure, the NAc, as well as a control area, the caudate. The caudate is a brain structure that forms part of the basal ganglia. It is part of the nigrostriatal brain pathway and is believed to be involved in body posture, accuracy and speed of movements, and approach-attachment behaviors (Villablanca, 2010). Like the NAc, the caudate also contains medium spiny neurons that express dopamine receptors (Purves, Augustine, & Fitzpatrick, 2001). However, unlike the NAc, the caudate is not part of the mesolimbic pathway and is not believed to be directly involved in rewarding and addictive behaviors (Bohbot, Del Balso, Conrad, Konishi, & Leyton, 2013). Thus, the caudate served as a suitable control brain region since it is functionally different from the NAc, yet anatomically and biologically similar.

Procedure

Ovariectomy. The hamster's weight was recorded along with the date of surgery, type of surgery, and date of birth of the hamster. The hamster was anesthetized in a gas sealed chamber under 5% isoflurane in oxygen. Once the hamster was completely under the effects of the anesthesia, which occurred when the hamster was breathing slowly and no longer moving, it was quickly transported to the shaving station. The flanks of the hamster, or the sides of the area

where the rib cage meets the spine, were shaven. (The ovaries of the hamster are located under the flanks). Throughout the rest of the procedure, standard aseptic surgery technique was used.

The hamster was then transferred to the surgical area and placed faced down on a sterile drape. A nose cone tube, that contained anesthesia flowing from it, was placed in the mouth and nose area of the hamster to prevent the hamster from becoming conscious throughout the remainder of the surgery. The anesthesia was then turned down to 2.5% isoflurane in oxygen. Throughout the rest of the procedure, the breathing rate of the hamster was periodically examined. If the breathing rate increased at any point, then the anesthesia was increased to 3% and the procedure was stopped until it slowed again. To make sure that the hamster was completely unresponsive, a toe of the hamster was pinched. If no response by the hamster was observed, then the surgery was continued. However, if a reaction was observed in response to the pinching, then the surgery was stopped and the anesthesia was increased until the hamster was completely unresponsive to the pinching.

Once the hamster was unresponsive, 3 alternating scrubs of 70% ethanol and betadine were performed on each of the shaved areas to disinfect the area and prevent bacteria and other pathogens from entering the wound site. The hamster was then given a subcutaneous injection of an opioid derivative (0.1cc Butorphanol), as well as two other subcutaneous injections of a local anesthetic (0.5cc of Bupiviacaine) to each of the shaven areas where the incisions were performed. The hamster was then covered with a sterile drape. Using a surgery scissor, a small incision of the skin on one of the shaven areas was performed and the skin was then stretched using forceps. Then another small incision was performed through the muscle to expose the fat pad, which contained the ovaries attached to it. The fat pad was gently pulled out through the muscle wall and the ovary was removed by clamping the fat pad with a heated hemostat. The

heated hemostat cauterized the area right outside the ovary and separated the ovary from the fat pad. A scalpel was then used to completely disconnect the ovary from the fat pad. The fat pad was placed back inside the body of the hamster and the muscle wall was sutured with an absorbable suture. The skin was then stapled with a surgical stapler. The same procedure was then repeated on the other shaven side to remove the second ovary.

After both ovaries were removed and the skin on both sides was stapled, the anesthesia was turned off. The hamster was then placed, still covered with the drape, back in its cage. The hamster woke up around a minute after being placed back in the cage. The hamster was checked every 15 minutes in the hour following surgery to monitor for signs of pain and discomfort. The hamster was given opiate injections (0.1cc Butorphanol) once a day for 3 days for post-surgical pain management, and was monitored every day following surgery for signs of pain, discomfort, or infection (L. E. Been et al., 2015; Staffend et al., 2014).

Gonadal hormones injections cycles. Before testing for mating behavior, ovariectomized female hamsters were given a subcutaneous injection of estrogen (10ug/0.1ml dissolved in cotton seed oil) at 48 hours and again at 24 hours prior to the sexual experience. Also, at 4 hours prior to the sexual experience, another subcutaneous injection of progesterone (500ug/0.1ml dissolved in cotton seed oil) was given to the ovariectomized female hamsters. These injections, over the course of two days, had the purpose of mimicking the hormonal effects that occur during ovulation in female Syrian hamsters. This procedure was repeated before every sexual experience (L. E. Been et al., 2015; L. Been, 2015; Staffend et al., 2014).

Sexual experiences and mating behavior analysis. A male hamster was removed from his cage and placed in a cage containing a female hamster. A ten-minute timer was started at the moment when the male hamster was placed in the female hamster's cage. The entire ten-minute

sexual experience was recorded on a video camera. Once the ten-minute timer went off, the male hamster was removed from the female hamster's cage and placed back in his cage. To score the sexual experience, a stopwatch was started every time the female hamster entered in lordosis, which is the position of sexual receptivity for female hamsters characterized by the arching of the spine. The stopwatch was stopped when the female hamster switched out of lordosis but was continued every time she went back into the lordosis position. Also, the number of mounts the male hamster performed was recorded, as well as the number of intromissions and ejaculations. Mounts were defined as the physical act of the male getting on top of the female accompanied by rapid pelvic thrusting. Intromissions, or the insertion of the penis, were characterized by the thrusting behavior of the male hamster, while ejaculations were characterized by a latency period of around 1 minute after an intromission, when the male did not continue to mount. The time the female hamster spent in lordosis (lordosis duration) was recorded, along with the latency period it took the female hamster to display lordosis. This exact procedure was repeated once a week for the six weeks of sexual experiences. Improvement in mating efficiency were calculated by measuring differences in lordosis duration and latency and number of mounts, intromissions, and ejaculations across six weeks of sexual experiences. Any hamster that did not display sexual behavior during the experiences or showed very different behavior compared to the majority of the hamsters was removed from the analysis and the remainder of the experiment (L. E. Been et al., 2015; Staffend et al., 2014).

Anesthetized rapid decapitation. After the final sexual experience, all animals were given an overdose of isoflurane (5% isoflurane in oxygen). Once the animals were suspected to be completely under the effects of the anesthetizing agent, which was noted by the slow breathing rate, a toe of the hamsters was strongly pinched using tweezers. If there was no

response or movement from the animal, then the hamster was decapitated using a guillotine. The brain of the hamster was then separated from the skull and tissue punches were taken (L. E. Been et al., 2015).

Tissue punches. 1-mm thick coronal sections containing the brain regions of the NAc and the caudate were taken from the brain of the hamsters. Bilateral tissue punches (1-mm in diameter) were then immediately collected from the two brain regions. Tissue punches of the NAc contained both the core and shell of the NAc. Tissue punches of the caudate contained the dorsal medial caudate. All of the punches collected from the NAc and the caudate were flash-frozen for Western blotting (L. E. Been et al., 2015).

Western blotting. Samples of tissue from the tissue punches were homogenized in 1% sodium dodecyl sulfate processing buffer. Then the delta FosB protein was quantified using the Bio-Rad protein DC assay. The total amount of protein was then loaded into different wells of a 12% to 15% polyacrylamide gradient gel and was transferred to a nitrocellulose membrane. The membrane was then blocked in 5% nonfat dried milk in Tris-buffered saline. The membrane was then incubated overnight in a primary antibody against delta FosB. The next day, the membrane was incubated for 1 hour with a secondary antibody against the primary antibody conjugated to horseradish peroxidase. The blot was then visualized using chemiluminescent detection, imaged, and optical density was quantified using FluorChem HD software. In order to calculate the levels of delta FosB across the membrane, the delta FosB protein levels first had to be normalized to a loading control. Normalizing was accomplished by dividing the delta FosB levels by levels of a control protein whose expression was not expected to change throughout the experimental procedure. This allowed the researchers to ensure that changes visualized in delta FosB levels between the different experimental conditions were actual changes in protein expression and not

variations in the amount of overall protein sample that was added across the membrane. The loading control that was used was Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a protein that is found in most cells and plays significant roles in glycolysis, transcription, apoptosis, DNA replication, and RNA transport. Since GAPDH is involved in many important everyday cellular mechanisms, it is expressed at high and relatively constant levels (Kinoshita, 2013). These properties made GAPDH a suitable loading control (Staffend et al., 2014; L. E. Been et al., 2015).

