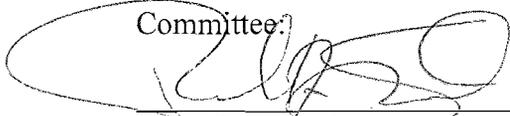


THE EFFECTS OF ADOLESCENT NICOTINE EXPOSURE ON DENDRITIC
MORPHOLOGY IN THE BED NUCLEUS OF THE STRIA TERMINALIS

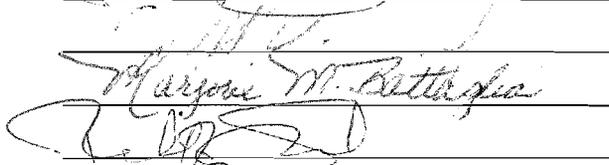
by

Kelsey Brown
A Thesis
Submitted to the
Graduate Faculty
of
George Mason University
in Partial Fulfillment of
The Requirements for the Degree
of
Master of Arts
Psychology

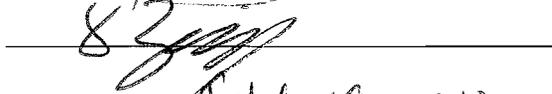
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Date:

July 19, 2012

Summer Semester 2012
George Mason University
Fairfax, VA

THE EFFECTS OF ADOLESCENT NICOTINE EXPOSURE ON DENDRITIC
MORPHOLOGY IN THE BED NUCLEUS OF THE STRIA TERMINALIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of
Arts at George Mason University

by

Kelsey Brown
Bachelor of Science
George Mason University, 2010

Director: Robert F. Smith, Professor
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Summer Semester 2012
George Mason University
Fairfax, VA

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DEDICATION

This is dedicated to my loving and supportive parents, Gail and Robert Brown, my sister, Ashley Paragone, my brother in law Scott Paragone, and my loving boyfriend James Smith

ACKNOWLEDGEMENTS

I would like to thank the faculty of the psychology department at George Mason University, and particularly my committee members Dr. Robert Smith, Dr. Craig McDonald, and Dr. Marjorie Battaglia. I would also like to thank Kathryn Taylor, Daniel Ehlinger, and Gina Fernandez for their help and support in the completion of this thesis

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ABSTRACT

THE EFFECTS OF ADOLESCENT NICOTINE EXPOSURE ON DENDRITIC MORPHOLOGY IN THE BED NUCLEUS OF THE STRIA TERMINALIS

Kelsey Brown, M.A.

George Mason University, 2012

Thesis Director: Dr. Robert F. Smith

Nicotine has been shown to increase the risk of anxiety related disorders in adulthood (Slawecki, Gilder, Roth, & Ehlers, 2003). Early nicotine exposure may negatively affect the development of the adolescent brain (Morissette, Tull, Gulliver, Kamholz, & Zimering, 2007). The bed nucleus of the stria terminalis has been shown to have major projections to the PVNmp that regulates the brain and body's reaction to stress as well as influences activity of the HPA axis. This study investigated morphological changes of dendrites on neurons located in the bed nucleus of the stria terminalis following adolescent nicotine exposure. Male Sprague-Dawley rats were administered nicotine (0.5mg/kg/day) 3 days a week for 2 consecutive weeks, starting at postnatal day P (32). 17 days following the end of dosing, at postnatal day P (60), brains were processed for Golgi-Cox staining and neurons were digitally reconstructed for branch order and Sholl (Sholl, 1953) analysis. Nicotine pretreatment produced an increase in the number of

bifurcations and total length of dendrites. Nicotine pretreatment also increased the average length of dendrites, suggesting that growth of existing branches is occurring as well as formation of new branches. Sholl analysis also revealed an increase in the number of intersections with concentric spheres, increased amount of dendritic material within concentric spheres, and an increase of dendritic branching within concentric spheres occurring between 20-300 μ m from the soma in dendrites.

INTRODUCTION

As smoking prevalence increases among adolescents, the neurological risk it places upon them becomes detrimental. Early nicotine exposure may negatively affect the development of the adolescent brain, heightening sensitivity to nicotine effects and increasing the risk of anxiety related disorders in adulthood (Morissette et al., 2007). However, small amounts of nicotine have shown to have some anxiolytic effects. This places smokers in a more vulnerable position to the addictive nature of nicotine and creates a situation that will make it harder to quit as they mature into adulthood. Anxious individuals are more likely to smoke, however studies show that smoking also increases the risk of having an anxiety disorder. Anxiety, being a future-orientated state involving perceived uncontrollability and unpredictability over dangerous events or the person's emotional response to those events (Morissette et al., 2007). Adolescent nicotine exposure is associated with an increased risk of developing agoraphobia, panic disorder, generalized anxiety disorder, and an increase in the onset of panic attacks. Nicotine's long lasting impact on anxiety-related behavior, following the end of the withdrawal period, highlights the predisposition of increased anxiety in adulthood. Smoking shows to alleviate anxiety, but it doesn't do so across all situations. Nicotine has shown to have a profile similar to that of alcohol and diazepam in the anxiolytic effect observed during

acute or chronic administration of nicotine, but anxiogenic effects were found during withdrawal and continued after the withdrawal period ended (Morissette et al., 2007).

