THE EFFECT OF D1 ANTAGONISTS ON THE DENDRITIC MORPHOLOGY OF THE DORSAL STRIATUM IN ADOLESCENT RATS PRIOR TO THE INJECTION OF NICOTINE

by

Julian Burke

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

Committee:

_____ Director

_____

_____

_____

_____ Department Chairperson

_____ Dean, College of Humanities and Social Sciences

Date: _____ Fall Semester 2015
George Mason University
Fairfax, VA

The Effect of D1 Antagonists on the Dendritic Morphology of the Dorsal Striatum in Adolescent Rats Prior to the Injection of Nicotine

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

by

Julian Burke
Masters of Arts
George Mason University, 2015

Director: Craig McDonald, Professor
Department of Psychology

Fall Semester 2015
George Mason University
Fairfax, VA

ACKNOWLEDGEMENTS

I would first like to thank my family for providing me with all of the opportunities that have lead me to where I am today. I am truly grateful for their love and support in all that I do.

I would also like to express my sincere gratitude to my committee director, Dr. Craig McDonald, for his guidance and support throughout this process. I also wish to gratefully acknowledge the members of my Master's committee, Dr. Robert Smith, Dr. Jennifer Brielmaier, and Dr. Linda Chrosniak, and express my sincere gratitude to them. Additionally, special thanks go to Dr. Dan Ehlinger and Dr. Hadley Bergstrom for their invaluable consultation and guidance. Together, their encouragement and feedback inspired me each step of the way.

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

THE EFFECT OF D1 ANTAGONISTS ON THE DENDRITIC MORPHOLOGY OF THE DORSAL STRIATUM IN ADOLESCENT RATS PRIOR TO THE INJECTION OF NICOTINE

Julian Burke, M.A.

George Mason University, 2015

Thesis Director: Craig McDonald

The purpose of this research study was (1) to verify whether nicotine results in the same dendritic remodeling in the dorsal striatum that has been shown to occur in the nucleus accumbens (NAcc) (Ehlinger et al., 2014) and (2) whether the use of D1-class receptor (D1DR) antagonists prior to the injection of nicotine would prevent the hypothesized dendritic remodeling. Rats were divided into four groups, each having been injected with either a D1DR antagonist (SCH23390 hydrocholride) or saline solution 20 minutes prior to the injection of either nicotine or saline solution. This treatment occurred every other day over a period of fourteen days (P28-P42). After a Golgi-Cox staining, neurons were reconstructed and three morphological parameters (length, intersections, and bifurcations) were analyzed. While neither hypothesis was supported, significant differences within the dorsal striatum were found between the neurons of the dorsomedial (DMS) and dorsolateral (DLS) regions, with neurons in the DLS having greater dendritic length. In

addition, an interaction between location and nicotine treatment was found, suggesting nicotine differentially affected neurons in the DLS and DMS. Specifically, there was a tendency for nicotine to decrease dendritic length in the DMS, but increase dendritic length in the DLS. These findings suggest that the DLS and DMS act antagonistically, and that nicotine exposure could promote inflexible habit learning (e.g. addiction), which is mediated by the DLS, at the expense of DMS-mediated goal-oriented learning.

CHAPTER ONE

Introduction and Literature Review

Smoking is the most common preventable cause of death in the United States and is also responsible for over 87% of lung cancer deaths. In addition to the 69 cancer causing chemicals in tobacco smoke, 181 other harmful chemicals have been identified (The National Cancer Institute, 2011). There are over 480,000 deaths per year caused by smoking in the United States. Despite these statistics, about 42 million Americans still smoke (U.S. Department of Health and Human Services, 2014). The underlying factor for this continued use is due to the addictive qualities of nicotine (Picciotto & Kenny, 2013). Over the past years, studies have indicated the majority of smokers began smoking during adolescence. In just three years the number of adults who began smoking during adolescence increased from 80% to 88% (Centers for Disease Control and Prevention, 2012; U.S. Department of Health and Human Services, 2014). This is especially problematic, as the brain has not finished developing until adulthood and is more prone to the damaging effects of nicotine (Brielmaier, McDonald & Smith, 2007; Centers for Disease Control and Prevention, 2012). It is therefore pertinent to study adolescents when examining the effects of nicotine on the brain.

Previous studies emphasized that adolescent brains are more susceptible to the rewarding effects of nicotine, which is mainly due to the fact that the brain has not yet fully developed (National Institute on Drug Abuse, 2012; Smith, 2003; U.S. Department

of Health and Human Services, 2014). Although various human studies have found certain behavioral effects of nicotine beneficial, such as increased motor abilities and working memory (Heishman, Kleykamp, & Singleton, 2010), animal studies have indicated that these beneficial effects come at a cost, such as anxious behavior in adolescents that lasts well into adulthood (Smith et al., 2006). Nicotine has also been shown to improve learning at low doses at the expense of diminishing the ability to unlearn or extinguish a learned behavior such as fear conditioning. This also gives insight into why quitting may be so difficult for many smokers, as they are unable to unlearn their conditioned responses(Smith et al., 2006).

Interestingly, several studies have indicated that the severity of nicotine withdrawal symptoms are minimal during adolescence compared to the more negative symptoms that adult smokers exhibit (Natividad, Buczyncski, Parsons, Torres, & O'Dell, 2012; O'Dell et al., 2006; Smith et al., 2008). This in turn allows adolescents to receive the enhanced and rewarding effects of nicotine without the consequence of any major negative withdrawal symptoms. Therefore, adolescents may perceive that they are actually not addicted and thus have no reason to quit smoking.

The mesocorticolimbic dopamine system is responsible for many functions including movement, however, it is also plays a role in addiction. This system originates in both the ventral tegmental area (VTA) as well as the substantia nigra pars compacta (SNc) which projects dopamine to various subcortical structures, most notably the dorsal and ventral striatum (Arias-Carrión, Stamelou, Murillo-Rodríguez, Menéndez-González, & Pöppel, 2010; Nestler, 2001). The dorsal striatum is comprised of two nuclei, the

caudate and putamen (CPu) while the accumbens (NAcc) composes the ventral striatum (Bear, Connors, & Paradiso, 2007; Haines, 2013). Although both areas contribute to addiction, the NAcc has garnered much attention as evidenced by a vast number of recent studies that focus not only on structural changes, but also many more aspects. Many of the studies examining the structural changes (Brown & Kolb, 2001; Ehlinger et al., 2014; McDonald et al., 2007) have indicated a growth in the dendritic complexity in the NAcc after the introduction of nicotine.