Statistical analysis. To analyze significant differences in protein levels (specifically the delta FosB levels) between sexually experienced and sexually naive groups, one-way ANOVAs, $p<.05$, were used. Similarly, to analyze significant differences in protein levels between males and females of the same experimental group, one-way ANOVAs, $p<.05$, were used (L. E. Been et al., 2015). Finally, to analyze significant differences in mating behavior between the different weeks of sexual experiences, repeated measures one-way ANOVAs, $p<.05$, were used.

Results

Mating Behavior

There was no significant difference in mean lordosis duration across the 6 weeks of sexual experiences, $F(1, 33)=1.70$, $p=.17$ (Figure 1). Similarly, the results also showed that there was no significant difference in mean lordosis latency across the 6 weeks of sexual experiences, $F(1, 33)=2.17$, $p=.20$ (Figure 2).

There was no significant difference in the mean number of mounts across the 6 weeks of sexual experiences, $F(1, 36)=.10$, $p=.99$ (Figure 3). Similarly, the results demonstrated that there was no significant difference in the mean number of intromissions across the 6 weeks of sexual experiences, $F(1, 36)=.32$, $p=.90$ (Figure 4). The results also showed that there was no

significant difference in the mean number of ejaculations across the 6 weeks of sexual experiences, $F(1, 36)=2.01$, $p=.16$ (Figure 5).

Taken as a whole, the results indicate that the sexual behavior of hamsters did not significantly change across the 6 weeks of sexual experiences. Thus, the hypothesis that if hamsters are allowed to have prior sexual experiences, then male and female hamsters will increase their mating efficiency by spending more time actively having sex and performing more sex-related behaviors was not supported.

Delta FosB levels

NAc vs caudate comparisons. There was a significant difference in mean delta FosB levels in the NAc between male hamsters that received sexual experiences and male hamsters that were sexually naïve, $F(1, 4)=25.20$, $p=.04$. Specifically, the results showed that the sexually experienced males ($M=.19$, $SD=.07$) had significantly higher mean delta FosB levels in the NAc than sexually naïve males ($M=.55$, $SD=.07$) (Figures 6, 14, and 15). The results also demonstrated that there was no significant difference in mean delta FosB levels in the caudate between sexually experienced males ($M=.40$, $SD=.29$) and sexually naïve males ($M=.22$, $SD=.11$), $F(1, 8)=.98$, $p=.36$ (Figure 7). Similarly, the results for the female hamsters revealed that there was a significant difference in mean delta FosB levels in the NAc between hamsters that received sexual experiences and hamsters that were sexually naïve, $F(1, 12)=21.43$, $p=.001$. Specifically, the results showed that the sexually experienced females ($M=.65$, $SD=.11$) had significantly higher mean delta FosB levels in the NAc than sexually naïve females ($M=.34$, $SD=.12$) (Figure 8). The results also demonstrated that there was no significant difference in mean delta FosB levels in the caudate between sexually experienced females ($M=.45$, $SD=.21$) and sexually naïve females ($M=.35$, $SD=.17$), $F(1, 12)=.85$, $p=.38$ (Figure 9). Taken as a whole,

the results indicate that male and female hamsters that received 6 weeks of sexual experiences had significantly greater levels of delta FosB in the NAc than male and female hamsters that did not receive the 6 weeks of sexual experiences and thus were sexually naïve. Therefore, sexual experience led to an increase in delta FosB expression in the NAc of both male and female hamsters. The results also indicate that there was no significant difference in delta FosB levels in the caudate between male and female hamsters that received 6 weeks of sexual experiences and male and female hamsters that were sexually naïve. Thus, sexual experience did not affect the expression of delta FosB in the caudate of both male and female hamsters. The hypothesis that if delta FosB plays a critical part of the molecular pathway underlying neuroplasticity in the NAc following sexual experiences, then sexual experiences should lead to an up-regulation of delta FosB in the NAc was thus supported.

Female vs male comparisons. There was no significant difference in mean delta FosB levels in the NAc between sexually experienced male (M=.55, SD=.07) and female (M=.65, SD=.11) hamsters, $F(1, 8)=1.51$, p=.27 (Figure 10). Similarly, the results demonstrated that there was no significant difference in mean delta FosB levels in the caudate between sexually experienced males (M=.40, SD=.29) and females (M=.45, SD=.21), $F(1, 11)=.11$, p=.75 (Figure 11). The results also showed that there was no significant difference in mean delta FosB levels in the NAc between sexually naive males (M=.19, SD=.07) and females (M=.34, SD=.12), $F(1, 8)=2.71$, p=.15 (Figure 12). Finally, the results revealed that there was no significant difference in mean delta FosB levels in the caudate between sexually naive males (M=.22, SD=.11) and females (M=.35, SD=.17), $F(1, 9)=1.33$, p=.29 (Figure 13). Taken as a whole, the results indicate that there was no significant difference in delta FosB levels in the caudate and the NAc between males and females. Therefore, the expression of delta FosB in the NAc and the caudate was not

significantly different between male and female hamsters. Thus, the hypothesis that if the reward pathway in male and female brains react similarly to sexual experiences, then the predicted up-regulation of delta FosB should not be significantly different between the sexes was also supported.

Discussion

The current study was the first to examine the neural changes at the molecular level that occur in the NAc of both male and female Syrian hamsters in response to repeated sexual experiences utilizing sensitive protein quantifying techniques. We found that repeated exposure to sexual experiences led to an up-regulation of delta FosB in the NAc of both male and female hamsters. This was evident since male and female hamsters that were sexually experienced had significantly greater levels of delta FosB in the NAc compared to male and female hamsters that were sexually naïve. Therefore, our first prediction that delta FosB plays a critical part of the molecular pathway underlying neuroplasticity in the NAc following sexual experiences was supported. In addition, the current research was also the first to specifically study sex differences in the neural changes involved in the NAc in response to repeated sexual experiences. We found that a similar neurological response to sexual experiences in the NAc likely exists between sexes. This was evident since sexually experienced males and females did not have significantly different levels of delta FosB in the NAc. Thus, our second prediction that the reward pathway in male and female brains react similarly to sexual experiences was supported. The current investigation also examined differences in mating behavior of both male and female hamsters across the 6 weeks of sexual experiences. We found that male and female hamsters did not improve their mating behavior across the 6 weeks. This was evident since the mating behavior (lordosis duration and latency and number of mounts, intromissions, and ejaculations) of the

hamsters did not significantly differ between the six weeks of sexual experiences. Therefore, the prediction that male and female Syrian hamsters improve their mating behavior after exposure to sexual experiences was not supported.

Delta FosB up-regulation in the NAc following sexual experiences

Both male and female hamsters that were exposed to 6 weeks of sexual experiences had significantly higher levels of delta FosB in the NAc than hamsters that were sexually naïve. Considering that the only difference between the sexually experienced and naïve groups of hamsters was the exposure to sexual experiences, this result suggests that six weeks of sexual experiences lead to an up-regulation of delta FosB in the NAc of both male and female hamsters. This finding is consistent with the current literature, which indicates that expression of delta FosB in the NAc is increased in response to sexual experiences. In male rats for example, Wallace and colleagues demonstrated that 8 to 10 weeks of sexual experiences led to significant increases in delta FosB levels in the core and shell regions of the NAc (Wallace et al., 2008). Similarly, Pitchers and colleagues showed that repeated exposure to sexual experiences led to an accumulation of delta FosB in the NAc of adult male Sprague Dawley rats (Pitchers et al., 2010). In female Syrian hamsters, Meisel and Mullins found that there were higher levels of delta FosB/FosB staining in the core of the NAc of sexually experienced females compared to sexually naïve females, thereby demonstrating that sexual experience led to increases in delta FosB expression in the NAc (Hedges et al., 2009; Meisel & Mullins, 2006). Since delta FosB is a biomarker for neuroplasticity, this result also suggests that repeated exposure to sexual experiences leads to neuroplastic changes in the NAc of male and female hamsters. This is consistent with the current literature which implies that exposure to sexual experiences leads to

neuroplastic changes, in the form of dendritic spine density, in the core and shell regions of the NAc (Meisel and Mullins, 2006).

