Nicotinic Acetylcholine Receptors and Cocaine Reward

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CERTIFICATE OF APPROVAL

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Abstract

Drug abuse is harmful to individuals, their families and a costly drain on scarce societal resources. Treatment for drug dependence can be difficult and produces mixed results. Therefore, it is incumbent upon researchers in the field of drug abuse to evaluate models and methods of testing procedures intended for therapeutic intervention. Animal models of drug dependence have become more sophisticated in representing the human condition of drug dependence. One such model is the long-access protocol of drug self-administration. Rats allowed an extended period of time to self-administer cocaine significantly escalate their drug consumption as compared to animals with only limited drug access. This is a robust phenomenon that is hypothesized to model the loss-of-control aspect in human drug dependent individuals. More than likely several neurotransmitter systems are involved in the shift from drug use to drug dependence.

Nicotine and cocaine are drugs commonly co-abused in human drug users. Experimental evidence suggests that the co-occurrence of these two drugs in drug users is more than a coincidence. Cocaine exerts its primary rewarding effects by acting within the mesoaccumbens reward pathway in the brain. Nicotine, the prototypical agonist of the nicotinic acetylcholine receptor (nAChR) has been reported to enhance the rewarding experience of cocaine and possibly inducing an increase cocaine intake. Therefore, I have hypothesized that nicotinic receptors are critically involved in the experience of cocaine reward. This includes the escalated intake of cocaine that is observed in animals with long-access to self-administer the drug. Furthermore, this thesis will detail a

possible mechanism and site specific location within the mesoaccumbens pathway that the nAChRs may be exerting their effects.

Experimental data indicated that systemic blockade of nicotinic receptors blunted the effect of long-access on the rat's daily cocaine consumption. The data was such that animals would not escalate their cocaine intake during periods of nAChR antagonism. Further research revealed that once animals had significantly increased their cocaine intake, as a function of long-access, antagonism of nAChRs in the ventral tegmental area (VTA) significantly decreased, but did not completely eliminate, cocaine self-administration. Given that nicotinic receptors in the VTA disrupted cocaine intake, a final experiment was conducted to elucidate a possible mechanism by which nAChRs exert influence over VTA dopamine projection neurons.

The findings in this thesis implicate the neuronal nicotinic receptors—particularly nAChRs in the VTA— as being functionally involved in cocaine self-administration. These findings may be of importance for clinicians and therapists when treating patients for cocaine dependence.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 DRUG ABUSE AND SOCIETY

Drug abuse in America is a complex problem; one that society must confront. The Office of National Drug Control Policy (ONDCP) estimates the monetary cost of non-prescription drug use—alcohol, the chemical components of tobacco and illicit drugs—in our society is roughly $484 billion per year. This number is derived from the cumulative cost of lost employment productivity health related problems as well as the added cost of the criminal justice system. By way of comparison, the economic cost of diabetes in America was only $131 billion in 2002. However, these financial considerations alone cannot account for the untold damage to individuals, families and communities that are affected by drug abuse. The overview of findings from the 2007 "National Survey on Drug Use and Health" from the Substance Abuse and Mental Health Services Administration (SAMHSA) indicated that 22.6 million Americans—or 9.2% of the total population—over the age of 12 admitted to current drug use and almost as many as could be classified as substance abusers. Nationally, the prevalence rate of cocaine use amongst all persons older than 12 was 2.4% in 2006.

There are many perspectives from which to target the study of addiction: societal, psychological and biological to give a few examples. At the level of the individual, drugs of abuse act within the body's own biological reward system; thus the impetus for understanding the neurobiology of drug dependence, ultimately, with an eye towards treatment and recovery for drug addicted individuals.

1.2 IDENTIFYING AND DEFINING DRUG USE DISORDERS

When speaking of drug use disorders terms such as addiction, dependence, use and abuse are commonly bandied about. However, a clinical difference is recognized between casual drug users and drug dependent patients. Along the drug use spectrum there are a few fundamental states: drug use, drug abuse and finally drug dependence or addiction. In this thesis, drug addiction and drug dependence will be used interchangeably.

The Diagnostic and Statistical Manual, fourth revision (DSM IV) is the standard by which clinicians use to distinguish drug use from drug abuse and drug dependence (American Psychiatric Association, 1994). Under the guidelines set forth by the DSM IV an individual must meet at least three of the following seven criteria at any time within the previous twelve months in order to be considered drug dependent:

1. Tolerance: need for increased amounts of drug in order to induce intoxication or attenuated response to the same amount of drug.
2. Exhibit withdrawal symptoms that result in clinically significant signs of distress or signs of social and/ or occupational impairment. A strong desire (craving) to re-administer the drug is commonly present as well.
3. Substance is taken in greater than intended amounts and done so over longer periods of time.
4. A recurring but failed attempt to reduce substance usage.
5. Greater amount of time spent in pursuit of substance, using substance or avoiding the negative affective withdrawal.

6. Occupational and/ or social duties neglected or reduced as a function of drug use.
7. Continual substance use in spite of negative consequences—physical, social or legal—that results from drug use.

Drug use disorder in humans is a complex interaction of biology and environmental influences. The definitions presented above speak specifically to human drug use; however, in a reductionistic fashion, many of the criteria of human drug dependence can be experimentally studied in the laboratory. This thesis will focus on one animal model used to study two characteristics of drug use disorder: first, substance is taken in greater amounts over time and second, greater amounts of time are spent in pursuit of the substance.

Many therapies have been instituted to aid in the recovery from drug addiction. Although marginal success has been achieved with each, it seems the long term prognosis is often one of relapse for the drug user, despite long periods of abstinence (Dackis and O'Brien, 2001; Wagner and Anthony, 2002; Kreek et al, 2002; Tate et al, 2008). It is therefore incumbent upon clinicians and researchers to understand the full biological and social spectrum of drug use disorder. This thesis will discuss one aspect of the biological underpinning of drug use disorder. What follows will center on animal models that will be used to investigate the neurobiology of the drug dependent state. The anatomy and physiology of two primary nuclei within the brain reward circuit: the nucleus accumbens (NAc) and the ventral tegmental area (VTA); on the primary neurotransmitters and their

associated receptors that act within the reward circuit and finally the hypothesis and rationale that served as the basis of this thesis.

2. DRUGS OF ABUSE & THE MESOLIMBIC SYSTEM:

Drugs of abuse include stimulants, depressants, benzodiazepines, opiates and hallucinogens. They all exert their influence either directly or indirectly within and upon the mesocorticolimbic circuit; ultimately resulting in increased extracellular DA in the NAc (Wise, 1996).

COCAINE

Cocaine is an alkaloid crystal derived from the leaves of the coca plant (*Erythroxylon coca*). Both a powerful stimulant of the central nervous system as well as a topical anesthetic, cocaine has a high liability for abuse and dependence. Cocaine's pharmacology is such that it acts as an antagonist at plasma membrane-bound monoamine transporters, i.e. blockade of the DA transporter (DAT) the norepinephrine transporter (NET) and the serotonin transporter (SERT; Boja at al., 1992; Miller et al, 2001; Hall et al, 2004; Sora et al, 2001). The practical result of transport blockade is to keep the various neurotransmitters active in the synaptic cleft for a longer period of time. Dopamine transporters are densely expressed in the NAc and VTA. Cocaine acts to enhance extracellular DA in the NAc via blockade of DATs (Fujita et al, 1994; Freed et al, 1995).

An indirect route of cocaine action exists within the VTA (Einhorn et al, 1988) to increase intra-tegemental DA neurotransmission—again by blocking VTA DAT, which

in turn results in activation of the inhibitory DA D2 autoreceptors as well as the excitatory DA D1 receptors, located presynaptically on GLU terminals (Cameron & Williams, 1993).

NICOTINE

Nicotine, the prototypical agonist of the nAChR, is both rewarding and addictive. Animals will readily learn to self-administer nicotine (Clark, 1969; Slifer & Balster, 1985; Donny et al, 1999; DeNoble & Mele, 2006) and in humans, nicotine intake (generally in the form of cigarette smoking) is highly reinforcing and difficult to stop (George & O'Malley, 2004; Harvey et al, 2004; Mitrouska et al, 2007), providing further evidence of the involvement of nAChRs in motivated behaviors (Palmatier et al, 2007). Activation of the nicotinic receptor results in an enhancement of DA neurotransmission to the NAc (Corrigall et al, 1992; Nisell et al, 1994a; Schilstrom et al, 1998; Sziraki et al, 2002; Kosowski et al, 2004;). Primarily this is mediated at the level of the VTA by acting on DA dendrites expressing the nicotinic receptor, and especially at the presynaptic level by enhancing the release of GLU from projections originating in the mPFC (Fu et al, 2000; Grillner & Svensson, 2000; Nisell et al, 1994b; for discussion of rat prefrontal cortex see Uylings et al, 2003). Nicotine stimulates DA VTA neurons with greater efficacy than DA neurons in the SN (Mereu et al, 1987). This may be a function of greater nAChR expression in the VTA vs. the SN (Keath et al, 2007). This evidence argues more in favor of the hedonic value of nicotine rather than its motor activating affects. Given the postsynaptic, but more often than not, presynaptic location of nicotinic acetylcholine receptors in the meso-accumbens pathway it has been proposed

they exert their influence primarily as a function of volume transmission of ACh (Mansvelder et al, 2002; 2003).

Cocaine and nicotine are commonly co-abused drugs in human addicts (Henningfield et al, 1990; O'Brien, 1997). This relationship is more than coincidental, given the ubiquity of cholinergic projections to, and nAChR within, the mesocorticolimbic pathway (Butcher et al, 1975; Smith and Parent 1984; Smith et al, 2004a, and b). The functional significance of cholinergic fibers within the DA system can be observed on many levels. In human cocaine addicts, systemic agonism of the nicotinic receptor enhances cravings for cocaine (Reid et al, 1998), whereas systemic antagonism of these same receptors decreases cue induced cravings for cocaine (Reid et al, 1999). Furthermore, in animal studies nicotine has been shown to reinstate cocaine-seeking behavior following extinction and site-specific antagonism of nicotinic receptors has been shown to suppress responding for cocaine. Furthermore, cholinergic interneurons in the NAc are activated as a function of cocaine sensitization protocols (Berlanga et al, 2003).

2.2. ANIMAL MODELS OF DRUG SEEKING BEAHVIOR

Methods of studying drug abuse in animals at the systems level include passive (respondent conditioning; for review see Tzschentke, 2007) and active (operant conditioning) behavioral paradigms. Although both methods have pros and cons, there exist certain advantages to using operant behavior when modeling human drug use in animals. Primarily the motivational aspect of drug use, which may be separate from the pharmacological aspect alone, cannot be overlooked. Mark and colleagues demonstrated

that animals self-administering cocaine exhibited greater ACh release in the NAc than pair matched animals passively receiving identical amounts of cocaine. It has become apparent that both short and long-lasting neurobiological adaptations occur as a function of motivated drug intake (Stefanski et al, 2004; Jacobs et al, 2005; for review see Jacobs et al, 2003).

LONG-ACCESS and the ESCALATION OF DRUG INTAKE

The loss of control of drug use in rats self-administering cocaine was first reported by Ahmed and Koob in 1998. This protocol allows rats self-administering cocaine on short-access (ShA) or 1hr schedule and long-access (LgA) or 6hr time period in which to consume drug. The resultant drug consumption by the LgA rats was one of "escalated" cocaine intake; such that animals consumed significantly more drug in their 1st hour when compared to their ShA counterpart controls. This phenomenon is thought to model the loss of control aspect of human drug dependence, as defined by the DSM IV. Although employed chiefly in rats for the study of cocaine abuse, escalation of drug intake in the long-access protocol has been shown to be a robust phenomenon. It is replicable across species, such as monkeys (Carroll et al, 2005) and drug classes such as psychostimulants, hallucinogens and opiates (Ahmed and Koob, 1999; Lenior and Ahmed, 2006; Kitamura et al, 2006; Carroll et al, 2005). However, it should be noted that under the LgA protocol nicotine failed to induce an escalated drug intake response from the animals (Paterson and Markou, 2003).

The LgA protocol of drug intake is hypothesized to model another diagnostic criterion of human drug dependence: a greater amount of time spent in pursuit of the drug. Using LgA experimental procedures increases the motivation of the animal, as is evidenced by an increase in the amount of work performed to gain access to the drug (Paterson and Markou, 2003). This last point is of particular interest, given that activation of nicotinic receptors has been shown to facilitate excessive cocaine seeking behaviors (Bechtholt and Mark, 2002), an apparent phenotypic similarity of escalated cocaine intake. Certainly nicotinic receptors play a role in enhanced cocaine seeking and perhaps are fundamentally necessary for the production of escalated cocaine intake.