The Amygdala has been shown to play a major role in the regulation of anxiety and was previously thought to be the primary controller of the behaviors implicating fear and anxiety. Recent work has shown that there is involvement of the bed nucleus of the stria terminalis (BNST) in the generation of these emotional states. The BNST is considered to be part of the extended amygdala and its cell morphology and neurotransmitters closely resemble that of the central nucleus of the amygdala and it is thought to work in a close manner to the amygdala (Pêgo, Morgado, Cerqueira, Almeida, & Sousa, 2006). Both the BNST and the central nucleus receive sensory information from the basolateral nucleus of the amygdala, and send efferent axons to various hypothalamus and brainstem regions that are involved in fear and anxiety (Pêgo et al., 2006). They differ in the two main functions; the BNST may respond to signals that will actually trigger anxiety, while the central nucleus of the amygdala may be more responsible for generating a state of fear (Pêgo et al., 2006). Previous studies looked at volume and neuronal numbers of the BNST in relation to heightened levels of anxiety and determined there was no significant increase in volume or neuronal numbers in animals displaying heightened anxiety (Pêgo et al., 2006). There may be morphological differences in the dendritic formation of neurons in the BNST in relation to heightened levels of anxiety. To date, this idea of a positive correlation between anxiety and dendritic branching has not been studied within the BNST, but it has been looked at in the basolateral amygdala (Vyas, Jadhav, & Chattarji, 2006). Cells in the basolateral

amygdala have been observed to have elongated dendrites and an increase in bifurcations as a result of stress, and this lengthening and branching shows to last for a number of weeks following the stress, leading to the theory that the changes may carry on into adulthood (Vyas et al., 2006). The central nucleus of the amygdala and the medial amygdala areas receive input from the basolateral amygdala neurons. Since there is an increase in dendritic branching, there may be an increase in output from the basolateral amygdala as a result of increased stress and levels of anxiety.

The central nucleus and the BNST receive sensory information from the basolateral nucleus of the amygdala and then respond to emotionally significant stimuli; however the specific behaviors they elicit are different (Davis, 1998). The BNST responds more to input related to anxiety, where as the central nucleus of the amygdala is involved more in fear and not as much in anxiety. This differentiation was noted through corticotrophin-releasing hormone (CRH) infusion in combination with chemical lesions of both the amygdala and the BNST (Davis, 1998). CRH is known to be released during periods of stress or anxiety. Some of this release may come from CRH containing neurons in the central nucleus of the amygdala that project and act on receptors in the BNST (Davis, 1998). CRH showed to produce an increase in startle that had a slow onset and decay which was not dependent on prior fear condition and is blocked by anxiolytic compounds (Davis, 1998). Chemical lesions of the amygdala failed to block CRH-enhanced startle; lesions of the BNST completely blocked CRH-enhanced startle. Local infusion of CRH directly into the BNST, of animals without a lesion, produced a rapid and large increase in the startle amplitude (Davis, 1998). Lesions and chemical

inactivation of the BNST significantly decreased both light-enhanced startle and CRH-enhanced startle without having an effect on fear-potentiated startle (Davis, 1998).

The relationship between anxiety and smoking is one of interest, especially in the situation of adolescent smoking leading to early nicotine exposure. Several studies have reported a greater sensitivity to nicotine's effects in adolescent animals, compared to adult animals when tested during drug administration. There are reports stating that there has been observed lasting behavioral and molecular effects of nicotine exposure specific to the adolescent period that was measurable weeks and even months following the last dose of nicotine. The long term addictive effects on adolescents may relate to emotional behavior changes as a result of nicotine exposure, such as anxiety (Smith et al., 2006). Rats that received nicotine during mid-adolescence showed a decrease in exploration and activity compared to rats with no prior exposure to nicotine when tested in adulthood. This finding is consistent with anxiogenic and depressive profiles that state that rats will have a smaller tendency to explore when anxiety levels are increase (Smith et al., 2006). Open-field studies have shown increase anxiety-like behavior in adult male Sprague-Dawley rats treated with nicotine during adolescence when tested in adulthood, proving the long lasting effect that nicotine places on anxiety related behavior (Smith et al., 2006). Early adolescent exposure to nicotine has also produced anxiety-like behaviors that were detectable into adulthood, while comparable nicotine exposure in adult rats failed to produce such effects (Smith et al., 2006).

Previous studies looked at the effects of periadolescent nicotine administration on dendritic morphology of medium spiny neurons from the nucleus accumbens shell (C. G.

McDonald et al., 2005). Medium spiny neurons are the principle cells in the nucleus accumbens and they create the output path of the corticomesolimbic reward circuitry, which plays a major role in addiction. Prior studies on the medium spiny neurons in the nucleus accumbens had shown that plastic changes occurred to the cells in response to administration of nicotine (C. G. McDonald et al., 2005). Changes seen in the medium spiny neurons were an increase in dendritic lengths and spiny density; many of which were long lasting changes showing that adolescent nicotine exposure can have effects on the adult brain. Changes in dendritic morphology within the basolateral amygdala following adolescent nicotine exposure have also been studied. Adolescent nicotine exposure led to an increase in basilar dendritic length in the basolateral amygdala (Bergstrom, 2009). This remodeling suggests that the same might be true for the remodeling of dendrites in the BNST with an increase in anxiety.

The Bed Nucleus of the Stria Terminalis

The BNST is a compilation of twelve nuclei that surround the caudal part of the anterior commissure deep within the brain. Studies have suggested its role in coordinating activity of the autonomic, neuroendocrine, and somatic motor systems (Dumont, 2009). Its wide range of function has been further noted in lesion studies showing its role in physiological fear, food intake regulation, social behavior, and goal orientated behavior (Dumont, 2009). Its various functions places it as a structure of high interest in understanding the basis for psychological states such as anxiety, addiction, and anorexia (Dumont, 2009).

A main focus of our research is the BNST's role in anxiety-related behaviors observed in adult rats following adolescent nicotine exposure. Research has shown that pathological states, such as anxiety disorders and addiction, may be a result of maladaptive processes occurring in regions of the brain highly associated with stress response and reward (McElligott & Winder, 2009). The BNST has recently been associated with these processes through its role in conditioned and unconditioned fear response, anxiety related behaviors, drug dependences, and stress induced drug-seeking (McElligott & Winder, 2009). These various behaviors are believed to be regulated by the limbic and cortical region's glutamatergic projections to the BNST (McElligott & Winder, 2009). Alterations in these various connections are assumed to be the underlying mechanism for anxiety disorders and addiction. This creates the foundation for the idea that adolescent nicotine exposure could cause morphological changes within the BNST and from the BNST to the paraventricular nucleus of the hypothalamus (PVN) and the Ventral Tegmental Area (VTA); causing changes in anxiety-related states and drug-abuse.