While the current research corroborates previous research, it should be noted that it was the first to investigate the effect that repeated sexual experiences had on the expression of delta FosB in the NAc of both male and female Syrian hamsters while utilizing western blotting technique. Utilizing both sexes in the experimental procedure is imperative not only because there might be underlying differences between males and females that would not be identified by research only looking at males or females, but also because there are very few studies involving neuroplasticity and brain differences in animal models that have used both sexes to test their hypotheses. This research also differed from the studies mentioned above in the use of western blotting. Western blotting technique, in contrast to immunohistochemistry, allows for the detection of very small amounts of protein down to the nano-molar level. Western blotting also allows for an easier interpretation of background. In immunohistochemistry, it is challenging to differentiate between background and weak levels of protein staining. Thus, western blotting is a more quantitative and sensitive technique than immunohistochemistry, and therefore allowed for more precise results to be obtained in this study than in the studies mentioned above ("Antibodies, Proteins, Kits and Reagents for Life Science", 2016; L. Been, 2015; Ricks, 2009).

Caudate is functionally different from the NAc

Both male and female hamsters that were sexually experienced did not have significantly different levels of delta FosB in the caudate than males and females that were sexually naïve. Again, since the only difference between the sexually naïve and experienced groups was the exposure to sexual experiences, this result suggests that the six weeks of sexual experiences did not lead to an up-regulation of delta FosB in the caudate of either male or female hamsters.

Considering delta FosB is a marker for neuroplasticity, this result also suggests that exposure to sexual experiences does not lead to neuroplastic changes in the caudate of male and female Syrian hamsters. This finding is consistent with the literature which indicates that exposure to sexual experience does not lead to neuroplastic changes in the caudate region of the brain (Meisel and Mullins, 2006).

The caudate forms part of the basal ganglia and the nigrostriatal brain pathway. This pathway is involved in movement coordinated behaviors such as maintaining body posture and controlling the accuracy of movements (Villablanca, 2010). The caudate is very anatomically and biologically similar to the NAc since it contains mostly medium spiny neurons that express dopamine receptors (Purves, Augustine, & Fitzpatrick, 2001). However, the caudate is considered functionally distinct to the NAc, since, unlike the NAc, it is not involved in rewarding behaviors and is not part of the mesolimbic pathway (Bohbot, Del Balso, Conrad, Konishi, & Leyton, 2013). The caudate was thus a suitable control region since it is anatomically and biologically comparable to the NAc but it is very functionally dissimilar. Therefore, any changes observed in the NAc in response to rewarding behaviors, should not correlate with any changes in the caudate. Consequently, the result that exposure to sexual experiences did not lead to changes in delta FosB levels in the caudate not only validates the use of the caudate as the control region for anatomical specificity in the current study, but also provides confidence that the observed changes in delta FosB in the NAc between sexually experienced and naïve hamsters were only associated with reward pathways and were not occurring throughout the brain. This suggests that the observed changes in delta FosB are more likely based on the rewarding nature of sexual experiences, and that sexual experiences themselves do not lead to neuroplastic changes throughout the brain globally.

Up-regulation of delta FosB is analogous between males and females

Sexually experienced males and females, as well as sexually naïve males and females, did not differ from each other in levels of delta FosB in the NAc and the caudate. This result, obtained in the first study to specifically examine sex differences in delta FosB levels in the NAc following sexual experiences, suggests that the brain and specifically the reward pathways of male and female Syrian hamsters change and react in the same ways to rewarding experiences and behaviors. In fact, this result suggests that the mechanism leading to neural changes in the NAc in response to rewarding experiences may be highly conserved between males and females and thus is the same between sexes.

This result, although expected, could be surprising for multiple reasons. The first is that male and female Syrian hamsters perform very dissimilar behaviors during a sexual experience. Specifically, males are very active, as they mount and thrust the females and groom themselves, while females are relatively immobile throughout the entire experience. This dissimilarity in mating behavior could lead researchers to assume that different levels of neuroplastic changes would exist between male and female Syrian hamsters. The result could also be surprising because the only study available in the literature that examined neuroplastic changes between males and females found that there were differences in the neuroplastic changes that occurred in the hippocampi of male and female rats following stressful experiences (Shors, Falduto, & Leuner, 2004). Based on this finding, researchers may assume that differences in neuroplastic changes between males and females exist throughout the brain of animals.

Although the result that there were no differences in a marker of neuroplastic changes in males and females in response to sexual experiences was expected, several alternative explanations for this result could exist. One possibility could be that there are different

mechanisms or slightly different pathways that lead to delta FosB expression between males and females, but the end result, which is the increased expression of delta FosB, remains the same between sexes. Therefore, since the current investigation only examined the end result, levels of delta FosB, it would have been impossible to notice these differences. Another possibility could be that males and females increase the expression of delta FosB over different time courses. For example, the mechanisms in one sex may lead to rapid increases in delta FosB expression at an early time point but an eventual plateau later on, while in the other sex may be characterized by an incremental up-regulation of expression of delta FosB. However, at a later time point, the levels of delta FosB in the NAc of both males and females are the same. Therefore, since the current investigation only examined differences in delta FosB levels at the end of the 6 weeks of sexual experiences, it would not have been possible to observe this sort of difference.

Syrian hamsters do not improve the efficiency of their mating behavior

In the mating behavior analysis, males did not significantly differ in the number of mounts, intromissions, and ejaculations, while females did not significantly differ in the duration and latency of lordosis, across the 6 weeks of sexual experiences. This result suggests that both male and female hamsters did not significantly improve their mating efficiency between the six weeks of sexual experiences. One alternate explanation for this lack of improvement in mating efficiency could be that 6 weeks of sexual behavior is not sufficient to produce noticeable and quantifiable improvements in mating behavior. Although this result was not expected, other studies such as the Bradley and Meisel 2001 study have obtained similar results (Bradley and Meisel, 2001). In their study, the researchers exposed female Syrian hamsters to 6 weeks of sexual experiences and compared the average lordosis durations between the first and the last week of the sexual experiences. They found that sexual experience did not significantly affect

the duration of lordosis. Similarly, in another study, Bradley and colleagues compared the lordosis latencies and durations between female Syrian hamsters that had prior sexual experience and female Syrian hamsters that did not have prior sexual experience (Bradley et al., 2004). The researchers found that sexual experience did not significantly affect the latency of lordosis, however, they also found that sexual experience actually resulted in a slightly shorter (although significant) lordosis duration compared to no prior sexual experience. The results of the current study, along with the results of the Bradley and Meisel 2001 study and the Bradley and colleagues study, suggest that Syrian hamsters do not improve the efficiency of their mating behavior.

Weaknesses and Limitations

Despite its many interesting results, this study had several weaknesses and limitations. The first of these was the relatively small sample size. There were only 32 hamsters studied in this investigation, which limits the researcher's ability to account for variability within the research subjects. Having such a small sample size also allows for outliers and confounds to have a greater influence on the acquired data, thereby decreasing the reliability of the results obtained. The second was that during the experimental procedure, tissue punches included both the core and shell regions of the NAc. Both regions were thus grouped together into the analysis of the data and it was not possible to investigate molecular differences in response to sexual experiences between the core and the shell regions. Our results are therefore generalized to the entire NAc, and not specific to the regions within the NAc. The possibility exists that the up-regulation of delta FosB in response to sexual experiences that was evident in the current research was limited to only the core or the shell of the NAc. Unfortunately, this question remains unanswered.

The third limitation of the current study was that only adult Syrian hamsters were used throughout the experimental procedure. Therefore, the results can only be generalized to a very specific age and type of animal and the current study fails to account for the possibility that the results obtained might be different in younger or older hamsters or in other species of mammals. Utilizing older and younger Syrian hamsters as well as other species of mammals would allow for more generalizable results, which is the ultimate goal of this research, to generalize to humans. It is important to note, however, that research into younger hamsters would only involve adolescent hamsters, as pre-pubescent hamsters are not sexually mature, and therefore cannot be studied in relation to sexual experience. Adolescent hamsters would be very interesting to investigate because adolescence is a time of great plasticity due to sexual maturation, and therefore, greater up-regulation of delta FosB and differences may be found between the subjects of study (L. Been, 2015). Finally, the current study did not measure any long term outcomes of the neuroplastic changes that occurred in the NAc in response to repeated sexual experiences. By sacrificing all of the test subjects right after the 6 weeks of sexual experiences, it was impossible to assess if the up-regulation of delta FosB in the NAc persists after the sexual experiences and for how long. The possibility exists that the increased expression of delta FosB subsides and returns to normal or pre-sexual experience levels shortly after the last sexual experience. This would suggest that delta FosB might not necessarily be responsible for the long term neuroplastic changes that are associated with addictive behavior, but instead for the short term neuroplastic changes. This is a possibility considering delta FosB is induced very rapidly after a rewarding experience, and degrades after a couple of weeks, while the behavioral changes seen in addiction persist long after that (Nestler et al., 2001). By not measuring long term outcomes, the current study was not able to account for this possibility.