As mentioned above, there are dopamine projections to both the dorsal and ventral striatum. Dopamine (DA), similarly to the NAcc, is regarded as the neurotransmitter responsible for the pleasurable effects of drugs (Carlson, 2009). Not surprisingly, the NAcc has been found to be especially sensitive to the dopamine-stimulant effect of acute nicotine (Di Chiara, 2000) which may contribute to the addictiveness of the drug. While dopamine has many other functions as well as five subtypes of receptors (Beaulieu & Gainetdinov, 2011), the D1-class receptors (D1DR) have been linked to the regulation of dendritic growth (Sunahara et al., 1990). Particularly, it is the combination of D1DRs and the cAMP-PKA pathways that upregulate intracellular signaling pathways within the NAcc, which in turn have displayed a role in dendritic plasticity. D2-type receptors (D2DR) on the other hand have been found to inhibit the cAMP-PKA pathway (Self, 2004). In our previous study, we found that the dendritic remodeling (increase in length and bifurcations) in the NAcc that follows the introduction of nicotine is dopamine dependent, specifically the D1DR

(Ehlinger et al., 2014). These results shed light on the possible underlying mechanisms of nicotine addiction.

Although the NAcc is under the spotlight for addiction studies, the dorsal striatum has provided researchers with additional evidence that may broaden the current understanding of addiction acquisition as well as the maintaining of acquired addictions. The dorsal striatum is generally associated with movement, but has also been linked to learning and addiction (Balleine, Delgado & Hikosaka, 2007; Vollsädt-Klein et al., 2010). Behaviorally, the dorsal striatum may help cause addiction through *cueing,* which may be due to the fact that the dorsal striatum aids in habitual learning. Cues in this case are simply stimuli that elicit a conditioned reaction. In a study conducted by Nummenmaa et al. (2012), the researchers examined differences in brain activation in humans (using an fMRI) of morbidly obese and normal-weight participants in cueing. Although this study did not examine drug effects, the aforementioned reward circuit has been shown to function similarly on both obesity and drug addiction. The results of the study indicated that morbidly obese people show an increased activation in the dorsal striatum when cued with appetizing foods, and exceeded the activation of participants of normal weight. In addition, the study demonstrated that there was increased connectivity of the dorsal striatum with the amygdala and posterior insula, which are associated with aspects of emotional regulation. What was notable about the secondary result was that this was evident even when participants were instructed to complete behavioral tasks unrelated to the hedonic stimuli and were not deliberately paying attention to the content of the cues (appetizing foods). This result, coupled with the fact that the NAcc is

responsible for the *rewarding* effects of a stimulus (food/drugs/etc.), may account for the continuation of the stimulus due to emotionally charged cues.

Another study conducted by DePoy et al. (2013) that examined mice, exemplifies supporting evidence for cueing using drugs (alcohol) rather than food cues in obesity. The authors define drug addictions as, the "degradation of executive control over behavior and increased compulsive drug seeking" (p. 14783). In other words, behavior shifts from the executive functions of the prefrontal cortex (PFC) to the automatic learned behaviors of the dorsal striatum. Their results indicated that it is the dorsal striatum that is responsible for changing learning and regulation of rewarding behavior. In their case, the administration of alcohol caused an increase in dendritic length in addition to an increase in bifurcations in the dorsal striatum that the authors suggest causes it to *prime* the continuation of the addictive behavior. The combination of both cueing and priming for rewarding substances, whether it is food or drugs, illustrates how this learned behavior may be difficult to break. This can make it especially difficult for adolescents as recent evidence indicates that both a preference for novelty and dopamine receptor density in the entire striatum peaks during adolescence (Churchwell, et al., 2012). In other words, adolescents are more likely to initiate drug use, are more susceptible to the addictive qualities of nicotine, and are likely to be more influenced by smoking cues, which in turn reinforces the addictive behavior. The fact that adolescents give in to peer pressure and make decisions based on emotion due to their underdeveloped PFC also plays a major role in their susceptibility to nicotine use (Blakemore & Robbins, 2012; Centers for Disease Control and Prevention, 2012).

Past research with rats has shown that nicotine causes an increase in length and bifurcations of the dendrites of medium spiny neurons (MSNs) in the NAcc and is D1DR dependent (Brown & Kolb, 2001; Ehlinger et al., 2014; McDonald et al., 2007). Although there are several studies examining the dendritic morphology of the NAcc after nicotine exposure, nicotine research on the dendritic morphology of the dorsal striatum is rather limited. However, a few studies have shown evidence that the dorsal striatum may exhibit similar reactions to the NAcc as a result of nicotine. For example, some human studies report that nicotine is the cause for increased dorsal striatal volume (Das, Cherbuin, Anstey, Sachdev, & Easteal, 2012; Janes, Park, Farmer, & Chakravarty, 2014). While increase in striatal volume does not necessarily indicate an increase in dendritic complexity, it is a valid hypothesis that warrants further study. Interestingly, in the study conducted by Das et al., (2012), greater volume in the dorsal striatum signified earlier initiation of smoking. The change in striatal volume could be due to increased dendritic length and bifurcations of its cells, or a predisposition to smoking, which calls for further investigation.

While the research examining increased dendritic growth (specifically in length and bifurcations) in the dorsal striatum due to nicotine has been lacking, studies on the effects of other drugs may provide evidence for dendritic alterations within this area. The previously mentioned study conducted by Depoy et al. (2013) indicates that chronic alcohol consumption produces dendritic changes in the dorsal striatum of mice similar to those observed for nicotine in the NAcc in rats. Specifically, Depoy et al. (2013) found that the introduction of chronic intermittent ethanol (CIE), which simulated drinking

behavior associated with alcohol abuse, caused the dendrites of the dorsostriatal cells to exhibit an increase in both length as well as the amount of branching when compared to the control group (no ethanol). Although the study did not focus on dopamine as an influencing factor, alcohol has been associated with dopamine release that parallels dopamine release through nicotine (Heinz et al., 2004). In addition, the authors provided evidence from previous research that alcohol has the same effect on dendritic morphology in the ventral striatum (NAcc). Therefore, it would seem reasonable to hypothesize that nicotine might have a similar effect on the dendrites of the dorsostriatal cells.