Previous research has shown changes in the medium spiny neurons of the nucleus accumbens has been seen as a result of periadolescent nicotine exposure. The changes that were seen were increases in dendritic lengths and spine density, which showed to be long lasting changes that carried on into adulthood (C. G. McDonald et al., 2005). These dendritic changes seen in the nucleus accumbens were linked to the psychomotor sensitization which is linked to the process of addiction (C. G. McDonald et al., 2005). Structures involved in the reward circuit are the ventral tegmental area, nucleus

accumbens, amygdala, hippocampus, and the bed nucleus of the stria terminalis.

Forebrain structures; Amygdala, Hippocampus, medial prefrontal cortex and the bed nucleus of the stria terminalis has been shown to play a role in the regulation of activity in the Hypothalamic-Pituitary-Adrenal (HPA) axis (Choi et al., 2007). The bed nucleus has been seen to have major projections to the PVNmp, which shows that it plays a role in the regulation of the HPA axis by triggering adrenocorticotropin (ACTH) secretion from the anterior pituitary (Choi et al., 2007). The HPA axis plays a major role in the reaction to stress. Interruption of the structures that regulate the activity of the HPA axis have been seen in the etiology of many stress related disorders including post-traumatic stress disorder and major anxiety disorders (Choi et al., 2007).

The Bed nucleus can be divided into two major divisions, the anterior division and the posterior division which regulate the PVNmp activity differently, which will have different affects on the activity in the HPA axis (Choi et al., 2007). The anterior division has been shown to send the heaviest projections to the PVNmp and they have been shown to stimulate the HPA axis. The posterior division also project heavily to the PVNmp, however its projections are GABAergic and have been seen to inhibit the HPA axis (Choi et al., 2007).

The bed nucleus is necessary for efficient relay of cortical excitation to dopamine neurons of the Ventral Tegmental Area (Jalabert, Aston-Jones, Herzog, Manzoni, & Georges, 2009). It has been shown that increasing the glutamatergic drive in the BNST enhances dopamine neuronal activity and produces characteristic bursting patterns. A small subgroup of the BNST projects directly to the VTA with a monosynaptic input and

exerts a strong excitatory influence on dopamine neurons mediated by NMDA and AMPA glutamatergic receptors (Jalabert et al., 2009). The BNST plays a critical role in responses to both stress/anxiety and in the reward mechanisms that involve excitatory glutamatergic transmission (Jalabert et al., 2009). The BNST's role in addictive behaviors such as drug seeking and stress induced relapse make it an important target for research with addictive substances like nicotine. Nicotine's influence of dendritic morphological changes in structures involved in the reward-circuitry activated by addictive drugs leads to the idea that nicotine may influence dendritic changes in all structures associated with the reward circuit. The role of the BNST in the reward circuit places it in a position to have its neuron's dendritic morphology influenced by nicotine exposure, which may be the underlying mechanism for the increase in anxiety-related behaviors observed in adulthood following adolescent nicotine exposure.

METHODS

Animals and Drugs

Animal subjects ($N=17$) were Male Sprague-Dawleys (Harlan, Indianapolis, IN) and arrived at our facility at postnatal day 21 (P21). Animals were group housed (4-5 per cage) with a 12-hour light cycle. All work to date in our laboratory has utilized an ad libitum feeding schedule. All nicotine administration took place during the light cycle. All housing and experimental procedures were carried out in accordance with the *National Institute of Health Guide for the Care and Use of Laboratory Animals*, in addition to requirements of George Mason University. Rats were given 10 days to adjust to the housing conditions before experimentation took place. Rats were randomly assigned to receive injections of nicotine (0.5 mg/kg, free base) or saline control via subcutaneous injections. Subcutaneous administration was used rather than continuous infusion regimen because smoking cigarettes during adolescents has been described as intermittent, low-intensity. Rats were administered nicotine or saline via subcutaneous injections 3 times per week for two weeks during adolescence (P32-43). The accepted age range for adolescence in rats conservatively ranges from P28-P45 (Spear, 2000). Neurobehavioral systems reach maximal maturation by P60, and rats are considered adults at this age (Herlenius & Lagercrantz, 2004). 17 days following the end of dosing all animals underwent intracardial perfusion at P60.

Neuroanatomy

17 days following the end of dosing, Rats (P60) were deeply anesthetized with ketamine solution (1.0mg/kg) and perfused intracardially with 0.9% NaCl. Brains were then removed and placed in Golgi-Cox solution, which was prepared according to the recipe of Glaser and Van der Loos for two weeks at room temperature. Following Golgi-Cox immersion, brains were stored in a 30% sucrose solution until vibratome sectioning (200 μ m sections). Sections were then stained using the protocol of Gibb and Kolb (1998). Briefly, sections were first alkalinized in ammonium hydroxide, developed and fixed using Kodak Rapid Fix, dehydrated through a series of ethanols, and cleared in a solution of 1/3 xylene, 1/3 100% alcohol, and 1/3 chloroform. Golgi-stained neurons were then reconstructed in 3-dimension using NeuroLucida interfaced to an Olympus BX51 light microscope. To eliminate bias, an experimenter blind to drug history traced all neurons under a 60X objective. Neurons were selected for tracing only if they were well impregnated and possessed unobstructed dendrites that could be followed without interruption. Examples of digital reconstruction of neurons from the BNST are shown in Figure 1.