3 ANATOMY OF THE MESOLIMBIC CIRCUIT

The mesocorticolimbic system (see Figure 1) is commonly referred to as the endogenous reward circuit of the brain. Neural activity within this system participates in appetitive behaviors such as eating, drinking, sex, motivational or goal directed movement (Schmidt, 1983) and selective attention (Piazza et al, 1988; Nieoullon, 2002). In addition to controlling goal directed behaviors the mesocorticolimbic circuit is functionally important as a center for reward prediction error processing and the relative importance of novel stimuli (Schultz et al, 1993, 1998; Hooks and Kalivas, 1995; Yun et al, 2004; Bayer and Glimcher, 2005; Pessiglione et al, 2006). Under normal circumstances this system facilitates a perception of satiety following activation by natural stimuli. However, drugs of abuse prevent the mesolimbic circuit from activating normal satiety mechanisms, or homeostasis, and instead force the system into an artificial state of unending activation without habituation.

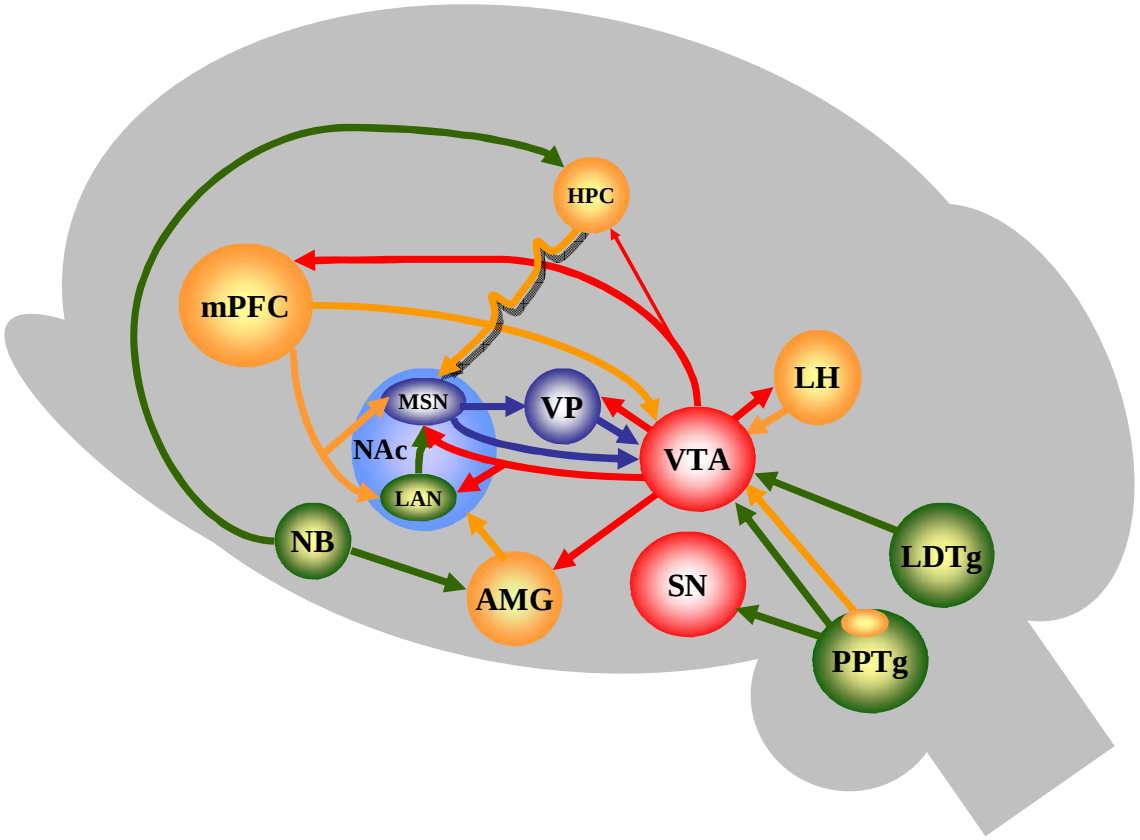


Figure 1.1: Sagital section from the rat brain. Red=Dopamine; Blue=GABA; Green=ACh; Orange=GLU. Abbreviations: SN, substantia nigra; VTA, ventral tegmental area; PPTg, pedunculopontine tegmental nucleus; LDTg, lateral dorsal tegmentum; AMG, amygdala; NAc, nucleus accumbens; LAN, large aspiney neurons; MSN, medium spiny neurons; HPC, ventral subiculum hippocampus; LH, lateral hypothalamus; mPFC, medial prefrontal cortex; VP, ventral pallidum; NB, nucleus basalis.

Drugs of abuse share the same neural-substrate with the common result of enhanced dopaminergic tone in the nucleus accumbens. This may or may not be preceded by activation of dopaminergic cell bodies in the VTA. For example nicotine activates DA cell bodies in the VTA resulting in greater DA release in the NAc. However, the action of amphetamine results in the release of DA into the NAc by de-vesicularization, a process independent of neuronal cell body activation. The anatomy and pharmacology of the NAc and the VTA will be discussed in some depth to qualify the rationale for this thesis.

3.1 MESOLIMBIC PROJECTIONS

3.1.1 VENTRAL TEGMENTAL AREA PROJECTIONS TO NAc:

The medial forebrain bundle (MFB) is comprised of a dense collection of ascending and descending axonal fibers running between the mid-brain septal nuclei and the basal forebrain regions (see Figure 1). The significance of these axonal fibers with regards to brain-reward was seminally described by the electrophysiological work performed by Olds and Milner in 1954. Animals with electrodes implanted in the MFB increased or maintained at a high rate of responding, self-administration of an electrical stimulation of this area. It was deduced that stimulation of MFB fibers was both an important and necessary neural substrate for positive reinforcement. Projections from VTA to the NAc—a key component of the medial forebrain bundle—are the most fundamental circuit in the neurobiological reward pathway of the brain. Further work determined catecholaminergic neurotransmitters in general (Parent & Poirier, 1969; Clavier & Routtenberg, 1976) and DA specifically, as being the primary neurotransmitter involved

in VTA-NAc communication (Koob et al, 1975; Fibiger et al, 1987; Fiorino et al, 1993). In time DA cell bodies were immunohistochemically localized to the VTA—traditionally known as the A10 region of the mesopontine (Dahlstroem & Fuxe, 1964; Kizer et al, 1976; Oades and Halliday, 1987). Using radioactive tracers Haglund and colleagues (1979), convincingly demonstrated that VTA DA fibers project primarily to the NAc, olfactory tubercules, the amygdala and the prefrontal cortex. Furthermore, the amygdala as well as the entorhinal cortex receives DA innervation from both the substantia nigra (SN) as well as the VTA. Follow up studies by others in the field elucidated that projections from the VTA terminate primarily in the dorsal portion of the NAc, (Chronister et al, 1980; Swanson, 1982). The SN is located more lateral to the VTA and constitutes the other primary source of midbrain DA. Although beyond the scope of this thesis, the importance of the SN cannot be overlooked and therefore will be briefly discussed here. Originally termed the A9 region of the mesopontine, cell bodies within the SN send DA projections to limbic and basal structures and in turn receive reciprocal input from the basal ganglia nuclei (Kizer et al, 1976). The SN is fundamentally important in movement—motivated and goal directed movement—as well as hyperlocomotion following psychostimulant administration (Wise & Bozarth, 1987; Amalric & Koob, 1993). Although some overlap of cell bodies exists with the VTA, the SN remains a distinct nucleus, one which both innervates and receives projections from the VTA (Beckstead et al, 1979). However, despite the importance of the SN, the remainder of this thesis will center on the ventral tegmental area as the source of midbrain dopamine.

Although DA is the primary output of the VTA to forebrain sites it is not the exclusive output. Bockstaele and Pickel (1995) first described those non-dopaminergic cells within the VTA that contain the inhibitory neurotransmitter, gamma-amino-butyric-acid (GABA) and project to the NAc. Other research has further demonstrated that these GABA cells within the VTA project to the prefrontal cortex (PFC) as well (Car and Sesack, 2000). Recent work in this field has also provided evidence of possible glutamate projection neurons within the VTA, although this remains speculative (Yamaguchi et al, 2007).

As with other biological systems the VTA-NAc circuit includes a negative feedback loop, which serves to tone down activity of VTA neurons. Excitation of neuronal cells within the NAc results in an inhibitory signal from the NAc back to the VTA, thus resulting in a reduction of the excitatory input. Early work by Yim and Mogenson detailed that the negative feedback loop in the mesolimbic circuit is mediated by GABA arising from the NAc, which resulted in an inhibition of DA neuronal cell bodies in the VTA (Yim and Mogenson, 1980). More recent evidence comes by way of the research performed by Rahman and McBride, which suggests that both GABA and cholinergic receptors in the NAc control inhibition of dopaminergic cell bodies within the VTA (Rahman & McBride, 2002).

3.1.2 LDTg AND PPTg PROJECTIONS TO THE VTA:

As a prominent part of the cholinergic reticular activating system the pedunculopontine tegmental nucleus (PPTg) and the lateral dorsal tegmentum (LDTg) send efferent

connections to the basal ganglia, thalamic nuclei and cortical regions of the brain (Cornwall et al, 1990). Collectively, the makeup of these two nuclei is heterogeneously composed of cholinergic and non-cholinergic—glutamate and GABA—cells (Honda & Semba, 1995; Steininger et al, 1997; Takakusaki et al, 1996; Clements & Grant, 1990; Ford et al, 1995). The VTA and SN both receive cholinergic innervation from the PPTg and the LDTg, traditionally known as Ch5 and Ch6 respectively (Mesulam et al, 1983; Manaye et al, 1999). Whereas the LDTg sends cholinergic projections exclusively to the VTA, the PPTg sends the majority of its cholinergic projection to the SN leaving only a small fraction of cholinergic projections to the VTA. In the VTA the pontine nuclei projections terminate on specific neurons in such a fashion as to promote DA excitation by attenuating the release of the inhibitory neurotransmitter GABA i.e. an indirect activation of DA cells (Omelchenko & Sesack, 2005). Acetylcholine (ACh) from the PPTg and LDTg acts on ligand gated nicotinic acetylcholine receptors (nAChR) in as well as the metabotropic muscarinic ACh receptors (mAChR) within the VTA, together the nAChR and mAChR play a crucial role in modulating dopamine cell firing (Garzon et al, 1999; Yeomans and Baptista, 1997; Corrigall et al, 2002). Further evidence suggests that the PPTg, despite its paucity of efferent connections to the VTA, is critically important for processing reward related stimuli by modulating ACh release (Lanca et al, 2000; Chen et al, 2006). The pontine nuclei also send cholinergic and non-cholinergic projections to other brain nuclei as well. For example the PPTg and LDTg send projections to the thalamas, basal forebrain and the globus pallidus. Ultimately, as part of the ascending reticular activating system the PPTg and LDTg are instrumental for arousal

and attention. Furthermore, the pontine nuclei serve as an intermediary link between cortical and cerebellar communication (Schwarz & Their, 1999).

3.2 NEUROTRANSMITTER SYSTEMS WITHIN the VTA and NAc

3.2.1 VENTRAL TEGMENTAL AREA NEUROTRANSMITTER SYSTEM:

The ventral tegmental area is positioned medial to the SN and ventral to the red nucleus. However, its boundaries are not cytologically distinct. Rather, it is defined by the boundaries of adjacent nuclei as well as its projection fields. Cells in the VTA are roughly comprised of 60% DA and 40% GABA neurons and a small but unknown amount of GLU cells (Margolis et al, 2006; van Bockstaele & Pickel 1995; Lavin et al, 2005). The VTA sends projections to the NAc shell, amygdala, prefrontal cortex, lateral hypothalamus and the LDTg. Neuronal cells within the VTA receive input from the prefrontal cortex, lateral hypothalamus, SN, pontine nuclei, the NAc and ventral pallidum. The VTA also receives serotoninergic projections from the raphe nucleus as well as noradrenergic input from the locus ceruleus. Neurons within the VTA have traditionally been labeled as either Type I or Type II based upon their electrophysiological and morphological properties (Phillipson, 1979; Sarti et al, 2007). Type I cells are dopaminergic cells and present a wide action potential, slow electrical conductance along the axon and a slow firing rate interspersed with occasional burst firing. Type II cells are GABAergic with faster action potentials, higher firing rate and faster electrical conduction along the axon (Guyenet & Aghajanian, 1978).