In our previous study, D1 dopamine was found to be a factor underlying the increased dendritic length and bifurcations of MSNs commonly associated with the introduction of nicotine to the NAcc. Comparable research has not been conducted on the dorsal striatum, however, there is evidence that D1DRs may also regulate dendritic morphology in this area. Just as with the NAcc, nicotine binds to neurons within the VTA, which in turn send dopamine to both the dorsal striatum and NAcc (Di Chiara, 2000; Picciotto & Kenny, 2013). In general, both the VTA and SNc project dopamine to the striatum (Arias-Carrión et al., 2010; Nestler, 2001). A study conducted by Fasano and Brambilla (2002) found that while both D1DRs and D2DRs play a role in terms of synaptic plasticity within the dorsal striatum, D1DRs are specifically required for long-term potentiation (LTP). Not only are there dopaminergic projections to D1DR containing neurons in the dorsal striatum, but it also includes a similar cell composition with the NAcc. MSNs are the primary cell type within the dorsal striatum and they have

been shown to express high levels of D1DRs (Surmeier, Ding, Day, Wang, & Shen, 2007).

The aforementioned DA system plays an important role in addiction of both adolescents and adults.However, current research has indicated that this system undergoes structural changes during adolescence, which can cause even more vulnerabilities to nicotine. A review of the literature by Spear (2000) examines in great detail the differences during the developmental transition between adolescence and adulthood. During adolescence for both rats and humans, there areroughly one-third to one-half times more D1DRs in the striatum than in adults as well as an overproduction of DA. In rats, this change peaks at P30 before declining at around P40. In other words, there is a developmental overproduction of DA receptors in both humans and rodents during adolescence which could be another explanation as to why nicotine has such a strong influence on adolescents when compared to adults.

The dorsal striatum in the rat brain is a relatively large structure that lacks any visibly apparent structural landmarks within its initial boundaries. Although it appears to be a single structure, the dorsal striatum can actually be divided into two parts, the dorsolateral (DLS) and dorsomedial striatum (DMS) (Jedynak, Uslaner, Esteban, & Robinson, 2007). While the research on differences of the effect of nicotine in these two areas is lacking, there has been research focusing on the functional differences between these two structures. For example, a study conducted by Fanelli, Klein, Reese, and Robinson (2013) examined neuronal activity differences during self-administration of alcohol in rats. The researchers found that goal-directed behavior is associated with the

DMS while habit formation is associated with the DLS. Using extracellular recordings from chronically implanted electrodes, they noted that the DMS was most active when presented with alcohol-predictive cues whereas the DLS was most excited right before the lever press for alcohol. Although a similar research approach for nicotine has not been conducted, one could speculate that due to the localized functions of the dorsal striatum (DLS vs. DMS), the hypothesized dendritic growth within the dorsal striatum due to nicotine may also illustrate such localization. In other words, it is possible that the medial and lateral dorsal striatum may exhibit differential nicotine-induced neural plasticity, which warrants further investigation.

Purpose of the Study

A review of past research has illustrated that nicotine causes dendritic growth by increasing dendritic length as well as causing an increase of bifurcations of MSNs in the NAcc which is D1 dependent (Ehlinger et al., 2014). While the NAcc has received considerably more research attention regarding dendritic remodeling, the dorsal striatum has not received as much attention. Evidence suggests that the dorsal striatal volume increases in relation to nicotine usage(Das, Cherbuin, Anstey, Sachdev, & Easteal, 2012; Janes, Park, Farmer, & Chakravarty, 2014). It stands to reason that this difference in volume may have been due to an increase in dendritic complexity (increased length and/or bifurcations. In addition, other drugs such as alcohol have been shown to increase dendritic length in the dorsal striatum (DePoy et al., 2013)

Thus, the purpose of this study was to examine whether dorsal striatal neurons increase in dendritic complexity as a result of nicotine. Samples from both the

dorsomedial and dorsolateral striatum were analyzed, which provide a basis for future studies and discussion regarding possible observable differences. The second purpose of this study was to examine whether nicotine induced dendritic remodeling in the dorsal striatum is D1DR dependent. It was hypothesized that (1) injectionof nicotine will result in an increase of dendritic length and bifurcations of MSNs within the dorsal striatum of adolescent rats and (2) the use of a D1DR antagonists in adolescent rats before nicotine exposure will prevent the occurrence of dendritic growth in the dorsal striatum.

CHAPTER TWO

Method

This study is a new investigation using the tissue of the study conducted by Ehlinger et al. (2014). Relevant information from the previous study will be provided below in addition to the methods for the current study.

Animals and Experimental Groups

Previously stained tissue slices of thirty-two male adolescent (P28) Sprague-Dawley rats were analyzed for the current study. Rats received pretreatment/treatment injections of saline, nicotine, and D1DR antagonists (SCH23390). The study consisted of four groups: (1) Saline pretreatment/ saline treatment, (2) SCH pretreatment/ saline treatment, (3) saline pretreatment/ nicotine treatment, (4) SCH pretreatment/ nicotine treatment. Groups one and two served as the two control measures for the experimental groups, three and four. The thirty-two rats were randomly and evenly segregated into the four groups and were housed in groups of 4-5 per cage. Cages provided a 12-hour light/dark cycle as well as free access to food and water.

Procedures

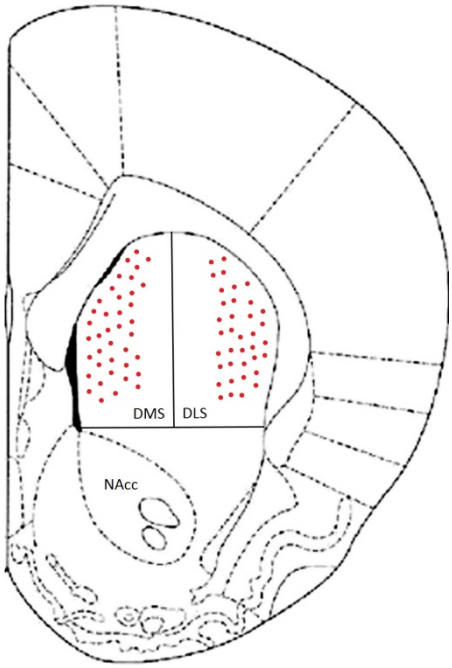
For the pretreatment, rats were administered subcutaneously (SC) with either .9% NaCl at 1ml/kg or SCH23390 hydrochloride in .9% NaCl, .05mg/kg at 1ml/kg. This occurred every other day (8 times) in a 14 day period (P28-P42) every other day. Rats were then injected with the treatment drug (either .9% NaCl at 1ml/kg SC or Nicotine

hydrogen tartrate in .9% NaCl, dose .5mg/kg, at 1ml/kg SC) twenty minutes after each pretreatment injection.