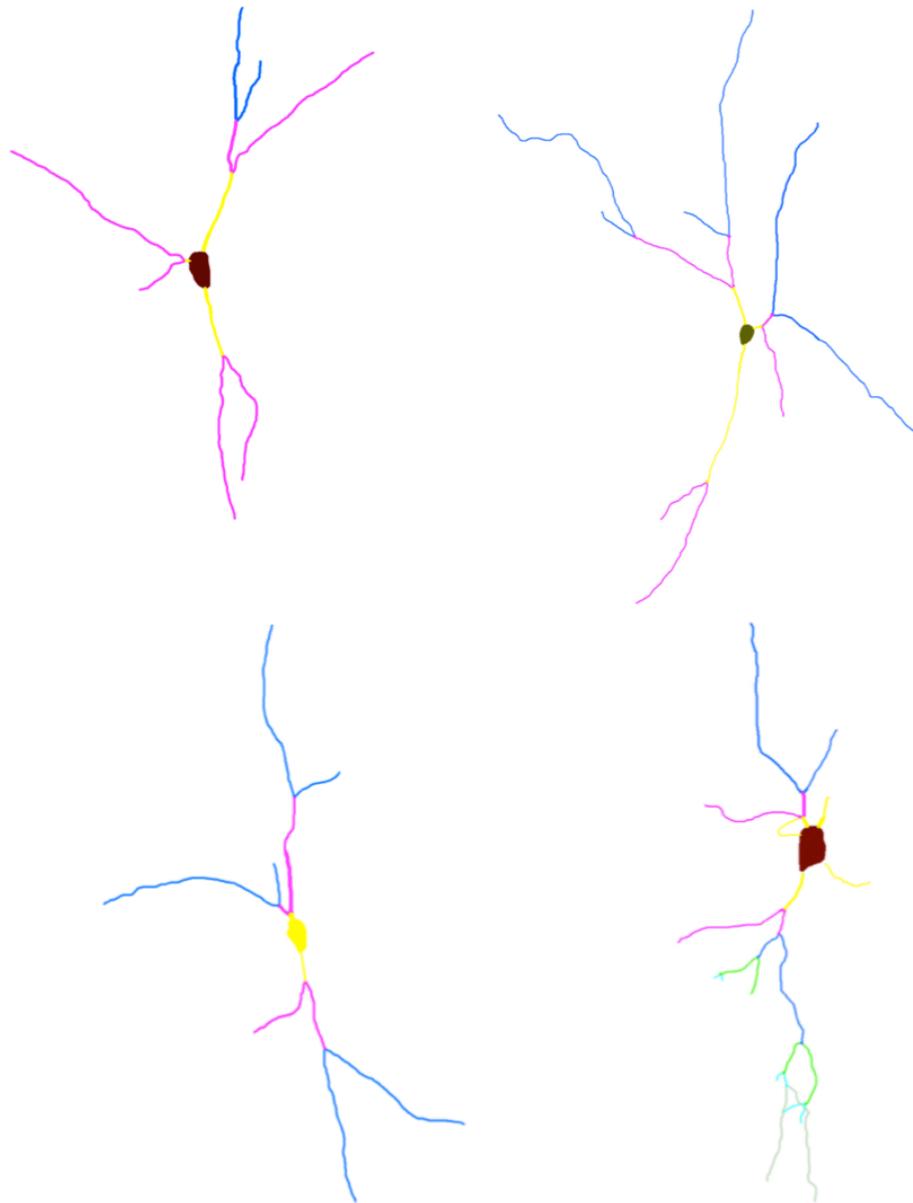


Figure 1: Representative of neuronal reconstruction from saline pretreatment (left) and nicotine pretreatment (right)

Morphometric Analysis

Morphological measurements will be obtained using NeuroExplorer software (Microbrightfield Biosciences, Williston, VT). The Morphometric parameters that were examined included the total number of branches, the number of branches as a function of

the branch order, the length of the dendrite, the total length of the dendrite as a function of the branch order, and the mean of the dendrite length as a function of the branch order. The Sholl analysis was implemented to analyze the distribution of the dendritic branching and the total length of the dendrites. The Sholl analysis observed the number of intersections, bifurcations, and total length of the dendrite between equidistant concentric spheres originating from the soma (0 μ m) and measuring 20 μ m in radial distance.

A specific cell type has not been identified by previous research as the primary occupant within the BNST, rather the BNST is occupied by a variety of different cell types (A. J. McDonald, 1983). To ensure that one specific cell type was not influencing the effect of our data a Principal Component Analysis (PCA) was conducted on the 83 neurons that were reconstructed. The PCA was based on various descriptives taken about each neuron, these included tree totals, nodes, terminals, total length of the dendrite, total volume, area of the soma, and max ferret of the soma.

Three to Six neurons from each animal were reconstructed. A mean value of each of the morphological measurements mentioned above was calculated for each animal. Statistical analysis was based on the mean value for each animal rather than each cell being treated as an independent measure. Violations of the assumption of normal distribution were corrected for by sqrt transformation of the data. An ANOVA with treatment (nicotine vs. saline) as the between the group factors and branch order as within the group factors was conducted for analysis of total number of dendrites, total number of branches, and branch order data. Violations of the assumption of sphericity were corrected using the Greenhouse-Geisser correction for the degrees of freedom. A

superscripted number “1” proceeding an *F* value indicates Greenhouse-Geisser-corrected value for degrees of freedom throughout the thesis. Following a significant interaction, independent sample *t*-tests on branch orders were conducted. To avoid false significance due to multiple comparisons the *Bonferroni* correction was implemented in the analysis. *P*-values that were below the *Bonferroni* criteria of significance or approaching $p = .05$, are noted as statistical trends. Therefore, for the analysis of morphometric parameters within branch orders, $p < .01$ was considered significant.

