5-2014

Role of the Ventral Tegmental Area and Ventral Tegmental Area Nicotinic Acetylcholine Receptors in the Incentive Amplifying Effect of Nicotine

Ashley B. Sheppard
East Tennessee State University

Role of the Ventral Tegmental Area and Ventral Tegmental Area Nicotinic Acetylcholine Receptors in the Incentive Amplifying Effect of Nicotine

---

A dissertation

presented to

the faculty of the Department of Psychology

East Tennessee State University

In partial fulfillment

of the requirements for the degree of

Doctor of Philosophy in Psychology

---

by

A. Brianna Sheppard

May 2014

Matthew I. Palmatier, PhD, Chair

Russell W. Brown, PhD

Richard M. Kostrzewa, PhD

Eric W. Sellers, PhD

Jon R. Webb, PhD

Keywords: goal-directed behavior, incentive amplifying effect, nicotine, nicotinic acetylcholine receptors, reinforcement enhancing effect, ventral tegmental area

ABSTRACT

Role of the Ventral Tegmental Area and Ventral Tegmental Area Nicotinic Acetylcholine Receptors in the Incentive Amplifying Effect of Nicotine

by

A. Brianna Sheppard

Nicotine has multiple behavioral effects as a result of its action in the central nervous system. Nicotine strengthens the behaviors that lead to nicotine administration (primary reinforcement), and this effect of nicotine depends on mesotelencephalic systems of the brain that are critical to goal directed behavior, reward, and reinforcement. Nicotine also serves as a 'reinforcement enhancer' – drug administration enhances behaviors that lead to other drug and nondrug reinforcers. Although the reinforcement enhancing effects of nicotine may promote tobacco use in the face of associated negative health outcomes, the neuroanatomical systems that mediate this effect of nicotine have never been described. The ventral tegmental area (VTA) is a nucleus that serves as a convergence point in the mesotelencephalic system, plays a substantial role in reinforcement by both drug and nondrug rewards and is rich in both presynaptic and postsynaptic nicotinic acetylcholine receptors (nAChRs). Therefore, these experiments were designed to determine the role of the VTA and nAChR subtypes in the reinforcement enhancing effect of nicotine. Transiently inhibiting the VTA with a gamma amino butyric acid (GABA) agonist cocktail (baclofen and muscimol) reduced both primary reinforcement by a visual stimulus and the reinforcement enhancing effect of nicotine, without producing nonspecific suppression of activity. Intra-VTA infusions of a high concentration of mecamylamine a nonselective nAChR antagonist, or methylycaconitine, an α7 nAChR antagonist, did not reduce the reinforcement enhancing effect of nicotine. Intra-VTA infusions of a low concentration of mecamylamine and

dihydro-beta-erythroidine (DHβE), a selective antagonist of nAChRs containing the *β2 subunit, attenuated, but did not abolish, the reinforcement enhancing effect of nicotine. In follow-up tests replacing systemic nicotine injections with intra-VTA infusions (70mM, 105mM) resulted in complete substitution of the reinforcement enhancing effects – increases in operant responding were comparable to giving injections of systemic nicotine. These results suggest that *β2-subunit containing nAChRs in the VTA play a role in the reinforcement enhancing effect of nicotine. However, when nicotine is administered systemically these reinforcement enhancing effects may depend on the action of nicotine at nAChRs in multiple brain nuclei.

DEDICATION

This work is dedicated to my dad Alvin Sheppard. His belief in me kept me going when I was ready to give up on myself. His unconditional love continues to inspire me as I follow my dreams. I miss you and love you more than words can express.

"Go confidently in the direction of your dreams. Live the life you have imagined."

-Henry David Thoreau

ACKNOWLEDGEMENTS

I would like to thank my mentor, Dr. Matt Palmatier, for helping me achieve my professional goals by being a great advisor, dissertation committee chair, and friend—my gratitude is unending. I would like to thank my family for their constant love and support. I would like to thank my committee members, Drs. Russ Brown, Eric Sellers, Jon Webb, and Rich Kostrzewa for their suggestions and insightful comments during the preparation of this document. I would also like to thank Tifani Fletcher for comments, suggestions, and moral support while we worked on our degrees. Thank you, Angela Hanley and Dr. Terry Harrison for your help with histological verification of cannula placements. I would also like to thank past and present members of the Associative Pharmacology Lab who have helped me with various aspects of this project—a special thanks for making work fun. This science was supported by grants from the East Tennessee State University School of Graduate Studies and the Johnson Center for Basic Cancer Research at Kansas State University.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Tobacco related health consequences account for one in five deaths annually and are currently the leading cause of preventable death in the United States (Center for Disease Control, 2011). Dependence on cigarettes, cigars, and smokeless tobacco products represents a costly behavioral problem in terms of both health care expenses and lost productivity, estimated at $48 billion per year (CDC, 2008). It is estimated that 5.1 million years of potential life are lost annually as a result of smoking cigarettes in the United States alone (CDC, 2008). Although the total consumption of cigarettes has decreased by 27% since 2000, the consumption of tobacco products other than cigarettes has increased by 123% during this time—2000 to 2011 (CDC, 2012). Many college-aged adults have self-identified as social smokers, smoking mostly at night and on weekends in settings such as bars and parties, that now represent a subgroup among the adult U.S. population that has shown a steady increase in smoking behavior since the 1990s (Debevec & Diamond, 2012; Moran, Wechsler, & Rigotti, 2004).

These trends in tobacco product use indicate that antismoking campaigns have succeeded in reducing the social acceptability of smoking cigarettes (Alamar & Glantz, 2006) but not overall rates of tobacco dependence that remains a major social health problem. Nicotine is recognized as the main addictive component of tobacco responsible for the development and maintenance of tobacco dependence (see Bevins & Caggiula, 2009). Therefore, the motivation to obtain nicotine, known as the primary reinforcing effects of the drug, has traditionally served as the major focus of research in understanding the underlying mechanisms of tobacco dependence and developing drug cessation treatments (Le Foll & Goldberg, 2009).

Effects of Nicotine on Goal-Directed Behavior

Primary Reinforcing Effects

Animal models of clinical behavioral disorders and psychiatric illness are essential for elucidating and quantifying changes at behavioral, cellular, and molecular levels to objectively define human psychopathology and create more effective clinical interventions (Kaffman & Krystal, 2012). Laboratory rats and mice have traditionally been used to model human drug dependence because these models allow for the use of invasive procedures that can identify behavioral, anatomical, neurochemical, and pharmacological effects of substances. Drugs that are abused by humans are also typically self-administered by laboratory mice and rats (O'Dell & Khroyan, 2009) and nonhuman primates (Le Foll, Wertheim, & Goldberg, 2007). Drug self-administration, a goal-directed behavior, is accomplished in animal models by implanting an intravenous (iv) jugular catheter and allowing lab animals to respond for intravenous drug infusions.

Preclinical drug self-administration is the current gold-standard for evaluating the primary reinforcing effects of drugs (Le Foll & Goldberg, 2009). When given unlimited access, monkeys (Deneau, Yanagita, & Seevers, 1969; Johanson, Balster, & Bonese, 1976) and rats (Bozarth & Wise, 1985) will self-administer cocaine at high rates, even to the point of death. In monkeys, nicotine doses 10 times greater than those of cocaine are required to produce consistent intravenous drug self-administration, 300µg/kg/infusion versus 30 µg/kg/infusion, respectively (Goldberg & Spealman, 1982). Nicotine is self-administered at a very low rate by laboratory rats (Palmatier et al., 2006)—rats self-administer approximately 10 infusions per hour of 60µg/kg/infusion nicotine (Palmatier et al., 2006), the peak dose for reliable nicotine self-administration in this species (Donny et al., 1998). These cocaine (Bozarth & Wise, 1985;

Deneau et al., 1976; Goldberg & Spealman, 1982) and nicotine (Palmatier et al., 2006) self-administration studies indicate that the motivation to obtain nicotine is low and nicotine is a weak primary reinforcer, especially when compared to other abused psychostimulants such as cocaine and amphetamine (Le Foll & Goldberg, 2009).

Low rates of nicotine self-administration in preclinical models suggest that this drug should have low abuse liability in humans. However, approximately 42.1 million people in the U. S. alone self-administer nicotine via smoking each year (CDC, 2012). The discontinuity between nicotine's abuse liability as measured in animal models and the abuse liability of the drug observed in humans suggests that the primary reinforcing effects of nicotine are not sufficient to explain tobacco dependence in people. Initial studies of nicotine's abuse liability suggested that the cues associated with drug delivery were important for maintaining nicotine self-administration in preclinical models (Goldberg, Spealman, & Goldberg, 1981) providing a potential explanation for why nicotine, a weak primary reinforcer, has a high abuse liability in people.

Early research in squirrel monkeys showed that removal of cue lights associated with nicotine delivery during self-administration significantly decreased operant responding to obtain nicotine infusions despite the continued availability of the drug (Goldberg et al., 1981). Caggiula and colleagues (2001, 2002) investigated the importance of nonpharmacological environmental stimuli associated with nicotine self-administration as a potential explanation for understanding the high prevalence of dependence to this weak primary reinforcer. For example, nicotine self-administration decreased when operant chamber lights previously associated with drug delivery were absent (Caggiula et al., 2001). Responding on the lever for nicotine infusions returned to baseline when light cues were reintroduced into the nicotine-infusion contingency (Caggiula et

al., 2001). These results suggest incentive stimuli play an important role in maintaining nicotine self-administration. Incentives are external stimuli that can come to control approach (or avoidance) behaviors through learning mechanisms and are capable of producing a conditioned motivational state (Cardinal, Parkinson, Hall, & Everitt, 2002). As a primary reinforcer, nicotine produces conditioned reinforcement through associative learning processes in that interoceptive, contextual, and discrete external cues become conditioned stimuli through reliable pairing with the pharmacological effects of the drug (Bevins & Palmatier, 2004). Nicotine may alter what is learned about the incentive value of external stimuli that predict nicotine availability by enhancing attentional control of cues associated with smoking (Chiamulera, 2005). Therefore, the conditioned reinforcing effects of nicotine have also received a considerable amount of attention as a potential explanation for the persistence of nicotine dependence despite well-known negative health outcomes associated with tobacco product use (Bevins, 2009).

The importance of discrete external stimuli in maintaining smoking behavior is supported by clinical research in dependent smokers. For example, Rose and colleagues (2003) directly assessed the importance of incentives to smokers in deriving satisfaction from the smoking experience. Researchers asked participants to abstain from smoking overnight and then administered nicotine intravenously (iv) in either a continuous or a pulsed manner—pulsed to simulate the rate at that nicotine would cross the blood-brain barrier if participants had taken puffs of a cigarette. These methods allowed researchers to eliminate the external incentive cues normally associated with smoking including the sight, scent, taste, and feel of holding a cigarette. Results showed that iv nicotine administration was not sufficient to reduce cravings, negative mood resulting from withdrawal, or the cumulative puff volume taken from a preferred brand cigarette following iv administration procedures (Rose, Behm, Westman, Bates, & Salley, 2003).

Perkins and colleagues (1994) observed an increase in the number of cigarette puffs made in response to seeing a lit cigarette after overnight abstinence suggesting that individuals attempting to quit are highly susceptible to the influence of smoking-related incentives. In smokers, exposure to smoking related cues results in automatic processing of salient smoking related stimuli, increases in drug-seeking behavior (Warren & McDonough, 1999) and increases in self-reported craving (Ordonana, Gonzalez-Javier, Gomez, & Amor, 2012; Shiffman et al., 2012). This research showing that nicotine-associated stimuli induces cue reactivity in smokers is analogous to preclinical studies assessing the ability of nicotine-associated stimuli to elicit goal-directed behavior (see Chiamulera, 2005 for review).

In preclinical models, the ability of nicotine-associated cues to elicit goal-directed behavior, behavior in that a contingency exists between an action and an outcome with the outcome viewed as a goal (Cardinal et al., 2002), extends beyond the opportunity to self-administer the drug. Acute nicotine administration increases responding for non-nicotine reinforcers such as visual stimuli (VS—turning off all lighting in the operant chamber for 60 seconds) (Chaudhri et al., 2006a) and sucrose in rats (Palmatier, O'Brien, & Hall, 2012). Acute nicotine administration also increases responding for nicotine-conditioned stimuli (Chaudhri et al., 2006b; Palmatier et al., 2007b). Increased motivation to respond for non-nicotine stimuli following acute nicotine administration suggest more than one effect of nicotine on reinforcement (Chaudhri et al., 2006a; Palmatier et al., 2006). A dual reinforcement model that posits nicotine increases behaviors that lead to reinforcement by non-nicotine stimuli that is not contingent on nicotine self-administration has been proposed to help explain the multiple effects of nicotine on reinforcement (Caggiula et al., 2009; Chaudhri et al., 2006a).

Dual Reinforcing Effects

According to the dual reinforcement model, nicotine has multiple effects on reinforced behavior including primary reinforcing and reinforcement enhancing effects (Caggiula et al., 2009; Chaudhri et al., 2006a). Nicotine, acting on the brain, increases the frequency or probability of behaviors that lead to nicotine delivery (primary reinforcement). However, a more robust effect of nicotine is a drug-enhanced motivation to obtain other non-nicotine stimuli (reinforcement enhancing effect) (Caggiula et al., 2009; Chaudri et al., 2006a). Donny and colleagues (2003) showed that nicotine, either injected by the experimenter or self-administered by the rat, increased responding for a visual stimulus. Lever-pressing for the VS decreased when nicotine administration was discontinued in these subjects suggesting that potentiated responding (reinforcement enhancing effect) for the nondrug reinforcer resulted from the acute pharmacological effects of nicotine (Donny et al., 2003).

Palmatier and colleagues (2006) used a 2-lever concurrent choice task in that rats could respond for either nicotine or a visual stimulus (1 sec cue light on followed by 1 min termination of all chamber lighting) as a reinforcer to directly test the dual reinforcement hypothesis. One group of rats could respond on one lever to self-administer nicotine infusions or on the alternative lever that resulted in presentation of the VS. Rats in this group responded for nicotine infusions at low rates comparable to a separate group responding only for nicotine infusions (alternate lever had no programmed consequence for the nicotine only group), confirming that nicotine served as a primary reinforcer. However, responding for visual stimuli increased multiplicatively, with rates of responding for the VS significantly higher than rates of responding exhibited by a group of rats lever-pressing for the VS alone. These results confirmed that self-administered nicotine could potentiate responding for a nondrug reward (Palmatier et al., 2006).

This experiment provided support for the dual reinforcement hypothesis by demonstrating that the primary reinforcing and the reinforcement enhancing effects of nicotine could be observed in the same paradigm but be behaviorally dissociated.

The degree to that nicotine increases the motivation to respond for a non-nicotine reinforcer depends on the dose of nicotine (Palmatier et al., 2008a), repeated exposure to nicotine (Palmatier et al., 2007a), and the desirability of the non-nicotine stimulus (ability of the stimulus to support responding) (Palmatier et al., 2007a, 2012; Raiff & Dallery, 2008). In rats with ad libitum access to water, acute systemic nicotine administration increased responding for a 20% sucrose solution (high desirability) without increasing responding for a 0% sucrose solution (water; low desirability) confirming that nicotine enhanced motivation for a non-nicotine reinforcer—behavior was goal-directed and not simply a result of behavioral activation (Palmatier et al., 2012). Acute nicotine increases the rate of responding for nicotine-conditioned stimuli, representing an additional, dissociable effect of the drug on reward beyond secondary conditioned reinforcement (Guy & Fletcher, 2013; Jones, Raiff, & Dallery, 2010; Palmatier et al., 2007b). Nicotine also increases approach to incentives, salient stimuli that can elicit attention and motivational states (Berridge & Robinson, 1998), associated with noncontingent rewards such as sucrose (Palmatier et al., 2013), drinking water in water-restricted rats (Guy & Fletcher, 2013; Olausson, Jentsch, & Taylor, 2003), and social interactions (Thiel, Sanabria, & Neisewander, 2009) in Pavlovian conditioning paradigms.

Several findings examining the effects of nicotine on reward processing in human samples are in agreement with the results of preclinical studies. Acute administration of nicotine increases the motivation to obtain alcohol and the amount of alcohol consumed (Barrett, Tichauer, Leyton, & Pihl, 2006), the perceived attractiveness of faces (Attwood, Penton-Voak, &

Munafo, 2009), and the motivation to obtain monetary rewards (Buhler et al, 2010). Experimental research has recently demonstrated the reinforcement enhancing effect of nicotine in human samples providing additional support for the dual reinforcement model of nicotine dependence (Perkins & Karelitz, 2013a, 2013b).

Perkins and Karelitz (2013a) used a computer software program that manipulates schedules of reinforcement to measure the effects of nicotine on motivation to respond for nondrug reinforcers, approximating response behavior seen in preclinical studies. In a counterbalanced within-subjects design, participants responded significantly more for a music reinforcer after smoking a nicotine containing cigarette compared to rates of responding for the same reward after smoking a denicotinized cigarette or not smoking (Perkins & Karelitz, 2013a). The reinforcement enhancing effect of nicotine were present in both dependent and nondependent smokers, suggesting that this effect of nicotine is distinct from withdrawal-related motivation to smoke (Perkins & Karelitz, 2013a). As seen in preclinical studies (Palmatier et al., 2007a, 2008a, 2012; Raiff & Dallery, 2008) the degree to that nicotine increased responding for a non-nicotine stimulus in smokers, specifically a preferred music reinforcer, also depended on the dose of nicotine and the desirability of the non-nicotine stimulus (Perkins & Karelitz, 2013b).

Two recent meta-analyses of experiments investigating the underlying biological substrates of smoking cue reactivity, analogous to preclinical studies assessing the ability of nicotine-associated stimuli to elicit goal-directed behavior, have implicated a role for the ventral striatum, anterior cingulate cortex, left amygdala, and right temporal parietal junction (Kuhn & Gallinat, 2011) and extended visual system nuclei (Engelmann et al, 2012) in eliciting behavioral responses to external smoking cues. Neuroadaptations in motivation-related nuclei such as the ventral tegmental area, the nucleus accumbens (NAc; a nucleus within the ventral striatum), and

ventral pallidum (VP) play an important role in the initial attribution of salience to external stimuli associated with drug administration and the promotion of goal-directed behavior in that drug self-administration is the goal (Grace, 2000; Kalivas & Barnes, 1993; Koob & Volkow, 2010). The importance of nicotine-conditioned stimuli in the maintenance of smoking behavior is well established; however, the recognition that the acute pharmacological effects of nicotine acting on the brain enhances behaviors that lead to other drug and nondrug rewards has been more recent (see Bevins & Caggiula, 2009; Chiamulera, 2005 for reviews).

Incentive Amplifying Effect

Nicotine-enhanced responding in operant conditioning paradigms may reflect an enhancement of incentive motivation. Incentive motivation is a conditioned motivational state elicited by incentive stimuli capable of initiating, maintaining, and potentiating reward-seeking behavior (Cardinal et al., 2002). Traditionally, incentives refer to stimuli and cannot always be dissociated from other motivational forces in operant conditioning. In operant conditioning, the manipulandum (e.g., nose-key) is the most salient discrete stimulus associated with reward delivery and should be considered an incentive. Experiments using other behavioral paradigms have demonstrated that nicotine increases Pavlovian conditioned approach responses (Guy & Fletcher, 2013; Palmatier et al., 2013) and conditioned reinforcement (e.g. Guy & Fletcher, 2013; Olaussen et al., 2003) supporting the hypothesis that nicotine enhances incentive motivation. The title of this document reflects what we believe to be the underlying behavioral mechanism responsible for the reinforcement enhancing effect of nicotine – increased incentive motivation. However, the hypothesis that increased approach towards and engagement with the nose key operant cannot be directly tested in the current paradigm. Therefore, the term "reinforcement enhancing effect" is used in place of "incentive amplifying effect" throughout the

remainder of this manuscript to more accurately describe the effect of nicotine on reinforcement investigated in the present studies.

Proposed Neural Substrates Underlying the Reinforcement Enhancing Effect of Nicotine

Initial investigations into the neural mechanisms underlying the reinforcement enhancing effect of nicotine suggest this effect of the drug on reinforcement is mediated by nicotinic acetylcholine receptors (Guy & Fletcher, 2013; Liu, Palmatier, Caggiula, Donny, & Sved, 2007). Nicotinic acetylcholine receptors (nAChRs) are cation channels composed of combinations of α and β subunits in a pentameric configuration (Mansvelder, Mertz, & Role, 2009). Homomeric α7 and heteromeric α4β2 nAChR subtypes are the most abundantly expressed nAChR subtypes in the central nervous system (Lodge & Grace, 2006; Mansvelder et al., 2009). However, the β2 subunit can be found in combination with multiple α subunits including α3, α4, α5, and α6 (Mansvelder et al., 2009). Studies by Liu and colleagues (2007) showed that systemic administration of the nonselective nAChR antagonist mecamylamine (MEC) and the *β2-subunit containing nAChR antagonist dihydro-β-erythriodine (DHβE—* indicating specificity for β-subunit) prior to noncontingent intravenous nicotine administration attenuated responding for a nondrug visual stimulus. In water restricted rats, systemic nicotine administration prior to Pavlovian conditioning sessions in that a compound light and tone compound stimulus was paired with presentations of water as a reward increased approach to the compound conditioned stimulus (Guy & Fletcher, 2013). In the same rats acute systemic nicotine administration also potentiated operant responding for the light and tone compound stimulus as a conditioned reinforcer (CR) and increased responding for the CR was attenuated by systemic MEC and DHβE antagonist administration (Guy & Fletcher, 2013).

Nicotine self-administration was reduced by systemic administration (intraperitoneal) of the α7 nAChR antagonist methylylcaconitine (MLA) (Markou & Paterson, 2001). However, systemic MLA administration did not reduce to motivation to respond for a visual stimulus (Liu et al., 2007), a nicotine-conditioned reinforcer (Guy & Fletcher, 2013), or brain stimulation reward (Markou & Patterson, 2001). Palmatier and colleagues (2008b) have also shown that systemic administration of the metabotropic glutamate 5 receptor (mGluR5) antagonists 3-[(2-methyl-1, 3-thiazol-4-yl)ethynyl]pyridine (MTEP) and 2-methyl-6-(phenylethynyl)pyridine (MPEP) decreased both intravenous nicotine self-administration and nicotine-enhanced responding for a visual stimulus in a 2-lever concurrent choice task. However, mGluR5 antagonist administration did not attenuate the reinforcement enhancing effect when nicotine was administered by the experimenter (Palmatier, Liu, Donny, Caggiula, & Sved, 2008b). Attenuation of nicotine-enhanced responding for non-nicotine primary reinforcers (Liu et al., 2007) and conditioned reinforcers (Guy & Fletcher, 2013) by systemic MEC and DHβE, but not MLA (Guy & Fletcher, 2013; Liu et al., 2007; Markou & Patterson, 2001), MTEP or MPEP administration (Palmatier et al., 2008b), indicates activation of *β2-subunit containing nAChRs is important for the reinforcement enhancing of nicotine. The actions of nicotine at *β2-subunit containing nAChRs expressed by brain nuclei composing circuits that are important for producing motivated, goal-directed behavior (Grace, 2000; Kalivas & Barnes, 1993; Kalivas, Churchill, & Romanides, 1999; Koob & Volkow, 2010) may underlie the reinforcement enhancing effect of this drug.

Motive Circuit

It is well established that the mesocorticolimbic system plays an integral role in reward-related (Floresco, West, Ash, Moore, & Grace, 2003; Kalivas & Barnes, 1993; Koob, 1996; Robinson & Berridge, 1998, 2000) and drug-taking behaviors (Balfour, Wright, Benwell, & Birrell, 2000; Grace, 2000; Wise, 2002; Wise & Bozarth, 1987). Evidence also demonstrates the importance of the ventral striatopallidal system in behavioral activation (Kalivas & Barnes, 1993), particularly approach toward novel stimuli important for learning about cues that predict the presence or absence of reinforcers (Hooks & Kalivas, 1995). The mesolimbic and ventral striatopallidal afferent systems have been shown to work in conjunction to increase DA volume transmission (Belujon, & Grace, 2011; Lodge & Grace, 2006; Wise, 2002) that mediates motivated behavior (Carelli, 2002, 2004; Kalivas & Barnes, 1993). Interactions between these afferent systems may produce the reinforcement enhancing effect of nicotine while sustained nicotine use may result in neuradaptations that skew reward-based learning, shifting behavior from voluntary drug use to habitual drug-seeking (Koob & Volkow, 2010).

The motive circuit (see Figure 1) consists of mesocorticolimbic and striatopallidal nuclei and is an integration point where emotional and motivational information are translated into adaptive motor behavior (Kalivas & Barnes, 1993; Kalivas, Churchill, & Romanides, 1999). The motive circuit consists of three subcircuits: 1) limbic, 2) motor, and 3) thalamocortical (Kalivas et al., 1999). The limbic subcircuit includes projections from the NaC shell to the VP and receives afferents from limbic nuclei such the VTA, the NAc core and the medial VP (Kalivas et al., 1999). The limbic subcircuit sends and receives projections from the motor subcircuit that consists of the substantia nigra (SN), NAc core, and the dorsolateral VP (Kalivas et al., 1999). Together, the limbic and motor subcircuits are important for registering the value of

motivationally salient stimuli and translating this motivationally relevant information into adaptive behavior, respectively (Kalivas et al., 1999).

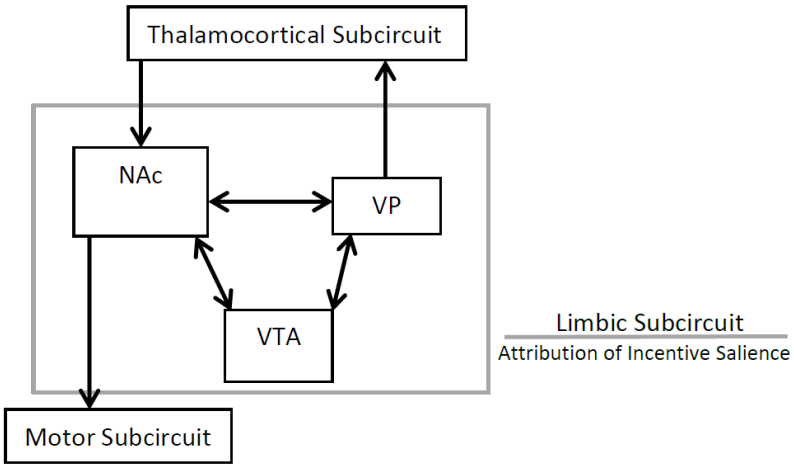


Figure 1. Motive Circuit The motive circuit consists of three subcircuits (limbic, motor, and thalamacortical) that work in conjunction to produce adaptive behavioral responses to motivationally relevant stimuli. The limbic subcircuit is hypothesized to attribute incentive value to external stimuli. The actions of nicotine on limbic subcircuit projections, in that the VTA is a central component, may result in the behavioral manifestation of incentive amplification. VTA-ventral tegmental area; NAc-nucleus accumbens; VP- ventral pallidum. *Adapted from Kalivas, Churchill, & Romanides, 1999.*

Motivationally relevant information from the limbic subcircuit is further modified by the thalamocoritical subcircuit that includes projections from the VP to the mediodorsal thalamus (MD) that receives information from prefrontal cortex (PFC) afferents including prelimbic and anterior cingulate cortices (Kalivas et al., 1999). Interestingly, the flow of information moves unidirectionally from the VP to the MD thalamus into the NAc core without reciprocal projections between these specific nuclei (Kalivas et al., 1999). This pallidal-thalamic projection may send information concerning the motivational salience of stimuli encoded by the limbic

subcircuit directly to the motor subcircuit. Such a direct projection may promote a strongly limbic-driven behavioral response that has not been refined by executive functions via the PFC, manifesting as a compulsion.