A prominent feature of the dopamine cells is their ability to encode certain salient aspects of stimuli based upon their temporal activation or firing patterns. Although the DA cell remains tonically active under normal conditions, motivationally relevant stimuli induce rapid "bursting" patterns of cellular activity. This burst firing pattern of activity is twice as effective in augmenting the release of DA in the NAc compared to tonic DA release (Suaud-Chagny et al, 1992; Overton and Clark, 1997) and is most likely controlled by GLU projections from the prefrontal cortex to the VTA (Murase et al, 1993) as well as projections from the LDTg (Lodge & Grace, 2006). It has been demonstrated that burst firing signals future expectancy and shifting of attentional focus (Cooper 2002; Omelchenko & Sesack, 2005; Lapish et al, 2007).

As a primary source of DA in the CNS, the VTA receives considerable input of other neurotransmitters onto its DA cells; neurotransmitters such as ACh, GABA, GLU and DA. This would be expected given DA cells in the VTA's central importance in appetitive and goal directed behaviors. The actions of each neurotransmitter in the VTA will be detailed in the following sections.

DA and the VTA

Dopamine neurons within the VTA release DA from their cellular body and dendritic regions following excitation. As such, this somato-dendritic release of DA can be considered a measure of DA cellular activation (Cragg & Greenfield, 1997; Adell & Artgas, 2004). The intra-VTA release of DA functions as an intra-nucleus negative-feedback signal by activation of inhibitory D2 autoreceptors located on the dendrites of

the DA cell. The net results of D2 activation are to inhibit the DA cell on which they reside (Einhorn et al, 1988; Brodie & Dunwiddie, 1990). In addition, D2 receptors co-localize to presynaptic GLU terminals that form synapses with DA cell dendrites in the VTA. Somato-dendritic release of DA and activation of these D2 receptors results in an attenuation of GLU release and therefore decrease DA cell activation (Koga and Momiyama, 2000). The excitatory dopamine D1 receptor is also present in the VTA. Located presynaptically on GABA terminals these receptors serve to enhance the release of the inhibitory neurotransmitter GABA; the net effect is a decrease in the tonic activation of the DA cell body (Cameron and Williams, 1995). Ultimately, the extracellular presence of DA in the VTA is a result of DA cell stimulation. The net effect of intra-VTA DA is one of inhibition: a toning-down of the excitability of DA cell bodies that lie within this nucleus.

GABA AND THE VTA

GABA released in the VTA comes from NAc GABA neurons projecting their terminals to the DA cell bodies within the VTA. This GABA projection completes a negative feedback circuit: VTA DA cells excite NAc GABA neurons, which in turn inhibit DA cells in the VTA. Many studies have confirmed the importance of the GABA neurotransmitter in the VTA. For example GABA microinjected into the VTA results in an inhibition of locomotion (Shank et al, 2007). GABA receptors have been localized to non-DA cells in the VTA and communicate reward related signals between DA and non-DA cells (Churchill et al, 1992; Laviolette and van der Kooy, 2001) and along with DA D2 receptors, promote a tonic inhibition of DA neurons (Westerink et al, 1996; Ikemoto

et al, 1997). Other GABA receptors, however, have been shown to be situated on VTA DA cell dendrites. To demonstrate the importance of these receptors on brain reward, Willick and colleagues microinjected GABA agonists into the VTA and observed a rightward shift of the intra-cranial self stimulation (ICSS) current-response curve (Willick & Kokkinidis, 1995). This may be explained by the results of Erhardt et al, (2002) that indicate the GABA receptors modulate the burst firing mode of DA neurons of the VTA. Ultimately however, the inhibitory properties of GABA in the VTA involve both dopaminergic and non-dopaminergic neurons (Stinus et al, 1982). It should also be noted that intra- VTA GABA terminals are involved in negative feedback loops. For example, DA D1 receptors located on GABA terminals enhance intra-VTA GABA release (Cameron and Williams, 1993). The VTA also has a rich projection of GABA interneurons as well, which tonically suppress DA cell firing (Car & Sesack, 2002)

As has been previously noted, the VTA sends GABA projections to the NAc (van Bockstaele and Pickel, 1995) and the prefrontal cortex (Car & Sesack, 2000) as well as the periaquaductal grey (PAG) and the dorsal raphe nucleus (Kirouac et al 2004). These GABA projections have been implicated in the non-dopamine dependent reward process.

GLUTAMATE and the VTA

The excitatory neurotransmitter glutamate (GLU) feeds into the VTA from the medial PFC (Car & Sesack 2000; Geisler et al, 2007; Wedzony et al, 2007; Takahada & Moghadamm, 1998) and the PPTg (Sesack et al, 2003; Charara et al, 1996; Blaha et al, 1996; Forster & Blaha, 2000). Glutamate in the VTA acts at AMPA/ NMDA and non-

NMDA receptors (Kretschmer, 1999) and serves to excite the DA cell bodies (Sesack et al, 2003; Sun et al, 2005). Furthermore, GLU input from the prefrontal cortex differentially alters DA cell activity in a way that promotes a switch from tonic to burst firing (Chergui et al, 1993; Takahada & Moghadamm 2000; Omelchenko & Sesack, 2005). In as much as GLU modifies the bursting potential of the DA cell, a reduction of GLU in the VTA returns DA cells to tonic levels of activation (Murase et al, 1993). Evidence suggests that the VTA itself may also contain a significant population of glutamatergic neurons (Chuhma et al, 2004; Laven et al, 2005). Using i*n situ* hybridization, Yamaguchi and colleagues determined that a small sub-population of cells in the VTA produced glutamate as their neurotransmitter; this was in exclusion of all other transmitters. Cells expressing mRNA for the vesicular glutamate transporter, VGluT1, were observed. These same cells rarely expressed markers for DA or GABA. The authors argued that these data indirectly provide evidence of glutamtergic cells within the VTA. However, the projection fields of the VTA GLU neurons remain unclear (Yamaguchi et al, 2007).

ACh AND THE VTA

In the VTA, activation of nAChR increases excitation of DA neurons and decreases the effect of inhibitory inputs onto those same cells (Mansvelder et al, 2002; Nisell et al, 1994a). The presence of ACh in the VTA is derived primarily from the LDTg and PPTg (Blaha et al, 1996; Car and Sesack 2000; Chen et al, 2006). The LDTg cholinergic terminals synapse exclusively onto mesoaccumbens VTA DA neurons. The PPTg sends the majority of its projections to the SN. However a small contingent of cholinergic

axons from the PPTg synapses onto VTA neurons (Omelchenko et al, 2006; Yeomans, 1995).

Cholinergic receptors expressed in the VTA are one of two sub-varieties: muscarinic and nicotinic receptors. As is the case with other receptor systems, activation of either the muscarinic or nicotinic receptors result in distinct, and in some cases disparate modulation of the cell on which they are localized.

Muscarinic cholinergic receptors (mAChRs) are slow acting; G-protein coupled receptors that generate excitatory post-synaptic potentials (EPSP's) via the M1, M3 and M5 subtypes and inhibitory post-synaptic potentials (IPSP's) through M2 and M4 activation. G-protein coupled mAChRs in the VTA appear to be instrumentally important in processing reward by modification of DA cell firing (Yeomanns and Baptista, 1997; Fink-Jensen et al, 1998; Rasmussen et al, 2000). Activation of the mAChRs the VTA results in an increase of DA release in the nucleus accumbens (Gronier et al, 2000; Moss et al, 2003).

Nicotinic cholinergic receptors (nAChRs) are fast acting, ligand gated and ionotropic in nature that generate rapid onset EPSP's. Nicotinic receptors consist of five protein subunits in a pentomeric conformation. Nicotinic receptors located on muscle cells are comprised of a combination of $\alpha 1$, $\beta 1$, γ and δ subunits. In brain, nAChR subunits are exclusively comprised of α and β proteins that exist in nine α and four β variants.

The αβ proteins surround a cation pore in a 2:3 stoichometic relationship (i.e. 2 α subunits: 3 β subunits). However, there exists a small sub-group of homopentameric receptors comprised of the α subunit alone (Pidoplichko et al, 2004). The subunit configuration imparts the binding specificity of the receptor to various agonists and antagonists (Klink et al, 2001; Papke et al, 2001; Connolly et al, 1992). ACh binds the α protein causing an allosteric conformational change resulting in increased cation permeability. The nicotinic channel is rapidly inactivated in a voltage-dependent fashion. In addition, repeated agonist exposure of nAChRs result in rapid desensitization and loss of function.

Located on DA cell bodies in the VTA (Clarcke et al, 1985) nicotinic receptors in this region appear to play a modulatory role for excitation of VTA DA neurons. However, nicotinic receptors are primarily located presynaptically and in the VTA have been localized to the glutamatergic terminals and serve to enhance glutamate release (Jones & Wonnacot, 2004). Thus, activation of nicotinic receptors may also be an indirect mechanism for excitatory modulation of the VTA DA neuron.

OTHER NEUROTRANSMITTERS IN THE VTA

Other prominent neurotransmitters that interact and affect VTA functioning include norepinephrine (NE), serotonin (5-hydroxytryptophan; 5HT), and endogenous opioid peptides. NE projects to the VTA and modulates DA cell firing both synaptically as well as extra-synaptically (Liprando et al, 2004). Slice recordings of DA neurons from the VTA indicate that NE receptors are located post synaptically on the dendrite region of the

cell. The functional importance of NE in the VTA is its ability to excite the DA cell, presumably through promoting the switch from single to burst firing (Arencibia-Albite et al, 2007; Pan et al, 2007).

5HT is fed into the VTA via the dorsal raphe nucleus which also sends 5HT projections to the NAc. Serotonin receptors have been co-localized to the DA cell body which results in an enhancement of excitation (Guan & McBride 1989; Yoshimoto & McBride, 1992; Nocjar et al, 2002). 5HT receptors have also been identified to localize presynaptically on the GABA terminal. Activation of receptors in this location results in the inhibition of GABA release and consequently an indirect activation of the DA cell (Cameron and Williams, 1995; Yan et al, 2004). Serotonin function in the VTA results in greater DA neurotransmission to the NAc (Yan et al, 2001; Filip et al, 2003; O'Dell and Parsons, 2004).

Opioid receptors in the VTA also have the ability to stimulate the DA neuron. However, their method of excitation is indirect. Opioid receptors decrease tonic firing of the GABA interneuron, the net effect being the release from inhibition—disinhibition—of the DA cell from the tonically active GABA interneuron (Gysling and Wang, 1983; Johnson & North, 1992; Devine et al, 1993). Additionally, opioid receptors in the VTA can affect downstream motivational behaviors as they pertain to drug abuse by facilitating DA cell excitation via receptors on presynaptic glutamate terminals (Sotomayor et al, 2006).

It should also be noted that the VTA receives excitatory input from the ventral bed nucleus of the stria terminalis (vBNST). Although the excitatory amino acid involved remains to be elucidated, Georges & Aston-Jones (2002) have demonstrated that its effects are mediated through both NMDA & non-NMDA receptors.

SUMMARY OF VTA FUNCTION

The VTA integrates multiple excitatory and inhibitory input signals and sends projections out to key substrates within the reward pathway (see Figures 1.1 and 1.2). Given the VTA's central role in appetitive behavior, motivation and drug abuse it is a likely target for therapeutic intervention when discussing treatment for drug dependence. One of the primary targets of VTA DA projections is to the NAc (Di Chiara & Imperato, 1988; Wise, 2002), The NAc also serves as an integration center for appetitive and reward related information coming in from other limbic areas such as the medial prefrontal cortex and the hippocampus. Furthermore, the NAc receives emotionally salient information regarding reward from the limbic system by way of the amygdala. Due to its basic role in processing activating stimuli the NAc will be discussed next.