A statistical analysis of data from the Sholl analysis was conducted on the mean values per animal as described for the above morphometric parameters. For the analysis of number of intersections, total length of dendrites, and number of bifurcations, an ANOVA with treatment (nicotine vs. saline) as the between-groups factor and the radial distance from the soma as the within-groups factor was conducted. Violations of the assumption of sphericity were corrected using the Greenhouse-Geisser correction for the degrees of freedom. Following a significant interaction, independent sample *t*-tests on distinct radii from the soma were conducted. To avoid false significance due to multiple comparisons the *Bonferroni* correction was implemented in the analysis. *P*-values that were below the *Bonferroni* criteria of significance or approaching $p = .05$, are noted as statistical trends. Therefore, for the analysis of morphometric parameters within branch orders, $p < .003$ was considered significant. A subscripted symbol “*” indicates significant prior to *Bonferroni* correction for all of the thesis. To avoid false values due to multiple comparisons, differences were only considered significant if the *p*-value for three consecutive points were $p < .05$. This limits the influence that any single radii has

on the analysis and ensures that parametric changes occur consecutively over an ample portion of the dendritic tree (Luck, 2005).

RESULTS

Cell Types

A Principal Component Analysis revealed that two components had Eigen values greater than 1.0. Component 1 explained 96% of the variance in tree totals, nodes, terminals, total length, total volume, area of the soma, and max ferret of the soma displayed by each neuron. Component 2 explained 2% of the variance in tree totals, nodes, terminals, total length, total volume, area of the soma, and max ferret of the soma displayed by each neuron. This indicates that 96% of the variability between cells can be grouped as one type of neuron. Without a distinction by the Principal Component Analysis of different cell types, we classified and analyzed all neurons further as one type of cell.

Branch Order Analysis

For dendrites, ANOVA with between-group factor (pretreatment) and repeated measure (branch order) revealed a significant main effect of pretreatment on number of branches ($F(1, 14) = 26.973, p < .0005$), which suggests that the nicotine pretreatment group shows an overall increased number of branches ($M = 7.155, SE = .343$) compared to the saline pretreatment group ($M = 4.99, SE = .159$). ANOVA also revealed a significant interaction of pretreatment x branch order on number of branches ($F(4, 56) = 4.040; p < .006$), which suggests that the increased number of branches following nicotine pretreatment is dependent on branch order of dendrites. Independent sample *t*-tests

revealed that nicotine pretreatment group had significantly increased number of 2nd order branches ($M=2.196, SE=.071$) compared to pretreatment saline ($M=1.907, SE=.036$), $t(11.667)=-3.634, p<.004$; 3rd order branches pretreatment nicotine ($M=1.7109, SE=.067$) compared to pretreatment saline ($M=1.265, SE=.074$), $t(14)=-4.452, p<.001$; and 4th order branches pretreatment nicotine ($M=.948, SE=.152$) compared to pretreatment saline ($M=.116, SE=.117$), $t(14)=-4.132, p<.001$. Independent sample *t*-tests also suggested a trend of increased number of 5th order branching in the nicotine pretreatment group ($M=.405, SE=.140$) compared to pretreatment saline ($M=0, SE=0$), $*t(8)=-2.886, p<.020$.

Figure 2

For dendrites, ANOVA with between-group factor (pretreatment) and repeated measure (branch order) revealed a significant main effect of pretreatment on total length, ($F(1, 14) = 19.974; p <.001$), which suggests that the nicotine pretreatment group shows an overall increase in total length ($M = 54.6, SE = 2.408$) compared to the saline pretreatment group ($M = 40.543, SE = 1.778$). ANOVA did not reveal a significant interaction of pretreatment x branch order on total length ($F(4, 56) = 1.513; p < .211$), which suggests that the increased total length following nicotine pretreatment is not dependent on branch order of dendrites. Figure 3

For dendrites, ANOVA with between-group factor (pretreatment) and repeated measure (branch order) revealed a significant main effect of pretreatment on average length ($F(1, 14) = 9.345; p < .009$), which suggest that nicotine pretreatment group shows an overall increase in average length of dendrites ($M=28.212, SE=1.37$) compared to saline pretreatment ($M= 22.72, SE= 1.009$). ANOVA also revealed a significant

interaction of pretreatment x branch order on average length ($F(2.468, 34.556) = 4.118$, $p < .018$), which suggests that nicotine pretreatment does alter average dendritic length. Importantly, this also suggests that the increased total length is the result increase in length of existing branches as well as an increase in formation of new branches. Independent sample t -tests revealed that nicotine pretreatment group had significantly increased the average length of 4th order branches ($M=3.721, SE= 1.86$) compared to pretreatment saline ($M=.833, SE=2.203$), $t(14)= -2.845, p<.013$; and 5th order branches pretreatment nicotine ($M=1.605, SE=1.63$) compared to pretreatment saline ($M=0, SE=.0$), $t(8)= -2.956, p<.018$. Figure 4

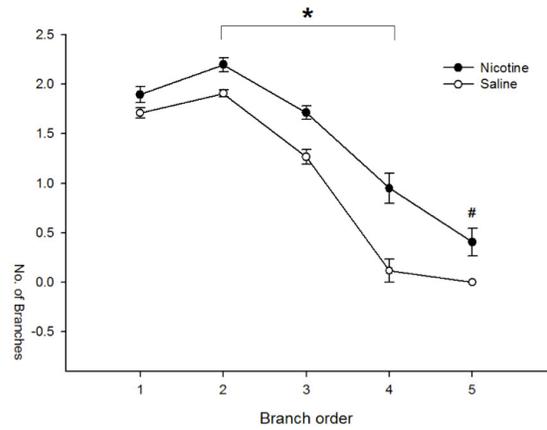
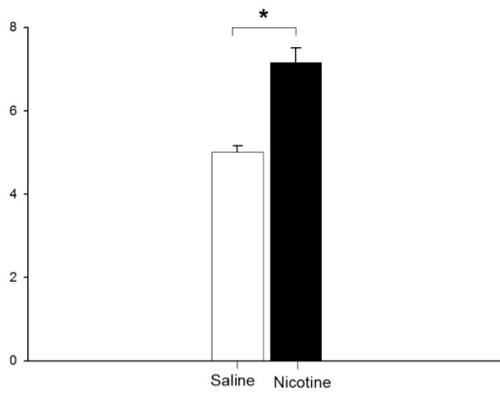