Future Directions

The results of this study, while interesting, still leave numerous questions unanswered regarding the neuroplastic changes that occur in response to rewarding behaviors that need to be resolved. The first of these involves the molecular pathway or signaling cascade that delta FosB is a part of. Research in addiction has demonstrated that drugs of abuse, and rewarding experiences in general, induce dopaminergic transmission from the VTA to the neurons of the NAc. This leads to increased activation of postsynaptic dopamine receptors in neurons of the NAc, which in turn is believed to lead to increases in delta FosB expression. Delta FosB then combines with Jun family proteins to form the activated AP-1 complex. This complex in turn regulates the expression of certain target genes. Activated delta FosB represses the expression of dynorphin, an opioid peptide, and induces the expression of GluA2, an AMPA glutamate receptor subunit, and the enzyme cyclin-dependent kinase-5 (Cdk5). The regulation of these targets has been linked to increases in dendritic spine density in the NAc. However, the process by which regulation of these targets leads to increases in dendritic spine density remains a mystery (Hilton, 2013; Nace & Tinsley, 2007; Nestler, 2012; Nestler, Barrot, & Self, 2001). Complete knowledge of this process could lead to a better understanding of how rewarding behaviors lead to long term changes in the brain and potentially addictive behaviors, and thus, the long term goal of future studies should be to understand this process.

In order to accomplish this goal, however, studies first need to focus on completing the delta FosB signaling pathway. This would involve identifying other downstream targets of not only the AP-1 complex, but also of the AP-1 targets themselves such as Cdk5, GluA2, and dynorphin. One way future studies could accomplish this is via Co-immunoprecipitation (Co-IP). This technique allows scientists to capture primary known targets as well as secondary targets

that interact with the primary targets using labeled antibodies ("Co-immunoprecipitation", n.d.). Researchers would first induce the delta FosB pathway in an animal model by exposing it to rewarding experiences. Then the researchers would collect tissue samples from the NAc of the animals and create a protein mixture by lysing or opening-up the cells. Labeled antibodies would then be made against the last known targets of the delta FosB pathway such as Cdk5, GluA2, and dynorphin. The antibodies would not only bind to these targets, but also allow for their own downstream targets to bind to them. The researchers would then be able to detect other proteins that interact with Cdk5, GluA2, and dynorphin, thereby identifying downstream targets of the pathway, as well as detecting where the primary targets localize throughout the cell. This would lead to a better understanding of the function of the last known targets and other downstream currently unknown targets.

Another way scientists could potentially identify unknown downstream targets of the delta FosB pathway is via western blotting. However, for this technique to be used researchers must be able to suspect a few proteins that could be potential downstream targets of the delta FosB pathway. Scientists would first induce the delta FosB pathway in an animal model and create a protein mixture in the same way as for Co-IP. Scientists would also create a protein mixture derived from control animals that did not have the delta FosB pathway induced. The proteins in the mixture would then be separated and transferred to a membrane. Primary antibodies against the suspected proteins, and labeled secondary antibodies against the primary antibodies, would be incubated with the membrane. Researchers would then be able to compare the levels of the suspected proteins between the control and the induced delta FosB samples. This would allow researchers to determine if the suspected proteins are being expressed in response to induction of the delta FosB pathway.

The second unanswered question that remains is if the up-regulation of delta FosB in the NAc in response to rewarding experiences is true for all mammals or just rodent species. The great majority of studies that have examined neuroplastic changes in the NAc in response to rewarding experiences have utilized rodents as model organisms. Therefore, our knowledge of how rewarding experiences lead to neuroplastic changes in the NAc, and more generally the delta FosB pathway itself, has largely been proven true in rodents. The possibility exists that the delta FosB pathway, or how the brain changes in response to rewarding experiences, is different in humans and other mammals.

Future studies should thus focus on investigating the delta FosB pathway and how the NAc changes in response to rewarding experiences in other species of mammals. This would involve a similar experimental design to the one utilized in the current study but with other species of mammals. The results could then be compared between species to see if any differences exist in the neuroplastic changes in the NAc in response to rewarding experiences. If similar results are obtained with many species of mammals, it would indicate that the delta FosB pathway and the way the NAc changes in response to rewarding behaviors is evolutionary conserved and is likely to be the same in humans.

Although the role that delta FosB plays in the NAc is relatively well understood, and there have been many studies that have investigated the topic, the role that delta FosB plays in other brain regions is less well understood (Nestler, 2012). For example, apart from the NAc, studies have only investigated the effect that increased expression of delta FosB has on behavior in the orbitofrontal cortex. These studies demonstrated that induction of delta FosB likely led to drug intake promoting behavior (Nestler, 2012; Winstanley, 2007). The question of what are the effects of increased expression of delta FosB in other brain regions remains unanswered. Thus,

future studies should also focus on investigating these effects. Results from these studies would provide a better understanding of the role of delta FosB in the brain and its effects on behavior.

Conclusion

The current research had the primary goals of studying the neuroplasticity involved in the NAc in response to sexual experiences and any neuroplastic differences that might exist between male and female Syrian hamsters. This study was able to demonstrate that exposure to the rewarding behavior of sexual experiences leads to an up regulation of delta FosB in the NAc of male and female hamsters. The study also demonstrated that male and female hamsters do not significantly differ in the way their reward pathways react and respond to the rewarding behavior of sexual experiences. In doing so, this research replicated the results obtained by multiple other studies that have investigated neuroplastic changes in the NAc in response to repeated sexual experiences, as well as produced new data regarding sexual differences in how the brain changes and responds to rewarding experiences. Therefore, the results obtained in this study serve as a basis for future research into how our brains change in response to rewarding behaviors.

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Appendix

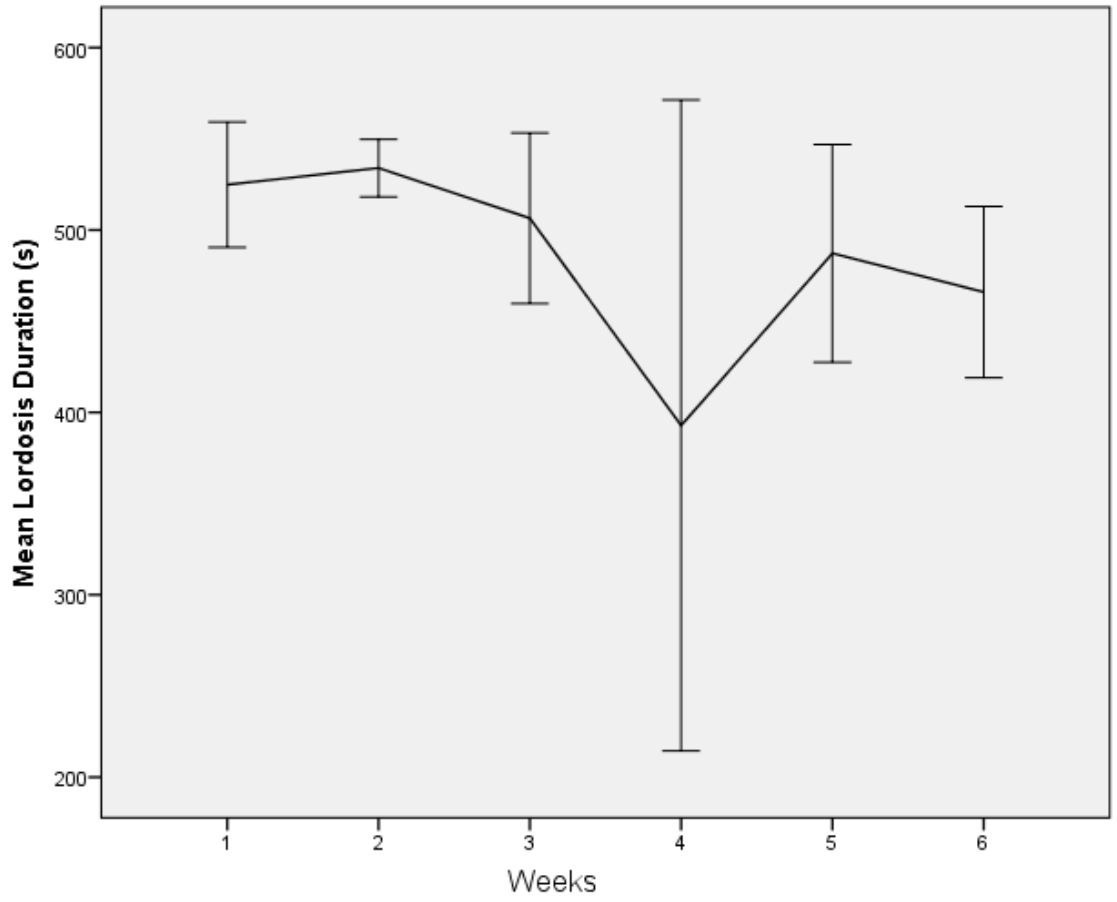

Figure 1. Mean (+/- standard error) lordosis duration in seconds of female Syrian hamsters across the 6 weeks of sexual experiences. The mean lordosis duration of female hamsters did not significantly differ across the 6 weeks of sexual experiences. * indicates significant differences between weeks, $P<.05$.