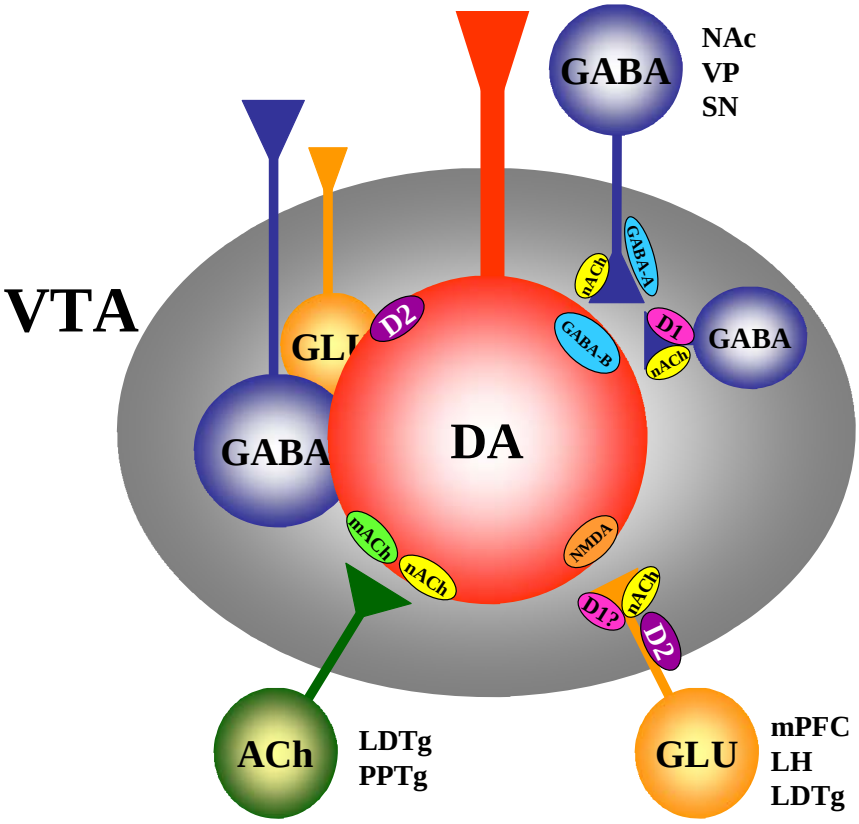


Figure 1.2: Intra VTA Circuit: Major axonal projections to and from the VTA as well as neurotransmitter receptors. Abbreviations: DA, dopamine; GLU, glutamate; ACh, acetylcholine; VTA, ventral tegmental area; LDTg, lateral dorsal tegmentum; PPTg, pedunculopontine tegmental nucleus; mPFC, medial prefrontal cortex; LH, lateral hypothalamus; NAc, nucleus accumbens; SN, substantia nigra VP, ventral pallidum. Colored receptors represent: Orange, NMDA receptor; Yellow, nicotinic ACh receptor; Green, muscarinic ACh receptor; Purple, DA D2 receptor; Magenta, DA D1 receptor; Blue, GABA receptor.

3.2.2 NUCLEUS ACCUMBENS

Once considered an adjunct to the striatum, the nucleus accumbens is now recognized as a distinct nucleus with associated subdivisions referred to as the core, shell and the rostral pole (Zham and Brog, 1992). Shell and core receive projections from the VTA and SN respectively. Furthermore, each sub-region of the NAc has distinct projections, although there is considerable overlap in terminal fields. NAc shell innervates the medial ventral pallidum (VP), the VTA, the rostral caudal hypothalamus and the extended amygdala. The core subdivision of the NAc sends projections to the dorsolateral VP, SN and the entopeduncular nucleus (Heimer et al, 1991). Projections from the lateral rostral pole largely follow core-like projections: globus pallidus, ventral pallidum, entopeduncular nucleus and parts of the lateral hypothalamus, VTA, dorsal SN pars compacta, structures. Medial rostral pole projections are "shell-like" in the nature of their innervation, activating the subcommissural ventral pallidum, lateral preoptic region, lateral hypothalamus and the bulk of the VTA (Zahm and Heimer 1990; see Figures 1 and 3 for an abbreviated projection diagram).

In reward related processing morphological distinction between the NAc shell and core becomes readily apparent (Fuchs et al, 2004; Ito et al, 2000). The function of the shell sub-region is that of motivation and goal planning. Conversely, the NAc core receives the majority of its DA input from the SN and the majority of its efferent project to the motor nuclei of the thalamus and back to the SN. Consequently, the NAc core is more involved in coordinating the motor activation associated with goal direction and motivation. The VTA sends its dopaminergic projections primarily to the NAc shell, and

in turn receiving projections from the same. For the purpose of this thesis the NAc shell will be the primary focus of this section.

DA and the NAc

As has been previously mentioned the NAc receives extensive DA projections from the VTA (Koob et al, 1975; Fibiger et al, 1987; Fiorino et al, 1993; see Figures 1 and 3). DA activity in the NAc is fundamental to motivated behaviors. A vast body of research has been devoted to dopamine's effect in the NAc (Damsma et al, 1992; Wenkstern et al, 1993; Wilson et al, 1995; Ikemoto & Panskeep, 1999; Salamone & Correa, 2002). The NAc has long been considered the primary site at which DA exerts appetitive and hedonic influence (Wise & Rompre, 1989; Wise, 1996; Salamone et al, 2003). Within the NAc cells express both the D1 and D2 type receptors (White and Wang, 1986), both of which have been shown to be fundamentally necessary in reward-related learning (Fenu et al, 2001; Yun et al, 2004; Schmidt and Pierce, 2006; Bari and Pierce 2005).

GABA and the NAc

As described by Mogenson and colleagues (1980) GABA cell bodies located within the NAc send their inhibitory projections to the ventral pallidum and the VTA. Parallel pathways exist and have distinct projection profiles based upon the origination of the GABA cell body in the core or shell region of the NAc (Zham and Heimer, 1990). The functional significance of the core/ shell subdivision is a function of their excitatory input as well as their output. The shell receives a larger portion of DA input from the VTA and in turn sends the majority of its GABA inhibitory projections back to the VTA. By

contrast the NAc core receives the preponderance of its DA from the SN and sends GABA back in as well. GABA cell bodies—termed medium spiny neurons (MSN) — comprise roughly 90% of the total population of neurons in the NAc. The MSN are the primary target for DA projections from the VTA, glutamate projections from the mPFC and cholinergic interneurons (refer to Figure 1.3).

GLUTAMATE and the NAc

Accumbal GLU is critically important for learning reward cues and goal directed behavior (Hauber et al, 2000) and has been shown to modulate drug seeking behavior (Di Ciano and Everitt, 2001; Bäckström and Hyytiä, 2007). The majority of GLU projections to the NAc come via the medial prefrontal cortex; the functional significance of these projections is both excitatory to cell bodies and serves to modulate both DA and ACh release (Segovia et al, 1997; Del Arco & Mora, 2005, 2008). Other GLU projections to the NAc come by way of the basal lateral amygdala (Kelly et al, 1982) and these GLU fibers have the ability to modulate DA neurotransmission within the NAc, independent of stimulation of DA cells in the VTA (Jackson & Moghaddam, 2001; Howland et al, 2002). A minor source of GLU input to the NAc comes via the ventral subiculum of the hippocampus. Terminating onto GABA cells this GLU action results in inhibition of DA cells within the VTA (Floresco et al, 2001) via the aforementioned negative feedback loop present in the in the mesolimbic circuit (see Figures 1 and 3).

ACh and the NAc

The major source of ACh in the NAc is from cholinergic interneurons, which in turn receive input from the VTA DA cells and mPFC GLU. Early studies pointed to the presence of ACh in DA regions of the brain (Butcher et al, 1975; Smith and Parent 1984). However, in the striatum the primary target of the ACh interneuron is the medium spiny neurons (de Rover et al, 2002; Gerfen, 1988; Izzo and Bolam, 1988). The medium spiny neurons of the NAc are also a primary target of midbrain DA neuronal projections. Thus it would appear that ACh interneurons modulate the synaptic targets of DA projections; DA cells in turn receive negative feedback, via GABA projections, from these same MSN (see Figure 3).

OTHER NEUROTRANSMITTERS and the NAc

CART peptide injected into the NAc blunts the locomotor stimulating effects of cocaine; this neuropeptide may be acting at the dopamine receptors in a homeostatic fashion to temper the effects of large amounts of extracellular DA (Jaworski et al, 2003; Hubert et al, 2008). 5HT activation in the NAc has been shown to enhance the activating effects of cocaine (Przegalinski et al, 2002a, b; Filip et al, 2003). Endogenous opioidergic peptides and their associated receptors in the NAc also play a role in hedonically motivated behaviors such as food intake (Majeed et al, 1986; Ward et al, 2006) and consumption of drugs of abuse (Martin et al, 2002) by modifying stimulated extracellular dopamine levels (Fuentealba et al, 2006).

In summary the nucleus accumbens is the nexus of several neurotransmitter systems. The NAc controls the propagation and selection of appetitive, motivated and goal directed

behaviors. Disruption of signals to and from the NAc often results in deficits in an animal's ability to seek and consume natural rewards e.g. food, water etc. Drugs of abuse, by their very nature, dramatically modify the normal function of this system. In this thesis I have examined how the mesolimbic system, specifically the ACh input to the DA signal, modifies drug seeking behavior.

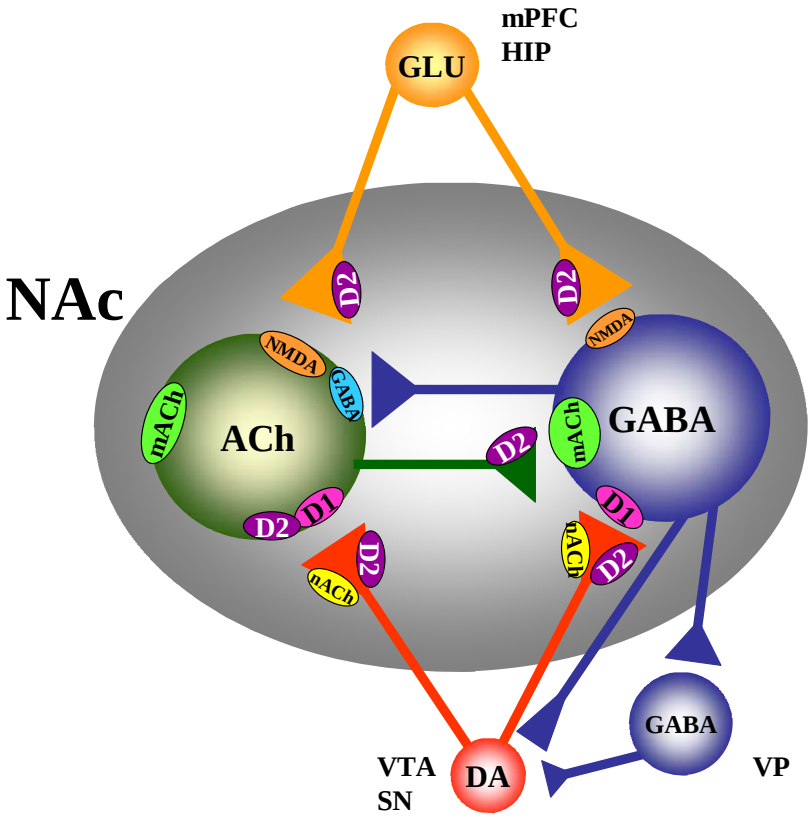


Figure 1.3: Intra-NAc circuitry: Major projections to and from the NAc. Abbreviations: DA, dopamine; GLU, glutamate; ACh, acetylcholine; VTA, ventral tegmental area; mPFC, medial prefrontal cortex; LH, lateral hypothalamus; NAc, nucleus accumbens; VP, ventral pallidum; HIP, hippocampus; SN, substantia nigra. Colored receptors represent: Orange, GLU receptor; Yellow, nicotinic ACh receptor; Green, muscarinic ACh receptor; Purple, DA D2 receptor; Magenta, DA D1 receptor; Blue, GABA receptor.

4. RATIONALE

As has been discussed, the mesocorticolimbic system is the central system for processing and facilitating behaviors related to reward. The ventral tegmental area and its dopaminergic projections to the nucleus accumbens form the core of the reward circuit. However, several neurotransmitters affect and in turn are affected by the meso-accumbens system. The excitatory neurotransmitter glutamate, contained in projection axons from the frontal cortex impinges upon both the NAc and the VTA. Whereas the inhibitory GABA signal from the NAc affects DA cell bodies within the VTA. Acetylcholine, both in the VTA and the NAc, appears to play an important role in modulating the DA signal originating in the VTA and ACh release from the pontine nuclei serves as a particularly important modulator of sensory signals terminating in the VTA.

Cocaine interacts within the endogenous meso-accumbens reward path in such a way as to continually enhance the DA signal. Many pathological neuroadaptations result as a consequence of cocaine's actions. An example of one such adaptation is the biological shift in the brain and consequently ability of an animal to take greater amounts of cocaine overtime and propensity to devote greater amounts of time spent in pursuit of the drug. Certainly these neurobiological and motivational changes involve the DA system. However, some evidence suggests involvement of the nicotinic acetylcholine receptors as well. Given the large body of evidence for the modulation of DA neurotransmission by AChRs there exists the possibility that cholinergic receptors—specifically nicotinic

receptors—may prove to be an underestimated therapeutic pharmacological target for the treatment of cocaine dependence.