The limbic, motor, and thalamocortical subcircuits are part of the mesocorticolimbic circuit and use gamma-aminobutyric acid (GABA) and glutamate as mediators of synaptic transmission with dopamine (DA) serving as a moderator of neuronal signaling (Kalivas et al., 1999). The functions of these three neurotransmitters can be further modified by cholingeric interneuron activity (Calabresi, Centonze, Gubellini, Pisani, & Bernardi, 2000). The reinforcement enhancing effect of nicotine is hypothesized to result from sensitized neural substrates underlying incentive motivation (Palmatier et al., 2013), an internal state indicating anticipation of reward that comes under the control of reward-related incentive cues (Saunders & Robinson, 2013). Therefore, projections within the limbic subcircuit important for attributing stimulus salience are likely targets for the effects of nicotine that produce reinforcement enhancement upstream of motor circuits.

Limbic Subcircuit: VTA-NAc-VP Connections

The VTA is a midbrain convergence zone receiving descending afferents from the PFC, NAc, VP, medial and lateral preoptic areas, lateral hypothalamus, and lateral habenula (Geisler & Zahm, 2005). Ascending VTA afferents include the dorsal raphe, periaqueductal gray, mesencephalic and pontine reticular formations (Geisler & Zahm, 2005) and the amygdala (Wise, 2002). Although VTA neuronal cell bodies are predominately dopaminergic, the posterior VTA and tail of the VTA (tVTA) contain GABAergic interneurons hypothesized to control DA release under certain conditions to modulate behavior (Olson & Nestler 2007; Tolu et al., 2013; see also Cicarelli et al., 2012 for speculation on VTA glutamatergic neurons). The tVTA receives

similar inputs to those of VTA DA neurons with the exception of an additional input from the NAc shell (Kaufling, Neinante, Pawlowski, Freund-Mercier, & Barrot, 2009). The projection from the NAc shell to the VTA receives glutamatergic projections from the amygdala regulated by medial PFC and hippocampal input (Wise, 2002), suggesting an important target for examining nicotine-induced neuroadaptations in incentive learning processes.

VTA DA neurons exhibit distinct population and burst physiological firing patterns that are altered through interactions between GABA and DA neurons (Floresco et al., 2003; Tolu et al., 2013). Population physiological firing activity involves spontaneous production of action potentials and is defined and quantified by the number of spontaneously active DA neurons in the VTA at a certain time (Floresco et al., 2003). These spontaneously active neurons maintain the dopaminergic tone of mesocorticolimbic circuitry (Floresco et al., 2003). Spontaneous firing of VTA DA neurons produces tonic DA release, maintaining steady-state levels of extracellular DA in axon terminals synapsing on the nucleus accumbens (Floresco et al., 2003). The nucleus accumbens is a major projection of VTA DA neurons that combines limbic and motor information to guide goal-directed behavior (Cheer et al., 2007; Floresco et al., 2003; Kalivas et al., 1999; McFarland & Kalivas, 2001; Wightman & Robinson, 2002). VTA-produced dopaminergic tone is regulated in part by GABAergic inputs from the VP (Floresco et al, 2003). Reduced GABA release from ventral pallidal terminals disinhibits at least a subset of VTA DA neurons, increasing the number of spontaneously active DA neurons and subsequently increasing tonic DA release (Floresco et al., 2003).

Burst physiological firing activity has been linked with the presentation of stimuli associated with rewards in primates (Schultz, 1998) that is regulated by glutamatergic and cholinergic inputs from the pedunculopontine tegmental nucleus (Floresco et al., 2003) as well as

GABA release from GABAergic VTA neurons (Tolu et al., 2013). Burst firing results in phasic DA release producing high levels of extracellular DA in the nucleus accumbens, dissociable from tonic levels of DA produced by disinhibition (Floresco et al., 2003).

Physiological firing patterns can be modified by the pharmacological and kinetic properties of nAchRs expressed by the VTA and its afferents (Mansvelder & McGehee, 2002). VTA DA neurons express a diverse combination of nicotinic receptors including α7 subunits and combinations of α3, α4, α5, α6, and β2, β3, and β4 (see Mansvelder et al., 2009 for review) that may integrate information about the intensity of incoming sensory and experienced-based predictive information to regulate DA release. The two most abundant subtypes are α7- and *β2-subunit containing nAChRs (Lodge & Grace, 2006). The α7 subtype is expressed pre-and peri-synaptically on glutamatergic terminals suggesting cholinergic regulation of PFC projections to the VTA (Mansvelder & McGehee, 2002). Nicotinic receptors containing the *β2-subunit are expressed on GABAergic projections from the VP to the VTA as well as DA and GABAergic VTA neurons (Mansvelder et al., 2009; Wise, 2002). Dopaminergic projections from the ventral tegmental area to the nucleus accumbens are critical to the primary and conditioned reinforcing effects of nicotine (Balfour, Wright, Benwell, & Birrell, 2000; Corrigal, Coen, & Adamson, 1994; Grace, 2000; Liechti, Lhuillier, Kaupmann, & Markou, 2007; Mansvelder & McGehee, 2002).

The nucleus accumbens is divided into core and shell regions based on histochemical differences (Groenewegen et al., 1999) and exhibits a topographic organization (Groenewegen et al., 1999; Kalivas et al., 1999), suggesting that information important for control of specific behaviors is localized (Cardinal et al., 2002; Kalivas et al., 1999; Wise, 2002). VTA DA afferents synapse onto nucleus accumbens GABAergic medium spiny neurons (MSNs) (Smith &

Bolam, 1990). These VTA-NAc connections integrate information concerning the motivational value of stimuli from multiple nuclei and the prefrontal cortex and translate this information into goal-directed behavior (Cardinal et al., 2002; Kalivas & Barnes, 1993; Carelli, 2002, 2004; Cheer et al., 2007; Kalivas et al., 1999). Nucleus accumbens MSNs send GABAergic projections back to the VTA and also to the VP (Groenewegen et al., 1999). NAc GABA release is regulated in part by cholinergic interneuron function (Bradfield, Bertran-Gonzalez, Chieng, & Balleine, 2013) and activation of DA D1- and D2-like receptor subtypes (Seamans & Yang, 2004).

D1 and D2-like receptors can have opposing physiological effects that function on different time courses to modulate the effects of GABA in a biphasic manner (Seamans & Yang, 2004). However, the exact mechanisms by that DA receptor subtypes are able to relay information concerning stimulus salience is currently unclear. D1-like receptors modulate n-methyl-d-aspartate receptor (NMDA-R) mediated excitatory postsynaptic potentials important for long-term potentiation that may regulate aspects of goal-directed behavior (Seamans & Yang, 2004). D2-like receptors are thought to reduce both presynaptic glutamate release and non-NMDA-R mediated depolarizations postsynaptically (Seamans & Yang, 2004), providing a potential mechanism for fine-tuning the intensity of stimulus salience initially encoded by D1-like receptor activity (Redgrave, Gurney, & Reynolds, 2008; Seamans & Yang, 2004).

The effects of nicotine at nAChRs expressed on limbic subcircuit nuclei such as the VTA, NAc, and VP play a critical role modulating DA release (Tolu et al., 2013; Zhao-Shea et al., 2011) that we hypothesize are necessary for the reinforcement enhancing effect of nicotine. Based on VTA-NAc-VP connectivity, nicotine's actions at VTA nAChRs would be expected to increase GABA release from NAc shell medium spiny neurons that should decrease GABA release from the VP onto the VTA, disinhibiting DA neurons. Disinhibition of these VTA DA

neurons should result in elevated levels of tonic dopamine release, increasing approach toward and responding for an available nondrug reward.

Proposed Functional Role of VTA in the Reinforcement Enhancing Effect of Nicotine

Support for the hypothesis that VTA-NAcS-VP GABAergic disinhibitory mechanisms play a role in the reinforcement enhancing effect of nicotine is provided by results showing that reduced VTA neuron burst firing, that would decrease tonic and phasic DA release (Floresco et al., 2003), results in disruption of learning about external cues that are predictive of reward (Zweifel et al., 2009). Activation of *β2-subunit containing nAChRs is necessary to shift VTA DA neurons from a population firing state (low frequency/low burst) to spontaneously active states that display distinct patterns of firing: high frequency/low burst, low frequency/high burst, or high frequency/high burst (Mameli-Engvall et al., 2006). Low frequency refers to a firing pattern in that action potential firing rates are lower than 5Hz and high frequency is defined as firing rates > 5Hz (Mameli-Engvall et al., 2006). Low burst indicates that the percent of spikes occurring with a burst (%SWB) are less than 20% of total spikes measured and high burst indicates that the %SWB is between 20%-60% of total spikes measured (Mameli-Engvall et al., 2006).

Noncontingent intravenous nicotine administration increases burst firing of VTA DA neurons (Mameli-Engvall et al., 2006; Tolu et al., 2013) that increases tonic and phasic DA release from the VTA to the nucleus accumbens (Adamantidis et al., 2011) through dissociable mechanisms (Floresco et al., 2003). Nicotine administration elicits three distinct burst firing patterns in VTA DA neurons: high frequency/low burst, low frequency/high burst, and high frequency/high burst (Mameli-Engvall et al., 2006). Nicotine-induced changes in burst firing patterns are potential mechanisms through that the drug affects motivational and associative

learning by rapidly increasing DA transmission in the nucleus accumbens in response to reward-associated cues (Erhardt, Schwieler, & Engberg, 2002; Exley et al., 2011; Farquhar, Latimer, & Winn, 2012; Schilstrom, Rawal, Mameli-Engvall, Nomikos, & Svensson, 2003). VTA-produced dopaminergic tone is regulated in part by GABAergic inputs from the VP (Floresco et al., 2003) and reduced GABA release from ventral pallidal terminals disinhibits at least a subset of VTA DA neurons, increasing the number of spontaneously active DA neurons and subsequently increasing tonic DA release (Floresco et al., 2003). Mameli-Engvall and colleagues (2006) have shown that *β2-subunit containing nAChRs are necessary for the three distinct nicotine-induced burst firing patterns observed in VTA DA neurons.

Nicotine administration shifted DA neurons from a resting to either high frequency/low burst or low frequency/high burst firing active states in wildtype and α7 knockout mice but not *β2 knockouts (Mameli-Engvall et al., 2006). Wild-type mice, but not α7 knockout mice, also exhibited a high frequency/high burst firing pattern (Mameli-Engvall et al., 2006). Selective re-expression of *β2-subunit containing nAChRs in the VTA of *β2 knockout mice using lentiviral transfection was required for noncontingent intravenous nicotine administration to shift VTA DA neurons from resting to spontaneously active states in that DA neurons exhibited high frequency/low burst or low frequency/high burst firing patterns (Mameli-Engvall et al., 2006). The presence of all firing patterns in wildtype mice and all but the high frequency/high burst firing pattern in α7 knockout mice suggests that nicotine-induced activation of α7 nAChRs shifts VTA DA neurons into a high frequency/high burst firing state (Mameli-Engvall et al., 2006). The failure of intravenous nicotine administration to shift VTA DA neurons from a resting state to any spontaneous active state in *β2 subunit knockout mice suggests that the actions of nicotine at this receptor subunit type are necessary to induce active states (Mameli-Engvall et al.,

2006). Shifting VTA DA neurons from resting to active states via activation of *β-subunit containing nAChRs would increase DA release at the NAc that mediates goal-directed behavior such as responding for drug or nondrug rewards (Carelli, 2002; 2004).

The importance of VTA *β2-subunit containing nAChRs for goal directed behavior is supported by studies showing that nAChR *β2 subunit knockout mice do not self-administer nicotine (Orejarena et al., 2012; Picciotto et al., 1998) and blocking VTA nAChRs attenuates NAc DA release (Nisell, Nomikos, & Svensson, 1994), intravenous nicotine self-administration (Corrigal, Coen, & Adamson, 1994), and intra-VTA nicotine self-administration in rats (Ikemoto, Qin, & Liu, 2006). Corrigal and colleagues (1994) used a combination of infusions of the nAChR *β2-subunit antagonist DHβE into the VTA and NAc and partial lesioning of pedunculopontine tegmental nucleus (PPTg) cholinergic inputs to the VTA to demonstrate that the pharmacological effects of nicotine acting within the VTA are necessary for nicotine self-administration. The authors demonstrated that VTA *β2-subunit containing nAChRs were necessary for nicotine self-administration (Corrigal et al., 1994); however, intravenous infusions of nicotine were reliability paired with a tone and light compound cue indicating nicotine availability. Systemic drug administration studies suggest an important role for VTA *β2-subunit containing nAChRs in the reinforcement enhancing effect of nicotine (Guy & Fletcher, 2013; Liu et al., 2007). However, the contributions of VTA *β2-subunit containing nAChRs to self-administration cannot be dissociated from the reinforcement enhancing effect of nicotine based on the findings of Corrigal and colleagues (1994). The present studies were designed to directly assess the functional role of the VTA and VTA nAChRs in the reinforcement enhancing effect of nicotine.

Research Questions Addressed

Nicotine has dual effects on reinforcement by increasing motivation to obtain more nicotine (Cagguila et al., 2001, 2002; Corrigal et al., 1994; Goldberg et al., 1981; Le Foll & Goldberg, 2009; Le Foll et al., 2007) and increasing motivated behaviors that maintain reinforcement by salient non-nicotine stimuli—reinforcement enhancing effect (Cagguila et al., 2009; Chaudhri et al., 2006a, 2006b; Palmatier et al., 2006, 2012). Animal and clinical studies suggest that the reinforcement enhancing effect of nicotine robustly increases behavior in a manner that is comparable to human tobacco use (Caggiula et al., 2009; Chiamulera, 2005; Perkins & Karelitz, 2013a, 2013b). The reinforcement enhancing effect of nicotine is hypothesized to be a motivationally driven effect (Palmatier et al., 2012, 2013) and the VTA plays a central role in both motivationally-driven behavior (Kalivas et al., 1999) and the reinforcing effects of nicotine (Corrigal et al., 1994; Mameli-Engvall et al., 2006). Therefore, the goal of this dissertation was to characterize the role of the VTA and VTA nAChR subtypes in the reinforcement enhancing effect of nicotine. This dissertation specifically addressed the following:

1) Establish the role of the VTA in the reinforcing effects of a visual stimulus by examining the effects of *in vivo* intracranial injection of GABA agonists on operant responding for a reinforcing sensory stimulus. A visual stimulus has been used extensively as a primary reinforcer to evaluate the reinforcement enhancing effects of nicotine (Chaudhri et al., 2006a; Donny et al., 2003; Liu et al., 2007; Palmatier et al., 2006, 2008b). No previous study has verified the role of the VTA in the primary reinforcing effects of the VS. Intra-VTA infusions of a $GABA_{A\&C}/GABA_B$ receptor subtype agonist cocktail of muscimol and baclofen into the VTA has been shown to transiently inhibit this nucleus in a drug-reinstatement paradigm (McFarland & Kalivas, 2001). Therefore, we tested the hypothesis that intracranial infusion of a

$GABA_{A\&C}$/$GABA_B$ receptor subtype agonist cocktail of muscimol and baclofen into the VTA would reduce responding for VS in both nicotine and saline pre-treated subjects.

2) Establish the role of VTA nAChRs in the reinforcement enhancing effect of nicotine by examining the effects of *in vivo* intracranial injection of a nonselective nAChR antagonist on operant responding for a reinforcing sensory stimulus. Systemic administration of the nonselective nAChR antagonist mecamylamine attenuates the reinforcement enhancing effect of nicotine (Guy & Fletcher, 2013; Liu et al., 2007). However, the role of VTA nAChRs in this effect of nicotine on reinforcement is unknown. We tested the hypothesis that intra-VTA infusion of the nonselective nAChR antagonist mecamylamine prior to systemic nicotine administration would reduce responding for the VS specific to rats receiving systemic nicotine.

3) Characterize the roles of VTA nAChR subtypes in the reinforcement enhancing effect of nicotine. Systemic administration of the nAChR *β2-subunit antagonist DHβE, but not the α7 nAChR antagonist MLA, reduces nicotine-enhanced responding for a nondrug visual stimulus (Liu et al., 2007) and nicotine-conditioned stimuli (Guy & Fletcher, 2013; Palmatier et al., 2007b). We hypothesized that attenuation of responding for a VS following intra-VTA administration of nAChR antagonists would be specific to rats receiving systemic nicotine. Based on previous research using systemic antagonist administration (Liu et al., 2007), intra-VTA administration of DHβE would be expected to significantly reduce responding for the VS in rats receiving systemic nicotine compared to rats receiving intra-VTA administration of MLA (α7 nAChR antagonist) and systemic nicotine. Homomeric α7 receptors regulate some forms of neuronal plasticity (Broide & Leslie, 1999) including ventral tegmental area neuron plasticity (Jin, Yang, Wang, & Wu, 2011) that may contribute to the acute reinforcement enhancing effect

of nicotine following chronic nicotine exposure. Therefore, we tested separate hypotheses that intra-VTA administration of *β2-subunit and α7 nAChR antagonists would attenuate the reinforcement enhancing effect of nicotine in a concentration-dependent manner without affecting rates of responding for the VS in rats pretreated with saline.

4) Establish that the acute pharmacological effects of nicotine in the VTA are sufficient to produce the reinforcement enhancing of nicotine. Intra-VTA nicotine administration produces conditioned reinforcing effects in the conditioned place preference paradigm (Laviolette, Alexson, & van der Kooy, 2002; Laviolette & van der Kooy, 2003) but does not produce strong primary reinforcing effects (Farquhar et al., 2012). No study to date has directly tested whether the acute pharmacological effects of nicotine acting in the VTA are sufficient to produce the reinforcement enhancing effect of nicotine. Therefore, we tested the hypothesis that intra-VTA nicotine administration concentration-dependently increases responding for the VS compared to responding for visual stimuli by rats receiving intra-VTA placebo administration and systemic saline.

CHAPTER 2

METHODS

Subjects

Male Sprague-Dawley rats (Charles River and Harlan, Inc.) weighing approximately 250-275g upon arrival were used in all studies. Subjects were housed individually in a temperature and humidity controlled room and maintained on a reversed 12:12 h light:dark cycle. Behavioral testing was conducted during the dark cycle for all experiments. Upon arrival, rats had free access to food for 4-6 days and were maintained on a restricted diet (100% body weight) throughout the remainder of the experiments. Rats were fed immediately after behavioral testing and had unlimited access to water in their home cages throughout the studies. All procedures were approved by the Institutional Animal Care and Use Committees at Kansas State University (Animal Welfare Assurance #: A3609-01) and East Tennessee State University (Animal Welfare Assurance #: A3203-01).

Apparatus

Experimental sessions were conducted in standard operant conditioning chambers housed in sound attenuating cubicles (Med Associates). Chambers were equipped with two illuminated nose-keys, a liquid dipper in a receptacle containing 0.1 ml dippers cups and two white house lights as an ambient lighting source. Nose-keys were located on each side of the receptacle, and one of the two ambient light sources was on one side of the chamber with an additional ambient light source located on the opposite wall of the chamber.

Drugs

The following intracranial (IC) active compound solutions, expressed in millimolar (mM) concentrations, were prepared in sterile phosphate-buffered saline (PBS): 0.1mM

muscimol/1.0mM baclofen GABA agonist cocktail (musc/bac; Sigma), 0.1mM and 1.0mM mecamylamine (MEC; Sigma), 20mM and 100mM dihydro-beta-erythrodine (DHβE; Tocris), 0.8mM and 8.0mM methyllycaconitine (MLA; Sigma), and 17mM, 35mM, 70mM, and 105mM nicotine (NIC—free base; Sigma). Figure 2 summarizes the types and concentrations of IC solutions used in the present experiments. IC compound concentrations were selected based on previous research using these compounds in a variety of reward-related behavioral tasks (See individual experiment descriptions below). Systemic administration of either (-)-Nicotine hydrogen tartrate salt (Sigma) dissolved in 0.9% saline to 0.40 mg/ml (free base) or isotonic saline served as a between-subjects factor. Systemic solutions were administered via subcutaneous (sc) injection at a volume of 1 ml/kg. The 0.40 mg/kg dose of nicotine (free base) was chosen based on previous dose-response data indicating that this dose is optimal for producing maximal operant responding for a visual stimulus (Palmatier et al., 2007a).

Stimuli

Rats were shaped to respond on nose-keys using a 20% sucrose solution (w/v; see Shaping Procedures). The primary reinforcer in all behavioral experiments was a visual stimulus (VS) operationally defined as extinguishing all lighting in the operant chamber (ambient light and illuminated nose-keys) for 30 seconds once the reinforcement schedule on the active nose-key was met. Previous research has shown that the visual stimulus maintains moderate levels of responding as a primary reinforcer (Chaudhri et al., 2006b; Palmatier et al., 2006).

Shaping Procedures

Illuminated nose-keys were the only source of chamber lighting during shaping sessions. Noncontingent dipper presentations occurred throughout each 60-minute session on a random 1-minute interval and nose-key lights were extinguished for 5s during each dipper presentation.

Rats were shaped to associate the activation of the dipper with access to 20% sucrose and to press nose-keys on a fixed ratio 1 (FR1) schedule—every press of either nose-key resulted in deliveries of 0.1ml sucrose into the receptacle by the dipper. Pressing either nose-key during shaping sessions resulted in two consecutive dipper presentations. Shaping procedures were considered completed when subjects earned a total of 100 sucrose reinforcers on the FR1 reinforcement schedule. The right key was designated the active nose-key operant during behavioral testing procedures using the VS as the primary reinforcer.

Presurgery Behavioral Testing

During each session rats were placed in an operant chamber and allowed to respond on the right nose-key for VS reinforcers on a FR3 schedule of reinforcement. Once rats met response stability criterion (<40% variability in mean number of VS earned for 3 of the previous 4 days), subjects were administered saline via subcutaneous injection (sc) 15 minutes prior to being placed in the operant chamber for two tests sessions. Rats were then randomly assigned to one of two systemic drug administration groups (Nicotine or Saline) with the constraint that mean active nose-key responding did not differ between groups on the FR3 schedule under placebo conditions. This procedure was used in order to match for individual differences in response rates prior to drug administration (Pastor, Andres, & Bernabeu, 2012). Rats received injections (sc) of either 0.40 mg/kg nicotine or saline 15 minutes prior to behavioral testing. After responding was stable following drug administration, rats were implanted with infusion cannula aimed at the posterior VTA.

Intracranial Cannula Implantation

Subjects were anesthetized using an injection of ketamine (80 mg/kg; intraperitoneal cavity) and Diazepam (5 mg/kg) or induction with isoflourane gas (inhalation) and maintained

using either Diazepam booster shots or isoflourane gas. Subjects were surgically implanted with 15 millimeter (mm) bilateral microinjection cannulas (mm from bregma) aimed at the posterior VTA (AP: -6.0; ML: +/- 0.75; D/V: -6.2) according to the atlas of Paxinos and Watson (2007) (Jaworski, Kimmel, Mitrano, Tallarida, & Kuhar, 2007). Cannulas (26 gauge hypodermic tubing, Small Parts) were secured to the skull using steel jeweler's screws and dental acrylic. Sixteen mm wires (33 gauge, Small Parts) were inserted into cannulas to prevent cannula obstruction. Rats were treated with 5 mg/kg of an antibiotic (Baytril) and 3 mg/kg of a nonsteriodal anti-inflammatory (Ketoprofen) administered via injection (sc) once daily for 3 days postoperatively. Rats were treated with 3 mg/kg of Baytril every other day until the study endpoint was reached.

Postsurgery Behavioral Testing

After 4-6 days of recovery, behavioral testing resumed and was conducted 7 days per week. Animals were handled and injected as would be performed during intracranial infusion tests. Injectors were not placed in the cannula to minimize tissue damage caused by multiple injector insertions until a postsurgery baseline level of responding was established. Subjects in all experiments then received 0.5 µL/hemisphere infusions of vehicle phosphate buffered saline (PBS) over a 120-second (s) period delivered through 17mm bilateral microinjectors (33 gauge hypodermic tubing, Small Parts) using a 10 µl Hamilton syringe on a digital pump (Stoelting). Injectors remained in place for an additional 60 s to allow diffusion of solution out of injectors. Once injectors were removed, subjects received designated sc injections (SAL or NIC) and obdurators were replaced. Fifteen minutes later, rats were placed in the operant chamber and allowed to respond for the VS on a FR3 schedule to establish a postsurgery baseline for VS responding. Postsurgical PBS infusion procedures were performed for all replications and were

designed to establish a postsurgery baseline rate of behavior including placebo infusion conditions.

After a baseline level of responding was established, postsurgery behavioral testing procedures were repeated using active compounds. The order of IC infusions of active compounds was counterbalanced using a Latin Square design—compounds used during a given replication appeared in each possible order at least once. Rats were placed in the operant chamber 15 minutes after designated systemic injections (GABA agonists and nAChR antagonists) or immediately after systemic saline administration (intra-VTA nicotine administration) and allowed to respond for the VS on an FR3 schedule of reinforcement. A washout period was implemented after each IC infusion to re-establish a baseline rate of responding for the visual stimulus. On washout days animals were handled and received systemic injections as would be performed during intracranial infusion tests. However, injectors were not placed in the cannula to minimize tissue damage caused by multiple injector insertions. Figure 2 summarizes postsurgery behavioral testing procedures.

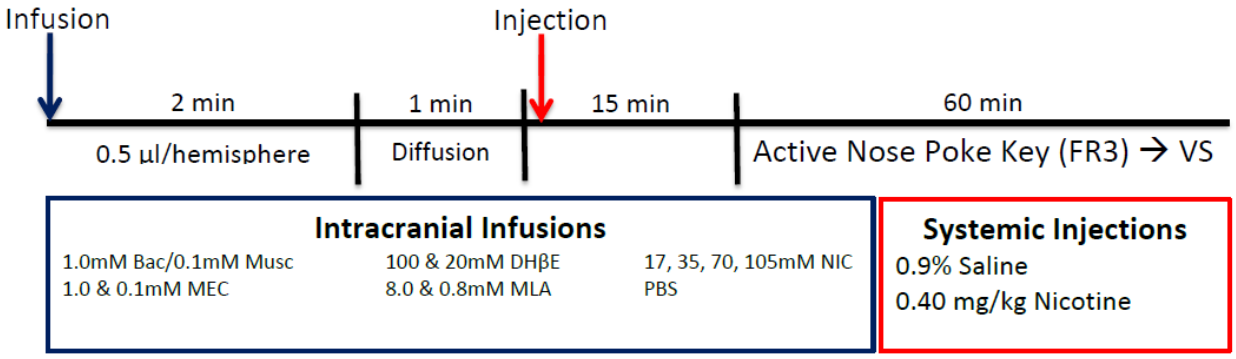


Figure 2. Schematic of postsurgery behavioral testing procedure. For Experiment 3, rats were placed into the operant chamber directly after the systemic saline injection was administered. *Abbreviations:* Bac-Baclofen; Musc-Muscimol, MEC-Mecamylamine, DHBE-Dihydro beta erythroidine; MLA-Methyllycaconitine; NIC-nicotine.