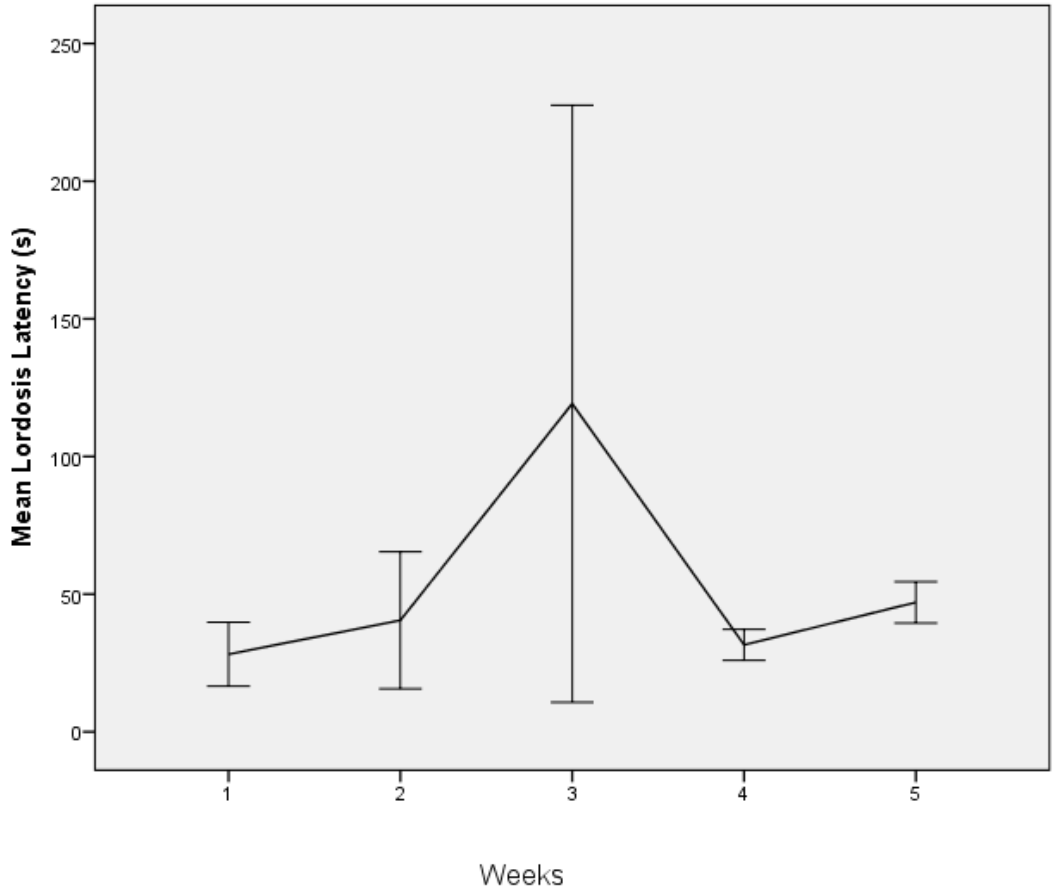

Figure 2. Mean (+/- standard error) lordosis latency in seconds of female Syrian hamsters across the 6 weeks of sexual experiences. The mean lordosis latency of female hamsters did not significantly differ across the 6 weeks of sexual experiences. * indicates significant differences between weeks, $P<.05$.

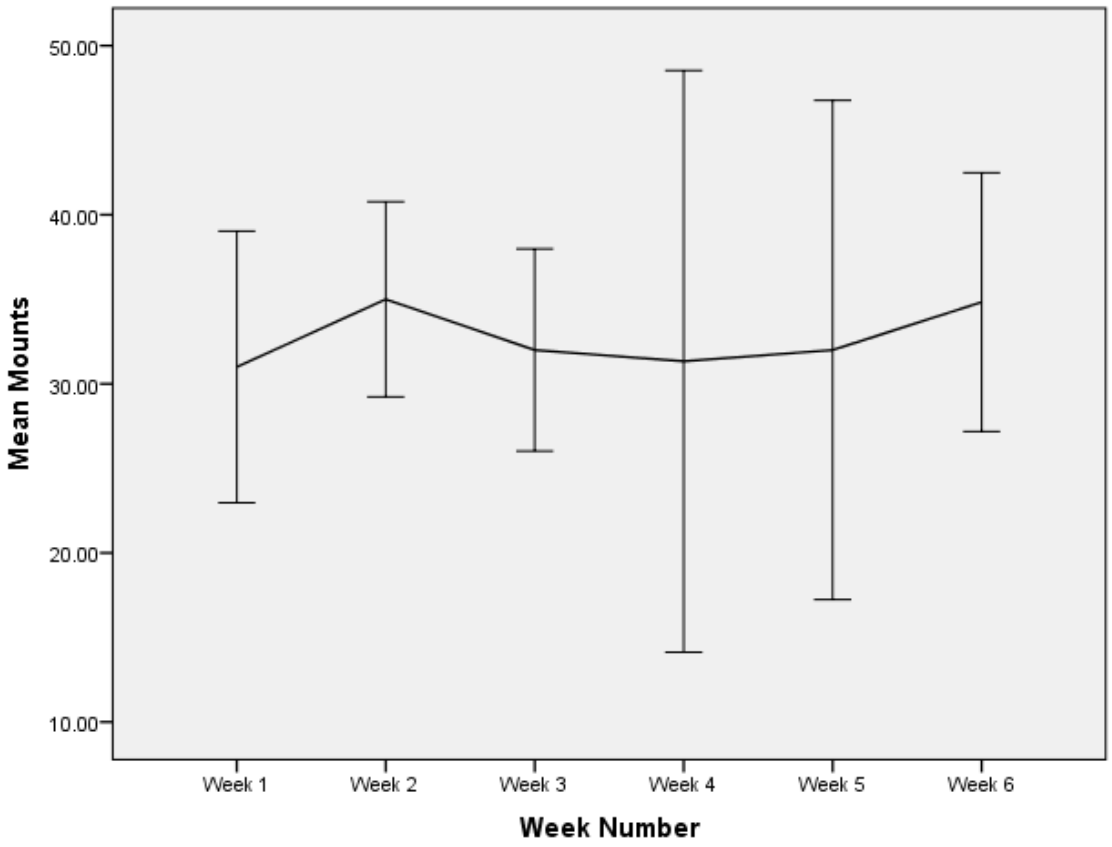

Figure 3. Mean (+/- standard error) number of mounts of male Syrian hamsters across the 6 weeks of sexual experiences. The mean number of mounts of male hamsters did not significantly differ across the 6 weeks of sexual experiences. * indicates significant differences between weeks, $P<.05$.