Figure 2: Branch order analysis of No. of Branches

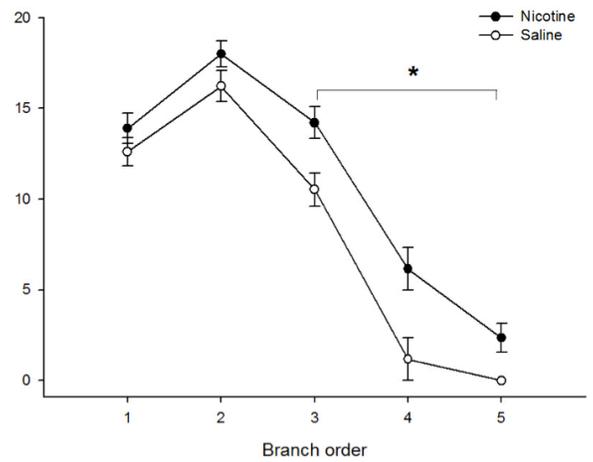
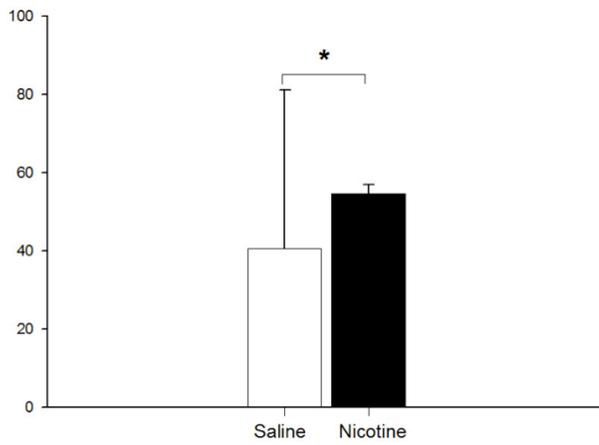


Figure 3: Branch order analysis of total length

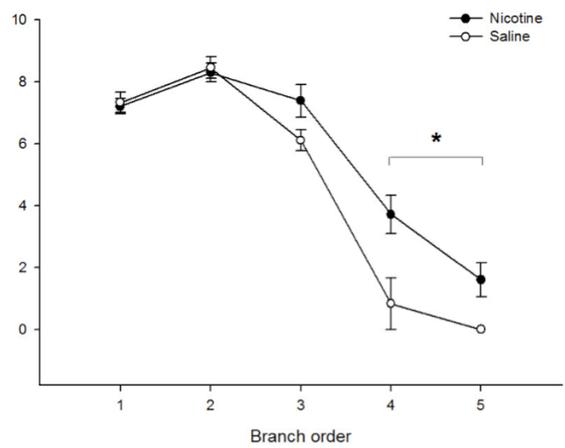
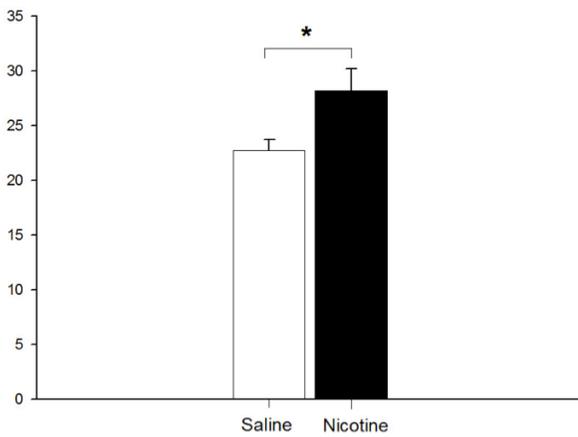


Figure 4: Branch order analysis of avg. length

Sholl Analysis

ANOVA with between-group factor (pretreatment) and repeated measure (radius) revealed a significant main effect of pretreatment on number of intersections ($F(1,14) = 11.503; p < .004$), which again suggests that nicotine pretreatment increases the overall number of intersections by the dendritic tree with the distinct radii. ANOVA also revealed a significant interaction of pretreatment x radius on intersections ($^1F(3.048, 42.668) = 3.048; p < .056$), which suggests that the increased intersections of dendritic tree with the distinct radii are dependent on the radial distance from the soma.

Independent sample *t*-tests revealed a trend towards increased intersections in the nicotine pretreatment group compared to the saline pretreatment group at 20 μm ($*t(14) = -2.468; p = .027$), 40 μm ($*t(14) = -3.047; p = .007$), 60 μm ($t(14) = -3.823; p = .002$), and 80 μm ($*t(14) = -3.320; p = .005$), from the soma. Figure 5

ANOVA with between-group factor (pretreatment) and repeated measure (radius) revealed a significant main effect of pretreatment on total length ($F(1,14) = 12.195; p < .004$), which again suggests that nicotine pretreatment increases the overall total length of the dendritic tree. ANOVA also revealed a significant interaction of pretreatment x radius on total length ($^1F(3.063, 42.88) = 3.951; p < .014$), which suggests that the increased total length of the dendritic tree is dependent on the radial distance from the soma. Independent sample *t*-tests revealed a trend toward increased total length in the nicotine pretreatment group compared to the saline pretreatment group at 40 μm ($*t(14) = -2.970; p = .010$), 60 μm ($*t(14) = -3.167; p = .007$), 80 μm ($t(10.62) = -4.4; p = .001$), and 100 μm ($*t(14) = -2.821; p < .014$), from the soma. Figure 6

ANOVA with between-group factor (pretreatment) and repeated measure (radius) revealed a significant main effect of pretreatment on number of bifurcations ($F(1, 14) = 24.823; p < .000$), which suggests that nicotine pretreatment increases the overall number of bifurcations on the dendritic tree. ANOVA also revealed a significant interaction of pretreatment x radius on number of bifurcations ($^1F(4.428, 61.99) = 3.076; p < .019$), which suggests that the number of bifurcations is dependent on the radial distance from the soma. Although independent t-test analysis failed to meet the criteria of significant differences at three-consecutive radii, there was a significant increase in the number of bifurcations in the nicotine pretreatment group compared to the saline pretreatment group at $40\mu\text{m}$ from the soma ($t(14) = -3.732; p = .002$), and a trend toward a significant increase at $120\mu\text{m}$ ($*t(11.878) = -2.667; p = .021$), and $180\mu\text{m}$ ($*t(8) = -2.9; p = .02$), from the soma. Without significance at three-consecutive radii, the interaction of pretreatment x radius on number of bifurcations was not considered to be significant.