The long-access paradigm has been proposed as a useful animal model to address the loss of control aspect of drug use. Yet to this date the involvement of nAChRs in LgA drug consumption has not been tested. Given the ability of the nicotinic receptor to modulate signal in the dopaminergic pathway it would seem highly probable that the nAChRs are functionally involved in the loss-of-control aspect of cocaine consumption. The purpose of this thesis is to investigate the role that nicotinic acetylcholine receptors play in the development and maintenance of escalated cocaine intake. Specifically I have hypothesized that nAChRs will alter cocaine self-administration, particularly escalated cocaine consumption under the extended access paradigm. Furthermore, this thesis will cover an experiment designed to elucidate a possible mechanism and site-specific location within the mesoaccumbens pathway in which nAChRs may be exerting their effect. Chapter 2 discusses the actions of nicotinic receptors in the development of escalated cocaine intake. Chapter 3 details site specific loci in the brain where these nAChRs act to modulate increased cocaine intake. Chapter 4 examines potential mechanism by which nAChRs receptors may exert their effect within the VTA. Finally, Chapter 5 will discuss the results of the data reported on within this thesis as it relates to the larger body of knowledge regarding the loss-of-control aspect of drug consumption.

CHAPTER 2

The nicotinic acetylcholine receptor antagonist mecamylamine prevents escalation of cocaine self-administration in rats with extended daily access

Introduction

Drug addiction is a complex, relapsing disorder that is characterized by abnormal behaviors and ideology centered on drug consumption (DSMIV 1994; McLellan et al. 2000). Similar to humans, laboratory animals demonstrate behaviors that are characteristic of pathological drug use under certain experimental conditions (Deroche-Gamonet et al. 2004). One such characteristic behavior is an escalation from moderate to excessive drug self-administration over time (Ahmed and Cador 2006; Ahmed and Koob 1998, 1999; Koob et al. 2004). The escalation effect has been demonstrated in several species (particularly in rats and monkeys) and with several drugs that carry a high potential for abuse in humans including cocaine (Ahmed and Koob 1998; Paterson and Markou 2003), methamphetamine (Kitamura et al. 2006), heroin, and phencyclidine (Ahmed et al. 2000; Carroll et al. 2005). A key component of the procedure needed for rats to show escalation of drug intake appears to be extended daily access to drug for self-administration. When rats are allowed to self-administer cocaine for 6 h per day, they show a progressive increase in drug consumption across sessions, an effect not observed in rats with shorter (1 h) daily self-administration. Moreover, the preponderance of the increase in drug consumption during longer access sessions occurs in the beginning of the drug availability period (Ahmed and Koob 1998). The neural substrates that underlie the

transition from moderate to high intake are not known. One candidate system is the mesolimbic dopamine (DA) pathway, which consists of DA neurons in the ventral tegmental area (VTA) that send projections to the nucleus accumbens (NAc; Nauta et al. 1978). The majority of drugs with high abuse potential in humans appear to share a common mechanism of action of increasing the amount of DA in the NAc (Di Chiara et al. 2004; Koob 2000; Salamone and Correa 2002; Wise and Rompre, 1989). In addition to DA, however, many neurotransmitters may play a direct or modulatory role in mediating reward and addiction including acetylcholine (ACh), serotonin, norepinephrine, glutamate and γ-aminobutyric acid (for review see Bardo 1998). All of these systems have inputs into the mesolimbic system, and the cholinergic neurons have a particularly intimate relationship with both the DA cell bodies and their associated terminal fields. In the cell body region of the VTA cholinergic axons from the pedunculopontine tegmental nucleus PPTg and the LDTg act in a regulatory fashion to modulate DA cell firing (Ikemoto et al. 1998; Picciotto and Corrigall, 2002; Pidoplichko et al. 2004; Yeomans and Baptista, 1997). Nicotine, like cocaine, has been shown to be an effective reinforcing stimulus for maintaining self-administration behavior in rodents (Corrigall and Coen 1989; Dadmarz and Vogel 2003; Donny et al. 2003; Kenny and Markou 2006; Liu et al. 2006), and many of its rewarding properties are mediated via the DA system (Berridge and Robinson 1998; Corrigall et al. 1994; Di Chiara and Imperato 1988; Haile et al. 2006). However, a growing body of research indicates that cocaine and nicotine, acting via the dopaminergic and cholinergic systems, respectively, may interact at both the cellular and systems level to modulate the reward process. Desai and Terry (2003) have demonstrated that nicotine can fully substitute for cocaine in discriminative

tasks in mice (Desai and Terry 2003). Bechtholt and Mark (2002) demonstrated that rats repeatedly treated with systemic nicotine increase cocaine-seeking behavior. Acute doses of nicotine have been reported to increase cravings for cocaine in human addicts (Reid et al. 1998), a finding that may be a function of the fact that nicotine and cocaine are often coabused drugs and that many conditioned factors contribute to the self-administration of these drugs (Caggiula et al. 2002; Henningfield et al. 1990).

Self-administration in animals is an important model of human drug-seeking behavior. In several studies, the release of ACh in the NAc and striatum has been linked to cocaine-seeking behavior (Berlanga et al. 2003; Mark et al. 1999). These data suggest that ACh may play a role in controlling cocaine self-administration (for review, see Smith et al. 2004), but the specific role of nicotinic cholinergic receptors in regulating the amount of drug an animal self-administers is presently unknown. In this experiment, we examined the effect of the nAChR antagonist mecamylamine (MEC) on cocaine self-administration. The objective was to determine the role of MEC-sensitive nAChRs in the development and/ or expression of escalated cocaine intake.

Methods

Subjects

Male Sprague-Dawley rats weighing 300 g at the beginning of operant conditioning were obtained from Charles River Laboratory (Willmington, MA). Upon arrival from the vendor rats were allowed at least 5 days to acclimate to their new surroundings before operant training began (see below). The animals were housed in clear plastic cages

(28×28×18 cm) covered with filter tops in a temperature-controlled environment (22°C) on a 12-h light/dark cycle (lights on at 06:00). The experiments began 2 h into the light cycle. Initially, the rats were pair-housed until the time of surgery, following which all the animals were individually housed. Rodent chow food (LabDiet; Richmond, IN) and water were available ad libitum in the home cage. Weights were collected and recorded every other day. Animal experimental procedures complied with the guidelines set forth in the "Guidelines for the Care and Use of Mammals in Neuroscience Research" (National Research Council of the National Academies 2003) and were approved by the Oregon Health & Science University's Institutional Animal Care and Use Committee.

Operant conditioning

The animals were trained in standard operant conditioning chambers (30×24×29 cm) housed inside of light- and sound-attenuating boxes (Med Associates, St Albans, VT). Each box was equipped with a dual lever system: a retractable active lever and a stationary inactive lever. Sessions began immediately after placement of the animal into the chamber. Illumination of the house light as well as the extension of the active lever indicated the initiation of a session. The rats learned to lever press for 45 mg food pellets (BioServ; Frenchtown, NJ) within 3– 4 days without the need for food deprivation. This is advantageous given that food-deprivation may alter behavior and associated underlying neural substrates (Bello et al. 2003; Pothos et al. 1995). Initially, the rats trained under a fixed ratio (FR) 1 schedule of reinforcement, in which each bar press resulted in the delivery of a single food pellet. A one-second time out was initiated following the delivery of the pellet and was signaled by the illumination of a stimulus light situated

above the active lever. When an animal was able to work for and receive 100 pellets in a 60 min session for two consecutive days the schedule of reinforcement increased to an FR3 schedule under the same contingencies. Once the rats had reached stable responding on this schedule, they were implanted with intravenous catheters.

Drugs

Cocaine HCl, supplied by the National Institute on Drug Abuse drug supply program (RTI International, Research Triangle Park, NC), was dissolved in physiological saline (0.9%) and pH adjusted to 7.0 with 1M NaOH. Mecamylamine was obtained from Sigma (St. Louis, MO).

Surgery

The animals were anesthetized with 0.15 cc of an anesthetic mixture comprised of 55.5 mg/cc ketamine, 5.5 mg/cc xylazine and 1.1 mg/cc acepromazine given IP and supplemented with ketamine (40 mg/kg, IP) as needed. Catheter construction was based on the method described in detail by Caine et al. (Caine et al. 1993). Briefly, catheters were made of micro-renathane tubing (0.25 mm OD× 0.12 mm ID; Braintree Scientific, Braintree MA) fitted over an external L-shaped stainless steel tube inside a threaded Teflon pedestal (Plastics One, Roanoke, VA). The pedestal was attached to a 15-mm diameter circle of polypropylene mesh (250 μm thick; Small Parts, Miami Lakes, FL) with cranioplastic cement. The tip of the catheter was tunneled 27 mm into the right or left jugular vein, just inferior to the intersection of the subclavian and external jugular veins. The distal end of the tubing was threaded subcutaneously to exit between the

scapulae. The animals were also fitted with an aluminum head shield that was cemented in place with cranioplastic cement and anchored to the skull with three 5-mm long stainless steel screws (size 00–80; Small Parts). Head shields were used as an attachment point for a tether connecting the rat to the fluid swivel. This system allowed animals to move freely about the cage while keeping tubing attached to the catheter out of reach. Catheters were flushed with a heparinized saline (70 u/cc) solution in the morning and Timentin antibiotic solution in the evening to prevent blood clotting and infection. If catheters exhibited a resistance to flow or leakage around the base during the heparin or Timentin flush or if rats scratched vigorously during the flush, then catheters were tested for patency. Catheters were also tested if lever pressing for cocaine dropped by more than 50% for two consecutive sessions. Catheter patency was tested with 0.05 ml of 500mg/ml the fast acting barbiturate, Brevital (methohexital sodium, Monarch Pharmaceuticals, Bristol, TN) injected directly into the catheter. Catheters were deemed to be intact and functional if the rat exhibited loss of muscle tone within 5 s of Brevital injection.

Self-administration

After at least 5 days of recovery from surgery, the rats were placed in operant conditioning chambers. A counterbalanced tether was attached to the head shield via alligator clip and an infusion line was attached to the IV catheter. The infusion line was connected to a 20 cc syringe filled with a cocaine solution and mounted in a syringe pump (Med Associates; Lafayette, IN) located on top of the sound-attenuating chamber. Lever presses on an FR3 reinforcement schedule resulted in the delivery of a 4-s infusion of cocaine solution (0.75 mg/kg per 120 μl infusion). Once cocaine acquisition sessions

began, rats were weighed daily, and the cocaine stock solution was adjusted for the weight of the animal to deliver a dose of 0.75 mg/kg. Coincident with the cocaine infusion, a 20-s timeout period was initiated, during which the active lever was retracted and the stimulus light above the lever illuminated. Timeout periods were necessary to prevent death from overdose of cocaine. Both active and inactive lever presses were recorded. The animals had twice-daily 1 h access sessions to cocaine (once in the morning and once in the afternoon) for 2 days to allow acquisition of drug self-administration behavior. After the acquisition period, single-daily 1 h sessions proceeded until responding reached a stability criterion of ±15% infusions for three consecutive days with a minimum of at least five infusions per hour.

Administration of mecamylamine

After stabilization on the 1 h daily access schedule rats were switched to 6 h per day access to cocaine for self-administration for the remainder of the experiment. On the first day of 6 h access to cocaine, rats were randomly assigned to one of three MEC treatment groups that received either 0, 7 μg per infusion or 70 μg per infusion of MEC intravenously and concurrently with each self-administered cocaine infusion by dissolving the MEC into the cocaine solution. To determine if repeated exposure to MEC had an effect on cocaine self-administration that was independent of extended drug access, a fourth group of rats remained on the limited, 1-h daily cocaine access schedule and received the highest concentration of MEC (70 μg per infusion) under the same protocol described above. There are limited data in the literature on doses of MEC administered intravenously. Therefore, we chose these doses based on the calculation

that, if rats self-administered an average of six infusions an hour, they would receive approximately 0.1 mg/kg MEC in the 7 μg group, up to 1.0 mg/kg of MEC in the 70 μg group. Pilot studies in our lab determined that this method of MEC administration did not cause loss of muscle tone or dystonia. Furthermore, food consumption was not interrupted either, as all animals continued to put on weight. MEC was added to the cocaine solution for five consecutive days of 6 h daily access for self-administration. This method of MEC delivery ensured that a constant amount of MEC, which has a half-life of 1.2 h (Young et al. 2001), was present throughout the course of the 6 h drug self-administration session. The nature of the escalation phenomenon is such that continuous long access to the drug of abuse is required. However, it remains unclear what optimal length of time is needed to induce escalated drug intake. Therefore, it was necessary to keep MEC active throughout the entire 6-h session to prevent possible confounding escalation effects that may have developed once MEC had been metabolized. Before beginning the daily experimental session on the sixth day of 6 h access MEC was removed from the cocaine solution and animals were allowed to self-administer an unadulterated cocaine solution for 6 h per day.