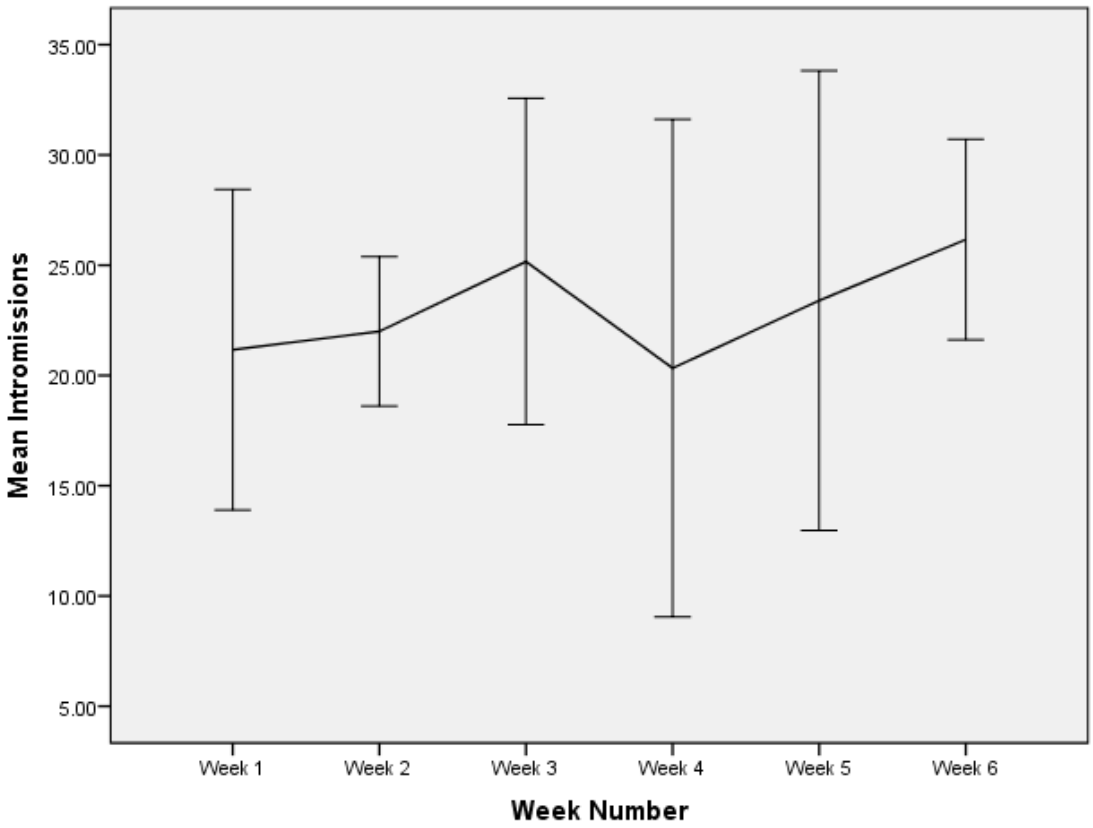

Figure 4. Mean (+/- standard error) number of intromissions of male Syrian hamsters across the 6 weeks of sexual experiences. The mean number of intromissions of male hamsters did not significantly differ across the 6 weeks of sexual experiences. * indicates significant differences between weeks, $P<.05$.

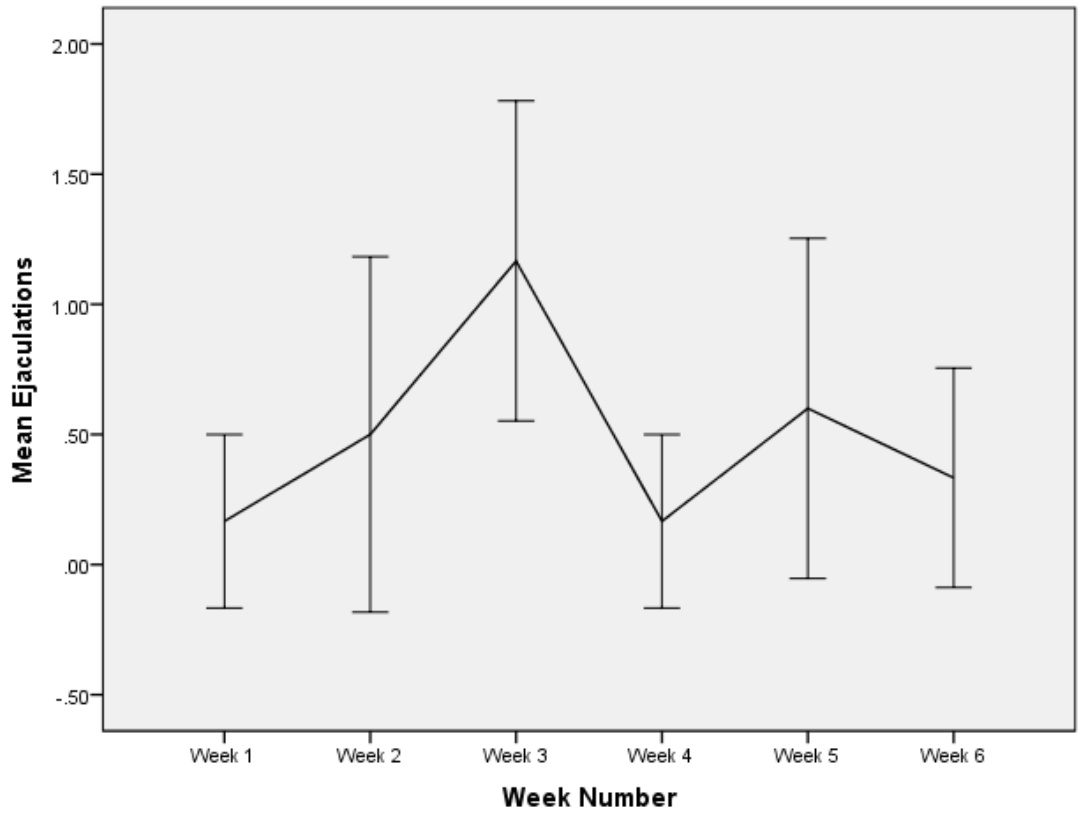

Figure 5. Mean (+/- standard error) number of ejaculations of male Syrian hamsters across the 6 weeks of sexual experiences. The mean number of ejaculations of male hamsters did not significantly differ across the 6 weeks of sexual experiences. * indicates significant differences between weeks, $P<.05$.

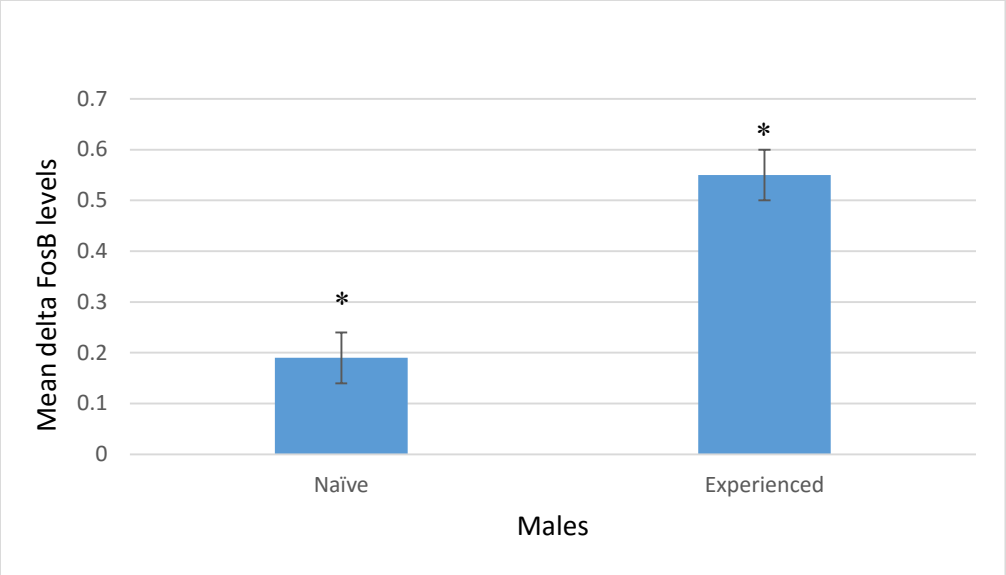

Figure 6. Mean (+/- standard error) delta FosB levels in the NAc of both sexually experienced and sexually naïve male Syrian hamsters. Sexually experienced males had significantly greater mean delta FosB levels in the NAc than sexually naïve males. Also important to note that these delta FosB levels are adjusted using GAPDH as a loading control. * indicates significant differences between male groups, $P<.05$.