Figure 7

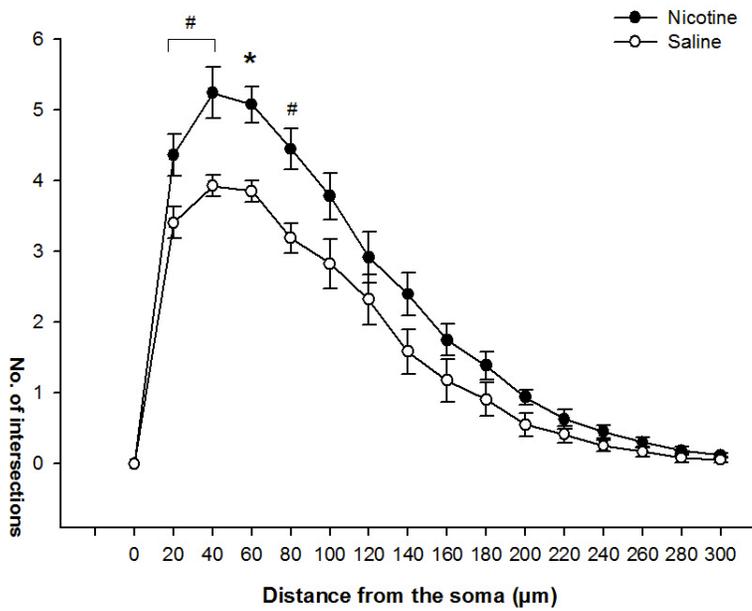


Figure 5: Sholl analysis on no. of intersections

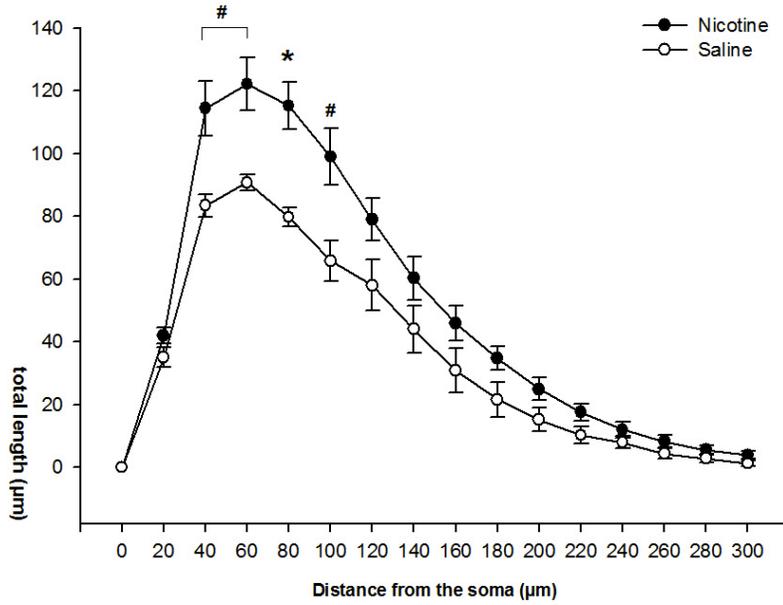


Figure 6: Sholl analysis on total length

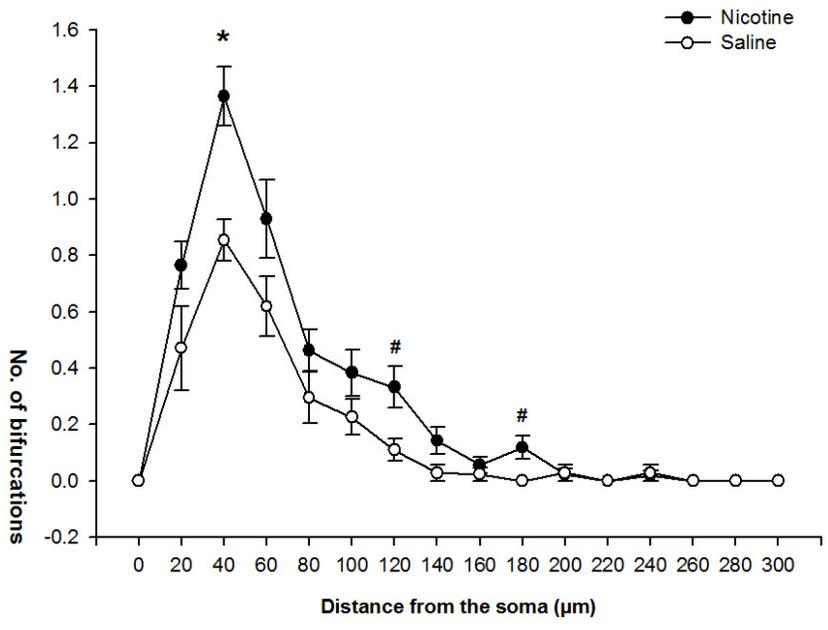


Figure 7: Sholl analysis on number of bifurcations

DISCUSSION

Adolescent nicotine exposure produced structural modifications of dendrites in the BNST. Dendritic morphological changes occurred in the form of new branching as well as lengthening of already existing branches. This alteration was also reflected in the Sholl analysis that displayed an increase of branching and lengthening of dendrites in animals that were exposed to nicotine during adolescence. To our knowledge no previous research has shown dendritic remodeling in the BNST following adolescent nicotine exposure.