Statistics

The number of active and inactive lever presses per hour, cocaine infusions and session time were recorded daily. A mixed-factor analysis of variance (ANOVA) compared responding between LgA mecamylamine treated groups (0, 7, 70 μg MEC) across days (MEC x Time). To determine if rats showed an escalation in cocaine self-administration with extended-access, a one-way ANOVA was conducted on the number of self-

administered cocaine infusions in the first hour of LgA. A second one-way ANOVA was performed on each individual group's total 6 h cocaine consumption across days.

Results

Most rats acquired stable responding for cocaine within 10 days of initial exposure to the drug. At least five infusions per hour in 1-h sessions with less then 15% variability over three consecutive days was required for animals to meet cocaine self-administration acquisition criteria. The first three data points shown in Fig. 2.1 represent baseline responding during 1 h cocaine access sessions. The remaining data points show the number of cocaine infusions in the first hour of daily 6 h access sessions for the long-access (LgA) groups of rats, and for the group that had continuous 1 h daily access (short-access; ShA). In Fig. 2.1, data are represented as a percentage of the average 1 h intake in baseline sessions to normalize between subjects variability in daily cocaine responding. The average, absolute numbers of cocaine infusions for each treatment group are presented in Table 1. A mixed-factor ANOVA on the first hour infusions revealed significant main effects of days ($F_{9, 250} = 9.66$, $P < 0.0001$) and drug treatment ($F_{3, 250} = 20.33$, $P<0.0001$) and a days × drug interaction ($F_{27, 250} = 1.69$, $P<0.05$). Bonferroni post-hoc tests revealed significant differences between the control group (0 μg MEC) and the LgA 70 μg MEC group on day 8($P<0.05$), the last day of MEC treatment. The control group (0 μg MEC) had significantly more cocaine infusions compared to continuous 1 h access animals on days 7 ($P<0.05$), 8 ($P<0.001$), 9 ($P<0.01$), and 10 ($P<0.001$). No significant differences were detected between the control group (0 μg MEC) and the 7 μg MEC group. However, the LgA 7 μg MEC group showed significantly more cocaine

infusions compared to the LgA 70 μg MEC access on day 6 ($P<0.05$). Rats in the LgA 7 μg MEC group had statistically higher cocaine intake compared to ShA 70 μg MEC group on days 5 ($P<0.05$), 6 ($P<0.01$), 7 ($P<0.05$), 8 ($P<0.01$), and 10 ($P<0.05$). Animals in the LgA 70 μg MEC group showed significantly higher cocaine intake relative to ShA 70 μg MEC animals only on day 9, the first day of MEC removal ($P<0.01$).

Figure 2.2a–d illustrate statistically significant differences in cocaine self-administration between MEC treatment groups and controls across three experimental time frames: 1 h cocaine access (days 1–3); the first 5 days of 6 h access plus MEC treatment (days 4–8) and the 2 days of 6 h access after MEC treatment (days 9–10). One-way ANOVAs revealed that control (0 MEC; Fig. 2a) and 7-μg MEC (Fig. 2.2b) groups showed significantly higher cocaine intake in the first hour of 6 h access sessions (days 4–8 and 9–10) compared to their respective 1 h access baseline (P's<0.0001). In contrast, animals in the 6-h access 70 μg MEC group showed significantly higher cocaine infusions only in the post-MEC phase (days 9–10; $P<0.0001$; Fig. 2.2c). ShA 70 μg MEC group on days 4–8 did not show any significant change in cocaine intake across phases (Fig. 2.2d).

Figure 2.3a shows the total number of daily cocaine infusions (6 h) in each group over the course of the experiment. In the first 3 days, all groups had 1 h access to cocaine for self-administration followed by 10 days of 6 h daily access for three groups (LgA). The ShA group remained on continuous 1 h daily access to cocaine. A mixed-factor ANOVA was performed on cocaine infusions for LgA groups. Significant main effects of days ($F_{9, 185} = 5.28$, $P<0.0001$) and drug treatment ($F_{2, 185} = 6.18$, $P< 0.001$) were detected, although

no significant days × drug treatment interaction was found. One-way ANOVAs were conducted on the number of cocaine infusions in 6 h access sessions based on the a priori hypotheses that extended access to cocaine would increase intake and MEC treatment would attenuate the escalation. One-way ANOVA followed by Bonferonni post-hoc tests indicated that animals in the LgA 0 μg MEC group showed escalation in cocaine intake ($F_{9, 70} = 3.32$, $P<0.01$) that reached statistical significance on the eighth day of 6 h access (compared to the first day of 6 h access). Animals in the 7 μg MEC group did not significantly increase their total 6 h intake over time. Animals in the LgA 70 μg MEC group significantly increased their 6-h cocaine intake ($F_{9, 53} = 2.77$, $P<0.001$); however, post hoc tests failed to find a day in which cocaine consumption was significantly greater than the first day of 6-h access. There were no differences in inactive lever responding between drug groups across any of the test days ($F_{9, 214} = 1.03$, $P>0.05$), by group ($F_{3, 214} = 2.44$, $P>0.05$) or an interaction of days × drug treatment ($F_{27, 214} = 1.27$, $P> 0.05$; Fig. 2.3b).

Discussion

In this study, we confirmed that male Sprague-Dawley rats increased their hourly self-administration of cocaine when allowed extended access time to self-administer the drug. When access time was increased from 1 to 6 h per day, rats in the control group increased their overall amount of cocaine consumed, as would be expected given the longer time for self-administration. However, animals significantly escalated their total intake by the eighth day of 6 h access and the majority of the escalation was seen in the first hour of the 6 h sessions (Table 1 and Fig. 3). This finding was consistent with the results of

previous work that has shown an escalation in cocaine self-administration using an extended access protocol in Wistar (Ahmed and Cador 2006; Ahmed and Koob 1998, 1999; Paterson and Markou 2003) and Sprague-Dawley rats (Ben-Shahar et al. 2004; Mantsch et al. 2001) as well in nonhuman primates using oral phencyclidine (Carroll et al. 2005). When MEC (70 μg/infusion) was added to the cocaine solution that was self-administered on the 6 h schedule, the rats did not show an escalation of cocaine intake. It is noteworthy that co-administration of MEC (70 μg) did not eliminate cocaine self-administration completely in this group, nor did it have an effect on self-administration in rats that were maintained on a 1 h access schedule throughout the study. Based on these findings, we concluded that antagonism of MEC-sensitive nAChRs blocked the (currently undetermined) process that underlies escalation of drug intake, without altering basal drug intake. When MEC was removed, rats that had previously experienced the 70 μg dose of MEC showed a gradual, daily increase in cocaine intake in 6 h sessions (Fig. 3). The pattern of escalation was remarkably similar to that exhibited by control animals that never received MEC, which suggests that the presence of MEC had prevented the development of escalation. An interesting finding was that when cocaine intake was measured in the first hour of the post-MEC sessions, rats that previously received the 70 μg dose of MEC demonstrated a strong increase in cocaine intake immediately after MEC was removed (Fig. 1). The amount of cocaine these rats self-administered in the first hour was identical to the amount control rats received, which initially suggested that the presence of MEC had prevented expression of escalation. However, it was apparent that the increase in self-administration was transient since cocaine intake in the remainder of the 6 h sessions (i.e., hours 2–6) was below the levels of control rats. Although a

compensatory response or reaction to the novelty of MEC removal may have contributed to the ephemeral increase in cocaine self-administration, the exact cause remains unknown.

Evidence presented in this study provides evidence for implicating MEC-sensitive nAChR in cocaine reward under LgA conditions, but not ShA cocaine reward. Our data indicates blockade of the nAChRs prevents an animal from escalating their drug intake despite conditions that would otherwise promote escalation of cocaine consumption. Rats treated with daily systemic nicotine show an increase in motivation to obtain cocaine (Bechtholt & Mark, 2002). Similarly, clinicians have observed that the more cigarettes a patient smokes while in drug treatment for cocaine abuse the less likely the success of the treatment (Patkar et al, 2003). Furthermore, our results are consistent with other work indicating the importance of nAChRs in determining the behavioral response to psychostimulants. Schoffelmeer and colleagues reported that MEC treatment blocked the induction of behavioral sensitization to cocaine and amphetamine. Co-administration of MEC also prevented the development of a sensitized dopamine efflux from NAc slices in rats treated with nicotine, cocaine or amphetamine (Schoffelmeer et al. 2002). Nicotine and cocaine show cross-sensitization of locomotor activation and this effect is greater in male than female rats and also in periadolescents compared to adults (Collins and Izenwasser 2004). Kitabatake et al, (2003) provided further support for the role of cholinergic involvement in the reward pathway. They have shown that the ablation of cholinergic cells within the striatum impairs reward-related learning. Others, such as Bechtholt and Mark (2002), have demonstrated that rats repeatedly treated with nicotine

increased their breaking point to self-administer cocaine on a progressive ratio schedule. Taken together, these data strongly support the hypothesis that cholinergic systems can affect self-administration behavior by altering cocaine reward.

Development and expression of escalation in drug intake may be related to the development of behavioral sensitization, where higher levels of activation are engendered by repeated exposure to drug (Robinson and Berridge 1993). Alternatively, increased intake during extended drug access may be a function of an increase in tolerance (Zernig et al, 2004). Cholinergic systems are integral components of the brain circuits that control neuroadaptation to repeated drug exposure, so the possibility that MEC prevented escalation in cocaine self-administration by altering sensitization is worth noting. Repeated activation of nAChRs by nicotine produces locomotor sensitization (Miller et al, 2001) and causes a sensitized neurochemical response in extracellular dopamine in the nucleus accumbens (Balfour et al, 1998). Moreover, nicotine sensitization is blocked by MEC, but not by the α7 nAChR antagonist α-bungarotoxin (Kempsill and Pratt, 2000), which suggests that nAChRs with a heteropentemeric conformation (i.e., those containing both α and β subunits) are involved. In the present study, we did not determine if MEC had an effect on sensitization to cocaine, but it is unlikely that an alteration in sensitization was the underlying cause of the ability of the 70 μg dose of MEC to block escalation. This proposition is based on the results of Ahmed and Cador (2006) found no correlation between the level of sensitization to cocaine and amount of cocaine self-administration by rats in an extended-access protocol similar to the one used in the present study (Ahmed and Cador 2006).

In addition to nAChRs, the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors plays an important role in the behavioral actions of psychostimulants. Sensitization of locomotor activity brought on by repeated treatment with stimulants is sensitive to blockade by antagonists of the NMDA receptor (Carlezon and Nestler 2002; Karler et al. 1994; Schenk et al. 1993), and this is also the case with sensitization induced by repeated stimulation of nAChRs with nicotine (Shoaib and Stolerman 1992). Therefore, NMDA receptors may be involved in the regulation of cocaine self-administration and may have been affected by treatment with MEC. In vitro assays have shown that MEC has noncompetitive antagonist properties at NMDA receptors (O'Dell and Christensen 1988), although its affinity is relatively low compared to other NMDA antagonists, such as MK-801 (Wong et al. 1986). As used in the present study, however, it is difficult to compare the potential activity of MEC at NMDA receptors to in vitro assays. In this experiment, rats received MEC intravenously and in small increments (a maximum 70 μg/ infusion) over a 6 h period. To our knowledge, this treatment regimen has not been used to test MEC activity at NMDA receptors so it is not possible to completely exclude a role for these receptors in the development of escalation of cocaine self-administration. Furthermore, although we did not test activation or inhibition of the muscarinic acetylcholine receptors others have found the MEC does not activate nor inhibit the mAChRs (De Sarno et al, 2003). There are limitations to this study, however. The method of MEC delivery was unique and yet necessary. It is unknown what the critical length of time is for inducing escalated consumption of cocaine. Because MEC has a half-life of approximately 90 min we employed this route of MEC administration to ensure a constant presence of the antagonist throughout the entire daily 6 hr experiment.