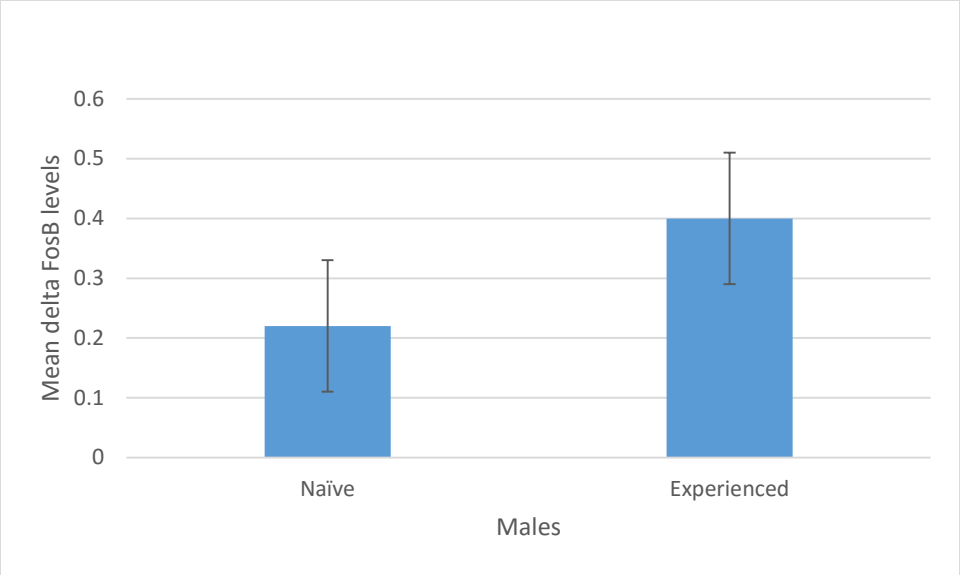

Figure 7. Mean (+/- standard error) delta FosB levels in the caudate of both sexually experienced and sexually naïve male Syrian hamsters. Sexually experienced males and sexually naïve males did not have significantly different mean delta FosB levels in the caudate. Also important to note that these delta FosB levels are adjusted using GAPDH as a loading control. * indicates significant differences between male groups, $P<.05$.

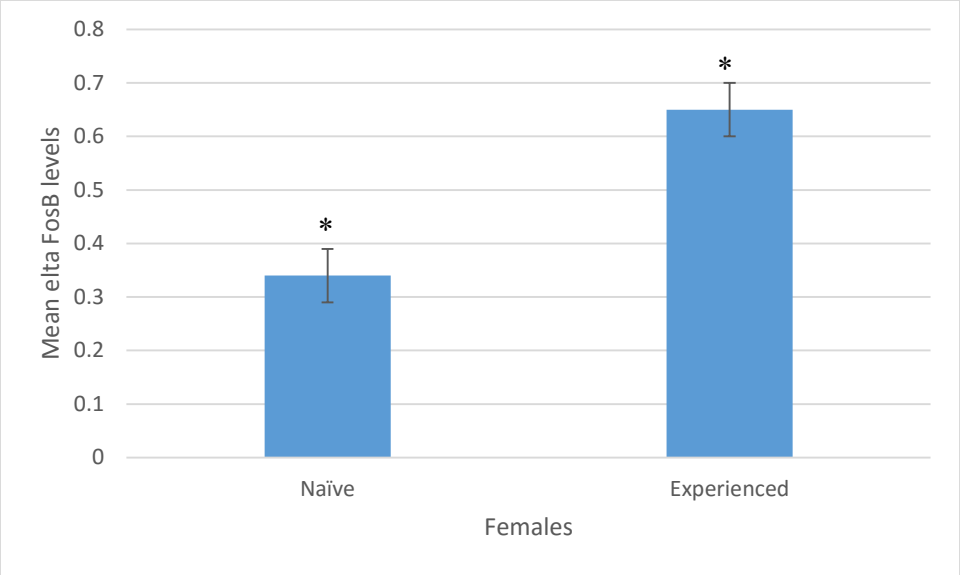

Figure 8. Mean (+/- standard error) delta FosB levels in the NAc of both sexually experienced and sexually naïve female Syrian hamsters. Sexually experienced females had significantly greater mean delta FosB levels in the NAc than sexually naïve females. Also important to note that these delta FosB levels are adjusted using GAPDH as a loading control. * indicates significant differences between female groups, $P<.05$.

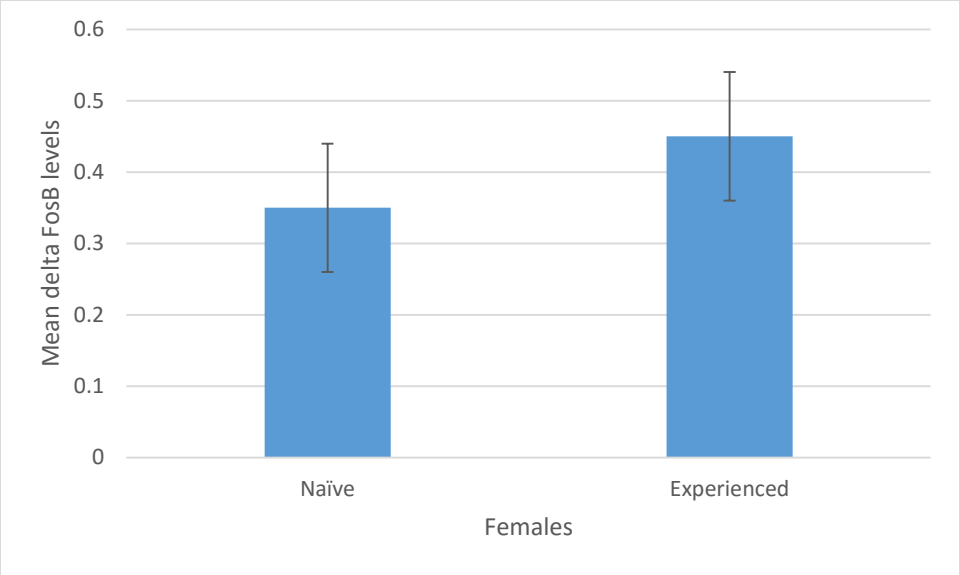

Figure 9. Mean (+/- standard error) delta FosB levels in the caudate of both sexually experienced and sexually naïve female Syrian hamsters. Sexually experienced females and sexually naïve females did not have significantly different mean delta FosB levels in the caudate. Also important to note that these delta FosB levels are adjusted using GAPDH as a loading control. * indicates significant differences between female groups, $P<.05$.

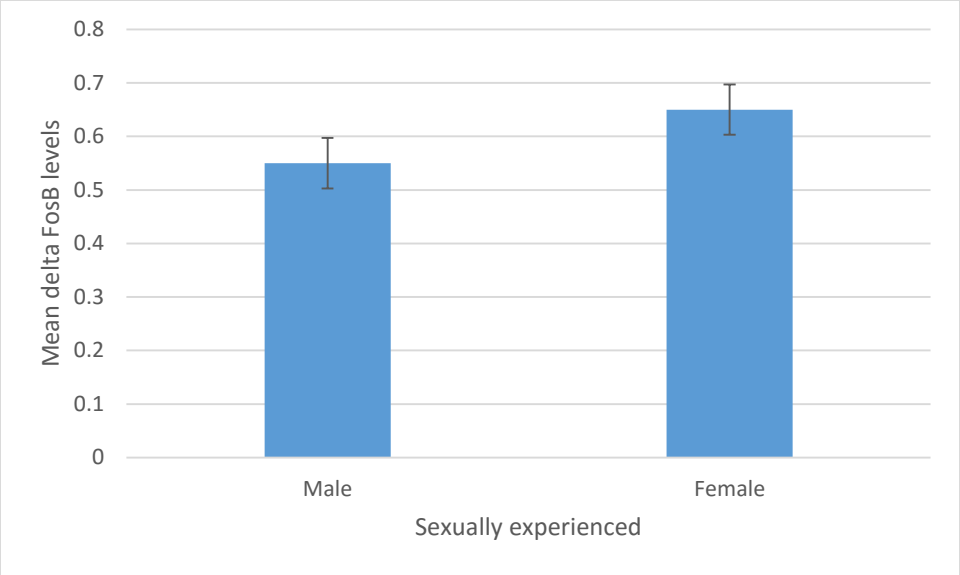

Figure 10. Mean (+/- standard error) delta FosB levels in the NAc of both male and female Syrian hamsters that were sexually experienced. Sexually experienced males and females did not have significantly different mean delta FosB levels in the NAc. Also important to note that these delta FosB levels are adjusted using GAPDH as a loading control. * indicates significant differences between sexually experienced groups, $P<.05$.