After the 14 day dosing period (P42), there was a 21-day abstinent period before the rats were sacrificed for staining (P63). Rats were deeply anesthetized using Ketamine/xylazine and perfused intracardially w/ .9% NaCl. After the brains were removed they were placed into Golgi-Cox solution on P63 for 14 days before they were transferred to 30% sucrose for about 5 days. The Golgi-Cox method, as performed by Ehlinger et al. (2014) was used as only the dendrites and soma of cells were stained (excluding axons). Lastly, after clearing the brains in CXA solution, they were sectioned at 200µm using a vibratome.

In the current study, neurons were examined using Neurolucida software (Microbrightfield Biosciences, Williston, VT, USA) at 60X objective by an experimenter blind to the treatment groups. Medium Spiny Neurons (MSNs) were randomly selected from the dorsal striatum for tracing. Two neurons were traced in both the dorsomedial and dorsolateral striatum for each hemisphere. The total amounted to 256 traced neurons. Due to the lack of physical landmarks within the dorsal striatum, to differentiate between dorsolateral and dorsomedial striatum a line was drawn separating medial from lateral by connecting the two most dorsal and ventral points of the oval shaped dorsal striatum. Only neurons that were located at least 50µmaway from the line were considered for tracing (fig. 1). Because the Golgi stain stained proportionately more cells as well as at a higher quality in the periphery of both the DMS and DLS, the vast majority of reconstructed neurons were from these areas.

Fig. 1 Boundaries for the dorsomedial (DM) and dorsolateral (DL) striatum. Red dots represent the region in which cells were collected.



Analysis

Neurons were analyzed using the Sholl analysis method in addition to examining the totals of dendritic length . Specifically, this analysis method used concentric spheres in 20µm increments radiating from the center of the soma to the outmost dendritic tip. By recording the dependent variables (intersections, nodes, and total dendritic length) the overall complexity of the neurons can be measured. To account for individual differences between neurons, the measurements from neuronsreconstructed in a specific brain region were averaged. For each dependent variable, separate mixed-omnibus ANOVA tests were used. Each ANOVA consisted of two between-group independent variables each containing two levels (pretreatment= saline or SCH23390; treatment= saline or nicotine) and one within-subjects independent variable (medial vs. lateral),which used the total

values for each parameter.Following the ANOVA, follow-up comparisons were made between groups using individual independent samples *t*-tests on the total values of the dependent variables. Following a significant interaction between pretreatment/treatment groups and Radius, independent samples *t*-tests were conducted at each specific radii between combinations of pretreatment/treatment groups to search for any significant differences at varying distances from the soma. To control for spurious significance values due to repeated individual *t*-tests the false discovery rate (FDR) correction was used for each radii point.

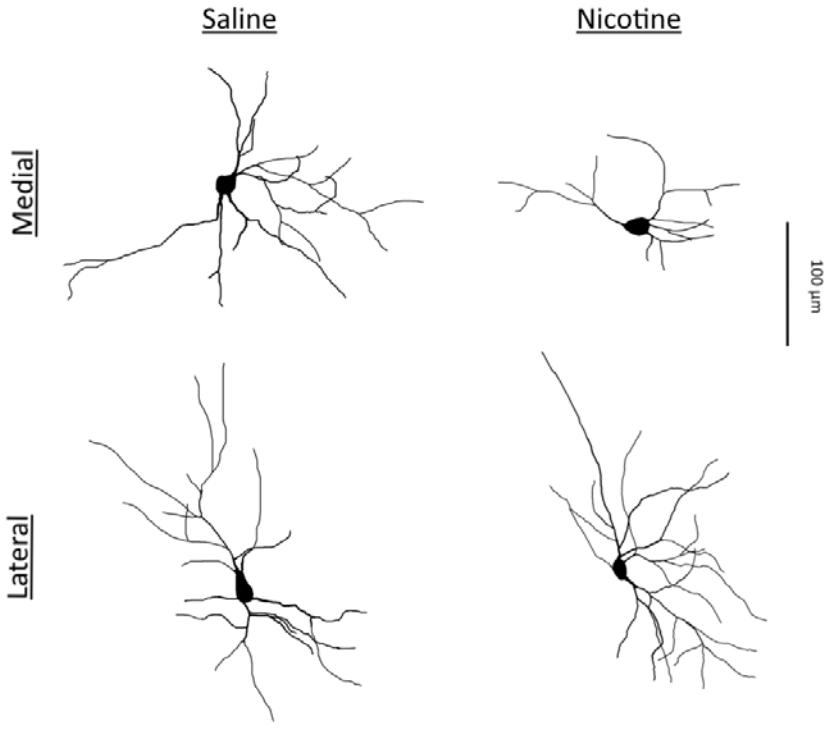


Fig. 2 Representative MSN reconstructions. *y*-axis, location. *x* axis, treatment group.

CHAPTER THREE

Results

Omnibus mixed ANOVA with between-subjects variables of pretreatment (saline; SCH23390) and treatment (saline; nicotine), and within-subjects variable (location) revealed non-significant interactions of pretreatment and treatment, for intersections, length, and bifurcations ($p>.05$) across both the dorsomedial and dorsolateral striatum. This suggests that combinations of the pretreatment and treatment did not significantly affect the dendritic morphology of MSNs in the dorsal striatum. However, there was a main effect of location (medial vs. lateral) for all three parameters (total length: $F(1,28) = 29.3$, $p< .001$; Intersections: $F(1,28) = 30.4$, $p< .001$;Bifurcations: $F(1,28) = 19.7$, $p< .001$). This revealed that the MSNs from the dorsolateral striatum exhibited greater dendritic length than those in the dorsomedial striatum as well as more intersections and bifurcations. In addition, the analysis revealed that there was an interaction between location and treatment on the MSNs of the dorsal striatum (total length: $F(1, 28) = 6.0$, $p = .02$; total intersections: $F(1, 28) = 6.2$, $p = .02$). Although non-significant the parameter bifurcations exhibited a trend in the same direction ($F(1,28) = 2.5$, $p = .128$). These interactions (fig. 3) suggest that nicotine enhances the effect of location by increasing the length of the dorsolateral striatal neurons while decreasing the length of the dorsomedial striatum.