However, we did not directly test the implications this route of MEC delivery may have had on learning the contingencies of self-administration. Future studies should employ a single sub-cutaneous injection of MEC. An advantage to this method would be better control over the dose of MEC each animal receives. However, it may be the case that a single bolus injection of MEC would offer a completely different cocaine self-administration profile than the data present here. Also, future work would be well served to include other nicotinic antagonists in order to assert with greater confidence that the blunted escalation effect was indeed due to nAChR antagonism. In this study we did not include animals self-administering cocaine on a ShA schedule and receiving 0μg MEC over an equivalent time course. Although this is the baseline condition of all animals in the experiment we did not maintain animals on ShA with no MEC. Other researchers have previously shown that animal self-administering cocaine on 1 hr daily time schedules do not significantly increase their drug consumption over time (Ahmed and Koob, 1998; 1999).

The extent of cholinergic involvement in the development and maintenance of psychostimulant-rewarded behaviors is presently unclear. Berlanga et al. (2003) suggested that cholinergic interneurons in the NAc are activated during the initial exposure to cocaine (Berlanga et al. 2003). In our studies, we found that blockade nAChRs prevented development of escalation of cocaine intake during periods of extended access; although we are not able to determine which brain site(s) were involved. Future experiments should address selective antagonism of nAChR in the NAc and the

VTA during the initial 5-day period of extended access to site specifically determine where MEC may be having an effect.

The findings of this study support the hypothesis that nAChRs play an integral role in controlling the transition to higher cocaine self-administration with prolonged access to the drug. Importantly, blockade of nAChRs did not eliminate cocaine self-administration but prevented the increase in cocaine consumption under conditions that normally promote an escalation of cocaine intake. This provides an opportunity for future investigations to determine the site of action of nAChRs in blocking the expression of escalation in cocaine self-administration.

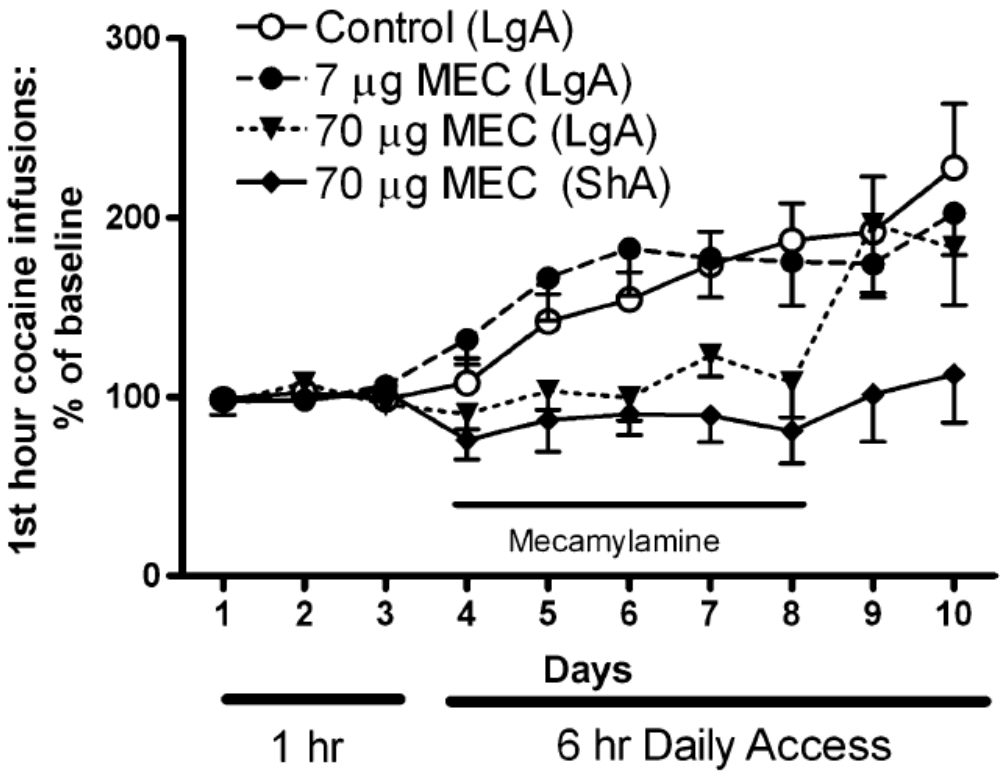

Control (LgA)
7 µg MEC (LgA)
70 µg MEC (LgA)
70 µg MEC (ShA)
1st hour cocaine infusions:
% of baseline
300
200
100
0
Mecamylamine
1
2
3
4
5
6
7
8
9
10
Days
1 hr
6 hr Daily Access

Figure 2.1

Fig. 2.1: Prevention of escalation of cocaine intake by co-administration of intravenous MEC. The number of cocaine infusions in daily 1 h sessions (days 1–3) and the first hour of 6 h self-administration sessions (days 4–10) were recorded and are represented as a percentage of the average of the last 3 days of cocaine intake on the 1 h access schedule. On days 4–8 animals received either 0 μg (open circles, solid line; n=9), 7 μg (closed circles, short dashed line; n=7), 70 μg (closed triangles, dotted line; n=7) or 70 μg–1 h only (diamond, solid line; n=7) per infusion of MEC dissolved into their cocaine solution for self-administration (indicated by the solid bar above the abscissa). For days 9–10 MEC was removed from the experiment and all animals had access to cocaine only. See text for significant differences between groups. Long-access (LgA): animals had 6 h access for self-administration on days 4–10. The short-access (ShA) group remained on 1-h daily access for cocaine self-administration throughout the experiment. Bars at the bottom of the figure indicate daily access time for cocaine self- administration in LgA groups.

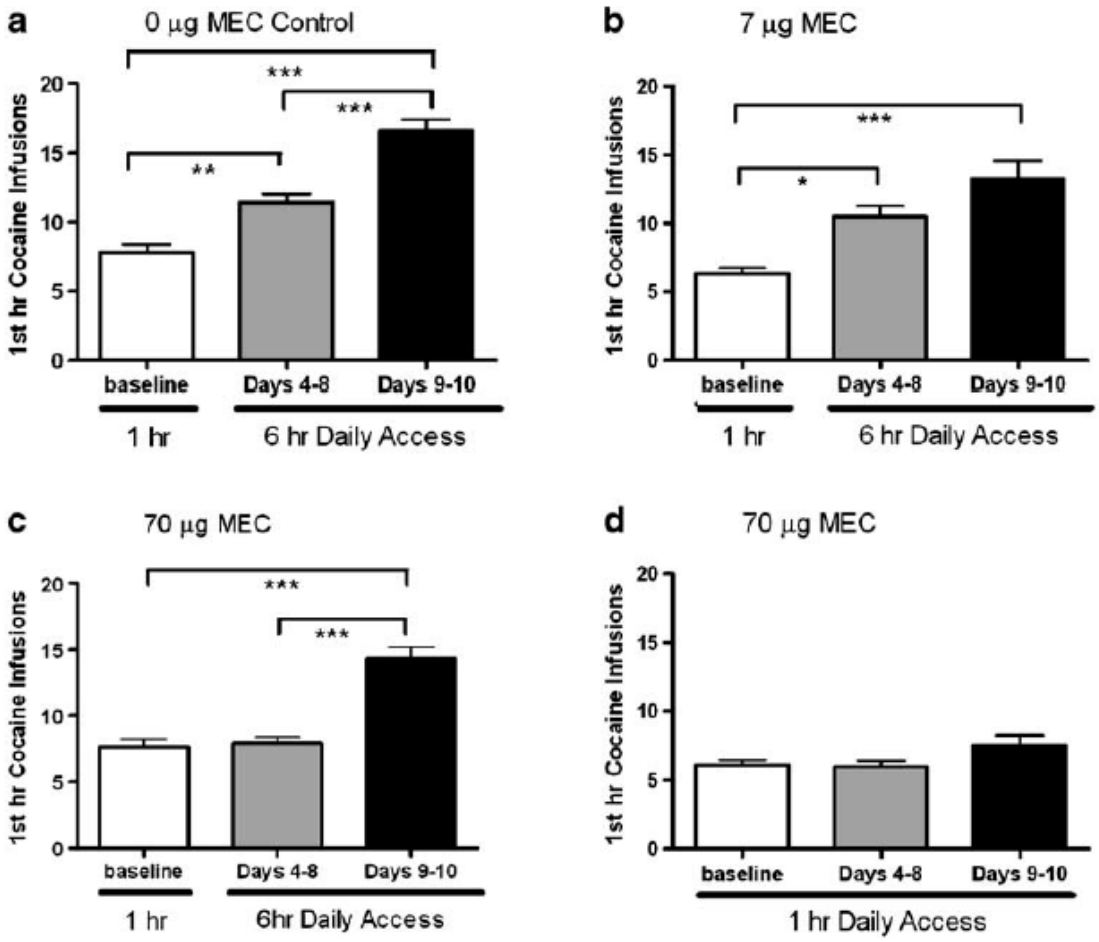

a
0 µg MEC Control
1st hr Cocaine Infusions
baseline
Days 4-8
Days 9-10
1 hr
6 hr Daily Access
b
7 µg MEC
1st hr Cocaine Infusions
baseline
Days 4-8
Days 9-10
1 hr
6 hr Daily Access
c
70 µg MEC
1st hr Cocaine Infusions
baseline
Days 4-8
Days 9-10
1 hr
6hr Daily Access
d
70 µg MEC
1st hr Cocaine Infusions
baseline
Days 4-8
Days 9-10
1 hr Daily Access

Figure 2.2

Fig. 2.2 a–d, Animals moved from 1 to 6 h per day access to cocaine increased their hourly intake over the course of 7 days (a–c). Data represent first hour cocaine infusions. Cocaine infusions were averaged across three experimental phases: Baseline= three-day average of 1 h per day access to cocaine (white bars); days 4–8=average cocaine intake in the first five days of 6 h per day access to cocaine (gray bars); days 9–10=average of the 2 days after MEC was removed from infusions in the MEC-treated groups (black bars; b–d). Asterisks represent significant difference between connected phases: * = ($P<0.05$); ** = ($P<0.001$; and *** = ($P<0.0001$).