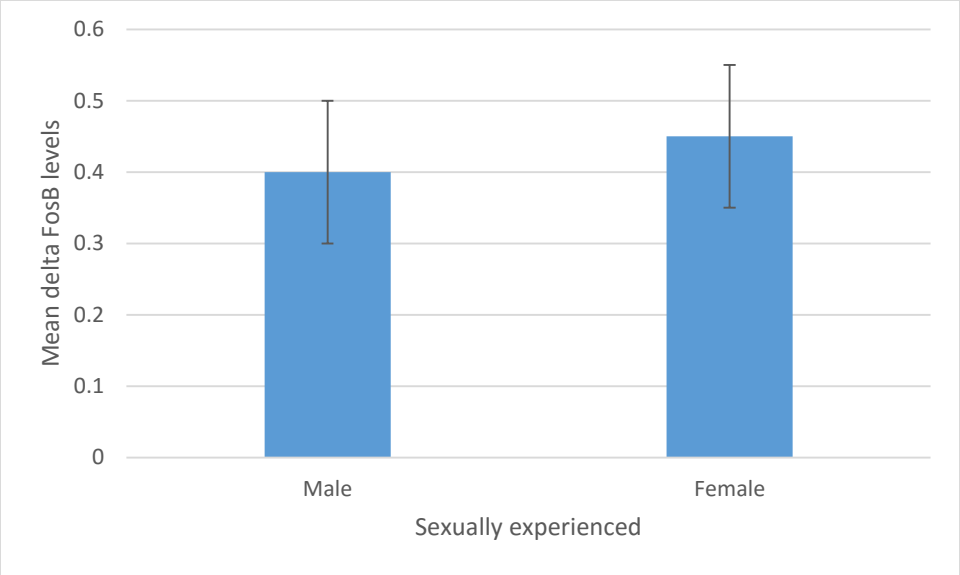

Figure 11. Mean (+/- standard error) delta FosB levels in the caudate of both male and female Syrian hamsters that were sexually experienced. Sexually experienced males and females did not have significantly different mean delta FosB levels in the caudate. Also important to note that these delta FosB levels are adjusted using GAPDH as a loading control. * indicates significant differences between sexually experienced groups, $P<.05$.

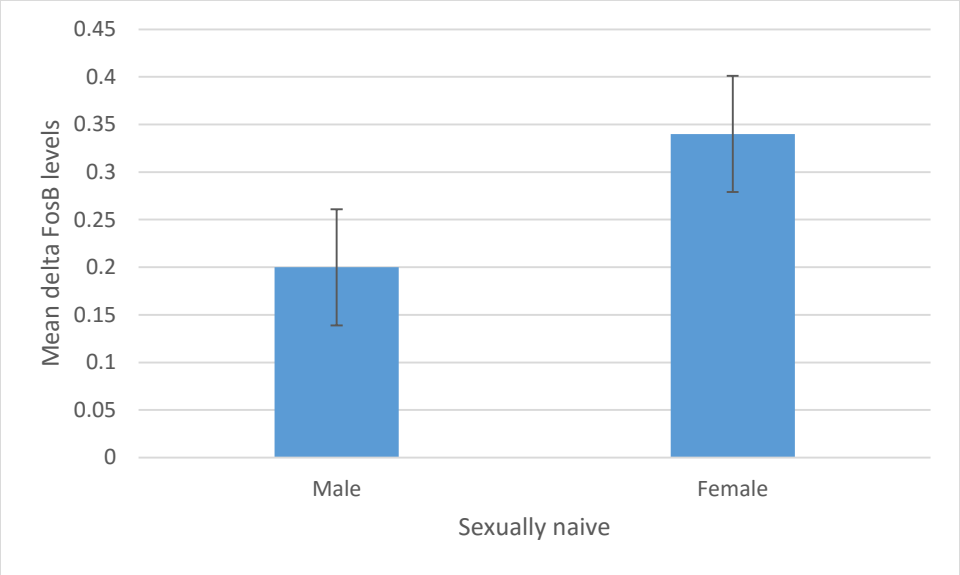

Figure 12. Mean (+/- standard error) delta FosB levels in the NAc of both male and female Syrian hamsters that were sexually naive. Sexually naive males and females did not have significantly different mean delta FosB levels in the NAc. Also important to note that these delta FosB levels are adjusted using GAPDH as a loading control. * indicates significant differences between sexually naive groups, $P<.05$.

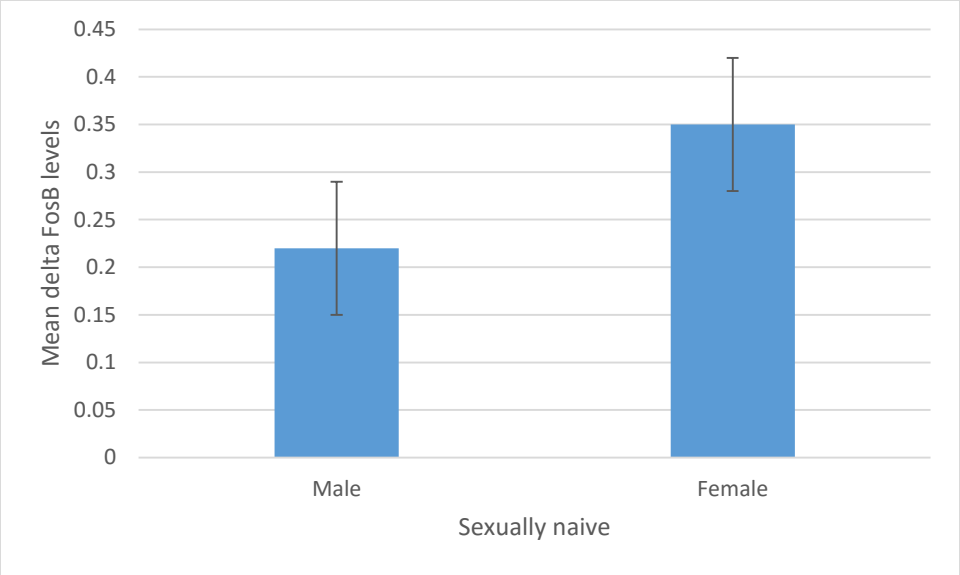

Figure 13. Mean (+/- standard error) delta FosB levels in the caudate of both male and female Syrian hamsters that were sexually naive. Sexually naive males and females did not have significantly different mean delta FosB levels in the NAc. Also important to note that these delta FosB levels are adjusted using GAPDH as a loading control. * indicates significant differences between sexually naive groups, $P<.05$.

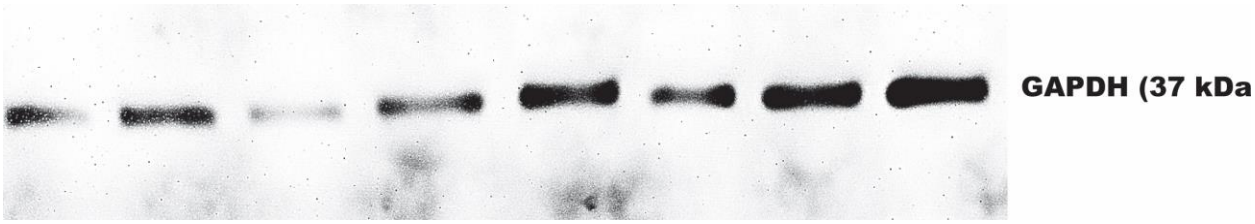

Figure 14. Representative image of the GAPDH bands on the western blot membrane. The image shows mostly strong bands of GAPDH, indicating the presence of GAPDH in the membrane samples.

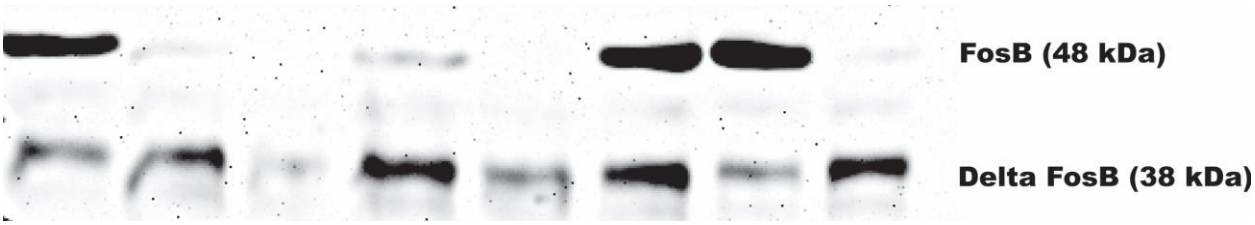

Figure 15. Representative image of the delta FosB/FosB bands on the western blot membrane. The image shows mostly strong bands of delta FosB, indicating the presence of delta FosB in the membrane samples.