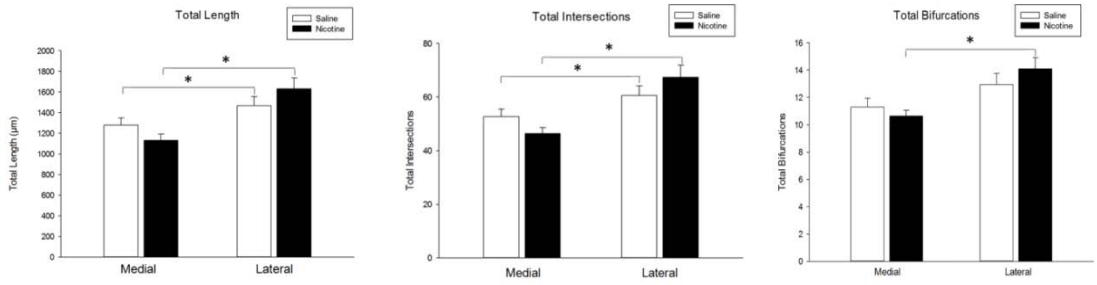


Fig. 3 Influence of the treatment on the three parameters of the dorsomedial and dorsolateral striatum

As a follow up to the ANOVA, independent samples t-tests were conducted to examine whether there were any significant differences between saline and nicotine treatment groups within the DLS and the DMS. Results indicated that the difference between saline and nicotine groups were not statistically significant for the totals of each parameter within both the DLS (length:$t(30) = -1.2$, $p > .05$; intersections: $t(30) = -1.2$, $p > .05$;bifurcations:$t(30) = -1$, $p > .05$) and the MLS (length:$t(30) = 1.6$, $p > .05$; intersections:$t(30) = 1.7$, $p > .05$; bifurcations:$t(30) = .9$, $p > .05$).

Paired samples t-tests were conducted to further explore the interaction identified in the ANOVA by examining the within group differences as a function of location. The groups defined as *saline* and *nicotine*below included the groups for the D1R1 antagonist. As expected, there were no significant differences between DLS and DMS with regards to the treatment groups (saline vs. nicotine) for the length and intersections parameters. However, the difference between the medial and lateral dorsal striatum for the total bifurcations significantly increased from 2 (M = 11.3, SD = 2.6; M = 13, SD = 3.2) for

the saline group ($t(15) = -2.1$, $p > .05$) to 4 (M = 10.6, SD = 1.7; M = 14.1, SD = 3.3) for the nicotine group ($t(15) = -3.9$, $p < .01$).

Paired-samples t-tests (medial vs. lateral) at each radius were conducted to determine the spatial distribution of significant differences in each of the morphological parameters (fig. 4). In order to prevent a type I error, the false discovery rate (FDR) correction was used. For length, the significantly different radii (by location) for the saline group ranged from R120 to R140, whereas the nicotine group included significantly different radii from R80 to R200 ($p < .05$). The saline group exhibited a significant number of intersections from R100 to R120 while the nicotine group exhibited significant differences at R80 through R180 ($p < .05$). Lastly, bifurcations did not exhibit any significant differences within the saline group, although in the nicotine group, there was a significant difference at one radius, R40 ($p < .05$). A noteworthy outcome of the analysis is that in the nicotine group for both length and intersections, the distribution of significant radii was much wider than in the saline groups. This is consistent with the aforementioned interaction which suggests that there is a larger difference in morphological complexity between the DMS and the DLS in the nicotine group.