Locomotor Activity

Behavioral test sessions were recorded for a subset of subjects using cameras mounted in the operant chambers (Trendnet). Videos were scored using an automated behavioral scanning system (Any Maze, Stoelting Co., Wood Date, IL). Comparisons of total distance traveled (meters), a dependent measure of locomotor activity, in a subset of 60-minute behavioral tests following baseline, GABA agonist, and nAChR antagonist (DHβE and MLA) infusion procedures were conducted to examine whether any attenuation of responding for the VS following intra-VTA infusion procedures resulted from a reduction in goal-directed behavior or locomotor suppression. The critical comparison for evaluating general locomotor suppression was a reduction in total distance traveled following intra-VTA active compound administration in rats receiving systemic saline compared to baseline locomotor activity as these rats would not experience psychostimulant induced locomotor activating effects (Vezina, McGehee, & Green, 2007).

Cannula Placement Verification

Brain tissue was extracted, fixed in a 37% formalin solution for ≥48hrs, and transferred to a 25% w/v sucrose/PBS solution until saturated. Tissue was then sectioned in 60 µm coronal slices using a cryostat (Leica) and cannula placements were verified by referencing a sterotaxic atlas (Paxinos & Watson, 2007). Subjects with bilateral cannula placement outside the posterior VTA (-5.4 to -6.24mm bregma range; Perroti et al. 2005; Rodd et al, 2008) were excluded from data analysis. Schematics illustrating cannula placement locations for rats meeting inclusion criteria are presented in Appendices A-J.

Research Design and Data Analysis

Systemic Drug x IC Drug x Response Mixed analyses of variance (ANOVAs) were conducted for each experiment with number of nose-key responses serving as the dependent measure. Systemic Drug served as the between subjects variable and type of nose-key response (active, inactive) as a within-subjects variable. IC Drug was treated as a within-subject variable in experiments when subjects completed tests for all concentrations of a specific compound. IC Drug was treated as a between-subjects variable when rats meeting inclusion criteria did not complete tests for all concentrations of a specific compound. Separate Systemic Drug x IC Drug Mixed ANOVAs were conducted to examine the effects of these factors on number of visual stimuli earned. Significant interactions were probed using simple effects analyses (Keppel & Zedeck, 1987) and t-tests where appropriate. Additional Repeated Measures and Between-Subjects ANOVAs and post hoc tests were used to examine the effects intra-VTA compound administration on general locomotor activity (total distance traveled) where appropriate. Alpha was set at $p<0.05$ for all statistical analyses.

IC Drug Baseline measures were calculated for each subject by averaging scores on each dependent measure (active and inactive nose-key responses and visual stimuli earned) across intra-VTA vehicle infusion (PBS) and wash-out days immediately preceding infusions of active compounds when response stability criterion had been met. Response stability criterion was defined as <40% variability in mean number of VS earned during postsurgery baseline procedures. The rationale for using this baseline measure was to decrease the total number of subjects needed to complete experiments by increasing the total number of intra-VTA infusions received by each subject while taking into account inherent variability in responding on nose-key operants.

Role of the VTA in the Primary Reinforcing Effects of the VS

Transient inhibition of the VTA using intracranial infusion of a 0.1 mM muscimol and 1.0 mM baclofen GABA receptor agonist cocktail followed by systemic drug administration was used to examine the role of the VTA in primary reinforcing effects of a visual stimulus used extensively to evaluate the reinforcement enhancing effect of nicotine (Chaudhri et al., 2006a; Donny et al., 2003; Liu et al., 2007; Palmatier et al., 2006, 2008b). This muscimol and baclofen mixture has been shown to transiently inactivate nuclei by increasing $GABA_{A\&C}$ (muscimol) and $GABA_B$ (baclofen) receptor subtype activity (Floresco et al., 2003; Gabriele & See, 2011; McFarland & Kalivas, 2001).

Statistical analysis used to evaluate the role of the VTA in the primary reinforcing effects of the VS consisted of a Systemic Drug (SAL, NIC) x IC Drug (Baseline; 1.0mM Bac/0.1mM Musc) x Response (Active; Inactive) Mixed Factorial ANOVA with systemic drug as the between subjects factor and number of nose-key responses made as the dependent variable. A separate Systemic Drug (SAL, NIC) x IC Drug (Baseline, 1.0mM Bac/0.1mM Musc) Mixed ANOVA was used to examine the effects of transient inhibition of the VTA on number of VS earned. Additionally, a Systemic Drug (SAL, NIC) x Session (Baseline, 1.0mM Bac/0.1mM Musc) Mixed ANOVA with Systemic drug serving as the between-subjects variable and total distance traveled (meters) as the dependent variable was conducted to examine whether intra-VTA administration of GABA agonist compounds suppressed general locomotor activity.

Role of VTA nAChRs in the Reinforcement Enhancing Effect of Nicotine

Intra-VTA administration of mecamylamine, a nonselective nAChR antagonist that functions by blocking open calcium channels (Roegge & Levin, 2006), was used to examine whether VTA nAChRs are directly involved in the reinforcement enhancing effect of nicotine.

Intra-VTA administration of 0.1mM of MEC was chosen as this concentration has been shown to attenuate nicotine-induced Fos expression at the nucleus accumbens (Schilstrom, de Villiers, Malmerfelt, Svensson, & Nomikos, 2000). A higher concentration (1.0mM MEC) was also used to examine the role of VTA nAChRs in the reinforcement enhancing effect of nicotine.

Statistical analyses to evaluate the role of the VTA nAChRs in the reinforcement enhancing effect of the nicotine consisted of a Systemic Drug (SAL, NIC) x IC Drug (Baseline, 0.1mM MEC, 1.0mM MEC) x Response (Active, Inactive) Mixed Factorial ANOVA with systemic drug as the between subjects factor and number of nose-key responses made as the dependent variable. A separate Systemic Drug (SAL, NIC) x IC Drug (Baseline, 0.1mM MEC, 1.0mM MEC) Mixed ANOVA was used to examine the effect of nonselective VTA nAChR blockade on number of visual stimuli earned. Video data for measuring total distance traveled was not available for mecamylamine infusion experiments.

Role of VTA *β2-subunit Containing and α7 nAChR Subtypes in the Reinforcement Enhancing Effect of Nicotine

Intra-VTA infusions of the selective nAChR subtype antagonists DHβE (20mM & 100mM) and MLA (0.8mM, 8.0mM) were used to target *β2-subunit containing and α7 nAChR subtypes, respectively. These specific antagonists were chosen to allow for comparisons of results to prior mechanistic work using systemic administration of these compounds in the reinforcement enhancing effect of nicotine (Liu et al., 2007). DHβE concentrations were chosen based on previous intra-VTA administration experiments examining the motivational valence of nicotine measured using CPP (Laviolette & van der Kooy, 2003) and intra-amygdala infusion of this compound in experiments examining the effects of nicotine on learning and memory (Addy, Nakijama, & Levin, 2003). MLA concentrations were chosen based on previous intra-VTA

antagonist experiments examining the role of α7 nAChR subtype in the effects of nicotine on responding for brain-stimulation reward (Panagais, Kastellakis, Spyraki, & Nomikos, 2000) and motivational valence of nicotine measured using the CPP paradigm (Laviolette & van der Kooy, 2003).

For DHβE and MLA infusion experiments, separate Systemic Drug (SAL, NIC) x IC Drug (Baseline, [Low], [High]) x Response (Active, Inactive) Mixed Factorial ANOVAs were conducted with number of nose-key responses made as the dependent variable. IC Drug was treated as a within-subjects variable in DHβE infusion experiments and as between-subjects variable in MLA infusion experiments as only four rats meeting inclusion criteria completed both α7 antagonist concentrations. Separate Systemic Drug (SAL, NIC) x IC Drug (Baseline, [Low], [High]) Mixed Factorial ANOVAs were also conducted for DHβE and MLA IC compounds with number visual stimuli earned as the dependent variable.

Cameras were not available in the operant boxes until later replications of experiments. Therefore, locomotor activity data were only available for a subset of rats receiving systemic saline and intra-VTA DHβE infusions (See Table 7). Descriptive statistics and a graphical depiction of data are presented in the results section but inferential statistical tests were not conducted because of the limited number of videos available for analysis. A Systemic Drug (NIC, SAL) x IC Drug (Baseline, [Low], [High]) Between-subjects ANOVA with total distance traveled (meters) as the dependent variable was conducted to examine whether intra-VTA MLA administration suppressed general locomotor activity.

Intra-VTA Nicotine

Nicotine was administered directly into the VTA to determine whether the acute pharmacological effects of the drug in this nucleus were sufficient to produce the reinforcement

enhancing effect of nicotine. One group of rats received systemic saline and intra-VTA infusions of PBS (SAL + 0mM) and served as a control group to compare rates of responding for the VS and number of VS earned for rats receiving intra-VTA infusions of different nicotine concentrations (17, 35, 70, & 105mM; free base) and systemic saline administration. A separate group of rats received systemic nicotine and intra-VTA PBS infusions (NIC + 0mM) to examine motivation for the VS following either systemic and intra-VTA nicotine.

Statistical analysis consisted of a Nicotine Dose (SAL+0mM, NIC+0mM, 17mM, 35mM, 70mM, 105mM) x Response (Active, Inactive) Mixed ANOVA with Nicotine Dose as the between subjects variable and number of nose-key responses made as the dependent variable. A separate Nicotine Dose (SAL+0mM, NIC+0mM, 17mM, 35mM, 70mM, 105mM) One-way ANOVA was conducted with number of visual stimuli earned serving as the dependent variable. Dunnett's t was used to make multiple comparisons with the SAL+0mM group as the control comparison to test the hypothesis that intra-VTA nicotine administration concentration-dependently increases responding for the VS compared to responding for the VS by rats receiving intra-VTA placebo administration and systemic saline.

CHAPTER 3

RESULTS

Role of the VTA in the Reinforcing Effects of a Visual Stimulus

The role of the VTA in the primary reinforcing effects of a visual stimulus was assessed by using intra-VTA administration of a 0.1 mM muscimol and 1.0 mM baclofen GABA receptor agonist cocktail followed by systemic drug administration. The hypothesis that intracranial microinjection of a GABA agonist cocktail of muscimol and baclofen into the VTA would reduce responding for a visual stimulus in both nicotine and saline pretreated subjects was supported. Descriptive statistics for transient inhibition experiments are presented in Table 1 (nose-key = NP; visual stimuli = VS; meters = m).

Table 1

Descriptive Statistics for Transient Inhibition Experiments

			Baseline		Muscimol and Baclofen	
Systemic Drug	Measure	n	*M*	*SEM*	*M*	*SEM*
Nicotine	Active NP	6	321.33	25.89	0.00	0.00
	Inactive NP	6	8.17	2.39	0.00	0.00
	VS Earned	6	72.67	6.01	0.00	0.00
	Total Distance Traveled (m)	4	115.58	22.03	63.29	22.19
Saline	Active NP	9	144.89	18.96	2.67	1.42
	Inactive NP	9	5.22	1.53	0.00	0.00
	VS Earned	9	39.22	6.77	0.78	0.43
	Total Distance Traveled (m)	6	21.23	8.56	27.34	16.95

Figure 3 illustrates the mean number of active nose-key responses made during baseline procedures (empty bars) and intra-VTA infusion of a 1.0mM 0.1mM muscimol and baclofen GABA agonist cocktail (black bars) in rats that received either systemic Nicotine (n=6) or Saline (n=9). As shown in the figure, responding for a visual stimulus was reduced in both Nicotine and

Saline groups following transient inhibition of the VTA using a GABA agonist cocktail (black bars) indicating an important role for this nucleus in the primary reinforcing effects of the VS. Mixed ANOVA yielded main effects of Systemic Drug, Response, and IC Drug (Fs≥31.60, ps<0.0001), significant two-way interactions between all factors (Fs≥28.37, ps<0.0001), and a significant Systemic Drug x Response x IC Drug three-way interaction, $F(1, 13) \geq 31.304$, $p < 0.0001$.

Simple effects analyses confirmed that rats receiving systemic Nicotine made more active nose-key responses (M = 321.33, SEM = 25.89) than the Saline group (M = 144.89, SEM = 18.96) and that transient inhibition of the VTA significantly reduced responding on the active nose-key for rats receiving either systemic Nicotine (M = 0.00, SEM = 0.00) or Saline (M = 2.67, SEM = 1.42), Fs$(1, 13) \geq 102.015$, ps < 0.05. Intra-VTA GABA agonist administration eliminated responding on the inactive nose-key in both systemic drug treatment groups (Figure 3 inset). However, the mean number of inactive nose-key responses made did not differ between groups under either IC Drug condition, Fs < 1. The mean number of visual stimuli earned by both systemic groups was also significantly reduced following intra-VTA GABA agonist administration (Figure 4). This was confirmed by Mixed ANOVA yielding significant main effects of Systemic Drug and IC Drug (Fs ≥ 11.11, ps ≤ 0.005) and a significant Systemic x IC Drug interaction, $F(1, 13) \geq 12.73$, $p = 0.003$.

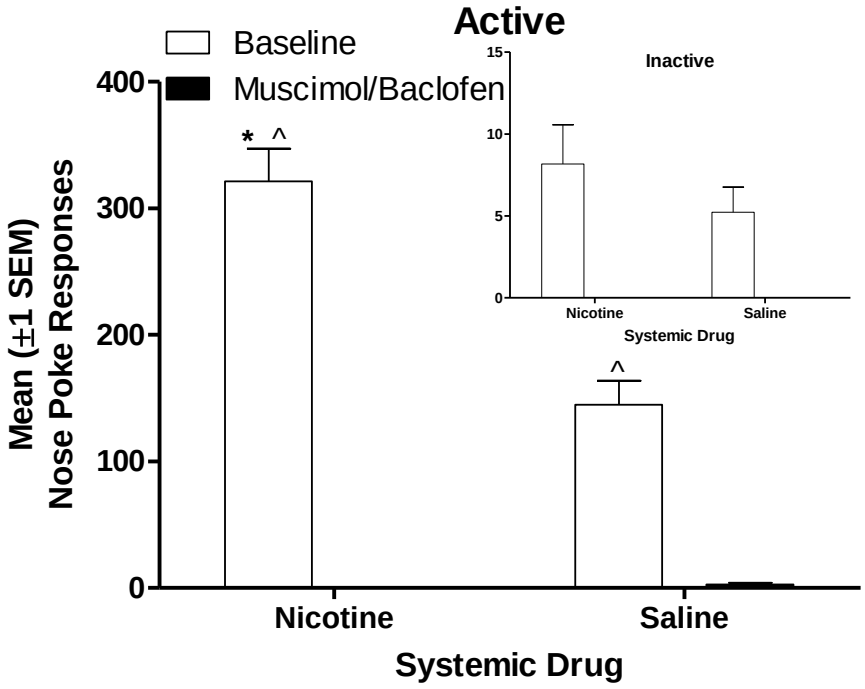


Figure 3: Transient inhibition of the VTA attenuated the primary reinforcing effects of the visual stimulus. Rats pretreated with Nicotine made significantly more active nose-key responses than rats pretreated with Saline following baseline procedures—$p < 0.05$, indicated by *. Intra-VTA GABA agonist infusion reduced responding on the active nose-key for both systemic drug treatment groups, ps < 0.05—indicated by ^. Mean inactive nose-key responses are shown in the figure inset. There were no significant differences in responding on the inactive nose-key between Nicotine and Saline groups under either intracranial drug infusion condition, $p > 0.05$.

Figure 5 shows the effects of intra-VTA GABA agonist administration on total distance traveled, a measure of general locomotor activity, in a subset of rats receiving either systemic Nicotine (n=4) or Saline (n=6). Total distance traveled was reduced in the Nicotine group to a greater extent than rats in the Saline group (*MD* = -52.29 and -14.17, respectively). However,

decreased primary reinforcing effects of the VS following transient inhibition of the VTA did not appear to result from general locomotor suppression as confirmed by a significant Mixed ANOVA Systemic x IC Drug interaction with total distance traveled as the dependent measure, $F(1, 8) = 5.57$, $p < 0.05$. Probing the interaction revealed that total distance traveled was significantly reduced in the Nicotine group, $F(1, 8) = 17.48$, $p < 0.05$ but not the Saline group, $F(1, 8) = 1.93$, $p>0.05$. Intra-VTA GABA induced reductions in total distance traveled specifically in the Nicotine group likely reflects a reduction in nicotine-induced locomotor activating effects.

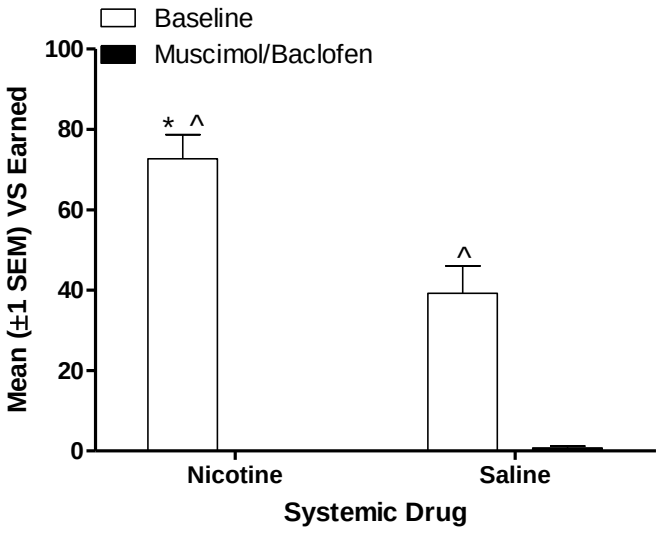


Figure 4. Intra-VTA infusion of muscimol and baclofen reduced the total number of visual stimuli earned. Rats pretreated wit Nicotine earned significantly more VS compared to rats pretreated with Saline following baseline procedures—$p < 0.05$, indicated by *. Intra-VTA GABA agonist infusion reduced the number of VS earned by both systemic drug treatment groups, ps < 0.05—indicated by ^.

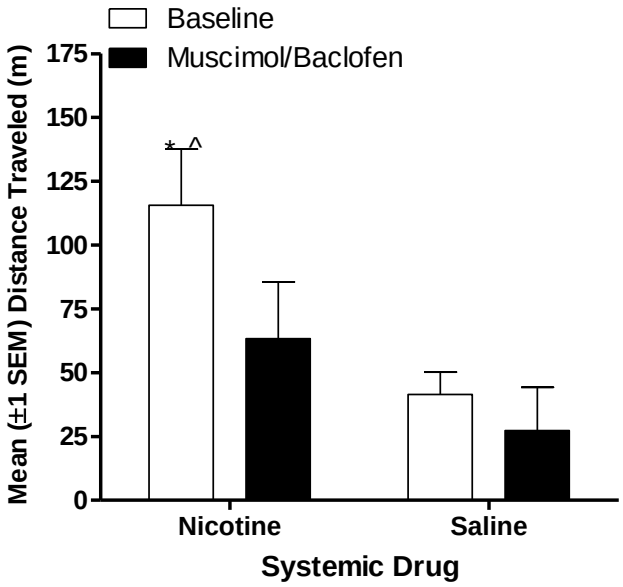


Figure 5. Transient inhibition of the VTA did not suppress general locomotor activity. Rats pretreated with Nicotine had significantly higher baseline rates of locomotor activity than rats in the Saline group—$p < 0.05$, indicated by *. Intra-VTA GABA infusion reduced total distance traveled in the Nicotine group ($p < 0.05$; indicated by ^) but not the Saline group ($p > 0.05$) reflecting a reduction in nicotine-induced locomotor activating effects.

Role of VTA nAChRs in the Reinforcement Enhancing Effect of Nicotine

Systemic administration of the nonselective nAChR antagonist mecamylamine attenuates the reinforcement enhancing effect of nicotine (Guy & Fletcher, 2013; Liu et al., 2007). We tested the hypothesis that intra-VTA infusion of MEC prior to systemic nicotine administration would attenuate motivation for the VS to levels reflective of responding supported by the primary reinforcing effects of the VS. As shown in Figures 6 and 7, rats receiving systemic Nicotine (n=12) made more active nose-key responses (Figure 6) and earned more visual stimuli (Figure 7) compared to rats receiving Saline (n=8) under all IC Drug conditions. Intra-VTA

administration of 0.1mM MEC, but not 1.0mM MEC, reduced responding on the active nose-key and the number of visual stimuli earned in rats receiving systemic Nicotine. Descriptive statistics for MEC infusion experiments are presented in Table 2.

Table 2

Descriptive Statistics for Intra-VTA Mecamylamine Infusion Experiments

		Baseline		0.1mM MEC		1.0mM MEC	
Systemic Drug	Measure	*M*	*SEM*	*M*	*SEM*	*M*	*SEM*
Nicotine (n=12)	Active NP	291.67	23.13	237.83	28.03	265.50	91.73
	Inactive NP	9.92	3.34	3.83	1.97	6.33	10.15
	VS Earned	69.83	4.2	57.83	5.98	64.08	18.87
Saline (n=8)	Active NP	144.5	21.63	137.88	27.59	157.88	16.06
	Inactive NP	7.86	2.61	8.00	4.04	5.63	2.07
	VS Earned	37.75	6.91	37.50	7.70	42.75	5.15

A Systemic Drug x Response x IC Drug Mixed ANOVA yielded significant main effects of Systemic Drug and Response, Fs $\geq$ 11.80, ps $\leq$ 0.003, but not IC Drug, $F(2, 36) = 3.04$, $p = 0.060$. Main effects were qualified by significant Systemic Drug x Response and Response x IC Drug two-way interactions, $Fs \geq 3.32$, ps ≤ 0.05. Simple effects analyses confirmed that all rats made significantly more active (Figure 6) than inactive nose-key responses (Figure 6 inset) under all IC conditions, $F(1,36) = 361.62$, $p < 0.05$. Responding on the inactive nose-key did not differ between systemic drug groups under any IC drug condition, Fs$(1,36) \leq 1.59$, ps > 0.05. Rats in the Nicotine group made significantly more active nose-key responses ($M = 291.67$, $SEM = 23.13$) than rats pretreated with Saline ($M = 144.50$, $SEM = 21.63$), $F(1, 36) = 157.067$, $p < 0.05$. Probing the significant Response x IC Drug interaction revealed that rates of responding on the active nose-key were significantly lower following intra-VTA administration of 0.1mM MEC compared to baseline rates of responding, $t(19)=2.49$, $p<0.05$. This effect is likely driven by

reduced responding on the active nose-key in the Nicotine group (*MD* = -53.84) compared to the Saline group (*MD* = -6.62).

As shown in Figure 7, rats receiving systemic Nicotine earned significantly more visual stimuli than the Saline group under all IC Drug conditions. This was confirmed by a Systemic Drug x IC Drug Mixed ANOVA yielding a significant main effect of Systemic Drug, $F(1,18) = 9.96$, $p = 0.005$. Compared to baseline conditions, a greater reduction in mean number of VS earned was seen in the Nicotine group (*MD* = -12.00) compared to the Saline group (*MD* = -0.25) following intra-VTA 0.1mM MEC administration. However, the main effect of IC Drug and the Systemic Drug x IC Drug interaction did not reach statistical significance, Fs ≥ 2.17, ps ≥ 0.062. Mean differences in active nose-key responses and VS earned suggested that reduced responding for the VS was driven by the effects of 0.1mM intra-VTA MEC in rats pretreated with Nicotine. Additional follow-up analyses using paired samples t-tests and Bonferroni's method to correct for alpha inflation (significance of alpha set at $p \leq 0.017$) were conducted for each systemic drug group to test the hypothesis that intra-VTA 0.1mM MEC significantly reduced responding on the active nose-key in rats pretreated with systemic Nicotine compared to the baseline condition. Results of the paired samples t-test for the Nicotine group confirmed that intra-VTA administration of 0.1mM MEC significantly reduced the mean number of active nose-key responses made compared to baseline conditions, $t(11) = 2.86$, $p = 0.015$. Compared to baseline conditions, intra-VTA administration of 0.1mM MEC did not significantly reduce the mean number of active nose-key responses made by the Saline group, $t(7) = 1.41$, $p = 0.20$.

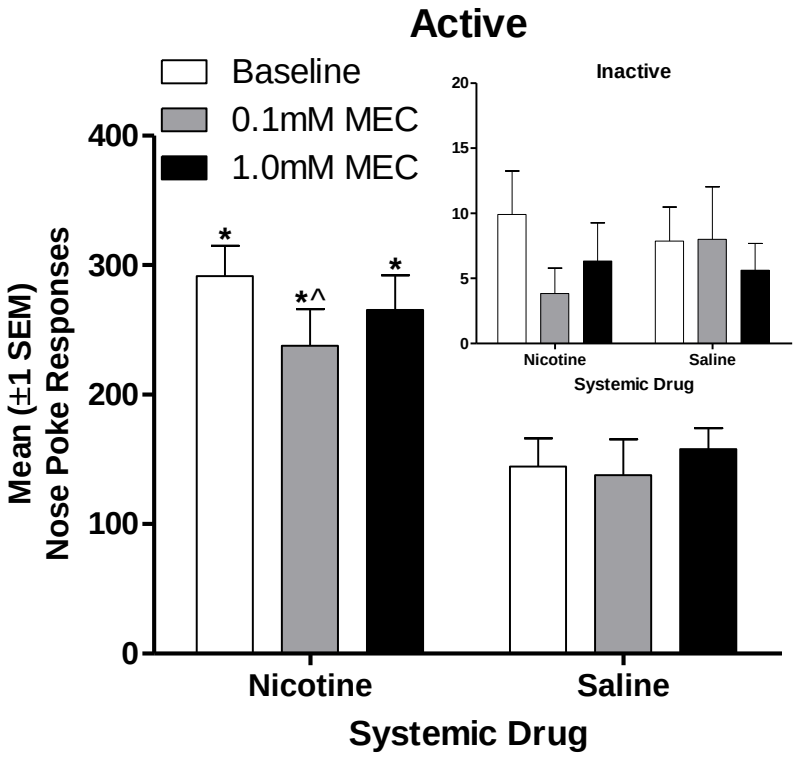


Figure 6. Intra-VTA Administration of a low, but not high, concentration of the nonselective nAChR antagonist mecamylamine attenuated the reinforcement enhancing effect of nicotine. Rats pretreated with Nicotine made significantly more active nose-key responses than rats in the Saline group under all IC Drug conditions—indicated by *. Infusing 0.1mM MEC (gray bars) into the VTA reduced the mean number of active nose-key responses only in rats pretreated with Nicotine. Attenuation of the reinforcement enhancing effect of nicotine by intra-VTA 0.1mM MEC infusion was confirmed by follow-up analyses using a paired samples t-tests that revealed that intra-VTA 0.1mM MEC infusions significantly reduced the mean number of active nose-key responses made by the Nicotine group compared to Baseline, $p < 0.017$—indicated by ^. Responding on the inactive nose-key did not differ between groups under any IC Drug condition, ps > 0.05 (inset).