Increase of Connectivity

The dendritic alterations found in the BNST, dendritic lengthening and increased branching, increases activity within the BNST via an increase in glutamate activity and may further increase excitatory output from the BNST. The BNST has a role in anxiety related behaviors, fear responses, behaviors associated with drug abuse and dependence, and stressed induced drug-seeking (McElligott & Winder, 2009). Cortical and limbic regions play a role in these behaviors by their glutamatergic inputs to the BNST, and alterations of these connections may result in drug addiction and anxiety-related behaviors (McElligott & Winder, 2009). Our study showed that following adolescent nicotine exposure there was a significant increase in branching and total length of dendrites located in the BNST. This correlates with the findings that adolescent nicotine

exposure leads to increased anxiety-related behaviors and nicotine preference in adulthood (Bergstrom, 2009; McDonald et al., 2005). This plasticity within the BNST may lead to an increase of synaptic input to the Ventral Tegmental Area (VTA) and the hypothalamic-pituitary-adrenal (HPA) axis. Major excitatory efferents from the BNST, medial prefrontal cortex (mPFC), laterodorsal and pedunclopontine tegmental nuclei synapse on the VTA; exciting dopamine (DA) neurons of the VTA(Jalabert et al., 2009). An increase of excitatory input to the VTA, resulting in an increase of DA activity, plays a role in the regulation of reward-directed behaviors associated with nicotine consumption.

The mPFC acts as a major component of the motivation network. This is noted by the mPFC providing 90% of the excitatory input to the VTA(Jalabert et al., 2009). A subdivision of the mPFC, the infralimbic cortex (ILCx), provides 10% of the excitatory input to the VTA, which is relayed through the BNST. Thus the BNST sends monosynaptic excitatory input to DA neurons within the VTA(Jalabert et al., 2009). The BNST also receives excitatory glutamatergic input from the ventral subiculum of the hippocampus (vSUB), which plays a role in the regulation of stress response and reward-directed behavior. Together the vSUB and the ILCx provide regulation of the BNST neurons that project on to the VTA and interact with the dopaminergic neurons(Jalabert et al., 2009). An increase of glutamate receptor activity in the BNST increases this input to the VTA and may underlie the behaviors associated with nicotine addiction and reward. Plasticity of the excitatory synapses from the BNST to the VTA may relate to operant learning associated with the BNST's role in the reward pathway.

The BNST's projection to the VTA excite DA neurons through the glutamatergic NMDA and AMPA receptors (Jalabert et al., 2009). The ILCx and vSUB also exert their regulation of the BNST through AMPA and NMDA receptors within the BNST. The ILCx and vSUB may have an inhibitory control over the BNST by sending glutamatergic input to GABA terminals within the BNST (Jalabert et al., 2009). Further studies have shown that the GABAergic system within the BNST is not affected by intra-BNST infusion of GABA or the introduction of NMDA/AMPA antagonist (Jalabert et al., 2009). Activation of DA neurons in the VTA is increased following an injection of glutamate into the BNST, while infusion of GABA into the BNST decreased activity of DA neurons in the VTA (Jalabert et al., 2009). Lengthening and increased branching of dendrites in the BNST may increase glutamatergic transmission to the VTA, which then increases DA neuronal activity. Increased DA activity in the VTA is associated with motivational behavior (Jalabert et al., 2009).

The BNST is involved in processing emotional and contextual stimuli and this interacts with the DA system and influences motivational behavior. This may underlie how stress can influence goal-directed behavior and reward systems. The ILCx regulates fear and drug memories after extinction through projection to the amygdala and the nucleus accumbens (NAc) (Jalabert et al., 2009). The BNST may parallel this memory circuit and further regulate the impact of information coming from the ILCx and vSUB in order to filter and amplify activation of DA neurons (Jalabert et al., 2009). The BNST relays input from stress sensitive areas of the cortex and limbic system to the HPA axis and to the brainstem nuclei (Oliveira et al., 2012). An increase in anxiety-related

behaviors has been associated with an increase in synapsin, a protein involved in the regulation of neurotransmission, and an increase in synaptic activity (Oliveira et al., 2012). Plastic changes in the BNST increases synaptic activity within the BNST and in the projections to the HPA axis, which creates an over activation of these brain regions. Increased activity in the BNST along with increased response of the HPA axis can lead to an increase of anxiety-related behaviors, as noted in the study conducted by Oliveria et al. The BNST regulates activity of the HPA axis by first regulating activity of the paraventricular nucleus of the hypothalamus (PVN) (Conrad & Winder, 2011). Activation of the PVN releases corticotropin-releasing hormone (CRH), which then activates the release of adrenocorticotrophic hormone (ACTH) into the bloodstream. This release of ACTH acts on the adrenal gland that then releases corticosterone (McElligott & Winder, 2009). The dorsomedial and fusiform nuclei within the BNST also express CRH; activation of these regions would excite the HPA axis and increase anxiety-related behaviors (Choi et al., 2007). Dysregulation of the HPA axis is commonly observed in patients with affective and substance abuse disorders (Conrad & Winder, 2011). Maladaptive forms of plasticity in regions that regulate the HPA axis have been noted to form following chronic stress, Etoh administration and now nicotine administration (Conrad & Winder, 2011). Nicotine exposure causes changes to occur in the HPA axis that leads to an increase in anxiety behaviors that can be seen well into adulthood.

Through our study the BNST has been shown to develop modifications that are consistent with the process of addiction and the manifestation of anxiety following adolescent nicotine exposure. The full extent of the BNST's role in addiction is not

wholly understood, but our current findings build upon this growing area of research and will help to guide future research in the hopes to fully understand the complexity of the BNST.

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CURRICULUM VITAE

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