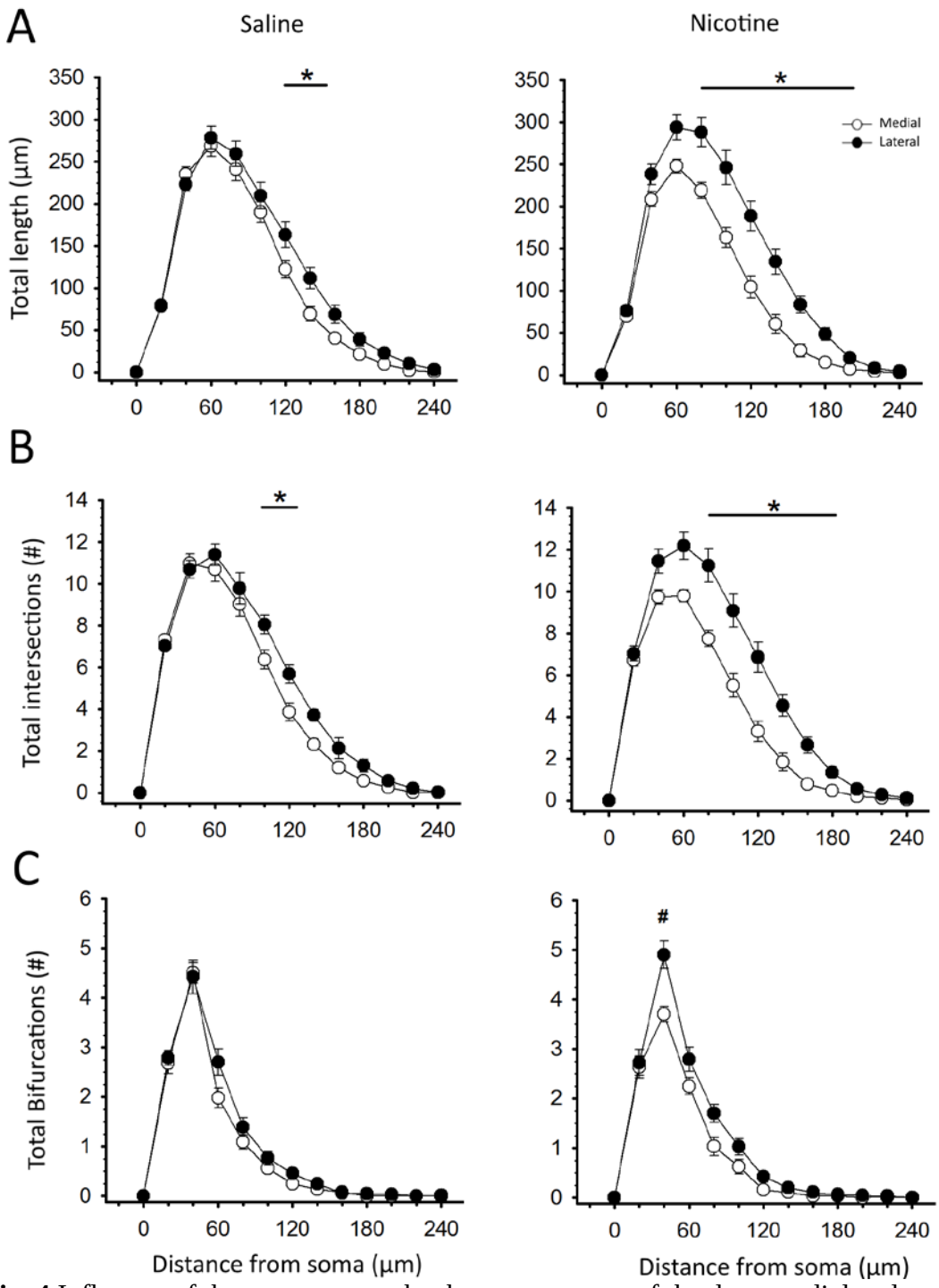


Fig. 4 Influence of the treatment on the three parameters of the dorsomedial and dorsolateral striatum as a function of distance from the soma. Total dendritic length (mean ± SEM), total intersections (mean ± SEM) and total bifurcations (mean ± SEM). *Significant difference between medial and lateral following FDR. #Significant between medial and lateral difference (p<0.05)

CHAPTER FOUR

Discussion

The purpose of this study was to (1) determine whether the medium spiny neurons of the dorsal striatum displayed similar dendritic growth after nicotine exposure as exhibited by neurons of the NAcc, and (2) ascertain whether this growth, if it occurred, was regulated by D1-type DA. The results indicate that the dendritic morphology did not emulate the dendritic remodeling as seen in the NAcc by Ehlinger et al. (2014). Because the neurons for this study were collected from the same animals as in the aforementioned study, these results provide a more direct comparison as no other potentially confounding variables were introduced through the use of a new sample.

Although there was no interaction between the pretreatment and treatment groups, the results exhibit other notable interactions and effects. One finding illustrates that, overall, DLS neurons have greater dendritic length than those from the DMS regardless of the drug group (saline or nicotine). However, the most notable result is the interaction between location (medial vs. lateral) and treatment (saline vs. nicotine). As stated before, the results indicate that the neurons of the DLS have greater dendritic length than the neurons of the DMS, which is true even for the control groups (saline, saline or antagonist, saline). While this structural difference was found in all groups, those within the nicotine treatment group tended to display greater differences. In other words,

nicotine treatment produced a trend toward increased dendritic length in DLS neurons and decreased the dendritic length in DMS neurons.

Past research has illustrated that there are distinct functional differences between the DLS and the DMS, namely the type of learning that is attributed to each region. The DLS is related to habit forming (less flexible), or stimulus-response (S-R) learning, while the DMS is related to goal oriented tasks (more flexible), or response-outcome (R-O) learning (Horvitz, 2009; Thorn, Atallah, Howe, & Graybiel, 2010). It is feasible that this functional difference could account for the trend of increased dendritic length of the neurons in the DLS, as these neurons are "primed" for habit acquisition or expression (addiction). This theory is supported by other recent studies which, although not nicotine based, have illustrated the importance of the DLS in habit formation with other addictive drugs such as alcohol and methamphetamines (DePoy et al., 2013; Jedynak et al., 2007). Future studies may want to focus on potential reasons for the decrease in dendritic length of the neurons in the DMS.

Results from this study suggest that the DMS and the DLS may have an antagonistic effect on one another. Although speculative, one possible reason may be that the more one becomes addicted to a substance (inflexible habit learning), the less activation is required from the flexible "goal" oriented region (DMS). If this were the case, quitting a substance would be substantially more difficult, as there are potentially fewersynaptic connections available for the new goal (quitting), while the connections for addiction are only strengthening. Also, this would further explain why such an antagonistic response is even necessary, as it would be physiologically more efficient to

transform a regularly reoccurring behavior into an automatic response than one that requires more intensive cognitive control. Similar effects between the DMS and DLS have also been exhibited in other studies in which early acquisition and performance of a rewarding task depend on the DMS while the DLS is responsible for overtraining, or habitual behavior (Balleine, Liljeholm, & Ostlund, 2009; Everitt & Robbins, 2013).

An area for future study is the effect of the duration of nicotine treatment as well as the duration of the abstinence period. Specifically, researchers could examine exactly when the DLS begins to increase in complexity. However, while the control could shift from the DMS to the DLS, previous research has also indicated that there is a similar transfer of control from the ventral striatum to the dorsal striatum overall (Everitt & Robbins, 2013; Vollsädt-Klein et al., 2010). This shift is displayed behaviorally as the difference between a hedonic vs. compulsive behavior. In other words, this too could be viewed as goal oriented (hedonic) or habitual (compulsive).

As noted above, while there was significant interaction between location and treatment, the DA antagonist prior to nicotine exposure did not have any effect. Although the dorsal striatum receives DA projections from the VTA, when compared to the NAcc, it does not receive the same amount of DA after nicotine exposure (Zhang et al., 2009). In fact, nicotine suppresses low-frequency dopamine release in both the dorsal striatum and NAcc in favor of increasing phasic bursts. However, this phasic bursting is much more predominant in the NAcc shell. This could explain why, in general, the neurons of the dorsal striatum did not exhibit any significant increase in dendritic length. In addition, this would potentially support other research which has indicated the shift of

control from the NAcc to dorsal striatum. Perhaps the focus on the NAcc during the phasic DA bursts is a mechanism underlying the effects of chronic nicotine exposure. Although these theories may explain the lack of pronounced dendritic remodeling of the neurons in the DMS, further research is required to determine what mechanisms explain the enhancement of the DLS neurons, as this study suggests the possibility that theydo not involve the D1-type DA.