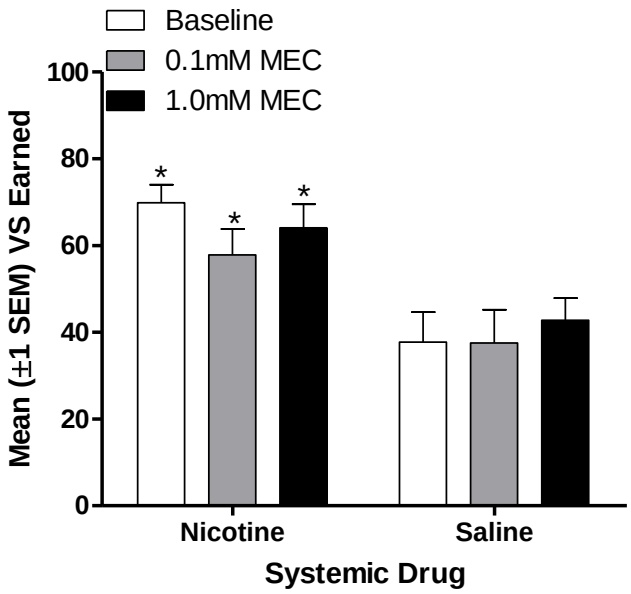


Figure 7. Intra-VTA mecamylamine did not reduce the number of VS earned in rats receiving systemic nicotine. Rats pretreated with Nicotine made significantly more active nose-key responses than rats in the Saline group under all IC Drug conditions—indicated by *.

Role of VTA *β2-subunit Containing and α7 nAChR Subtypes in the Reinforcement Enhancing Effect of Nicotine

*β2-subunit Containing nAChR Subtype

Systemic administration of the nAChR *β2-subunit antagonist DHβE reduces nicotine-enhanced responding for a nondrug visual stimulus reinforcer (Liu et al., 2007). Blocking *β2-subunit containing nAChRs in the VTA also reduced the responding for the VS in a concentration-dependent manner, an effect specific to rats treated with systemic Nicotine. As shown in Figure 8, Intra-VTA administration of DHβE reduced responding on the active nose-key in the Nicotine group (n=8) but not the Saline group (n=7). This was confirmed by a Systemic Drug x Response x IC Drug Mixed ANOVA that yielded significant main effects of Systemic Drug, Response, and IC Drug, Fs $\geq$ 21.60, ps $\leq$ 0.001 and significant two-way

interactions between all factors (Fs $\geq$ 10.83, ps $\leq$ 0.012) that were qualified by a significant Systemic Drug x Response x IC Drug interaction, $F(2, 26) = 4.59$, $p = 0.02$.

Probing the significant three-way interaction revealed that intra-VTA administration of 20mM (M = 242.75, SEM = 25.89) and 100mM DHβE (M = 205.63, SEM = 21.58) reduced the number of active nose-key responses made compared to the baseline condition (M = 312.38, SEM = 18.89) in the Nicotine group (ts $\geq$ 6.64, ps < 0.05). The mean number of active nose-key responses did not significantly differ between low and high DHβE concentrations, $t(7) = 1.57$, $p > 0.05$ (See Table 3). Simple effects analyses confirmed there were no significant differences in active nose-key responses under any IC Drug conditions in rats receiving systemic Saline, $F(1, 26) = 1.84$, $p > 0.05$. Mean number of inactive nose-key responses is presented in the Figure 8 inset. Although reductions in responding on the inactive nose poke were observed following DHβE administration, there were no significant differences in mean number of inactive nose-key responses made under any IC Drug condition for either Systemic Drug treatment group, Fs $\leq$ 2.31, ps > 0.05.

Mean number of visual stimuli earned also decreased following intra-VTA DHβE infusions in a concentration dependent manner (Figure 9). Mixed ANOVA yielded main effects of Systemic Drug and IC Drug, Fs $\geq$ 9.34, ps $\leq$ 0.002 but not a Systemic Drug x IC Drug interaction, $F = 1.95$, $p = 0.16$. Rats treated with systemic Nicotine earned more visual stimuli than rats treated with systemic Saline under all IC Drug conditions. Intra-VTA DHβE administration reduced total mean number of VS earned. Examining Table 3 indicates that the main effect of IC Drug is likely driven by reduced number of VS earned by the Nicotine group. A second potential explanation is that a low concentration of DHβE (20mM) may be sufficient to significantly attenuate the reinforcement enhancing effect of Nicotine ([Low] – Baseline MD = -

9.38; Saline *MD* = -1.14) while higher concentrations may reduce overall responding for the VS as a primary reinforcer (NIC [High] – Baseline *MD* = -20.63; Saline *MD* = -7.71). Alternatively, higher concentrations of *β2-subunit containing nAChR antagonists may produce general locomotor suppression. However, no effect on active nose-key responding (Figure 8) or total distance traveled (Figure 10) was observed following intra-VTA DHβE administration in rats receiving systemic Saline. Therefore, locomotor suppression is an unlikely explanation for the observed results.

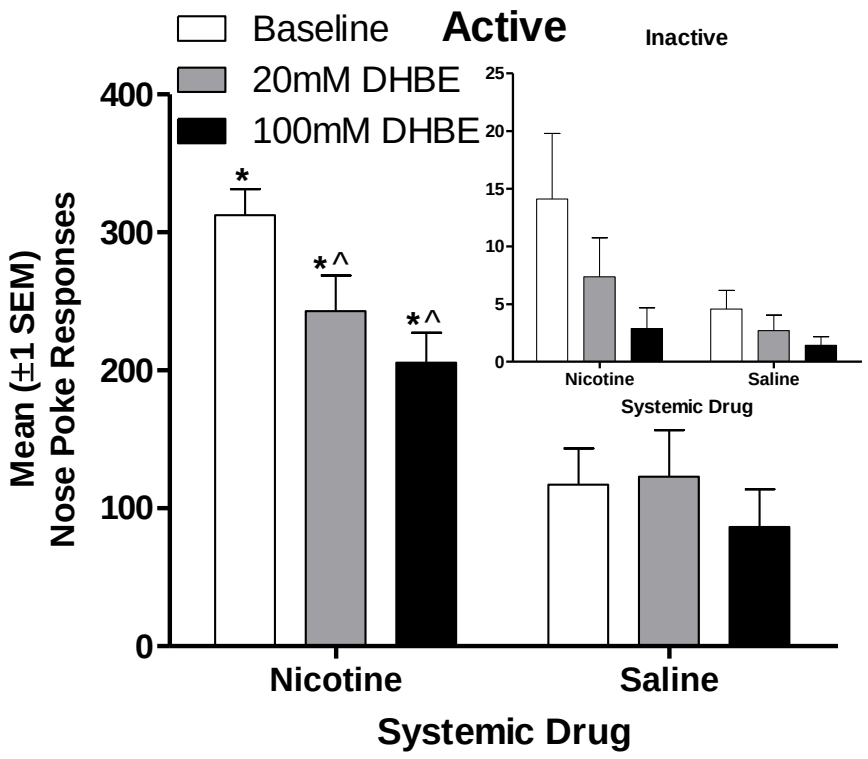


Figure 8. Blocking VTA *β2-subunit containing nAChRs attenuated the reinforcement enhancing effect of nicotine. Both DHβE concentrations tested reduced responding on the active nose-key in the Nicotine, but not the Saline, group. Rats treated with systemic Nicotine made significantly more active nose-key responses than rats treated with Saline under all IC drug conditions, respectively (ps < 0.05, indicated by *). Intra-VTA DHβE significantly reduced responding on the active nose-key in the Nicotine group compared to baseline—$p < 0.05$, indicated by ^. Responding on the inactive nose-key did not differ between groups under any IC Drug condition, ps > 0.05 (inset).

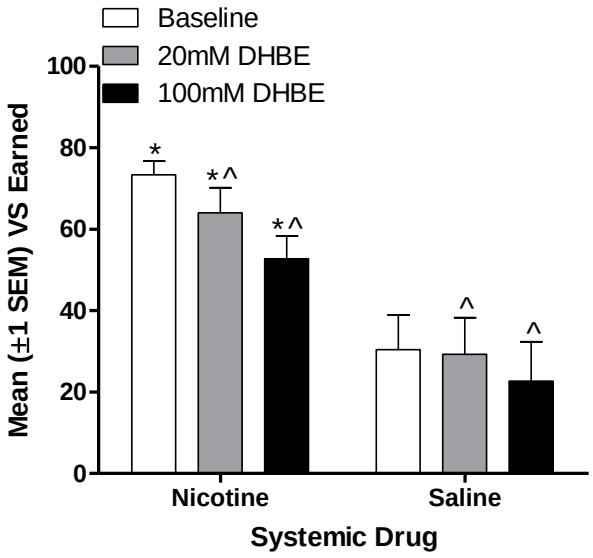


Figure 9. Intra-VTA administration of DHβE reduced the number of visual stimuli earned. Rats receiving systemic Nicotine earned significantly more VS than rats treated with Saline under all IC drug conditions (*ps* < 0.05, indicated by *). Intra-VTA DHβE infusions reduced the mean number of VS earned ($p < 0.05$, indicated by ^), an effect likely driven by Nicotine group.

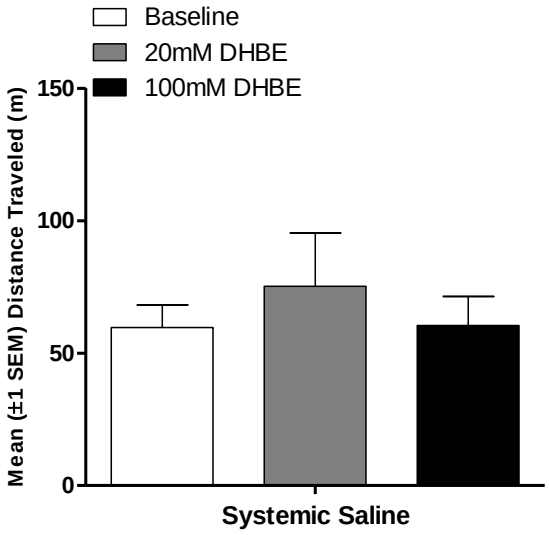


Figure 10. Blocking VTA *β2-subunit containing nAChRs did not affect general locomotor activity. Intra-VTA DHβE administration did not reduce total distance traveled in rats receiving systemic Saline at either concentration tested.

Table 3
Descriptive Statistics for Intra-VTA DHβE Infusion Experiments

		Baseline		20mM DHβE		100mM DHβE	
Systemic Drug	Measure	*M*	*SEM*	*M*	*SEM*	*M*	*SEM*
Nicotine (n=8)	Active NP	312.38	18.89	242.75	25.89	205.63	61.05
	Inactive NP	14.13	5.57	7.38	3.37	2.88	5.08
	VS Earned	73.38	3.32	64.00	6.21	52.75	15.75
Saline (n=7)	Active NP	117.00	26.40	123.00	33.57	86.43	27.51
	Inactive NP	4.57	1.62	2.71	1.32	1.43	0.75
	VS Earned	30.43	8.51	29.29	9.00	22.71	9.66
Saline		*n=6*		*n=4*		*n=3*	
	Total Distance Traveled (m)	59.67	8.56	75.27	20.04	60.47	10.90

α7 nAChR Subtype

Systemic administration of the α7 nAChR antagonist MLA does not reduce nicotine-enhanced responding for a visual stimulus (Lui et al., 2007) or a nicotine-conditioned stimulus (Guy & Fletcher, 2013). However, homomeric α7 receptors regulate some forms of neuronal

plasticity (Broide & Leslie, 1999) including ventral tegmental area neuron plasticity (Jin et al., 2011) that may contribute to the acute reinforcement enhancing effect of nicotine following chronic nicotine exposure. We tested the hypothesis that blocking VTA α7 nAChRs would attenuate the reinforcement enhancing effect of nicotine in a concentration-dependent manner without affecting rates of responding for the VS in rats pretreated with saline. Intra-VTA infusions of MLA did not reduce responding for the VS at either concentration tested (Figure 11) in either systemic treatment group (See Table 4).

Contrary to the research hypothesis, intra-VTA MLA did not significantly reduce the mean number of active nose-key responses made (Figure 11) or the mean number of VS earned (Figure 12) in rats receiving systemic Nicotine at either MLA concentration tested. A Systemic Drug x Response x IC Drug Mixed ANOVA, with Response (Active, Inactive) as the within-subjects variable, yielded significant main effects of Systemic Drug and Response, $Fs > 62.32$, $ps \leq 0.0001$, but not IC Drug, $F < 1$. Main effects were qualified by a significant Systemic Drug x Response two-way interaction, $F(1, 50) = 55.36$, $p<0.0001$. Probing the two-way interaction confirmed that rats in the Nicotine group made significantly more active nose-key responses than rats pretreated with Saline under all IC Drug conditions, $F(1, 50) = 124.70$, $p < 0.05$. There were no significant differences in mean inactive nose-key responses for either systemic drug group under any IC Drug condition, $F < 1$ (Figure 11 inset). For dependent variable VS earned, a Systemic Drug x IC Drug Between-Subjects ANOVA yielded a significant main effect of Systemic Drug, $F(1, 50) = 54.070$, $p < 0.0001$ but no main effect of IC Drug or a Systemic Drug x IC Drug interaction, $Fs < 1$. Rats in the Nicotine group earned significantly more VS than rats pretreated with Saline under all IC Drug conditions (Figure 12).

As seen in Figure 13, intra-VTA administration of MLA did not produce locomotor suppression. Although intra-VTA infusion of 0.8mM MLA increased locomotor activity in the Systemic Nicotine group (gray bars), this effect was not statistically significant ($p > 0.05$). A Systemic Drug x IC Drug Between-Subjects ANOVA with total distance traveled as the dependent measure yielded a significant main effect of Systemic Drug, $F(1, 26) = 12.32$, $p = 0.002$, but no main effect of IC Drug or a Systemic Drug x IC Drug interaction, $Fs < 1.21$, $ps \geq 0.31$.

Table 4

Descriptive Statistics for Intra-VTA MLA Infusion Experiments

		Baseline		0.8mM MLA		8.0mM MLA	
Systemic Drug	Measure	*M* (n)	*SEM*	*M* (n)	*SEM*	*M* (n)	*SEM*
Nicotine	Active NP	299.46 (13)	25.84	280.18 (11)	28.15	269.50 (10)	41.20
	Inactive NP	11.84 (13)	3.56	13.09 (11)	3.55	6.90 (10)	3.81
	VS Earned	66.54 (13)	5.26	65.73 (11)	6.99	64.5 (10)	8.28
	Total Distance Traveled (m)	131.85 (7)	23.73	176.22 (4)	50.00	118.34 (5)	27.27
Saline	Active NP	98.56 (9)	13.99	106.83 (6)	8.59	90.43 (7)	17.27
	Inactive NP	3.44 (9)	1.06	4.50 (6)	2.60	2.57 (7)	2.25
	VS Earned	25.56 (9)	5.38	27.17 (6)	3.52	22.71 (7)	5.31
	Total Distance Traveled (m)	59.67 (6)	8.56	81.89 (6)	13.11	68.71 (4)	23.65

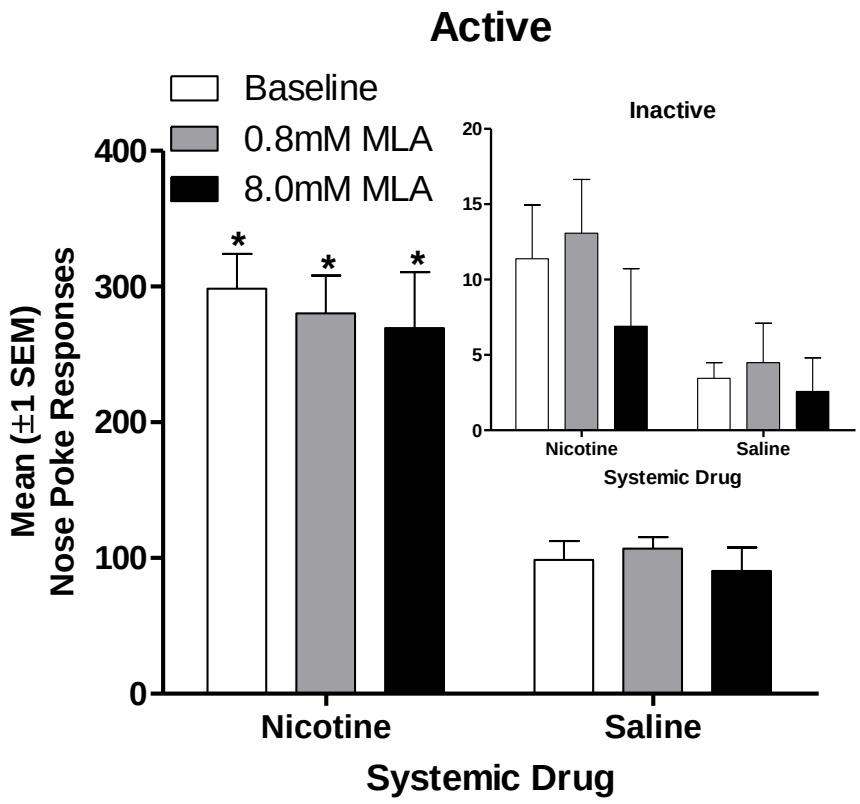


Figure 11. α7 nicotinic acetylcholine receptor antagonism did not attenuate the reinforcement enhancing effect of nicotine. Rats receiving Nicotine made significantly more active nose-key responses than rats in the Saline group under all IC Drug conditions, $p < 0.05$—indicated by *. Responding on the inactive nose-key did not significantly differ between systemic drug groups under any IC Drug condition, $p > 0.05$ (inset).

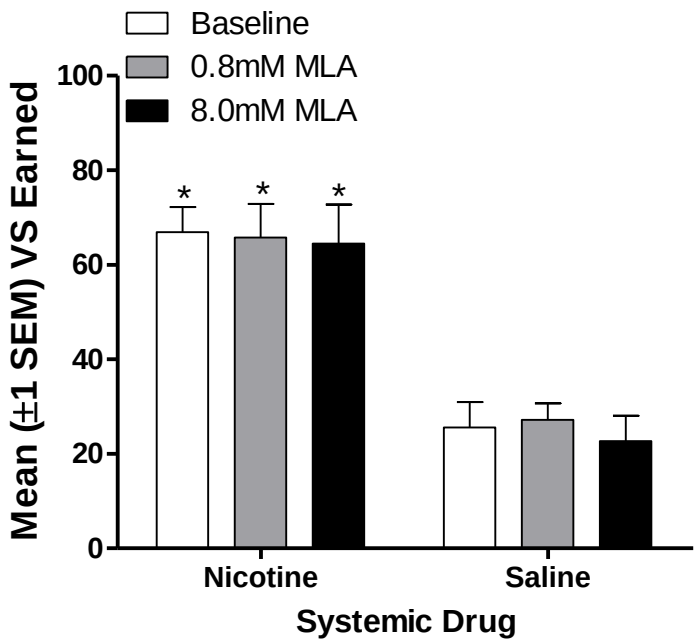


Figure 12. α7 nicotinic acetylcholine receptor antagonism did not reduce the number of VS earned. Rats pretreated with Nicotine earned significantly more VS than rats in the Saline group under all IC Drug conditions, $p < 0.05$—indicated by *).

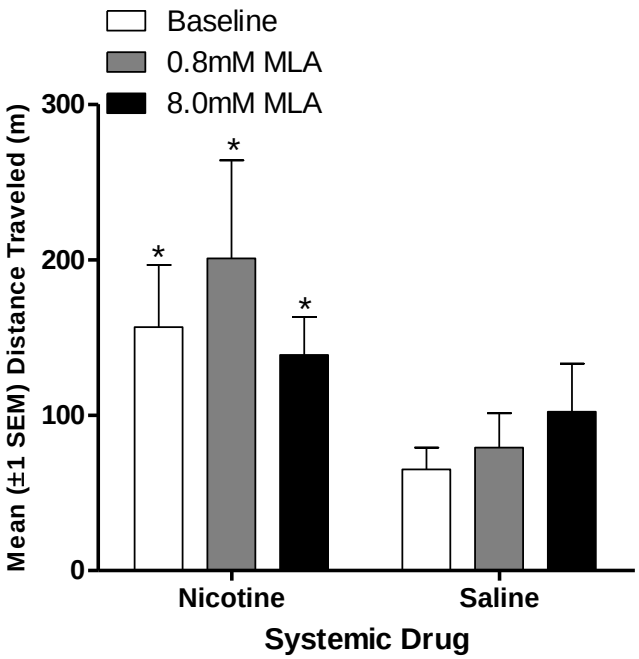


Figure 13. Blocking VTA α7 nAChRs did not produce general locomotor suppression. Mean total distance traveled was greater for rats treated with systemic Nicotine compared to systemic Saline under all IC Drug conditions, $p < 0.05$, indicated by *.

Intra-VTA Nicotine

No published study to date has directly tested whether the acute pharmacological effects of nicotine acting at the VTA are sufficient to produce reinforcement enhancement. Therefore, we tested the hypothesis that intra-VTA nicotine administration would increase responding for the VS compared to rats receiving intra-VTA placebo administration and systemic saline. Supporting the research hypothesis, nicotine administration directly into the VTA of rats chronically treated with systemic nicotine increased responding for the VS at higher concentrations (70mM & 105mM) compared to nicotine naïve rats (SAL + 0mM) (Figure 14).

Increased responding for the VS was confirmed by a Nicotine Concentration x Response Mixed ANOVA with nicotine concentration serving as the between-subjects variable. Results

yielded main effects of Nicotine Concentration and Response, *F*s ≥ 3.86, *p*s ≤ 0.008 and a significant Nicotine Concentration x Response interaction, $F(5, 32) = 3.36$, $p = 0.015$. A one-tailed Dunnett's post hoc analysis using the SAL + 0mM group as the control comparison confirmed that rats receiving systemic Nicotine (NIC + 0mM; *M* = 260.00, *SEM* = 48.05) or 70mM intra-VTA NIC (*M* = 232.60, *SEM* = 32.85) and 105mM intra-VTA NIC (*M* = 202.33, *SEM* = 51.64) followed by systemic saline made significantly more active nose-key responses than the control group (*M* = 71.57, *SEM* = 14.02), *p*s ≤ 0.038. There were no differences in responding on the inactive nose-key, $F < 1$.

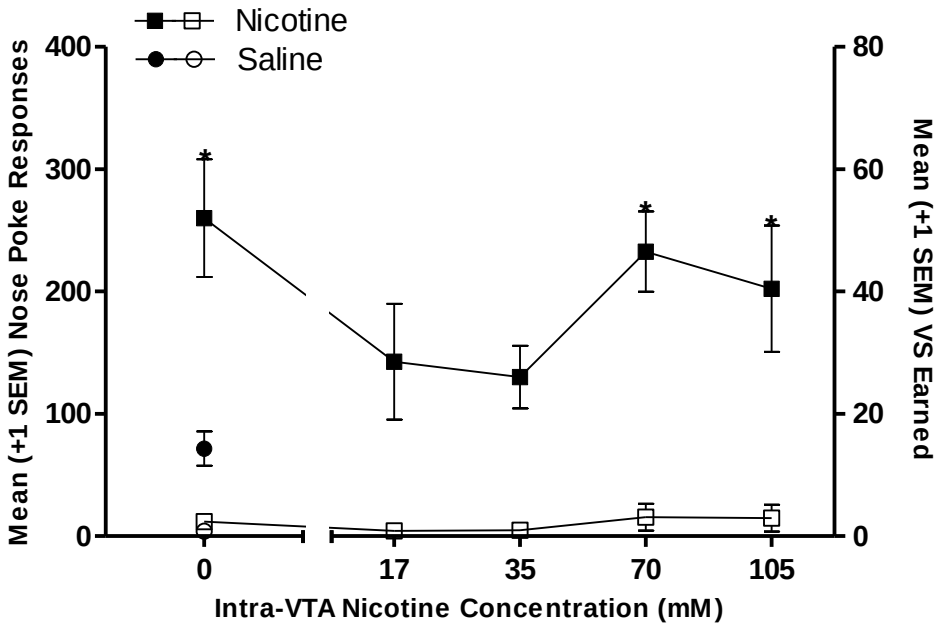


Figure 14. Intra-VTA nicotine is sufficient to produce reinforcement enhancement**.** Rats receiving 70mM or 105mM intra-VTA Nicotine infusions or systemic Nicotine (filled squares) made significantly more active nose-key responses (left Y-axis) and earned more VS (right Y-axis) compared to controls (SAL + 0mM; filled circles), *p*s ≤ 0.038—indicated by *. There were no differences in responding on the inactive nose-key (empty symbols), $p > 0.05$

Results of a one-way between-subjects ANOVA with visual stimuli as the dependent measure further supported the research hypothesis that the acute pharmacological effects of nicotine are sufficient to produce reinforcement enhancement, $F(5, 38) = 2.83$, $p = 0.032$. Results of a one-tailed Dunnett's multiple comparison (SAL + 0mM as comparison group) mirrored those for active nose-key responses with rats receiving either systemic Nicotine or high concentrations of intra-VTA Nicotine (70mM & 105mM) earning significantly more VS than nicotine naïve rats, ps ≤ 0.038. Descriptive statistics for intra-VTA Nicotine infusion experiments are presented in Table 5.

Table 5

Descriptive Statistics for Intra-VTA Nicotine Infusion Experiments

		Active NP		Inactive NP		VS Earned	
[Nicotine] Group	n	*M*	*SEM*	*M*	*SEM*	*M*	*SEM*
NIC + 0mM	7	260.00	48.05	12.00	5.22	58.28	9.05
17mM	5	142.60	47.30	4.40	1.81	36.80	14.55
35mM	8	130.13	25.65	4.75	3.07	35.88	8.35
70mM	5	232.60	32.85	15.60	11.07	59.80	7.51
105mM	6	202.33	51.64	14.83	10.94	51.67	13.69
SAL + 0mM	7	71.57	14.02	3.57	2.61	18.29	3.80

CHAPTER 4

DISCUSSION

The effects of nicotine in the brain increase the frequency or probability of responses that lead to nicotine (primary reinforcement; Caggiula et al., 2001, 2002; Corrigal et al., 1994; Goldberg et al., 1981; Le Foll & Goldberg, 2009; Le Foll et al., 2007). Nicotine also increases behaviors that lead to non-nicotine stimuli— the reinforcement enhancing effect (Caggiulla et al., 2009; Chaudhri et al., 2006a; Palmatier et al., 2006, 2012). Synergistic interactions between the acute pharmacological effects of nicotine and salient non-nicotine stimuli likely contribute to continued tobacco use despite associated negative health outcomes. The ventral tegmental area, a brain region important for goal-directed behavior, has been implicated in the primary reinforcing effects of nicotine (Corrigal et al., 1994; Tolu et al., 2013). The studies reported here are the first to examine the role of the VTA in the reinforcement enhancing effect of nicotine. We found that intra-VTA infusions of DHβE into the VTA significantly attenuated the reinforcement enhancing effect of nicotine without affecting basal responding for the visual stimulus (VS). The reinforcement enhancing effects of nicotine were also attenuated by a low (0.1 mM) but not a high (1.0 mM) intra-VTA infusion of the nonselective nAChR antagonist mecamylamine (MEC). Intra-VTA administration the α7 receptor antagonist MLA did not significantly affect responding. These results suggest that the *β2 subunit containing nAChRs in the VTA play at least a partial role in the reinforcement enhancing effects of systemic nicotine administration. However, the *β2-subunit antagonist only partially reduced the reinforcement enhancing effect of nicotine suggesting that other nAChR subtypes in the VTA or nAChRs in other brain nuclei should be investigated in future studies.