Conclusion

In conclusion, the hypotheses that (1) nicotine causes an increase in dendritic arborization of the MSN neurons in the dorsal striatum and (2) using a D1-type DA antagonist prior to nicotine exposure prevents the dendritic growth (which would also indicate that the growth is D1-type DA dependent) were not supported by the results. This is perhaps due to the fact that DA release from the VTA occurs mainly in the NAcc during nicotine exposure in addition to thepossibility that a different mechanism is responsible for dendritic remodeling in the dorsal striatum. While the hypotheses were not supported, results indicated dendritic length and branch number of neurons of the DLS were significantly greater than those in the DMS. Interestingly, there was also a significant interaction between location and treatment groups (saline vs. nicotine), suggesting that the difference between the DLS and DMS may increase after nicotine exposure. These results provide insight into potential theories of the underlying mechanisms for and interactions between the dorsal striatum and NAcc during and following nicotine exposure.

REFERENCES

Arias-Carrión, O., Stamelou, M., Murillo-Rodríguez, E., Menéndez-González, M., & Pöppel, E. (2010). Dopaminergic reward system: A short integrative review. *International Archives of Medicine, 3*, 24. doi:10.1186/1755-7682-3-24

Balleine, B. W., Delgado, M. R., & Hikosaka, O. (2007). The role of the dorsal striatum in reward and decision-making. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *27*(31), 8161–8165. http://doi.org/10.1523/JNEUROSCI.1554-07.2007

Balleine, B. W., Liljeholm, M., & Ostlund, S. B. (2009). The integrative function of the basal ganglia in instrumental conditioning. *Behavioural Brain Research*, *199*(1), 43–52. http://doi.org/10.1016/j.bbr.2008.10.034

Bear, M.F., Connors, B.W., & Paradiso, M.A. (2007). *Neuroscience: Exploring the brain* (3rd ed.). Baltimore, MD: Lippincott Williams & Wilkins.

Beaulieu, J.-M., & Gainetdinov, R. R. (2011). The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacological Reviews*, *63*(1), 182–217. doi:10.1124/pr.110.002642

Blakemore, S.-J., & Robbins, T. W. (2012). Decision-making in the adolescent brain. *Nature Neuroscience*, *15*(9), 1184–1191. doi:10.1038/nn.3177

Brielmaier, J. M., McDonald, C. G., & Smith, R. F. (2007). Immediate and long-term behavioral effects of a single nicotine injection in adolescent and adult rats. *Neurotoxicology and Teratology, 29*, 74-80. doi:10.1016/j.ntt.2006.09.023

Brown, R & Kolb, B. (2001). Nicotine sensitization increases dendritic length and spine density in the nucleus accumbens and cingulated cortex. *Brain Research, 899,* 94-100.

Carlson, N. R. (2009). *Foundations of physiological psychology* (7th ed.). Boston, MA: Pearson Education Inc.

Centers for Disease Control and Prevention. (2011). *Tobacco use: Targeting the nation's leading killer.* Retrieved from

http://www.cdc.gov/chronicdisease/resources/publications/aag/pdf/2011/Tobacco_AAG_2011_508.pdf

Centers for Disease Control and Prevention. (2012). *Youth and tobacco use.* Retrieved from http://www.cdc.gov/tobacco/data_statistics/fact_sheets/youth_data/tobacco_use/

Churchwell, J. C., Carey, P. D., Ferrett, H. L., Stein, D. J., & Yurgelun-Todd, D. A. (2012). Abnormal striatal circuitry and intensified novelty seeking among adolescents who abuse methamphetamine and cannabis. *Developmental Neuroscience*, *34*(4), 310–317. doi:000337724

Das, D., Cherbuin, N., Anstey, K. J., Sachdev, P. S., & Easteal, S. (2012). Lifetime cigarette smoking is associated with striatal volume measures. *Addiction Biology, 17*(4), 817–825. doi:10.1111/j.1369-1600.2010.00301.x

DePoy, L., Daut, R., Brigman, J. L., MacPherson, K., Crowley, N., Gunduz-Cinar, O., … Holmes, A. (2013). Chronic alcohol produces neuroadaptations to prime dorsal striatal learning. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(36), 14783–14788. doi:10.1073/pnas.1308198110

Di Chiara, G. (2000). Role of dopamine in the behavioural actions of nicotine related to addiction. *European Journal of Pharmacology, 393,* 295-314.

Ehlinger, D. G., Bergstrom, H. C., Burke, J. C., Fernandez, G. M., McDonald, C. G., & Smith, R. F. (2014). Adolescent nicotine-induced dendrite remodeling in the nucleus accumbens is rapid, persistent, and D1-dopamine receptor dependent. *Brain Structure & Function*. doi:10.1007/s00429-014-0897-3

Everitt, B. J., & Robbins, T. W. (2013). From the ventral to the dorsal striatum: devolving views of their roles in drug addiction. *Neuroscience and Biobehavioral Reviews*, *37*(9 Pt A), 1946–1954. http://doi.org/10.1016/j.neubiorev.2013.02.010

Fanelli, R. R., Klein, J. T., Reese, R. M., & Robinson, D. L. (2013). Dorsomedial and dorsolateral striatum exhibit distinct phasic neuronal activity during alcohol self-administration in rats. *The European Journal of Neuroscience*, *38*(4), 2637–2648. http://doi.org/10.1111/ejn.12271

Fasano, S., & Brambilla, R. (2002). Cellular mechanisms of striatum-dependent behavioral plasticity and drug addiction. *Current Molecular Medicine*, *2*(7), 649–665.

Gibb, R., Kolb, B. (1998). A method for vibratome sectioning of Golgi-Cox stained whole rat brain. *Journal of Neuroscience Methods, 79,* 1-4.

Haines, D. E. (2013). *Fundamental of neuroscience for basic and clinical applications* (4th ed.). Philadelphia, PA: Elsevier, Inc.