The present studies are also the first to illustrate that the VTA is necessary for the primary reinforcing effects of a visual stimulus and that the acute pharmacological effects of nicotine at the VTA are sufficient to produce a reinforcement enhancing effect. Transient inhibition of the VTA using a GABA agonist cocktail reduced the reinforcement enhancing effect in rats pretreated with nicotine as well as responding for the visual stimulus in rats receiving either systemic nicotine or saline without producing general locomotor suppression. Infusing nicotine directly into the VTA increased responding for the VS suggesting that the effects of nicotine at this nucleus are sufficient to produce the reinforcement enhancing effect.

Role of the VTA in the Primary Reinforcing Effects of a Visual Stimulus

The VTA is part of the limbic subcircuit important for attributing motivational value to salient stimuli and translating relevant motivational information into adaptive behavior via connections to thalamocortical and motor subcircuits, the three components of the motive circuit system (Kalivas et al., 1999). The dopaminergic projections from the ventral tegmental area to the nucleus accumbens are critical for the primary reinforcing effects of drugs of abuse including nicotine (Balfour et al., 2000; Corrigal et al., 1994; Grace, 2000; Liechti et al., 2007; Mansvelder & McGehee, 2002). Studies using bilateral or contralateral VTA dopaminergic lesions have shown that this nucleus is also important for the reinforcing effects of the non-nicotine stimuli such as sucrose (Shibata, Kameishi, Kondoh, & Torii, 2009) and intracranial self-stimulation (Fibiger, LePiane, Jakubovic, & Phillips, 1987).

Using a GABA agonist cocktail to transiently inhibit the VTA, we found that the visual stimulus served as a reinforcer. For rats pretreated with saline responding on the active nose-key occurred at higher rates than responding at the inactive nose-key. Nicotine administration enhanced reinforcement by the VS; rats receiving systemic nicotine made more active nose-key

responses and earned more visual stimuli than rats administered systemic saline under baseline conditions. Infusions of 0.1mM muscimol/1.0mM baclofen bilaterally into the VTA reduced responding on the active nose-key and the mean number of VS earned in both systemic drug treatment groups. Increasing inhibition in the VTA also eliminated responding on the inactive nose-key in both systemic drug groups suggesting that GABA agonist administration may have resulted in a general suppression of all behavior. However, rates of responding on the inactive nose-key were low under baseline conditions and there were no significant differences in mean number of inactive nose-key responses made by either systemic group during baseline or transient inhibition trials. More convincingly, Intra-VTA GABA agonist infusion did not significantly reduce the total distance traveled by the systemic Saline group during the GABA agonist trial compared to baseline levels of locomotor activity. Intra-VTA GABA agonist administration significantly reduced total distance traveled in Nicotine group reflecting a reduction in the locomotor activating effects of nicotine (Vezina et al., 2007). Together, these results support that the VTA is necessary for the primary reinforcing effects of the visual stimulus.

McFarland and Kalivas (2001) previously demonstrated that transient inhibition of the VTA blocks cocaine-induced reinstatement without producing locomotor suppression. In their study, locomotor activity was measured in an open-field arena following a 1-hour habituation period. The current study measured the effects of transient inhibition of the VTA on general locomotor activity within the reinforcement context of the operant chamber, eliminating the possibility that environmental novelty contributed to observed locomotor activity. The GABA agonist cocktail used by McFarland and Kalivas (2001) and the current study blocked GABA A, B, and C receptor subtypes to produce gross inhibition of the VTA that reduced specific

motivated behaviors without affecting general motor activity. Laviolette and van der Kooy (2001) have shown that VTA somatodendritic GABA receptor subtypes play differential roles in relaying motivational information in a conditioned place preference paradigm. More specifically, $GABA_B$ receptor subtypes have been shown to relay information about the motivational valence of a drug-conditioned context independent of DA receptor function while $GABA_A$ receptor subtypes work in conjunction with DA receptors to indicate rewarding and aversive information (Laviolette & van der Kooy, 2001).

A fruitful avenue of potential study for better understanding the role of the VTA in the primary reinforcing effects of non-nicotine stimuli and reinforcement enhancing effect of nicotine is to examine the contributions of GABA receptor subtypes in these reinforcement behaviors. For example, the posterior VTA, specifically targeted in the present study, expresses a higher density of $GABA_A$ receptors (Cicarelli et al., 2012) and receives projections directly from nucleus accumbens shell GABA releasing medium spiny neurons (Xia et al., 2011). Antagonizing pVTA $GABA_A$ receptors only may prevent disinhibition of VTA DA neurons, decreasing the physiological activity of VTA DA neurons that subsequently reduces the salience of stimuli. Additional studies examining the relay of motivational information within the VTA will help disentangle the role of this nucleus in the multiple reinforcing effects of nicotine.

Role of VTA nAChRs in the Reinforcement Enhancing Effect of Nicotine

Nonselective Antagonism

Systemic nicotine administration increased responding for a visual stimulus compared to systemic saline administration in all experiments. This result is in agreement with previous research showing that nicotine increases the motivation for non-nicotine reinforcers in an operant conditioning paradigm including visual and audio-visual stimuli (Chaudhri et al., 2006a, 2006b;

Liu et al., 2007; Palmatier et al., 2006, 2007a), sucrose (Palmatier et al., 2012), and nicotine-conditioned stimuli (Chaudhri et al., 2006a; Jones et al., 2010; Palmatier et al., 2007b; Raiff & Dallery, 2008). Previous research has shown that systemic MEC administration reduces responding for a visual stimulus (Liu et al., 2007) and nicotine-conditioned reinforcers (Guy & Flecther, 2013; Jones et al., 2010). Additionally, intra-VTA nicotine administration has been shown to induce Fos expression in the nucleus accumbens, a marker of drug-induced neuroplasticity. Fos expression is blocked by intra-VTA administration of 0.1mM MEC (Schilstrom et al., 2000).

Based on these findings, we hypothesized that intra-VTA administration of nAChR antagonists would attenuate the reinforcement enhancing effect of nicotine in a concentration-dependent manner. Follow-up analyses indicated that bilateral intra-VTA infusion of 0.1mM MEC reduced responding on the active nose-key by rats receiving systemic Nicotine. A higher concentration of mecamylamine (1.0 mM) did not significantly reduce responding for the VS in either systemic group. Mecamylamine can transiently NMDA-Rs at higher concentrations (Papke, Sanberg, & Shytle, 2001). If 1.0mM MEC were delivered at a high enough concentration to saturate VTA nAChRs, nonspecific antagonism of NMDA-Rs would be expected to reduce responding by both Nicotine and Saline groups. However, 1.0mM MEC infusions did not reduce responding compared to baseline condition, suggesting that antagonism of NMDA-Rs did not reach a level that could interfere with operant behavior. One potential explanation for a reduction at the low, but not high, antagonist concentration is that MEC has different affinities for nAChR subtypes that are differentially expressed by dopaminergic and GABAergic VTA neurons.

Yang and colleagues (2009) have identified three functionally distinct nAChR phenotypes expressed on VTA dopaminergic neurons. Using patch-clamp recordings and mRNA

RT-PCR in freshly harvested VTA neurons, the authors showed that individual VTA DA neurons predominately express one of three subtypes of nAChRs that are more sensitive to either α4β2 agonists and antagonists, α7 agonists and antagonists, or cysteine (*β2-subunit agonist) and MEC (Yang et al., 2009). Direct application of 0.001mM nicotine to the posterior (but not anterior) VTA activated nAChRs in wild-type mice without producing currents in α4* nAChR knockout-mice (Zhao-Shea et al., 2011). Analysis of nAChR subunit gene expression indicated that α4, α6, and β3 nAChR subtypes were predominantly expressed in pVTA DA neurons (Zhao-Shea et al., 2011) while α4β2* nAChRs are expressed on both DA and GABA VTA neurons (Grady et al., 2010). Mecamylamine may have a higher affinity for α4 and/or α6 + β2 subunit containing nAChRs expressed in the pVTA (Pidoplichko, DeBiasi, Williams, & Dani, 1997), reducing motivation when these receptors are occupied at low concentrations of MEC. Higher concentrations of MEC may occupy additional nAChR binding sites that have opposing functions, producing no net change in behavior. Intra-VTA administration of more selective α* and *β subunit agonists or antagonists would help elucidate the contributions of VTA DA and GABA neuron nAChR subtypes in the reinforcement enhancing effect of nicotine. Results from intra-VTA administration of two nAChR subtype specific antagonists, DHβE and MLA, to antagonize *β2-subunit containing subtypes and α7 subtypes, respectively, indicate that VTA *β2-subunit containing nAChRs mediate the reinforcement enhancing effect of nicotine.

***β2-subunit Containing Subtype**

Corrigal and colleagues (1994) concluded that VTA *β2-subunit containing nAChRs were necessary for nicotine self-administration. Intravenous infusions of nicotine were reliably paired with a light and tone compound cue indicating nicotine availability and acute administration of DHβE reduced responding for the combined nicotine and cue reinforcer.

Notably, Palmatier and colleagues (2006) have demonstrated the nicotine self-administration is behaviorally dissociable from the reinforcement enhancing effect of the drug and that the effects of MEC, a nonselective nAChR antagonist, differed in its ability to reduce responding for nicotine relative to responding for the VS (Palmatier, Liu, Caggiula, Donny, & Sved, 2007c). Therefore, manipulations that acutely reduce operant responding for nicotine may more likely reflect a reduction in the incentive-promoting or reinforcement enhancing effect of nicotine (e. g. Corrigal et al., 1994). The present experiments are the first to show that VTA *β2-subunit containing nAChRs mediate the reinforcement enhancing effect of nicotine in a paradigm that specifically isolates this effect of nicotine from its primary reinforcing effects.

Attenuation of the reinforcement enhancing effect of nicotine following intra-VTA DHβE administration is consistent with previous research showing that systemic administration of DHβE prior to systemic nicotine administration reduces responding for a visual stimulus (Liu et al., 2007) and a nicotine-conditioned reinforcer (Guy & Fletcher, 2013). The α4β2 receptor subtype is the most commonly expressed subtype of nAChR containing the *β2-subunit in the VTA (Mansvelder et al., 2002). This receptor is necessary for registering the aversive and rewarding effects of nicotine in context conditioning (Laviolette & van der Kooy, 2003) suggesting that this receptor subtype expressed in the VTA plays an important role in attributing motivational salience to cues predicting reward availability. The α4β2* nAChR subtype has the highest affinity for nicotine compared to α6β2*, α3β4*, and α7 receptor subtypes (Grady et al., 2010). The α4β2* receptor subtype is expressed on pVTA DA neurons (Yang et al., 2009) and regulates DA release in the mesoaccumbens pathway (Grady et al., 2010) important for initiating goal-directed behaviors (Carelli, 2002, 2004).

The β2*-containing nAChRs on dopaminergic VTA neurons express combinations of α4, α5, and α6 subunits while VTA GABA neurons predominately express $(\alpha4)_2(\beta2)_3$ receptors (Klink, de Kerchove d'Exaerde, Zoli, & Changeux, 2001; see also Nashmi & Lester, 2006) that regulate GABA release in this nucleus (Grady et al., 2010). A combination of activation and desensitization of α4β2* receptors on specific populations of GABAergic and dopaminergic neurons may be necessary for reward anticipation (Klink et al., 2001; Tolu et al., 2013; van Zessen, Phillips, Gudygin, & Stuber, 2012) and consumption behaviors (van Zessen et al., 2012). Using lentiviral re-expression of nAChR *β2-subunits on VTA neurons, Tolu and colleagues (2012) have shown that activation of at least a subset of GABAergic VTA neurons is necessary for increased VTA DA neuron burst firing. *In vivo* optogenetic stimulation of VTA GABA neurons during the presentation of a cue predicting a sucrose reward did not disrupt approach to the sipper spout or licking behavior to consume the reward (van Zessen et al., 2012). However, optogenetic activation of VTA GABA neurons following reward delivery decreased total reward consumption without affecting anticipatory approach behavior (van Zessen et al., 2012).

VTA GABA neuron activation during cue presentation increases GABA and DA release in the nucleus accumbens while suppressing physiological activity of VTA DA neurons in close proximity to stimulated GABA neurons (van Zessen et al., 2012). The composition and configuration of nAChRs differentially expressed on DA and GABA VTA neurons affect rates of desensitization and recovery from desensitization (Campling, Kuryatov, & Lindstrom, 2013; Girod et al., 1999). Together these results suggest that one or more populations of GABAergic neurons are tonically inhibiting DA neuron function while other subsets of GABAergic interneurons can selectively disinhibit DA neurons by suppressing tonically active VTA GABAergic neurons via different rates of nAChR desensitization and recovery. The net result of

these different desensitization profiles could be a net increase in DA neuron activity and anticipatory approach behavior. Additionally, desensitization of α4β2* nAChRs on GABAergic interneurons may reduce tonic inhibition on VTA DA neurons that would also produce a net increase in DA neuron burst activity.

Consistent with these hypotheses, concentrations of nicotine achieved by smokers (0.13μM) are capable of producing *in vivo* smoldering activation, desensitization of some nAChRs and activation of other nAChRs, only in α4β2* receptor subtypes (Campling et al., 2013). Additionally, VTA GABAergic neurons that synapse onto VTA DA neurons express α6* containing nAChRs on their terminal buttons (Yang et al., 2011) that may be another potential target for altering DA neuron population and burst firing patterns. Computational models incorporating data from studies of nAChR function expressed on DA and GABA neurons *in vitro* and *in vivo* support a combination of direct activation and disinhibition in nicotine reinforcement (Graupner, Maex, & Gutkin, 2013). Disentangling the functional roles of α4β2* receptor activation and desensitization on DA and GABA VTA neurons in anticipatory and consumatory behaviors represents an area of research with promise for pharmacologically targeting two facets of nicotine reinforcement—reinforcement enhancement and nicotine self-administration.

The present findings that blocking *β2-subunit containing nAChRs subtypes attenuates the reinforcement enhancing effect of nicotine and previous results from our lab showing that nicotine increases Pavlovian conditioned-approach as measured by sign-tracking behavior (Palmatier et al., 2013) suggests that the *β2-subunit containing nAChRs may be especially important for registering the salience of cues that elicit approach behavior and incentive motivation (Tomie, Grimes, & Pokorecky, 2008). A direct measure of the role of VTA nAChRs containing *β2-subunits in incentive motivation would be to repeat the present infusion protocols

using a sign-tracking/goal-tracking paradigm. Attenuation, but not elimination, of enhanced responding on the active nose-key in the Nicotine group suggests that nicotine may be acting at nAChRs in other brain regions that contribute to reward seeking behavior. Therefore, simultaneous administration of DHβE and nicotine directly into the VTA in a sign-tracking paradigm will provide additional specificity when defining the role of *β2-subunit containing nAChRs in this nucleus on incentive motivation. Given the importance of nicotine-associated incentives in satisfaction gained from smoking (Chiamulera, 2005; Rose et al., 2003) and craving (Ordonana et al., 2012; Shiffman et al., 2012; Warren & McConough, 1999), understanding the intricacies of nAChR function in incentive motivation is especially important for understanding underlying mechanisms of nicotine dependence.

α7 Subtype

Consistent with previous research using systemic administration of the α7 nAChR antagonist MLA (Guy & Fletcher, 2013; Liu et al., 2007), intra-VTA α7 nAChR antagonist administration did not significantly affect responding for the visual stimulus in either the nicotine or saline systemic drug treatment groups. A significant contribution of somatodendritic α7 nAChRs in the posterior VTA to the reinforcement enhancing effect of nicotine is not supported by the present results. Studies using mRNA detection have shown that somatodendritic expression of the α7 nAChR subtype occurs in less than half of VTA neurons (Klink et al., 2001; Nashmi & Lester, 2006); however, this subtype is highly expressed pre-, peri-, and extrasynaptically on terminals that modulate the release of other neurotransmitters such as GABA, glutamate, and acetylcholine in multiple brain areas (Girod et al., 1999) including glutamatergic projections to the VTA (Jones & Wonnacott, 2004) and in the nucleus accumbens

(Fu, Matta, Gao, & Sharp, 2000). Therefore, α7 nAChRs expressed on ascending or descending projections to the VTA may contribute to the acute reinforcement enhancing effect of nicotine.

Using microdialysis to deliver antagonists and extract dialysate *in vivo*, Fu and colleagues (2000) showed that intra-accumbens, but not intra-VTA, administration of MLA reduced striatal DA release following noncontingent intravenous nicotine administration. In the same study, Fu and colleagues (2000) showed that infusions of MEC into the VTA, but not NAc core, reduced striatal DA release. Simultaneous infusion of MEC into the VTA and MLA into the NAc core attenuated DA release more than administration of either nAChR antagonist alone. The authors concluded that stimulus-induced striatal DA release may be further increased by the effects of nicotine at α7 nAChR expressed in the nucleus accumbens (Fu et al., 2000). Based on these findings, administering MLA into the nucleus accumbens prior to systemic nicotine administration would be expected to attenuate of the reinforcement enhancing effect of nicotine as measured in the current paradigm. Contralateral antagonism of VTA *β2-subunit containing nAChRs and NAc α7 nAChRs simultaneously would also be expected to decrease responding for a visual stimulus compared to nucleus specific administration of either nAChR subtype antagonist alone.

Intra-VTA Nicotine Administration

Drugs with high abuse liability such as cocaine (Rodd et al., 2005), morphine, and ethanol (McBride, Murphy, & Ikemoto, 1999) are readily self-administered directly into the pVTA by rats. Previous studies investigating the primary reinforcing effects of nicotine using intra-VTA drug self-administration concluded that nicotine has weak primary reinforcing effects (Farquhar et al., 2011). More intra-VTA nicotine infusions were earned in rats responding on a lever that had previously been associated with non-nicotine reinforcers (food delivery and an

associated light conditioned stimulus) compared to rats with a drug-lever association only (no previous food reinforcement; Farquhar et al., 2011). The current experiments are the first to directly show that the acute pharmacological actions of nicotine are sufficient to enhance operant responding for a non-nicotine reinforcer. Intra-VTA administration of nicotine directly into the pVTA was sufficient to produce reinforcement enhancement. Responding for visual stimuli following 70mM and 105mM intra-VTA nicotine infusions directly into the VTA produced rates of responding on the active nose-key similar to systemic nicotine administration and significantly higher than responding for the VS in rats receiving intra-VTA and systemic placebo.

No published studies to date have provided information on brain nicotine concentrations during smoking in humans (Campling et al., 2013). Therefore, direct comparisons of the VTA nicotine concentrations producing reinforcement enhancement in the present studies and those observed in smokers is not possible. Peak venous blood nicotine concentrations have been estimated to be between 0.058-0.34nM (Benowitz, Kuyt, & Jacob, 1982; Campling et al., 2013) with brain concentration levels hypothesized to be significantly higher because of the inhalation route of administration via smoking. Higher nicotine brain concentrations following inhalation of cigarette smoke is supported by studies showing that nicotine levels in arterial plasma are more than double venous concentrations (Henningfield, Stapleton, Benowitz, Grayson, & London, 1993). *In vitro* studies indicate that the clinically relevant nicotine concentrations that can be achieved via smoking that are necessary to produce α4β2* nAChR desensitization at levels observed in human smokers (Brody et al., 2006) range between 0.1 μM and 0.18 μM (Campling et al., 2013), much lower than concentrations used in the present study. Studies using systemic nicotine administration suggest that 0.125mg/kg nicotine (0.77mM) approximates serum nicotine concentrations found in humans (Harris, Mattson, LeSage, Keyler, & Pentel, 2010). Systemic

nicotine concentrations (2.47mM) that produced similar rates of responding on the active nose-key for the VS as 70mM and 105mM intra-VTA concentrations used in the present study were also higher than *in vitro* (Campling et al., 2013) and *in vivo* (Harris et al., 2010) estimates for clinically relevant nicotine concentrations. Studies in clinical samples illustrating the ability of nicotine via smoking to increase responding for a nondrug music reinforcer (Perkins & Karelitz, 2013a; 2013b) and cue-elicited smoking behavior despite self-reported satiety (Hogarth, Dickinson, & Duka, 2010) supports the clinical relevance of the present studies to better understand the neural substrates of reinforcement enhancement involved in nicotine dependence. Investigating clinical behavioral disorders and human psychopathology in animal models is necessary to understand the behavioral, cellular, and molecular substrates underlying these conditions (Kaffman & Krystal, 2012). Metabolic and neuroanatomical differences between rats and humans prevent one-to-one comparisons between species (Kaffman & Krystal, 2012). However, continued investigations of the reinforcement enhancing effect in both preclinical and clinical samples is needed to understand the mechanism through that nicotine increases motivation to obtain non-nicotine reinforcers and the contribution of this reinforcement enhancing effect to nicotine dependence.

The current data confirm the involvement of the VTA in the reinforcement enhancing effect of nicotine; however, nicotine self-administered via smoking is distributed throughout the central nervous system and affects nAChRs on multiple nuclei involved in the reinforcing effects of the drug. Other nuclei within the limbic subcircuit of the motive circuit have reciprocal connections to the VTA (Groenewegen et al., 1999; Kalivas et al., 1999), specifically the NAc and VP, that are important for adaptive, goal directed behaviors (Carelli, 2002, 2004; Kalivas et al., 1999). For example, the VP has been shown to play an important role in cocaine (Root et al.,

2013) and alcohol (Kemppainen, Raivio, & Kiianmaa, 2012) self-administration as well as approach to food-associated incentive stimuli (Smith, Tindell, Aldridge, & Berridge, 2009) and predictive learning mechanisms (Leung & Balleine, 2013) supporting an especially important role of this nucleus in the reinforcement enhancing effect of nicotine that warrants additional research. A future study replicating the infusion procedures used in the current experiments will help elucidate the role of the VP and VP nAChRs in the reinforcement enhancing effect of nicotine.

Limitations

The current experiments are the first to confirm that acute pharmacological effects of nicotine acting on nAChRs within the VTA are sufficient for potentiating responding for a non-nicotine reinforcer and that *β2-subunit containing nAChRs expressed on this nucleus mediate this reinforcement enhancing effect of nicotine. Systemically administering nicotine adds external validity to the results as nicotine administration via smoking would also result in actions of the drug throughout the central nervous system. However, systemic drug treatment limits the specificity of conclusions that can be drawn about the contribution of nAChR subtypes expressed on the VTA. Simultaneous administration of nicotine and nAChR antagonists directly into the VTA is necessary to identify the unique contribution of VTA nAChRs by limiting the actions of nicotine to this nucleus.

Methyllycaconitine was used as a selective α7 nAChR subtype antagonist in the current studies; however, this compound has been shown to interact with α3/α6β2β3 nicotinic receptor subtypes (Mogg et al., 2002). Therefore, conclusions about the specific contribution of the α7 homomeric receptor subtype must also include consideration for these *β-subunit containing subtypes. In a similar vein, the *β-subunit containing antagonist nAChR DHβE shows a high

specificity for the α4β2* receptor subtype; however, the contributions of different α4β2* subunit stoichiometries cannot be assessed with this compound (Grady et al., 2010). Additionally, video data was only available for a subset of subjects; therefore, firm conclusions about the effects of blocking *β2-subunit containing nAChR subtypes on general locomotor activity or nicotine-induced locomotor activation cannot be drawn from the present experiments.

Previous research has shown that the reinforcement enhancing effect of nicotine is an effect on motivation (Palmatier et al., 2012). The fixed ratio schedule of reinforcement used the present studies (FR3) is not as strong a measure of motivation as a progressive ratio schedule (PR) in that subjects must make successively more responses to earn the next reinforcer, a technique commonly used to examine drug reinforcement (Richardson & Roberts, 1996). Although the PR schedule was not employed in the current studies to measure motivation to obtain the VS, research from our lab indicates that the mean breaking point, the highest number of responses a subject is willing to make to earn a single reinforcer used as a measure of motivation (Richardson & Roberts, 1996), is three for the VS (Sheppard & Palmatier, unpublished results), supporting the appropriateness of the FR3 schedule used in these experiments.

Translational Implications

Animal and clinical studies suggest that the reinforcement enhancing effect of nicotine robustly increases behavior in a manner that is comparable to human tobacco use (Carter & Tiffany, 1999; Chiamulera, 2005; Perkins & Karelitz, 2013a, 2013b). The current studies provide a groundwork for understanding the neural circuitry underlying the reinforcement enhancing effect of nicotine on behavior. For example, varenicline, a partial agonist with actions at α4β2* nAChRs and currently one of the most effective pharmacotherapies for smoking cessation,

potentiates responding for non-nicotine reinforcers at low doses while attenuating the reinforcement enhancing effect of nicotine at higher concentrations in preclinical models of nicotine dependence (Levin et al., 2012). The current studies suggest that VTA nAChRs are part of the underlying mechanism for the effects of varenicline, an empirical question that can be addressed by intra-VTA administration of varenicline in the behavior paradigm used in the current study. Identifying the neural substrates underlying the reinforcement enhancing effect of nicotine will increase our understanding of the development and maintenance of tobacco dependence and provide additional insight for intervention efforts.

Conclusions

The current series of experiments are the first to show that *β2-subuniting containing nAChR subtypes expressed somatodendritically on the VTA partially mediate the reinforcement enhancing effect of nicotine. Results of the present studies are also the first to show that the VTA is necessary for the primary reinforcing effects of a visual stimulus and that the acute pharmacological effects of nicotine at the VTA are sufficient to produce reinforcement enhancement. These results support previous findings that the *β2-subunit containing nAChRs are important for nicotine-enhanced responding for non-nicotine reinforcers (Guy & Fletcher, 2013; Liu et al., 2007) and provides additional insight into the neuroanatomical and pharmacological substrates underlying this effect of nicotine on reinforcement. These studies also highlight the need for additional research on different nAChR subtypes and subtype configurations as well as the effects of nicotine at other nuclei involved in motivated behavior in that the VTA is but one point within interconnected circuits.

REFERENCES

Adamantidis, A. R., Tsai, H. C., Boutrel, B., Zhang, F., Stuber, G. D., Budygin, E. A., … de Lecea, L. (2011). Optogenetic interrogation of dopaminergic modulation of multiple phases of reward-seeking behavior. *Journal of Neuroscience, 31,* 10829-10839. doi: 10.1523/JNEUROSCI.2246-11.2011.

Addy, N. A., Nakijama, A., & Levin, E. D. (2003). Nicotinic mechanisms of memory: effects of acute local DHβE and MLA infusions in the basolateral amygdala. *Cognitive Brain Research, 16,* 51-57. doi: 10.1016/SO926-6410(02)00209-4.

Alamar, B., & Glantz, S. A. (2006). Effect of increased social unacceptability of cigarette smoking on reduction in cigarette consumption. *American Journal of Public Health, 95,* 1359-1363. doi: 10.2105/AJPH.2005.069617.

Attwood, A. S., Penton-Voak, I. S., & Monafo, M. R. (2009). Effects of acute nicotine administration on ratings of attractiveness of facial cues. *Nicotine and Tobacco Research, 11,* 44-48. doi: 10.1093/ntr/ntn006.