Hatsukami, D. K., Stead, L. F., & Gupta, P. C. (2008). Tobacco addiction. *Lancet, 371*(9629), 2027–2038. doi:10.1016/S0140-6736(08)60871-5

Heinz, A., Siessmeier, T., Wrase, J., Hermann, D., Klein, S., Grüsser-Sinopoli, S. M., … Bartenstein, P. (2014). Correlation between dopamine D2 receptors in the ventral striatum and central processing of alcohol cues and craving. *American Journal of Psychiatry*. Retrieved from http://ajp.psychiatryonline.org/doi/10.1176/ajp.161.10.1783

Heishman, S. J., Kleykamp, B. A., & Singleton, E. G. (2010). Meta-analysis of the acute effects of nicotine and smoking on human performance. *Psychopharmacology, 210*(4), 453–469. doi:10.1007/s00213-010-1848-1

Janes, A. C., Park, M. T. M., Farmer, S., & Chakravarty, M. M. (2015). Striatal morphology is associated with tobacco cigarette craving. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *40*(2), 406–411. doi:10.1038/npp.2014.185

Jedynak, J. P., Uslaner, J. M., Esteban, J. A., & Robinson, T. E. (2007). Methamphetamine-induced structural plasticity in the dorsal striatum. *The European Journal of Neuroscience, 25*(3), 847–853. http://doi.org/10.1111/j.1460-9568.2007.05316.x

McDonald, C.G., Eppolito, A.K., Brielmaier, J.M., Smith, L.N., Bergstrom, H.C., Lawhead, M.R., & Smith, R.F. (2007). Evidence for elevated nicotine-induced structural plasticity in nucleus accumbens of adolescent rats. *Brain Research, 1151,* 211-218. doi: 10.1016/j.brainres.2007.03.019

National Institute on Drug Abuse. (2012). *Research report series: Tobacco/nicotine.* U.S. Department of Health and Human Services, National Institute of Health. NIH Publication Number 12-4342.

Natividad, L. A., Buczynski, M. W., Parsons, L. H., Torres, O. V., & O'Dell, L. E. (2012). Adolescent rats are resistant to adaptations in excitatory and inhibitory mechanisms that modulate mesolimbic dopamine during nicotine withdrawal. *Journal of Neurochemistry*, *123*(4), 578–588. http://doi.org/10.1111/j.1471-4159.2012.07926.x

Nestler, E. J. (2001). Molecular basis of long-term plasticity underlying addiction. *Nature Reviews. Neuroscience, 2*(2), 119–128. doi:10.1038/35053570

Nummenmaa, L., Hirvonen, J., Hannukainen, J. C., Immonen, H., Lindroos, M. M., Salminen, P., & Nuutila, P. (2012). Dorsal striatum and its limbic connectivity mediate abnormal anticipatory reward processing in obesity. *PloS One*, *7*(2), e31089. doi:10.1371/journal.pone.0031089

O'Dell, L. E., Bruijnzeel, A. W., Smith, R. T., Parsons, L. H., Merves, M. L., Goldberger, B. A., … Markou, A. (2006). Diminished nicotine withdrawal in adolescent rats: implications for vulnerability to addiction. *Psychopharmacology*, *186*(4), 612–619. http://doi.org/10.1007/s00213-006-0383-6

Olds, J & Milner, P. (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain.*Journal of Comparative and Physiological Psychology, 47*(6), 419-427. doi: 10.1037/h0058775.

Picciotto, M. R., & Kenny, P. J. (2013). Molecular mechanisms underlying behaviors related to nicotine addiction. *Cold Spring Harbor Perspectives in Medicine*, *3*(1), a012112. doi:10.1101/cshperspect.a012112

Self, D. W. (2004). Regulation of drug-taking and -seeking behaviors by neuroadaptations in the mesolimbic dopamine system. *Neuropharmacology*, *47 Suppl 1*, 242–255. http://doi.org/10.1016/j.neuropharm.2004.07.005

Smith, R. F. (2003). Animal models of periadolescent substance abuse. *Neurotoxicology and Teratology, 25,* 291-301. doi:10.1016/S0892-0362(02)00349-5.

Smith, L. N., McDonald, C. G., Bergstrom, H. C., Brielmaier, J. M., Eppolito, A. K., Wheeler, T. L., Falco, A. M. & Smith, R. F. (2006). Long-term changes in fear conditioning and anxiety-like behavior following nicotine exposure in adult versus adolescent rats. *Pharmacology, Biochemistry and Behavior*,*85*(1), 91-97. doi:10.1016/j.pbb.2006.07.014

Smith, A. E., Cavallo, D. A., Dahl, T., Wu, R., George, T. P., & Krishnan-Sarin, S. (2008). Effects of acute tobacco abstinence in adolescent smokers compared with nonsmokers. *The Journal of Adolescent Health: Official Publication of the Society for Adolescent Medicine*, *43*(1), 46–54. http://doi.org/10.1016/j.jadohealth.2007.12.004

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience and Biobehavioral Reviews, 24*(4), 417–463.

Sunahara, R. K., Niznik, H. B., Weiner, D. M., Stormann, T. M., Brann, M. R., Kennedy, J. L., Gelernter, J. E., et al. (1990). Human dopamine D1 receptor encoded by an

intronless gene on chromosome 5. *Nature,347*(6288), 80–83. doi:10.1038/347080a0

Surmeier, D. J., Ding, J., Day, M., Wang, Z., & Shen, W. (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends in Neurosciences, 30*(5), 228–235. doi:10.1016/j.tins.2007.03.008

U.S. Department of Health and Human Services. (2014). *The health consequences of smoking: 50 years of progress. A report of the surgeon general*. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health.

Vollstädt-Klein, S., Wichert, S., Rabinstein, J., Bühler, M., Klein, O., Ende, G., … Mann, K. (2010). Initial, habitual and compulsive alcohol use is characterized by a shift of cue processing from ventral to dorsal striatum. *Addiction (Abingdon, England), 105*(10), 1741–1749. http://doi.org/10.1111/j.1360-0443.2010.03022.x

Zhang, T., Zhang, L., Liang, Y., Siapas, A. G., Zhou, F.-M., & Dani, J. A. (2009). Dopamine signaling differences in the nucleus accumbens and dorsal striatum exploited by nicotine. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 29*(13), 4035–4043. http://doi.org/10.1523/JNEUROSCI.0261-09.2009

BIOGRAPHY

Julian C. Burke was born in Munich, Germany and grew up in Northern Virginia. He attended the Honors College at George Mason University, where he completed his undergraduate education in just three years and received his Bachelor of Arts Degree in Psychology in 2013. Julian continued his studies at George Mason University and went on to complete his Master of Arts Degree in Cognitive and Behavioral Neuroscience in 2015. He intends to work in research for a few years before pursuing his Doctorate Degree in this field.