Balfour, D. J., Wright, A. E., Benwell, M. E., & Birrell, C. E. (2000). The putative role of extra-synaptic mesolimbic dopamine in the neurobiology of nicotine dependence. *Behavioral Brain Research, 113,* 73-83. doi: 10.1016/S0166-4328(00)00202-3.

Barrett, S. P., Tichauer, M., Leyton, M., & Pihl, R. O. (2006). Nicotine increases self-administration in nondependent male smokers. *Drug and Alcohol Dependence, 81,* 197-204. doi: 10.1016/j.drugalcdep.2005.06.009.

Belujon, P., & Grace, A. A. (2011). Hippocampus, amygdala and stress: interacting systems that affect susceptibility to addiction. *Annals of the New York Academy of Sciences, 1216,* 114-121. doi: 10.1111/j.1749-6632.2010.05896.x.

Benowitz, N. L., Kuyt, F., & Jacob, P. (1982). Circadian blood nicotine concentrations during cigarette smoking. *Clinical Pharmacology and Therapeutics, 32*(6), 758-764.

Bevins, R. A. (2009). Altering the motivational function of nicotine through conditioning processes. In R. A. Bevins & A. R. Caggiula (Eds.), *The motivational impact of nicotine and its role in tobacco use* (pp. 111-129).

Bevins, R. A., & Caggiula, A. R. (ed.). (2009). *The motivational impact of nicotine and its role in tobacco use.* New York: Springer.

Bevins, R. A., & Palmatier, M. I. (2004). Extending the role of associative learning processes in nicotine addiction. *Behavioral and Cognitive Neuroscience Reviews, 3*, 143-158. doi: 10.1177/1534582304272005.

Bozarth, M. A., & Wise, R. A. (1985). Toxicity associated with long-term intravenous heroin and cocaine self-administration in the rat. *Journal of the American Medical Association, 254*(1), 81-83.

Bradfield, L. A., Bertran-Gonzalez, J., Chieng, B., & Balleine, B. W. (2013). The thalamostriatal pathway and cholinergic control of goal-directed action: interlacing new with existing learning in the striatum. *Neuron, 79,* 153-166. doi: 10.1016/j.neuron.2013.04.039

Brody, A. L., Mandelkern, M. A., London, E. D., Olmstead, R. E., Farahi, J., Scheibal, D., … Mukhin, A. G. (2006). Cigarette smoking saturates brain alpha 4 beta 2 nicotinic acetylcholine receptors. *Archives of General Psychiatry, 63*(8), 907-915. doi: 10.1001/archpsyc.63.8.907

Broide, R. S., & Leslie, F. M. (1999). The alpha7 nicotinic acetylcholine receptor in neuronal plasticity. *Molecular Neurobiology, 20*(1), 1-16.

Buhler, M., Vollstadt-Klein, S., Kobiella, A., Budde, H. Reed, L. J., Braus, D. F, … Smolka, M. N. (2010). Nicotine dependence is characterized by disordered reward processing in a network driving motivation. *Biological Psychiatry, 67,* 745-752. doi: 10.1016/j.biopsych.2009.10.029.

Caggiula, A. R., Donny, E. C., White, A. R., Chaudhri, N. Booth, S., Gharib, M. A., … Sved, A. F. (2001). Cue dependency of nicotine self-administration and smoking. *Pharmacology, Biochemistry, and Behavior, 70,* 515-530. doi: 10.1016/S0091-3057(01)00676-1.

Caggiula, A. R., Donny, E. C., Chaudhri, N., Perkins, K. A., Evans-Martin, F. F., & Sved, A. F. (2002). Importance of nonpharmacological factors in nicotine self-administration and smoking. *Physiology and Behavior, 77,* 683-687. doi: 10.1016/S0031-9384(02)00918-6.

Caggiula, A. R., Donny, E., C., Palmatier, M. I., Liu, X., Chaudhri, N., & Sved, A. F. (2009). The role of nicotine in smoking: a dual-reinforcement model. In R. A. Bevins & A. R. Caggiula (Eds.), *The motivational impact of nicotine and its role in tobacco use* (pp. 91-109).

Campling, B. G., Kuryatov, A., & Lindstrom, J. (2013). Acute activation, desensitization and smoldering activation of human acetylcholine receptors. *PLOS One, 8*(11), 1-12. doi: 10.1371/journalpone.0079653

Calabresi, P., Centonze, D., Gubellini, P., Pisani, A., & Bernardi, G. (2000). Acetylcholine-mediated modulation of striatal function. *Trends in Neuroscience, 23,* 120-126. doi: 10.1016/S0166-2236(99)01501-5.

Cardinal, R. N., Parkinson, J. A., Hall, J., & Everitt, B. J. (2002). Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neuroscience and Biobehavioral Reviews, 26,* 321-352.

Carelli, R. M. (2002). The nucleus accumbens and reward: neurophysiological investigations in behaving animals. *Behavioral and Cognitive Neuroscience Reviews, 1*, 281-296.

Carelli, R. M. (2004). Nucleus accumbens cell firing and rapid dopamine release signaling during goal-directed behaviors in rats. *Neuropharmacology, 47*(Supplement 1), 180-189.

Carter, B. L., & Tiffany, S. T. (1999). Meta analysis of cure reactivity in addiction research. *Addiction, 94*, 327-340. doi: 10.1046/j.1360-0443.1999.9433273.x.

Center for Disease Control (2008). Smoking-attributable mortality, years of potential life lost, and productivity losses-United States, 2000-2004. *Morbidity and Mortality Weekly Report*, 57, 1226-1228. Retrieved from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5745a3.htm.

Center for Disease Control (2011). *Smoking and Tobacco Use Fact Sheets.* Retrieved from *http://www.cdc.gov/tobacco/data_statistics/fact_sheets/index.htm.*

Center for Disease Control (2012). Consumption of cigarettes and combustible tobacco-United States, 2000-2011. *Morbidity and Mortality Weekly Reports,* 61, 565-569. Retrieved from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6130a1.htm?s_cid=%20mm6130a1.htm_w.

Chaudhri, N., Caggiula, A. R., Donny, E. C., Palmatier, M. I., Liu, X., & Sved, A. F. (2006a). Complex interactions between nicotine and nonpharmacological stimuli reveal multiple roles for nicotine in reinforcement. *Psychopharmacology, 184*, 353-366. doi: 10.1007/s00213-005-0178-1.

Chaudhri, N., Caggiula, A. R., Donny, E. C., Booth, S., Gharib, M., Craven, L., … Sved, A. F. (2006b). Operant responding for conditioned and unconditioned reinforcers in rats is differentially enhanced by the primary reinforcing and reinforcement enhancing effects of nicotine. *Psychopharmacology (Berlin), 189,* 27-36. doi: 10.1007/s00213-006-0522-0.

Cheer, J. F., Aragona, B. J., Heien, M. L. Seipel, A. T., Carelli, R. M., & Wightman, R. M. (2007). Coordinated accumbal dopamine release and neural activity drive goal-directed behavior. *Neuron, 54,* 237-244.

Chiamulera, C. (2005). Cue reactivity in nicotine an tobacco dependence: a "multiple-action" model of nicotine as a primary reinforcement and as an enhancer of the effects of smoking-associated stimuli. *Brain Research Reviews, 48,* 74-97. doi: 10.1016/j.brainresrev.2004.08.005.

Ciccarelli, A., Calza, A., Panzanelli, Concas, A., Glustetto, M., & Sassoe-Pognetto, M. (2012). Organization of GABAergic synaptic circuits in the rat ventral tegmental area. *PL0S One, 7,* e46250. doi: 10.1371/journalpone0046250.

Corrigal, W. A., Coen, K.M., & Adamson, K. L. (1994). Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. *Brain Research, 653,* 278-284. doi: 10.1016/0006-8993(94)90401-4.

Debevec, K., & Diamond, W. D. (2012). Social smokers: Smoking motivations, behavior, vulnerability, and responses to antismoking advertising. *Journal of Consumer Behavior, 11,* 207-216. doi: 10.1002/cb.1373.

Deneau, G., Yanagita, T., & Seevers, M. H. (1969). Self-administration of psychoactive substances by the monkey. *Psychopharmacologia, 16*(1), 30-48.

Donny, E. C., Caggiula, A. R., Mielke, M. M., Jacobs, K. S., Rose, C., & Sved, A. F. (1998). Acquisition of nicotine self-administration in rats: the effects of dose, feeding schedule, and drug contingency. *Psychopharmacology (Berlinin), 136*(1), 83-90.

Donny, E. C., Chaudri, N., Caggiula, A. R., Evans-Martin, F. F., Booth, S., Gharib, M. A., … Sved, A. F. (2003). Operant responding for a visual reinforcer in rats is enhanced by noncontingent nicotine: implications for nicotine self-administration and reinforcement. *Psychopharmacology (Berlin.), 169,* 68-76. doi: 10.1007/s00213-003-1473-3.

Engelmann, J. M., Versace, F., Robinson, J. D., Minnix, J. A., Lam, C. Y., Cui, Y., … Cinciripini, P. M. (2012). Neural substrates of smoking cue reactivity: a meta-analysis of fMRI studies. *NeuroImage, 60,* 252-262. doi: 10.1016/j.neuroimage.2011.12.024.

Erhardt, S., Schwieler, L., & Engberg, G. (2002). Excitatory and inhibitory responses of dopamine neurons in the ventral tegmental area to nicotine. *Synapse, 43,* 227-237. doi: 10.1002/syn.10044.

Exley, R., Maubourguet, N., David, V., Eddine, R., Evrard, A., Pons, S., … Faure, P. (2011). Distinct contributions of nicotinic acetylcholine receptor subunit α4 and subunit α6 to the reinforcing effects of nicotine. *Proceedings of the National Academy of Sciences (U.S.A), 108,* 7577-7582. doi: 10.1073/pnas.1103000108.

Farquhar, M. J., Latimer, M. P., & Winn, P. (2012). Nicotine self-administered directly into the VTA by rats is weakly reinforcing but has strong reinforcement enhancing properties. *Psychopharmacology (Berlin.), 220,* 43-54. doi: 10.1007/s00213-011-2452-8.

Floresco, S. B., West, A. R., Ash, B., Moore, H., & Grace, A. A. (2003). Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nature Neuroscience, 6,* 968-973. doi: 10.1038/nn1103.

Fu, Y., Matta, S. G., Gao, W., & Sharp, B. M. (2000). Local α-bungarotoxin-sensitive nicotinic receptors in the nucleus accumbens modulate nicotine-stimulated dopamine secretion in vivo. *Neuroscience, 101*(2), 369-375.

Gabriele, A., & See, R. E. (2011). Lesions and reversible inactivation of the dorsolateral caudate-putamen impair cocaine-primed reinstatement to cocaine-seeking rats. *Brain Research, 1417,* 27-35. doi: 10.1016/j.brainres.2011.08.2030.

Geisler, S., & Zahm, D. S. (2005). Afferents of the ventral tegmental area in the rat-anatomical substratum for integrative functions. *The Journal of Comparative Neurology, 490,* 270-294. doi: 10.1002/cne.20668.

Girod, R., Crabtree, G., Ernstrom, G., Ramirez-Latorre, J., McGehee, D., Turner, J., & Role, L. (1999). Heteromeric complexes of α5 and/or α7 subunits: effects of calcium and potential role in nicotine-induced presynaptic facilitation. *Annals of the New York Academy of Sciences, 868*(1), 578-590.

Goldberg, S. R., Spealman, R. D., & Goldberg, D. M. (1981). Persistent behavior at high rates maintained by intravenous self-administration of nicotine. *Science, 214,* 575-575. Retrieved from http://www.sciencemag.org/.

Goldberg, S. R., & Spealman, R. D. (1982). Maintenance and suppression of behavior by intravenous nicotine injections in squirrel monkeys. *Science, 214*(4520), 573-575.

Grace, A. A. (2000). The tonic/phasic model of dopamine system regulation and its implications for understanding alcohol and psychostimulant craving. *Addiction, 95,* S119-S128. doi: 10.1080/09652140050111690.

Grady, S. R., Drenan, R. M., Breining, S. R., Yohannes, D., Wageman, C. R., Fedorov, N. B., … Marks, M. J. (2010). Structural differences determine the relative selectivity of nicotinic compounds for native α4β2*, α6β2*, α3β4*, and α7 nicotine acetylcholine receptors. *Neuropharmacology, 58*(7), 1054-1066. doi: 10.1016/j.neuropharm.2010.01.013

Graupner, M., Maex, R., & Gutkin, B. (2013). Endogenous cholinergic inputs and local circuit mechanisms govern the phasic mesolimbic dopamine response to nicotine. *PLOS One Computational Biology, 9*(8), 1-15. doi: 10.1371/journal.pcbi.1003183

Groenewegen, H. J., Wright, C. I., Beijer, A. J. V., & Voorn, P. (1999). Convergence and segregation of ventral striatal inputs and outputs. *Annals of the New York Academy of Sciences, 877,* 49-63. 10.1111/j.1749-6632.1999.tb09260.x.

Guy, E. G., & Fletcher, P. J. (2013). Nicotine-induced enhancement of responding for conditioned reinforcement in rats: role of prior nicotine exposure and α4β2 nicotinic receptors. *Psychopharmacology (Berlin), 225*(2), 429-440. doi: 10.1007/s00213-012-2832-8

Harris, A. C., Mattson, C., LeSage, M. G., Keyler, D. E., & Pentel, P. R. (2010). Comparison of the behavioral effects of cigarette smoke and pure nicotine in rats. *Pharmacology, Biochemistry, and Behavior, 96*, 217-227. doi: 10.1016/j.pbb.2010.05.008

Henningfield, J. E., Stapleton, J. M., Benowitz, N. L., Grayson, R. F., & London, E. D. (1993). Higher levels of nicotine in arterial than venous blood after cigarette smoking. *Drug and Alcohol Dependence, 33*(1), 23-29.

Hogarth, L., Dickinson, A., & Duka, T. (2010). The associative basis of cue-elicited drug-taking in humans. *Psychopharmacology, 208*, 337-351. doi: 10.1007/s00213-009-1735-9

Hooks, M. S., & Kalivas, P. W. (1995). The role of mesoaccumbens-pallidal circuitry in novelty-induced behavioral activation. *Neuroscience, 64,* 587-597. doi: 10.1016/0306-4522(94)00409-X.

Ikemoto, S., Qin, M., & Liu, Z-H. (2006). Primary reinforcing effects of nicotine are triggered from multiple regions both inside and outside the ventral tegmental area. *Journal of Neuroscience, 18,* 723-730. doi: 10.1523/JNEUROSCI.4542-05.2006.

Jaworski, J. N., Kimmel, H. L., Mitrano, D. A., Tallarida, R. J., & Kuhar, M. J. (2007). Intra-VTA CART 55-102 reduces the locomotor effect of systemic cocaine in rats: an isobolographic analysis. *Neuropeptides, 41,* 65-72. doi: 10.1016/j.npep.2006.12.003.

Jin, Y., Yang, K., Wang, H., & Wu, J. (2011). Exposure of nicotine to ventral tegmental area slices induces glutamatergic synaptic plasticity on dopamine neurons. *Synapse, 65*(4), 332-338. doi: 10.1002/syn.20850

Johanson, C. E., Balster, R. L., & Bonese, K. (1976). Self-administration of psychomotor stimulant drugs: the effects of unlimited access. *Pharmacology, Biochemistry, and Behavior, 4*(1), 45-51.

Jones, J., Raiff, B. R., & Dallery, J. (2010). Nicotine's enhancing effects on responding maintained by conditioned reinforcers are reduced by pretreatment with meccamylamine but not hexamethonium in rats. *Experimental and Clinical Psychopharmacology, 18,* 350-358. doi: 10.1037/a0020601.

Jones, I. W., & Wonnacott, S. (2004). Precise localization of α7 nicotinic acetylcholine receptors on glutamatergic axon terminals in the rat ventral tegmental area. *The Journal of Neuroscience, 24*(50), 11244-11252. doi: 10.1523/JNEUROSCI.3009-04.2004

Kaffman, A., & Krystal, J. H. (2012). New frontiers in animal research of psychiatric illness. *Methods in Molecular Biology, 829,* 3-30. doi: 10.1007/978-1-61779-458-2_1.

Kalivas, P. W., & Barnes, C. D. (Eds). (1993). *Limbic motor circuits and neuropsychiatry.* Boca Raton, FL: CRC Press.

Kalivas, P. W., Churchill, L., & Romanides, A. (1999). Involvement of the pallidal-thalamocortical circuit in adaptive behavior. *Annals of the New York Academy of Sciences, 877,* 64-70. doi: 10.1111/j.1749-6632.1999.tb09261.x.

Kaufling, J., Veinante, P., Pawlowski, S. A., Freund-Mercier, M-J., & Barrot, M. (2009). Afferents to the GABAergic tail of the ventral tegmental area in the rat. *The Journal of Comparative Neurology, 513,* 597-621. doi: 10.1002/cne.21983.

Kemppainen, H., Ravio, N., & Kiianmaa, K. (2012). Role for ventral pallidal GABAergic mechanisms in the regulation of ethanol self-administration. *Psychopharmacology (Berlinin), 223*(2), 211-221. doi: 10.1007/s00213-012-2709-x

Keppel G., & Zedek, S. (1987). *Data analysis for research designs.* New York, NY: W.H. Freeman.

Klink, R., de Kerchove d'Exaerde, A., Zoli, M., & Changeux, J-P. (2001). Molecular and physiological diversity of nicotine acetylcholine receptors in the midbrain dopaminergic nuclei. *The Journal of Neuroscience, 21*(5), 1452-1463.

Koob, G. F. (1996). Hedonic valence, dopamine, and motivation. *Molecular Psychiatry, 1,* 186-189. Retrieved from http://www.nature.com/mp/index.html.

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology Reviews, 25,* 217-238. doi: 10.1038/npp.2009.110.

Kuhn, S., & Gallinat, J. (2011). Common biology of craving across legal and illegal drugs-a quantitative meta-analysis of cue-reactivity brain response. *European Journal of Neuroscience, 33*, 1318-1326. doi: 10.1111/j.1460-9568.2010.07590.x.

Laviolette, S. R., & van der Kooy, D. (2001). GABAA receptors in the ventral tegmental area control bidirectional reward signaling between dopaminergic and non-dopaminergic neural motivational systems. *European Journal of Neuroscience, 13*, 1009-1015. doi: 10.1046/j.1460-9568.2001.01458.x.

Laviolette, S. R., Alexson, T. O., & van der Kooy, D. (2002). Lesions of the tegmental pedunculopontine nucleus block the rewarding effects and reveal the aversive effects of nicotine in the ventral tegmental area. *Journal of Neuroscience, 22*, 8653-8660. Retrieved from http://www.jneurosci.org/.

Laviolette, S. R., & van der Kooy, D. (2003). The motivational valence of nicotine in the rat ventral tegmental area is switched from rewarding to aversive following blockade of the α7-subunit containing nicotinic acetylcholine receptor. *Psychopharmacology, 166*, 306-313. Retrieved from http://www.springer.com/biomed/neuroscience/journal/213.

Le Foll, B., & Goldberg, S. R. (2009). Effects of nicotine on experimental animals and humans: an update on addictive properties. *Handbook of Experimental Pharmacology*, *192*, 335-367. doi: 10.1007/978-3-540-69248-5_12.

Le Foll, B., Wertheim, C., & Goldberg, S. R. (2007). High reinforcing efficacy of nicotine in non-human primates. *PLoS One*, *2*, e230. doi: 10.1371/journal.pone.0000230.

Leung, B. K., & Balleine, B. W. (2013). The ventral striato-pallidal pathway mediates the effect of predictive learning on choice between goal-directed actions. *The Journal of Neuroscience, 33*(34), 13848-13860. doi: 10.1523/JNEUROSCI.1697-13.2013

Levin, M. E., Weaver, M. T., Palmatier, M. I., Caggiula, A. R., Sved, A. F., & Donny, E. C. (2012). Varenicline dose dependently enhances responding for nonpharmacological reinforcers and attenuates the reinforcement enhancing effects of nicotine. *Nicotine & Tobacco Research, 14*(3), 299-305. doi: 10.1093/ntr/ntr213

Liechti, M. E., Lhuillier, L., Kaupmann, K., & Markou, A. (2007). Metabotropic glutamate 2/3 receptors in the ventral tegmental area and nucleus accumbens shell are involved in behaviors related to nicotine dependence. *Journal of Neuroscience, 27*, 9077-9085. doi: 10.1523/JNEUROSCI.1766-07.2007.

Liu, X., Palmatier, M. I., Caggiula, A. R., Donny, E. C., & Sved, A. F. (2007). Reinforcement enhancing effects of nicotine and its attenuation by nicotinic antagonists in rats. *Psychopharmacology, 194*, 463-473. doi: 10.1007/s00213-007-0863-3.

Lodge, D. J., & Grace, A. A. (2006). The hippocampus modulates dopamine neuron responsivity by regulating the intensity of phasic neuron activation. *Neuropsychopharmacology, 31*, 1356-1361. doi: 10.1038/sj.npp.1300963.

Mameli-Engvall, M., Evrard, A., Pons, S., Maskos, U., Svensson, T. H., Changeux, J-P, & Faure, P. (2006). Hierarchical control of dopamine neuron-firing patterns by nicotinic receptors. *Neuron, 50*, 911-921. doi: 10.1010/j.neuron.2006.05.007

Mansvelder, H. D., & McGehee, D. S. (2002). Cellular and synaptic mechanisms of nicotine addiction. *Journal of Neurobiology, 53,* 606-617. doi: 10.1002/neu.10148.

Mansvelder, H. D., Keath, J. R., & McGehee, D. S. (2002). Synaptic mechanisms underlie nicotine-induced excitability of brain reward areas. *Neuron, 33*(6), 905-919.

Mansvelder, H. D., Mertz, M., & Role, L. W. (2009). Nicotinic modulation of synaptic transmission and plasticity in cortico-limbic circuits. *Seminars in Cell and Developmental Biology, 20,* 432-440. doi: 10.1016/j.semcbd.2009.01.007.

Markou, A., & Paterson, N. E. (2001). The nicotinic antagonist methyllycaconitine has differential effects on nicotine self-administration and nicotine withdrawal in the rat. *Nicotine & Tobacco Research, 3,* 361-373. doi: 10.1080/14622200110073380.

McBride, W. J., Murphy, J. M., & Ikemoto, S. (1999). Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. *Behavioral Brain Research, 101*(2), 129-152.

McFarland, K., & Kalivas, P. W. (2001). The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. *The Journal of Neuroscience, 21,* 8055-8063. Retrieved from http://www.jneurosci.org/.

Mogg, A. J., Whiteaker, P., McIntosh, J. M., Marks, M., Collins, A. C., & Wonnacott, S. (2002). Methyllycaconitine is a potent antagonist of alpha-conotoxin-MII-sensitive presynaptic nicotine acetylcholine receptors in rat striatum. *Journal of Pharmacology and Experimental Therapeutics, 302*(1), 197-204.

Moran, S., Wechsler, H., & Rigotti, N. A. (2004). Social smoking among US college students. *Pediatrics, 114,* 1028-1034. doi: 10.1542/peds.2003-0558-L

Nashmi, R., & Lester, H. A. (2006). CNS localization of neuronal nicotinic receptors. *Journal of Molecular Neuroscience, 30*(1-2), 181-184. doi: 10.1385/JMN/30:1-2-181

Nisell, M., Nomikos, G. G., & Svensson, T. H. (1994). Infusion of nicotine in the ventral tegmental area or the nucleus accumbens of the rat differentially affects accumbal dopamine release. *Pharmacology & Toxicology, 75,* 348-352. Retrieved from http://scialert.net/jindex.php?issn=1816-496x.

O'Dell, L. E., & Khroyan, T. V. (2009). Rodent models of nicotine reward: what do they tell us about tobacco abuse in humans? *Pharmacology, Biochemistry, and Behavior, 91*, 482-486. doi: 10.1016/j.pbb.2008.12.011.

Olausson, P., Jentsch, D., & Taylor, J. R. (2003). Repeated nicotine exposure enhances reward-related learning in the rat. *Neuropsychopharmacology, 28,* 1264-1271. doi: 10.1038/sj.npp.1300173.

Olson, V. S., & Nestler, E. J. (2007). Topographical organization of GABAergic neurons within the ventral tegmental area of the rat. *Synapse, 61,* 87-95. doi: 10.1002/syn.20344.

Ordonana, J. R., Gonzalez-Javier, F., & Gomez-Amor, J. (2012). Psychophysiological reactivity to environmental tobacco smoke on smokers and non-smokers. *Additive Behaviors, 37,* 838-843. doi: 10.1016/j.addbeh.2012.03.005.

Orejarena, M. J., Herrara-Solis, A., Pons, S., Maskos, U., Maldonado, R., & Robledo, P. (2012). Selective re-expression of β2 nicotinic acetylcholine receptor subunits in the ventral tegmental area of the mouse restores intravenous nicotine self-administration. *Neuropharmacology, 63*(2), 235-241. doi: 10.1016/j.neuropharm.2012.03.011

Palmatier, M. I., Evans-Martin, F. F., Hoffman, A. Caggiula, A. R., Chaudri, N., Donny, E. C., … Sved, A. F. (2006). Dissociating the primary and reinforcement enhancing effects of nicotine using a rat self-administration paradigm with concurrently available drug and environmental factors. *Psychopharmacology (Berlin.), 184,* 391-400. doi: 10.1007/s00213-005-0183-4.

Palmatier, M. I., Matteson, G. L., Black, J. J., Liu, X., Caggiula, A. R., Craven, L., … Sved, A. F. (2007a). The reinforcement enhancing effects of nicotine depend on the incentive value of nondrug reinforcers and increase with repeated drug injections. *Drug and Alcohol Dependence, 89,* 52-59. doi: 10.1016/j.drugalcdep.2006.11.020.

Palmatier, M. I., Liu, X., Matteson, G. L., Donny, E. C., Caggiula, A. R., & Sved, A. F. (2007b). Conditioned reinforcement in rats established with self-administered nicotine and enhanced by noncontingent nicotine. *Psychopharmacology, 195,* 235-243. doi: 10.1007/s00213-007-0897-6.

Palmatier, M. I., Liu, X., Caggiula, A. R., Donny, E. C., & Sved, A. F. (2007c). The role of nicotinic acetylcholine receptors in the primary reinforcing and reinforcement-enhancing effects of nicotine. *Neuropsychopharmacology, 32*(5), 1098-1108. doi: 10.1038/sj.npp.1301228

Palmatier, M. I., Coddington, S. B., Liu, X., Donny E. C. Caggiula, A. R. & Sved, A. F. (2008a). The motivation to obtain nicotine-conditioned reinforcers depends on nicotine dose. *Neuropsychopharmacology*, 55, 1425-1430. Retrieved from http://www.nature.com/npp/index.html.

Palmatier, M. I., Liu, X., Donny, E. C., Caggiula, A. R., & Sved, A. F. (2008b). Metabotropic glutamate 5 receptor (mGluR5) antagonists decrease nicotine seeking but do not affect the reinforcement enhancing effects of nicotine. *Neuropsychopharmacology, 33,* 2139-2147. doi: 10.1038/sj.npp.1301623.

Palmatier, M. I., O'Brien, L. C., & Hall, M. J. (2012). The role of conditioning history and reinforcer strength in the reinforcement enhancing effects of nicotine in rats. *Psychopharmacology (Berlin.), 219,* 1119-1131. doi: 10.1007/s00213-011-2439-5.

Palmatier, M. I., Marks, K. R., Jones, S. A., Freeman, K. S., Wissman, K. M., & Sheppard, A. B. (2013). The effect of nicotine on sign-tracking and goal-tracking in a Pavlovian conditioned approach paradigm in rats. *Psychopharmacology (Berlin), 226,* 247-259. doi: 10.1007//s00213-012-289-9

Panagais, G., Kastellakis, A., Spyraki, C., & Nomikos, G. (2000). Effects of methyllycoconitine (MLA), an α7 nicotinic receptor antagonist, on nicotine- and cocaine-induced potentiation of brain stimulation reward. *Psychopharmacology, 149,* 388-396. Retrieved from http://www.springer.com/biomed/neuroscience/journal/213.

Papke, R. L., Sanberg, P. R., & Shytle, R. D. (2001). Analysis of mecamylamine stereoisomers on human nicotinic receptor subtypes. *The Journal of Pharmacology and Experimental Therapeutics, 297,* 646-656.

Pastor, V., Andres, M. E., & Bernabeu, R. O. (2012). The effect of previous exposure to nicotine on nicotine place preference. *Psychopharmacology (Berlin), 2012.* doi: 10.1007/s00213-012-2928-1.

Paxinos, G., & Watson, C. (2007). *The rat brain in stereotaxic coordinates.* (6th ed.) [Computer software]. New York, NY: Elsevier.

Perkins, K. A., Epstein, L. H., Grobe, J., & Fonte, C. (1994). Tobacco abstinence, smoking cues, and the reinforcing value of smoking. *Pharmacology, Biochemistry, and Behavior, 47*, 107-112. doi: 10.1016/0091-3057(94)90118-X.

Perkins, K. A., & Karelitz, J. L. (2013a). Reinforcement enhancing effects of nicotine via smoking. *Psychopharmacology (Berlin), 228*(3), 479-486. doi: 10.1007/s00213-013-3054-4

Perkins, K. A., & Karelitz, J. L. (2013b). Influence of reinforcer magnitude and nicotine amount on smoking's acute reinforcement enhancing effects. *Drug and Alcohol Dependence, 133*(1), 167-171. doi: 10.1016/j.drugalcdep.2013.05.016

Perrotti, L. I., Bolanos, C. A., Choi, K. H., Russo, S. C., Edwards, s., Ullery, P. G., … Barrot, M. (2005). ΔFosB accumulates in a GABAergic cell population in the posterior tail of the ventral tegmental area after psychostimulant treatment. *European Journal of Neuroscience, 21*, 2817-2824. doi: 10.1111/j.1460-9568.2005.04110.x.

Picciotto, M. R., Zoli, M., Rimondini, R., Lena, C., Marubio, L. M., Pich, E. M., … Changeux, J. P. (1998). Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature, 391*(6663), 173-177.

Pidoplichko, V. I., DeBiasi, M., Williams, J. T., & Dani, J. A. (1997). Nicotine activates and desensitizes midbrain dopamine neurons. *Nature, 390*, 401-404.

Raiff, B. R., & Dallery, J. (2008). The generality of nicotine as a reinforcer enhancer in rats: effects on responding maintained by primary and conditioned reinforcers and resistance to extinction. *Psychopharmacology, 201*, 305-314. doi: 10.1007/s00213-008-1282-9.

Redgrave, P., Gurney, K., & Reynolds, J. (2008). What is reinforced by phasic dopamine signals? *Brain Research Reviews, 58*, 322-339. doi: 10.1016/j.brainresrev.2007.10.007.

Richardson, N. R., & Roberts, D. C. (1996). Progressive ratio schedules in drug self-administration studies in rats: A method to evaluate reinforcing efficacy. *Journal of Neuroscience Methods, 66*(1), 1-11.

Robinson, T. E., & Berridge, K. C. (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Research Reviews, 18*, 247-291. doi: 10.1016/0165-0173(93)90013-P.

Robinson, T. E., & Berridge, K. C. (2000). The psychology and neurobiology of addiction: An incentive-sensitization view. *Addiction, Supplement 2*, S91-S117. doi: 10.1046/j.1360-0443.05.8s2.19.x.

Rodd, Z. A., Bell, R. L., Kuc, K. A., Zhang, Y., Murphy, J. M., & McBride, W. J. (2005). Intracranial self-administration of cocaine within the posterior ventral tegmental area of Wistar rats: Evidence for involvement of serotonin-3 receptors and dopamine neurons. *Journal of Pharmacology and Experimental Therapeutics, 313*(1), 134-145.

Rodd, Z. A., Oster, S. M., Ding, Z. M., Toalston, J. E., Deehan, G., Bell, R. L., … McBride, W. J. (2008). The reinforcing properties of salsolinol in the ventral tegmental area: evidence for regional heterogeneity and the involvement of serotonin and dopamine. *Alcohol Clinical and Experimental Research, 32*, 230-239. doi: 10.1111/j.1530-0277.2007.00572.x.

Roegge, C. S., & Levin, E. D. (2006). Nicotinic receptor antagonists in rats. In E. D. Levin & J. J. Buccafusco (Eds.), *Animal models of cognitive impairment*. Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK1838/.

Root, D. H., Ma, S., Barker, D. J., Megehee, L., Striano, B. M., Ralston, C. M., …West, M. O. (2013). Differential roles of ventral pallidum subregions during cocaine self-administration behaviors. *Journal of Comparative Neurobiology, 521*(3), 558-588. doi: 10.1002/cne.23191

Rose, J. E., Behm, F. M., Westman, E. C., Bates, J. E., & Salley, A. (2003). Pharmacologic and sensorimotor components of satiation in cigarette smoking. *Pharmacology, Biochemistry, and Behavior, 76,* 243-250. doi: 10.1016/j.pbb.2003.07.002.

Saunders, B. T., & Robinson, T. E. (2013). Individual variation in resisting temptation: implications for addiction. *Neuroscience and Biobehavioral Reveiws, 37*(9 Pt A), 1955-1975. doi: 10.1016/j.neubiorev.2013.02.008

Schilstrom, B., de Villiers, S., Malmerfelt, A., Svensson, T. H., & Nomikos, G. G. (2000). Nicotine-induced Fos Expression in the nucleus accumbens and the medial prefrontal cortex of the rat: role of nicotinic and NMDA receptors in the ventral tegmental area. *Synapse, 36,* 314-321. doi: 10.1002/(SICI)1098-2396(20000615)26:4<314::AIDSYN8>3.0.CO;2-U.

Schilstrom, B., Rawal, N., Mameli-Engvall, M., Monikos, G. G., & Svensson, T. H. (2003). Dual effects of nicotine on dopamine neurons mediated by different nicotinic receptor subtypes. *International Journal of Neuropsychopharmacology, 6,* 1-11. doi: 10.1017/S1461145702003188.

Schultz, W. (1998). The phasic reward signal of primate dopamine neurons. *Advances in Pharmacology, 42,* 686-690. Retrieved from http://www.sciencedirect.com/science/bookseries/10543589.

Seamans, J. K., & Yang, C. R. (2004). The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Progress in Neurobiology, 74,* 1-57. doi: 10.1016/j.pneurobio.2004.10.002.

Shibata, R., Kameishi, M., Kondoh, T., & Torri, K. (2009). Bilateral dopaminergic lesions in the ventral tegmental area of rats influence sucrose intake, but not umami and amino acid intake. *Physiology and Behavior, 96*(4-5), 667-674. doi: 10.1016/j.physbeh.2009.01.002

Shiffman, S., Dunbar, M., Kirchner, T., Li, X., Tindle, H., Anderson, S., & Scholl, S. (2012). Smoker reactivity to cues: effects on craving and on smoking behavior. *Journal of Abnormal Psychology.* doi: 10.1037/a0028339.

Smith, A. D., & Bolam, J. P. (1990). The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurons. *Trends in Neuroscience, 13*(7), 259-265.

Smith, K. S., Tindell, A. J., Aldridge, J. W., & Berridge, K. C. (2009). Ventral pallidum roles in reward and motivation. *Behavioural Brain Research, 196*(2), 155-167. doi: 10.1016/j.bbr.2008.09.038

Thiel, K. J., Sanabria, F., & Neisewander, J. L. (2009). Synergistic interaction between nicotine and social rewards in adolescent male rats. *Psychopharmacology (Berlin), 204,* 391-402. doi: 10.1007/s00213-009-1470-2.

Tolu, S., Eddine, R., Marti, F., David, V., Graupner, M., Pons, S., … Maskos, U. (2013). Co-activation of VTA DA and GABA neurons mediates nicotine reinforcement. *Molecular Psychiatry, 18*(3), 382-393. doi: 10.1038/mp.2012.83.

Tomie, A., Grimes, K. L., & Pohorecky, L. A. (2008). Behavioral characteristics and neurobiological substrates shared by Pavlovian sign-tracking and drug abuse. *Brain Research Reviews, 58*(1), 121-135. doi: 10.1016/j.brainresrev.2007.12.003

van Zessen, R., Phillips, J. L., Budygin, E. A., & Stuber, G. D. (2012). Activation of VTA GABA neurons disrupts reward consumption. *Neuron, 73*(6), 1184-1194. doi: 10.1016/j.neuron.2012.02.016

Vezina, P., McGehee, D. S., & Green, W. N. (2007). Exposure to nicotine and sensitization of nicotine-induced behaviors. *Progress in Neuropsychopharmacology and Biological Psychiatry, 31,* 1625-1638. PMID: 17936462

Warren, C. A., & McDonough, B. E. (1999). Event-related brain potentials as indicators of smoking cue-reactivity. *Clinical Neurophysiology, 110,* 1570-1584. doi: 10.1016/S1388-2457-2457(99)00089-9.

Wightman, R. M., & Robinson, D. L. (2002). Transient changes in mesolimbic dopamine and their association with 'reward'. *Journal of Neurochemistry, 82*(4), 721-735.

Wise, R. A., & Bozarth, M. A. (1987). A psychomotor stimulant theory of addiction. *Psychological Review, 94,* 469-492. doi: 10.1037/0033-295X.94.4.469.

Wise, R. A. (2002). Brain reward circuitry: insights from unsensed incentives. *Neuron, 36,* 229-240. doi: 10.1016/S0896-6273(02)00965-0.

Xia, Y., Driscoll, J. R., Wilbrecht, L., Margolis, E. B., Fields, H. L., & Hjelmstad, G. O. (2011). Nucleus accumbens medium spiny neurons target non-dopaminergic neurons in the ventral tegmental area. *The Journal of Neuroscience, 31,* 7811-7816. doi: 10.1523/JNEUROSCI.1504-11.2011.

Yang, K., Hu, J., Lucero, L., Liu, Q., Zheng, C., Zhen, X., … Wu, J. (2009). Distinctive nicotinic acetylcholine receptor functional phenotypes of rat ventral tegmental area dopamine neurons. *Journal of Physiology, 587*(2), 345-361.

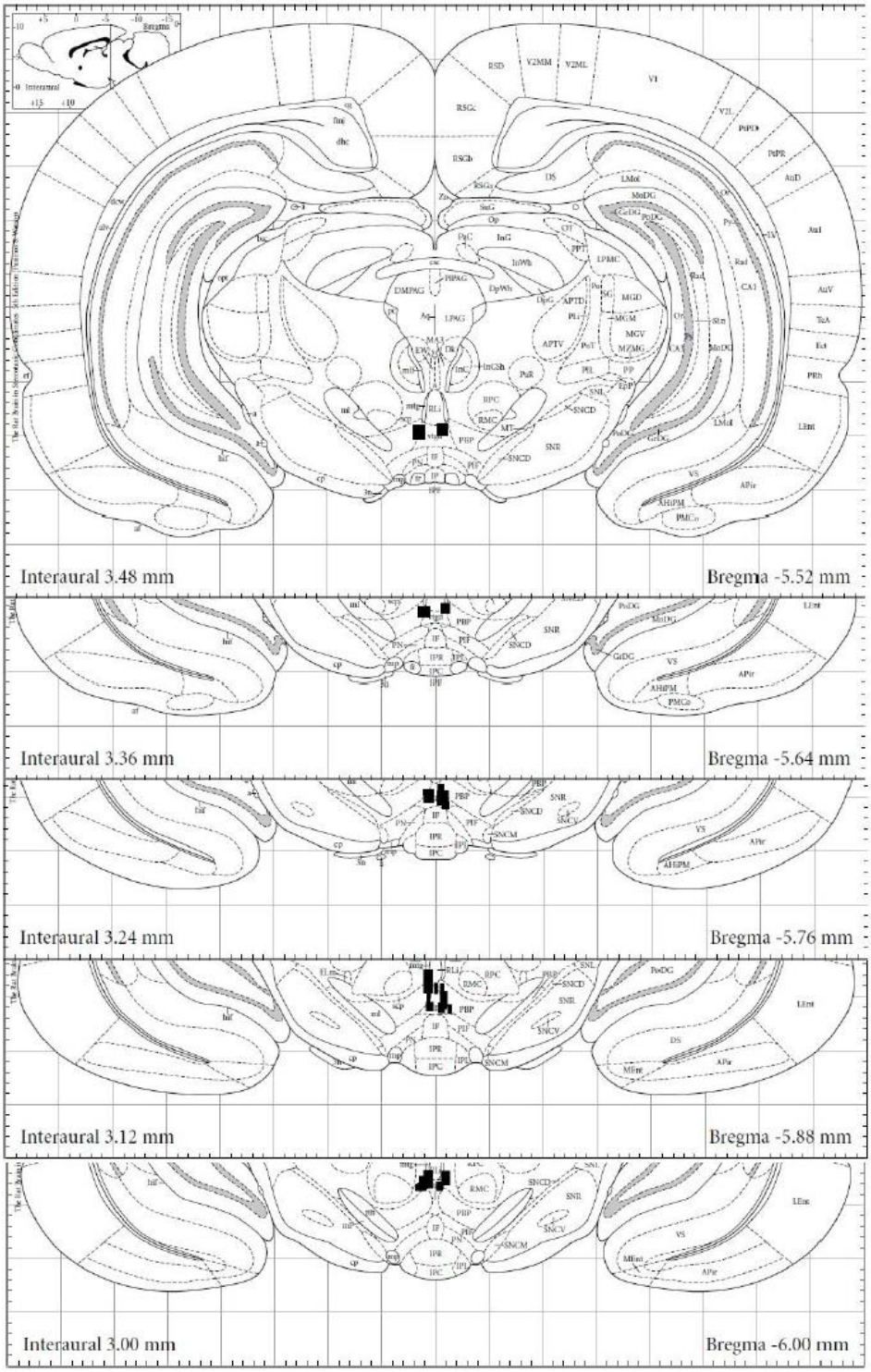
Yang, K., Buhlman, L., Khan, G. M., Nichols, R. A., Jin, G., McIntosh, J. M., … Wu, J. (2011). Functional nicotinic acetylcholine receptors containing α6 subunits are on GABAergic neuronal boutons adherent to ventral tegmental area dopamine neurons. *Journal of Neuroscience, 31*(7), 2537-2548. doi: 10.1523/JNEUROSCI.3003-10.2011

Zhao-Shea, R., Liu, L., Soll, L. G., Improgo, M. R., Meyers, E. E., McIntosh, J. M., … Tapper, A. R. (2011). Nicotine-mediated activation of dopaminergic neurons in distinct regions of the ventral tegmental area. *Neuropsychopharmacology, 36*(5), 1021-1032. doi: 10.1038/npp.2010.340

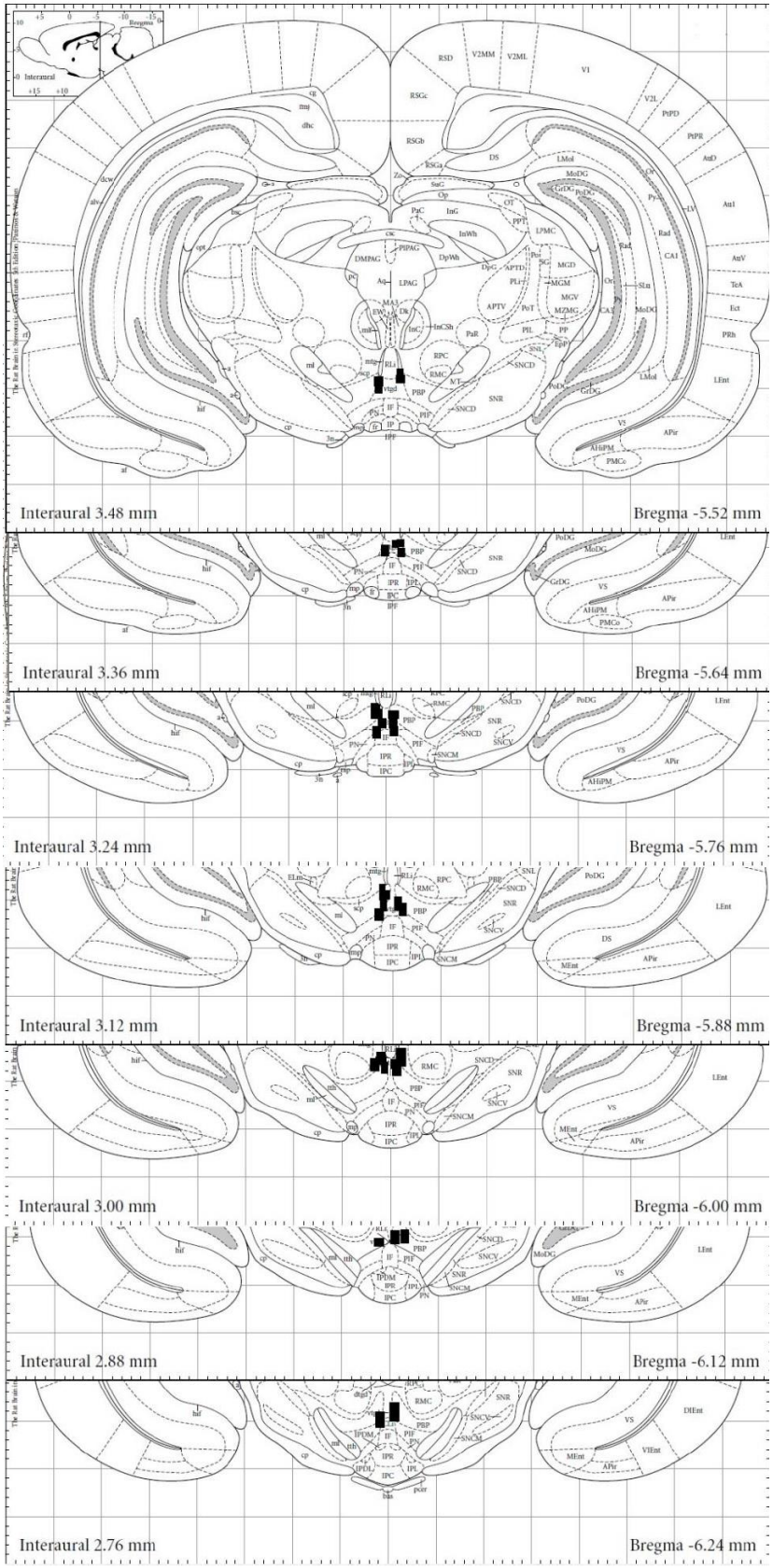
Zweifel, L. S., Parker, J. G., Lobb, C. J., Rainwater, A., Wall, V. Z, Fadok, J. P., … Palmiter, R. D. (2009). Disruption of NMDAR-dependent burst firing by dopamine neurons provides selective assessment of phasic dopamine-dependent behavior. *Proceedings of the National Academy of Sciences, 106*, 7281-7288. doi: 10.1073/pnas.0813415106.

APPENDICES

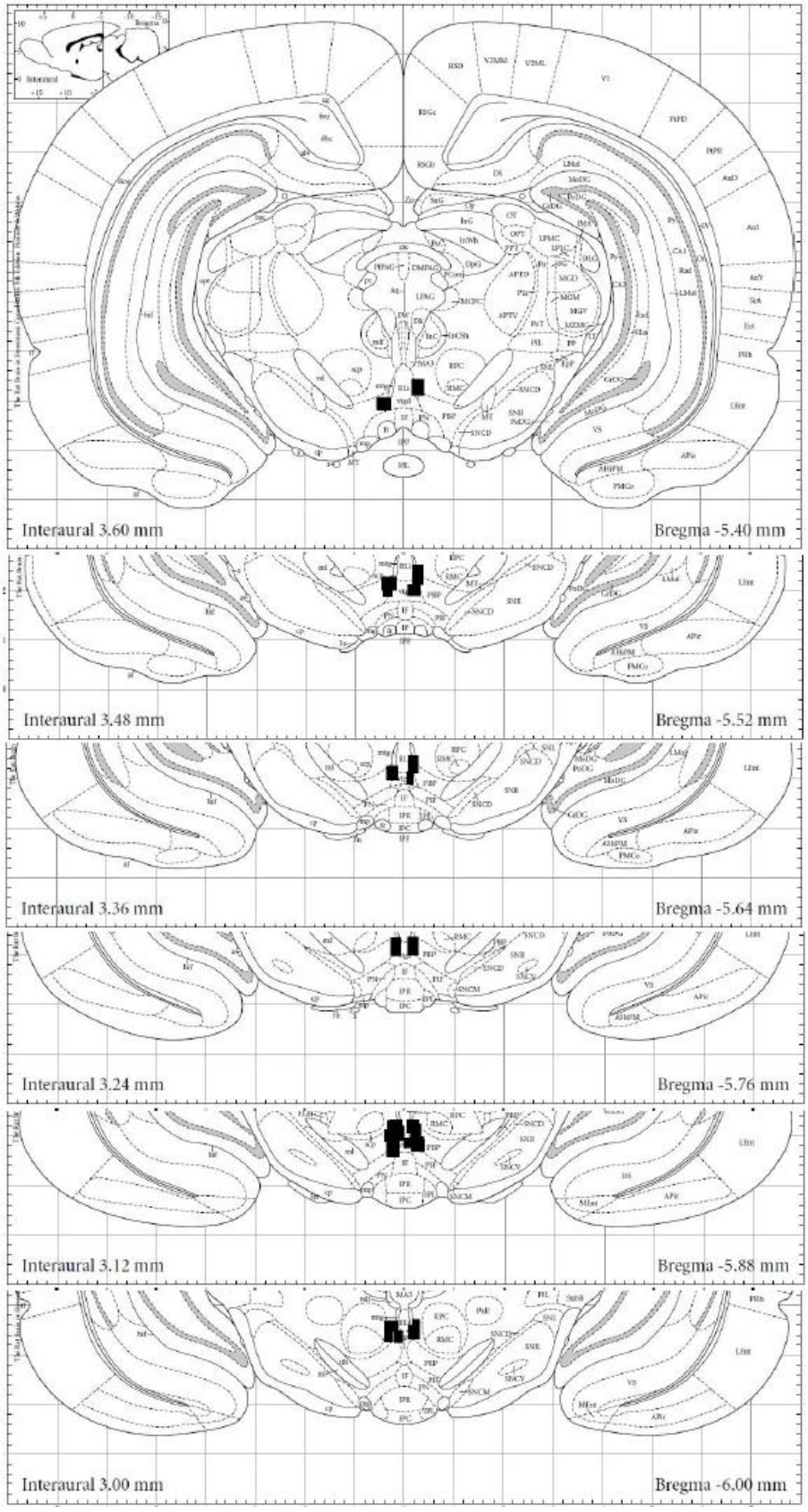
Appendix A: Cannula Placements—Muscimol and baclofen



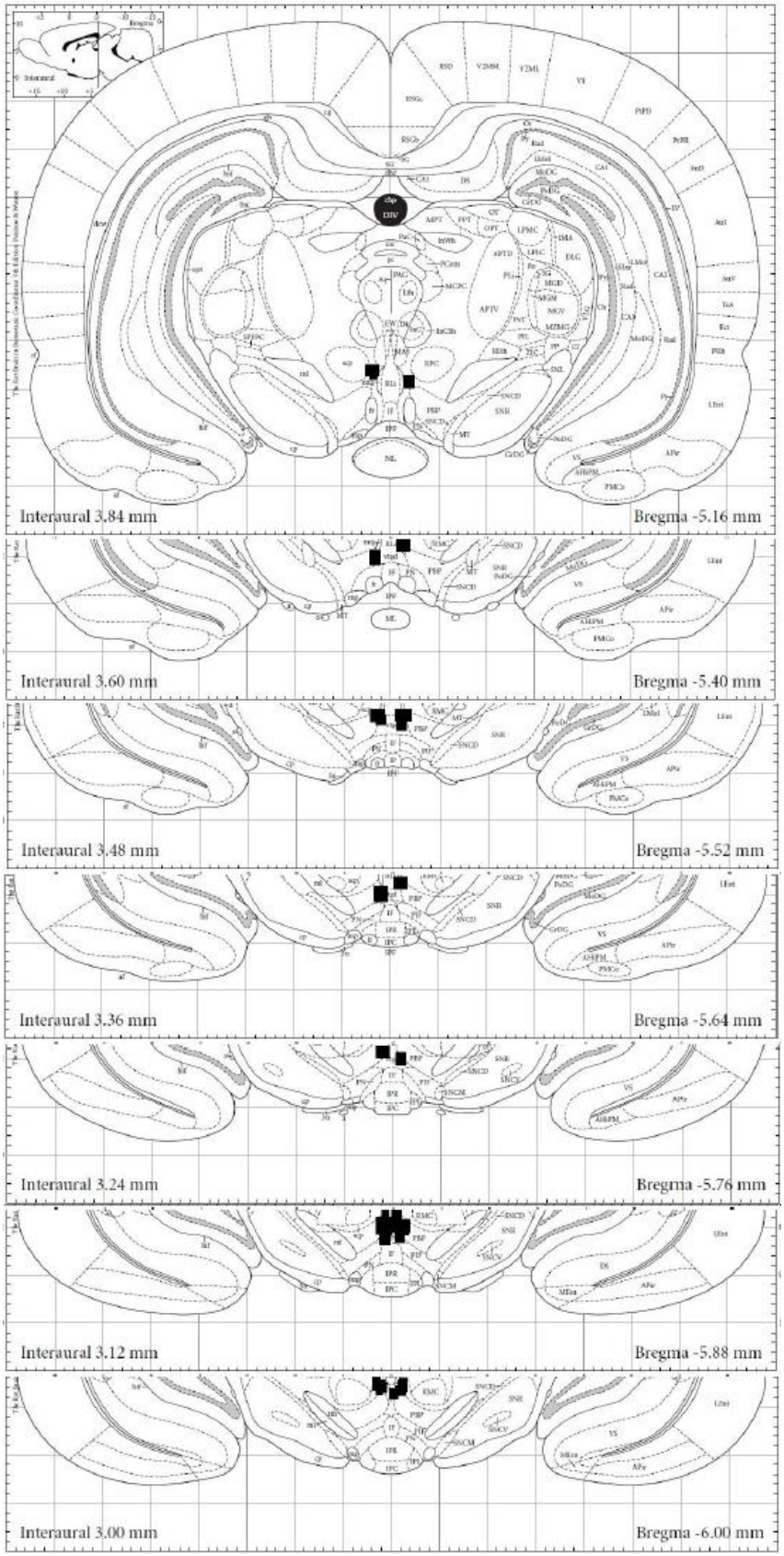
Appendix B: Cannula Placements—Mecamylamine



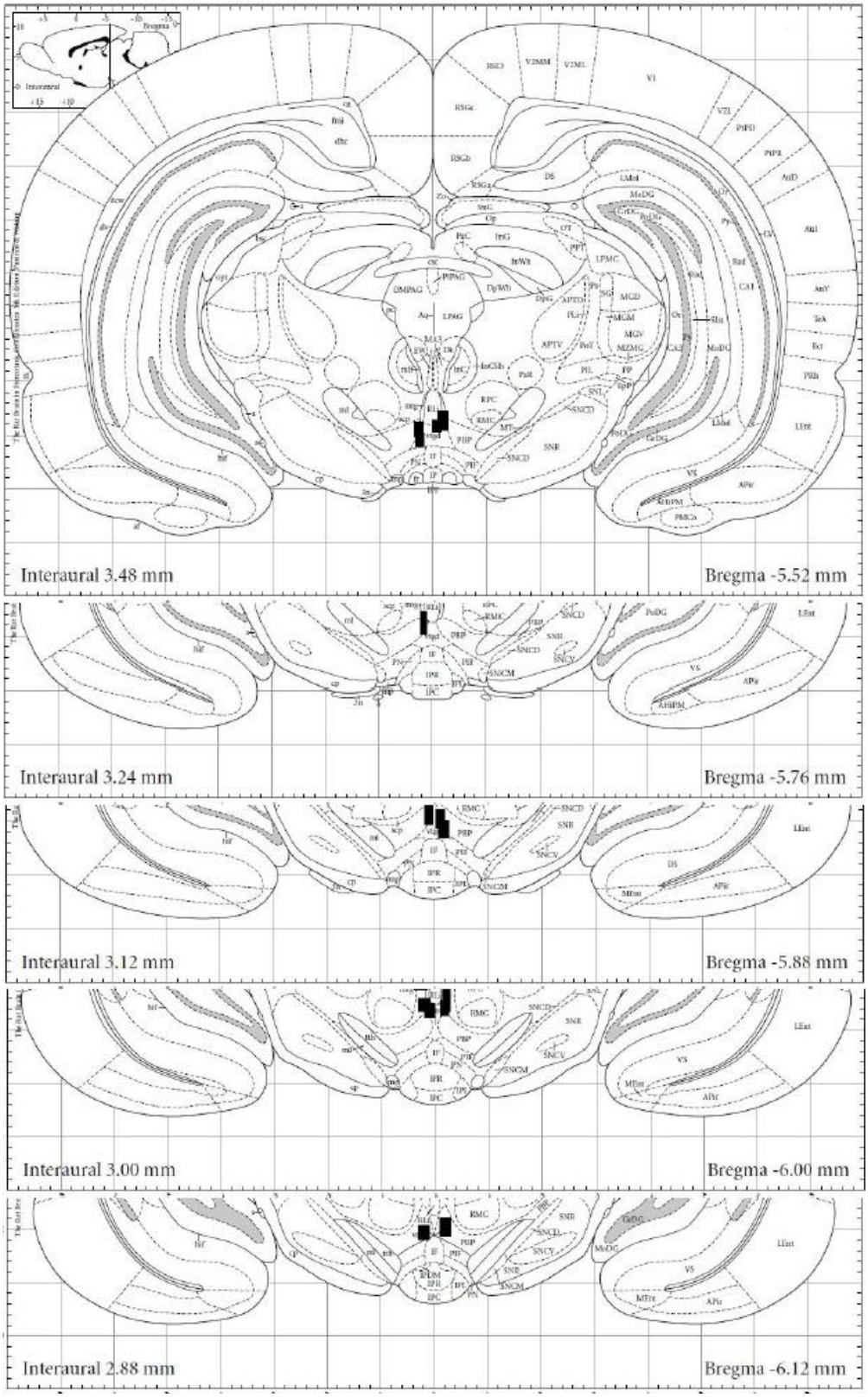
Appendix C: Cannula Placements—DHβE



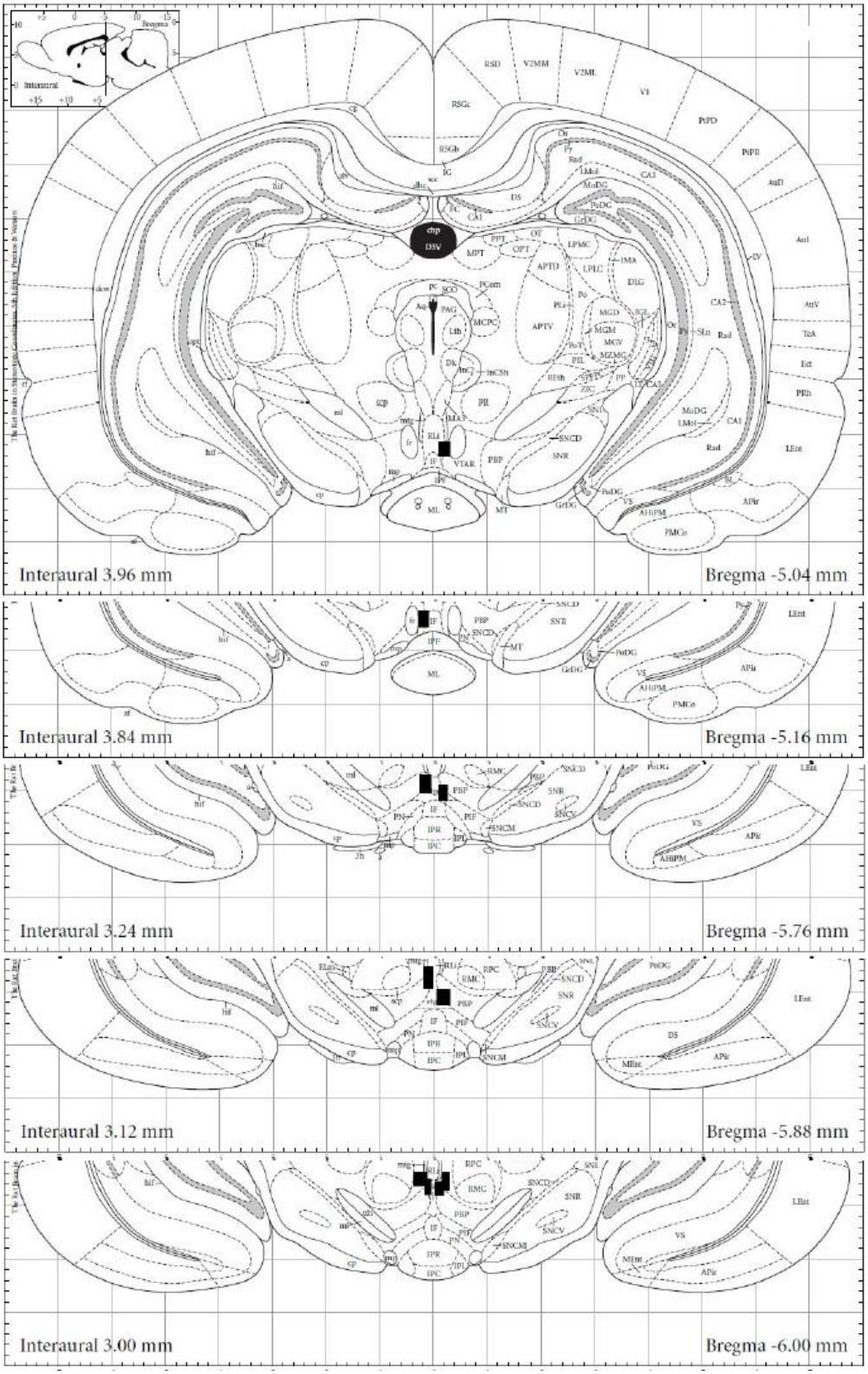
Appendix D: Cannula Placements—MLA



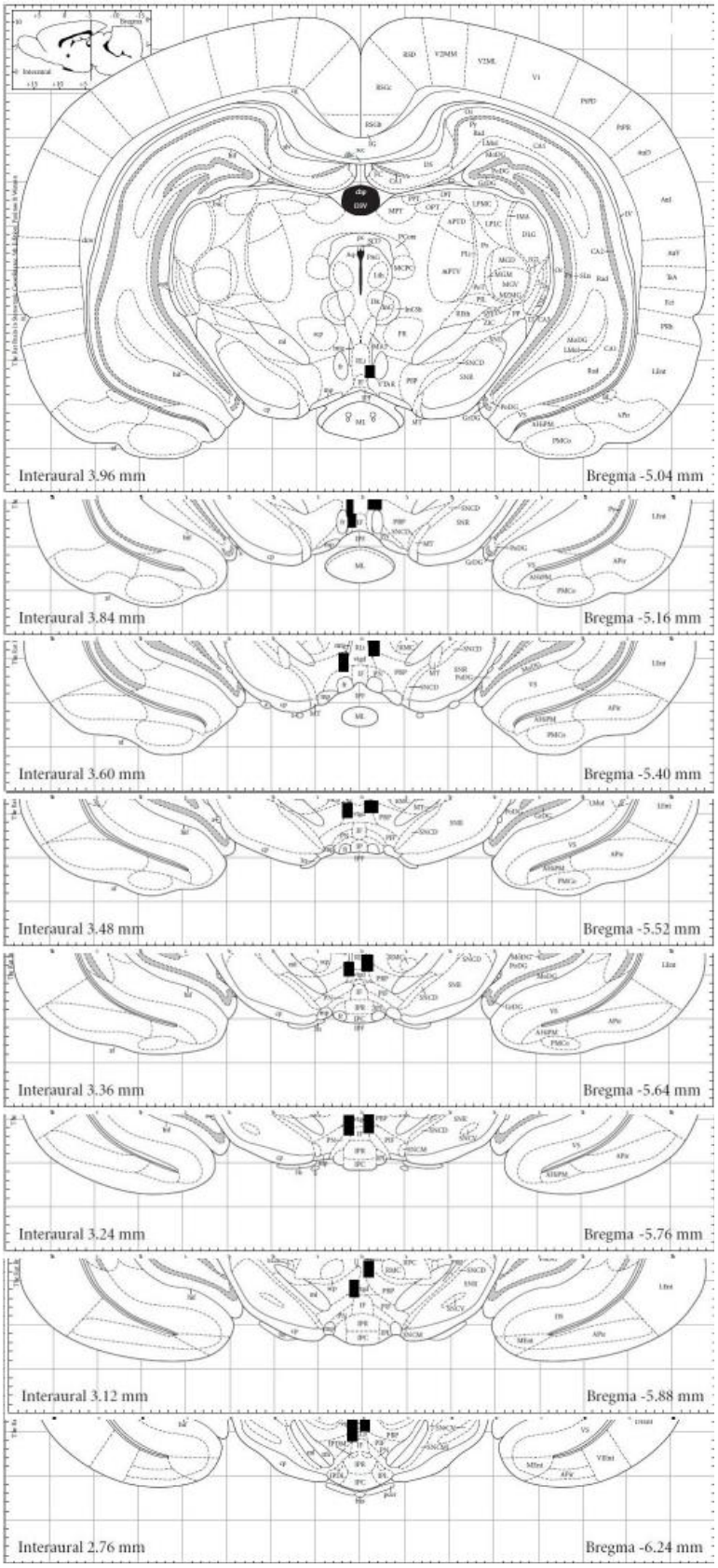
Appendix E: Cannula Placements—NIC + 0mM Intra-VTA NIC



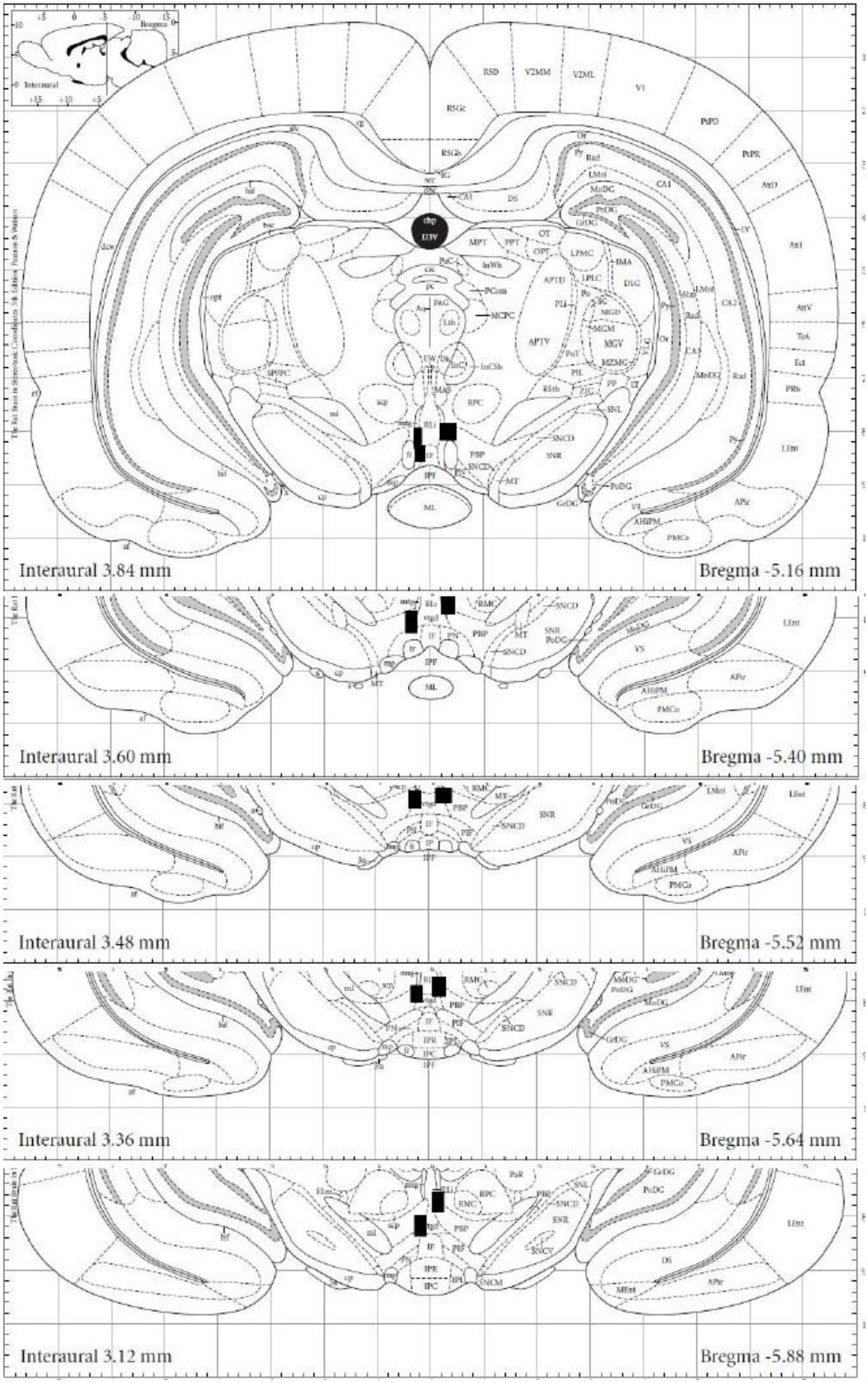
Appendix F: Cannula Placements—17mM Intra-VTA NIC



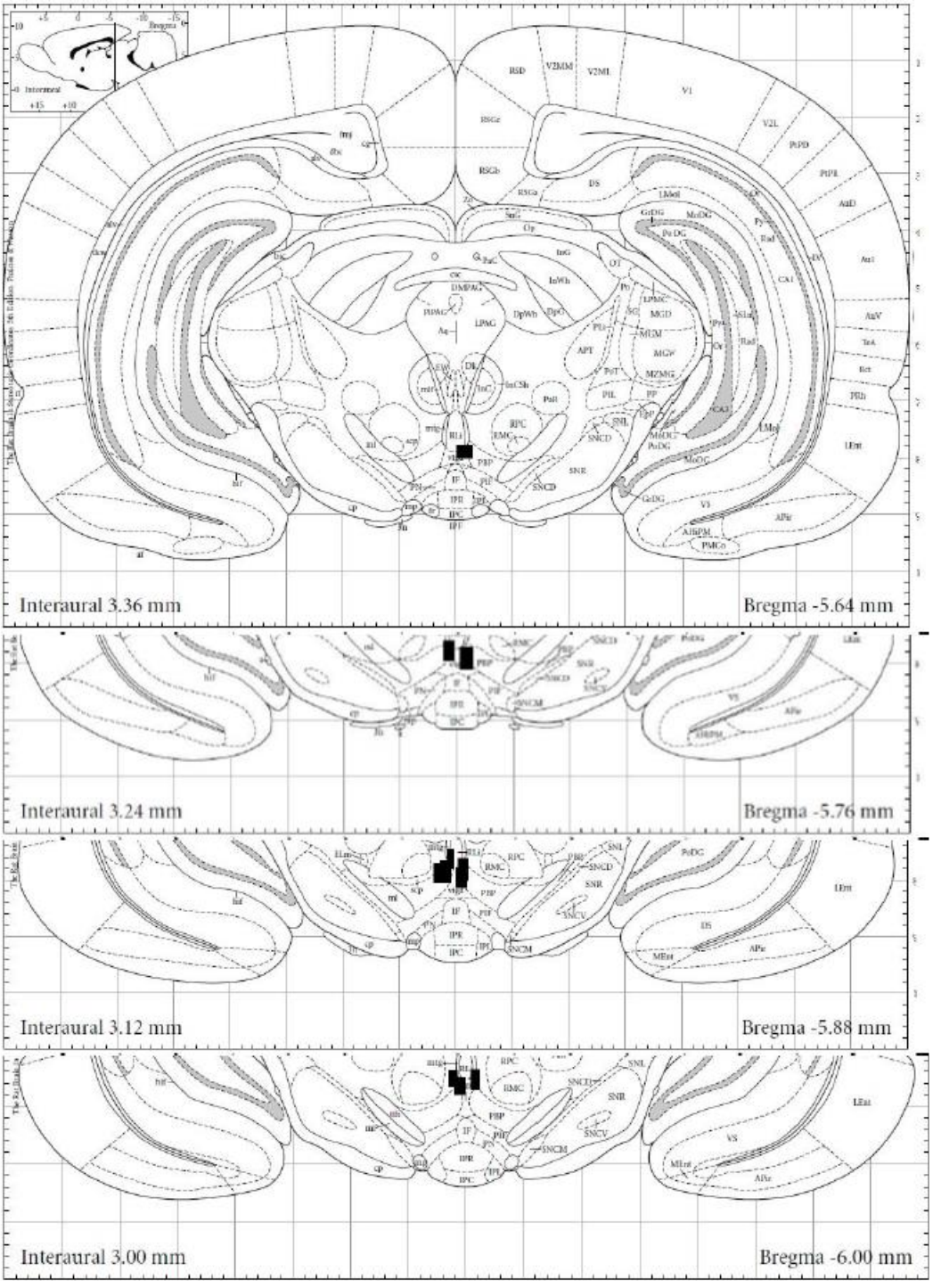
Appendix G: Cannula Placements—35mM Intra-VTA NIC



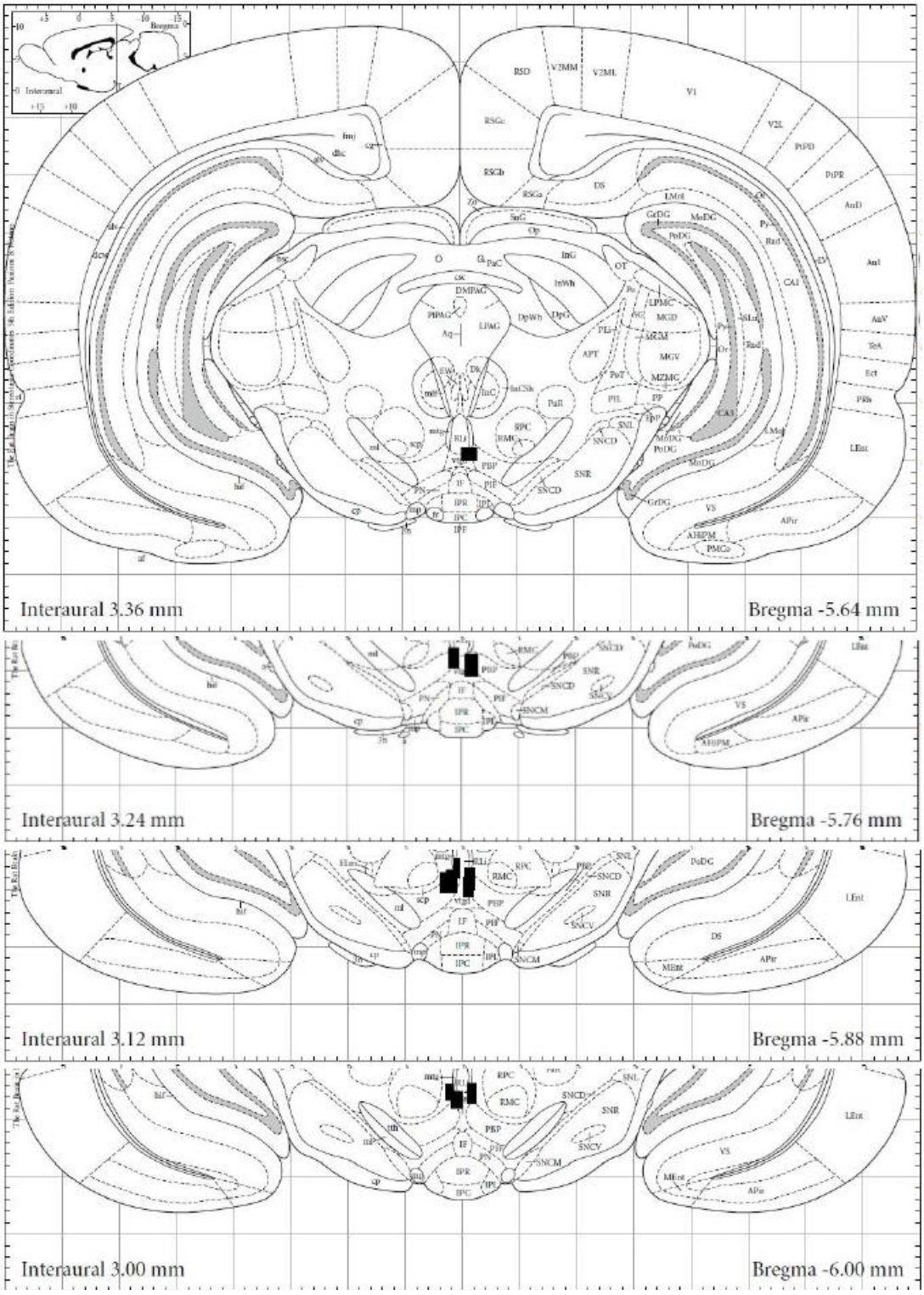
Appendix H: Cannula Placements—70mM Intra-VTA NIC



Appendix I: Cannula Placements—105mM Intra-VTA NIC



Appendix I: Cannula Placements—SAL + 0mM Intra-VTA NIC



VITA

A. BRIANNA SHEPPARD

Education:

Ph.D. Psychology, East Tennessee State University
Johnson City, Tennessee *2014*

M.A. Psychology, East Tennessee State University
Johnson City, Tennessee *2008*

B.S. Psychology, The University of Virginia's College at Wise
Wise, Virginia *2006*

Publications:

Palmatier, M. I., Marks, K. R., Jones, S. A, Freeman, K. S., Wissman, K. M., & **Sheppard, A. B.** (2013). The effect of nicotine on sign-tracking and goal-tracking in a Pavlovian conditioned approach paradigm in rats. *Psychopharmacology (Berlin.), 226*, 247-259. doi: 10.1007/s00213-012-2892-9

Sheppard, A. B. Gross, S.C., Pavelka, S.A., Hall, M.J., & Palmatier, M.I. (2012). Caffeine increases the motivation to obtain nondrug reinforcers in rats. *Drug and Alcohol Dependence, 124*, 216-222. doi: 10.1016/j.drugalcdep.2012.01.008

Brown, R. W., Hughes, B. A., Hughes, A. B., **Sheppard, A. B**., Perna, M. K., Ragsdale, W. L., Roeding, R. L., & Pond, B. B. (2012). Sex and dose-related differences in methylphenidate adolescent locomotor sensitization and effects on BDNF. *Journal of Psychopharmacology, 26*, 1480-1488. doi:10.1177/0269881112454227

Brown, R. W., Maple, A. M., Perna, M. K, **Sheppard, A. B**., Cope, Z. A. & Kostrzewa, R. M. (2012). Schizophrenia and substance abuse comorbidity: nicotine addiction and the neonatal quinpirole model. *Developmental Neuroscience, 34*, 140-151. doi: 10.1159/000338830

Cope, Z.A., Thompson, K.N., **Sheppard, A.B.**, Noel, D.M., Roane, D.S., & Brown, R.W. (2010).Neonatal quinpirole treatment enhances locomotor activation and dopamine release in the nucleus accumbens core in response to amphetamine treatment in adulthood. *Synapse, 64*, 289-300

Sheppard A. B., Lehmann, J., Cope, Z.A. & Brown, R.W. (2009). Sex differences in nicotine sensitization and conditioned hyperactivity in adolescent rats neonatally treated with quinpirole: role of D2 and D3 receptor subtypes. *Behavioral Neuroscience, 123,* 1296-1308

Correll, J.A., Noel, D. M., **Sheppard, A.B**., Thompson, K.N., Li, Y., Yin, D., & Brown, R.W. (2009). Nicotine sensitization and analysis of brain-derived neurotrophic factor in adolescent β arrestin-2 knockout mice *Synapse, 63,* 510-519

Edited Book Chapters:

Jones, J. W., **Sheppard, A. B**., Shoe, J. & Lee C. Y., "Hypothermic Machine Perfusion Preservation of Livers," in *Organ Resuscitation and Re-Engineering,* Artech House Publishing, Norwood MA, 2011

Research Grants and Scholarships:

Internal Research Grant ($800), School of Graduate Studies, East Tennessee State University, Project: "Role of ventral tegmental area and ventral tegmental area nicotinic acetylcholine receptors in the incentive amplifying effects of nicotine, *2013*

Graduate Student Summer Stipend Award ($3000), Johnson Center for Basic Cancer Research at Kansas State University, *2012*

Timothy R. Donaghue Graduate Scholarship ($3000/year), Kansas State University, *2010 & 2011*

Internal Research Grant ($500), School of Graduate Studies, East Tennessee State University, Project: "Nicotine conditioned context and nicotine sensitization," *2007*

Honors and Awards:

Excellence in Teaching Award for Graduate Teacher Associates, School of Graduate Studies, East Tennessee State University, *2014*

Teaching Award for Graduate Teacher Associates, Department of Psychology, East Tennessee State University, *2014*

Graduate Student Award: Animal Learning, Psychobiology and Comparative Psychology, Midwestern Psychological Association, *2012*

Outstanding Research Contribution in Psychology, Department of Psychology, The University of Virginia's College at Wise, *2006*