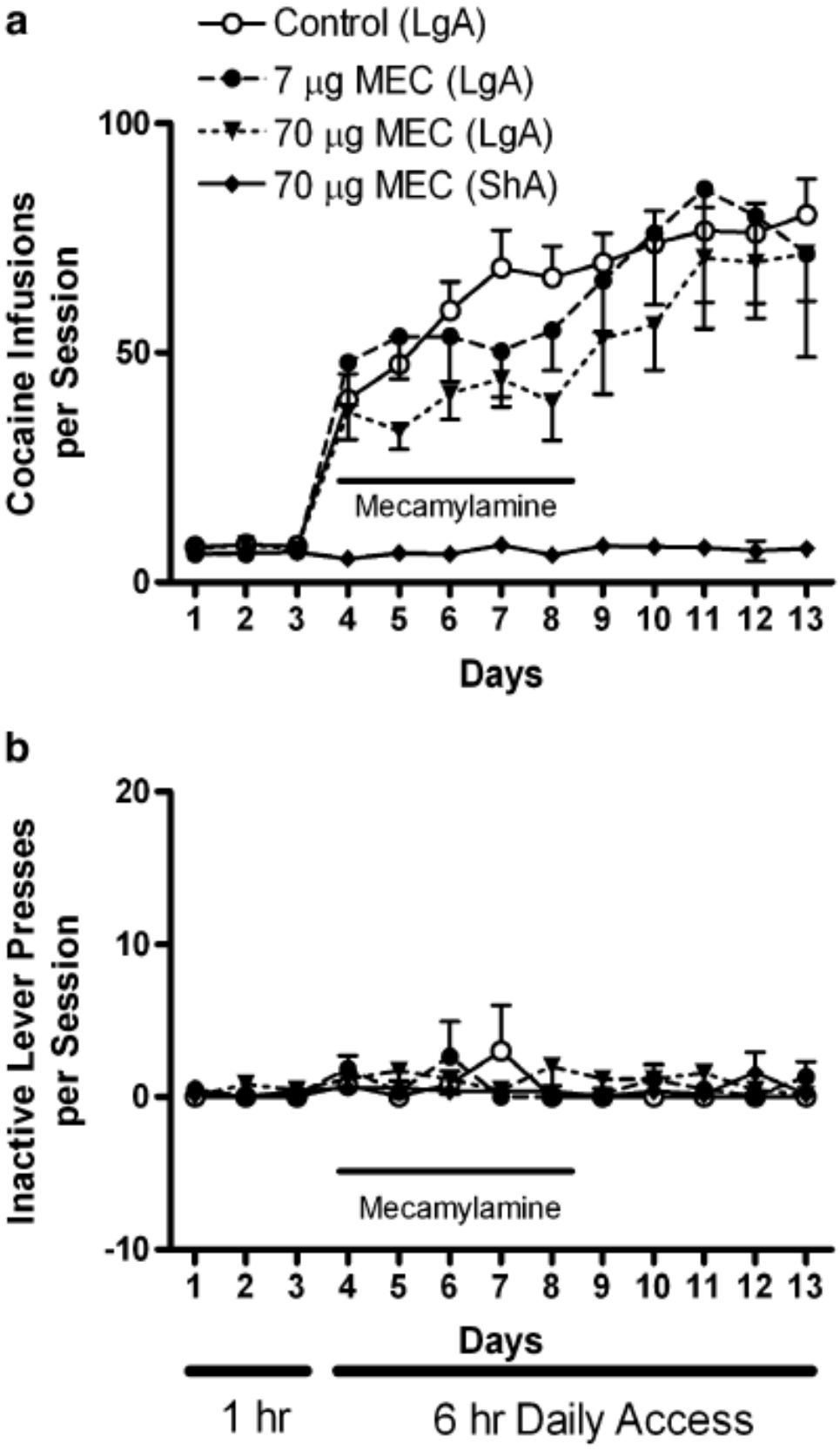

a
Control (LgA)
7 μg MEC (LgA)
70 μg MEC (LgA)
70 μg MEC (ShA)
Cocaine Infusions per Session
100
50
0
Mecamylamine
1 2 3 4 5 6 7 8 9 10 11 12 13
Days
b
Inactive Lever Presses per Session
20
10
0
-10
Mecamylamine
1 2 3 4 5 6 7 8 9 10 11 12 13
Days
1 hr
6 hr Daily Access

Fig.2.3: A) The total number of cocaine infusions plotted across 13 days. On days 1–3 animals had 1 h daily access to cocaine. Days 4–13 show total cocaine infusions during 6 h access sessions (LgA groups) and continuous 1 h access from the ShA group. On days 4–8 animals received either 0 μg (open circles, solid line; n=9), 7 μg (closed circles, dashed line; n=7), 70 μg (closed triangles, dotted line; n=7) or 70 μg during continuous 1 h access (filled diamonds, solid line; n = 7) of MEC dissolved into their cocaine solution for self-administration (area highlighted with Mecamylamine label and line). For days 9–13 MEC was removed and all animals had access to cocaine alone. B) Responding on inactive lever across all sessions. There were no statistically significant differences between the groups. Bars at the bottom of the figure indicate daily access time for cocaine self-administration in LgA groups.

Table 1 Mean daily values of cocaine self-administration for each treatment group

	Days									
Group: Mean (±SEM)	1	2	3	4	5	6	7	8	9	10
0 µg MEC (LgA)	7.6 (2.4)	8.2 (3.4)	7.8 (3.5)	8.5 (4.3)	10.6 (3.9)	11.2 (2.2)	13 (4.2)	13.7 (3.3)	14.1 (4.9)	16.2 (4.2)
7 µg MEC (LgA)	6.1(2.0)	6.1(1.7)	6.7(2.1)	8.3(3.5)	10.6(5.6)	11.4(4.9)	11.4(5.4)	10.7(4.6)	11(4.7)	13(6.3)
70 µg MEC (LgA)	7.1(2.8)	8.1(4.1)	7.1(3.2)	6.9(3.9)	7.0(1.0)	7.3(2.0)	9.3(2.8)	7.0(4.0)	13.3(5.1)	11.9(4.8)
70 µg MEC (ShA)	6.0(1.7)	5.8(1.5)	6.6(1.9)	4.6(1.5)	5.3(2.9)	5.6(1.8)	5.6(2.1)	5.5(3.4)	7.0(4.7)	7.3(4.0)

Table 2.1: Long-access groups (LgA) received 6 h daily access to cocaine beginning on day 4. Numbers for LgA groups represent cocaine infusions in the first hour of 6-h access sessions. The short-access group (ShA) was maintained on 1 h per day access to cocaine throughout the experiment.

CHAPTER 3

Decreased high-level cocaine self-administration by antagonism of nicotinic acetylcholine receptors in the ventral tegmental area but not the nucleus accumbens of rats

Introduction

Addiction to drugs of abuse results in a costly drain on scarce societal resources and often results in untold damage to the lives of individuals and their families (Cartwright, 2008; Office of National Drug Policy, 2001). In order to more effectively treat drug addiction it is necessary to more fully understand how repeated, high-level drug intake affects neurological function.

Substance related disorders include both physiological and psychological dependence and the treatment for addiction is likely to be equally complex (DSMIV, 1994). Due to the multifaceted nature of substance dependence, it has become increasingly useful to study, in a reductionist fashion, each characteristic independently. One prominent characteristic of addiction is the significant increase in drug consumption that occurs during the transition from casual drug use to habitual drug abuse and dependence. In the laboratory, rats that are given long periods of daily access to drugs for self-administration show a similar transition from moderate to escalated drug intake compared to animals that have limited daily access. The extended drug access paradigm may therefore be a useful model to study the neurobiological substrates of escalating drug use.

Escalation of drug intake was first described by Ahmed and Koob (1998), who found that rats previously trained to self-administer cocaine in daily 1hr sessions significantly increased their rate of drug intake when they were allowed six-hour of daily access to cocaine for self-administration (Ahmed & Koob, 1998). Although there remains some debate over the experimental conditions that produce escalation in drug intake (for review, see Zernig et al, 2007), the phenomenon has been replicated in several laboratories and has been demonstrated for several drugs in addition to cocaine (Ben-Shahar et al, 2006; Carroll et al, 2005; Ferrario et al, 2005; Paterson & Markou, 2003; Mantsch et al, 2004; Kitamura et al, 2006; Knackstedt & Kalivas, 2007; Hansen & Mark, 2007).

Nicotine and cocaine are commonly co-abused drugs in humans (Wiseman et al, 2005; Wiseman, 1998). This relationship may have to do with how each drug affects the neurobiology of the reward pathways in the brain. Indirect evidence suggests that activation of nicotinic acetylcholine receptors (nAChRs) modulates both the psychological and physiological components of cocaine reward. In the clinic, nicotine can induce cravings for cocaine in humans (Reid et al, 1998) and cue-induced cravings for cocaine can be attenuated by antagonism of nAChRs with mecamylamine (MEC; Reid et al, 1999). In animal studies activation of nicotinic receptors increases cocaine-seeking behavior (Bechtholt & Mark, 2002) whereas antagonism of nAChRs preferentially decreases self-administration of cocaine but not food (Levin et al, 2000). Place preference studies also indicate that nicotinic receptors may play an important role in the modulation of cocaine reward (Zachariou et al, 2001).

The meso-accumbens dopamine (DA) pathway is the primary reward circuit in the brain and is potently activated by drugs that have high abuse potential in humans, albeit through several different mechanisms (Wise, 1996; Koob, 1998). Cocaine acts as an indirect agonist for DA receptors, primarily by blocking the dopamine transporter's (DAT) ability to eliminate dopamine from the synaptic cleft. In contrast, a broad spectrum of evidence demonstrates that nicotine, acting at nAChRs on dopaminergic cell bodies within the VTA and GABAeric cells within the NAc, increases the DA cell excitability and transmission (McKay et al, 2007; Nisell, 1994; Pidoplichko, 1997; Dani et al, 2003; Fagen et al, 2007; Zanetti et al, 2007). Predictably, behavioral studies have demonstrated that both cocaine and nicotine are highly rewarding drugs, which can maintain self-administration behavior alone or in conjunction with each other (Ikemoto et al, 2006; Epping-Jordan et al, 1999; Alderson et al, 2006).

Previous work in our lab has demonstrated that systemic antagonism of nAChRs with MEC prevents an escalation of cocaine intake when rats are transitioned from 1 hr to 6 hr per day drug access (Hansen & Mark, 2007). The goal of the present study was to determine if MEC could reduce a previous established, high-level of cocaine self-administration using site-specific injections within the mesoaccumbens reward pathway. Based upon the anatomical location of nAChRs within the NAc and VTA (Alcantara et al, 2003; Keath, 2007; see pages 23 and 29 of this thesis), we hypothesized that antagonism of nAChRs in these areas would decrease the amount of cocaine consumed by rats on a 6 hr daily-access schedule.

Methods

Animals

Male Sprague-Dawley rats were acquired from Charles River Laboratory (Willmington, MA) and upon arrival were given five days to acclimate to their new environment. Animals had an initial weight of 300g on average and were initially housed in pairs until surgery, at which point they were individually housed. Home cages were made of hanging clear Plexiglas cages (28x28x18 cm) covered with filter tops and housed in a temperature-controlled environment (22°C). The animal housing room was set to a 12-hour light/dark schedule (lights on at 06:00). Rodent chow (LabDiet; Richmond, IN) and water were available *ad libitum* throughout the experiment. All experimental procedures complied with the guidelines set forth in the "Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research" (National Research Council of the National Academies, 2003) and were approved by the Oregon Health & Science University's Institutional Animal Care and Use Committee.

Operant Conditioning

Animals were trained in operant conditioning chambers (30 x 24 x 29cm) which were housed within sound and light attenuating boxes (Med Associates Inc.; St Albans, VT). Each operant chamber was equipped with two separate levers: a retracting active lever and a stationary inactive lever. Sessions began immediately after the rat was placed in the operant chamber. The illumination of a house light and the extension of the active lever bar signaled the beginning of each daily session. Pressing on the active lever resulted in a retraction of the lever and the delivery of a 45 mg food pellet (BioServ;

Frenchtown, NJ) along with the illumination of a stimulus light 2 cm above the lever. Initially animals were trained on a fixed-ratio 1 (FR1) schedule of reinforcement (one active lever press resulted in the delivery of a single food pellet). Once animals were able to earn 100 pellets in a 1-hr session the schedule of reinforcement was raised to FR3 for three days and finally FR6 for approximately three days until the time of intravenous catheter and microinjector guide shaft surgery (detailed below).

Drugs

Cocaine HCl was obtained from the National Institute on Drug Abuse drug supply program (RTI International, Research Triangle Park, NC), and was dissolved in physiological saline (0.9%) and adjusted to a pH of 7.0 with 1M NaOH. Mecamylamine HCl was obtained from Sigma (St. Louis, MO).

Catheter Surgery

Animals were anesthetized for surgery with a 1 cc/kg IP injection of an anesthetic mixture comprised of ketamine (55.5 mg/cc), xylazine (5.5 mg/cc) and Acepromazine (1.1 mg/cc) and supplemented with IP injections of ketamine as needed. Catheter construction was based upon methods described in detail by Caine and colleagues {Caine et al, 1993) and adapted for use in our lab (Hansen & Mark 2007). One incision was made on the ventral surface of the neck, 1 cm from the midline between the jaw and the shoulder blade. A second incision was made on the back between the scapulae. Catheter pedestals were inserted into the dorsal incision. The tip of the catheter tubing was threaded subcutaneously over the shoulder and exited from the ventral incision. The

jugular vein was isolated and an incision was made just distal to the juncture of the subclavian and external jugular vein branches. The catheter tip was inserted 27 mm into the vein and tied off at the entrance of the incision with 3-0 suture.

Intracranial guide shafts

After completing the jugular implant, animals were stereotaxically implanted with bilateral guide shafts (23 ga thin-walled stainless steel tubing, 10 mm long; Small Parts Inc.) that terminated 2.5 mm above either the NAc (A: 1.2 mm, L: +/- 1.0 mm, V: -4.0 mm) or the VTA (A: -6.0mm, L +/- 0.6mm, V: -4.3mm), relative to bregma (Paxinos & Watson, 1998). Rats were fitted with an aluminum head-shield anterior to the guide shafts and were cemented in place with cranioplastic cement anchored to the skull with 5 mm long stainless steel screws (size 00-80; Small Parts Inc.). Head shields protected guide shafts and injectors and served as an attachment point for a tether connecting the rat to the fluid swivel. Guide shafts were kept patent using 26 ga wire stylets. Following surgery rats were treated with 0.05cc sub-cutaneous of the analgesic Rimadyl (carprofen; Pfizer Animal Health, New York, NY). Catheters were flushed twice daily with 0.2 ml of a solution containing heparine (70 u/cc) in physiological saline in the morning and evening. For two weeks after surgery catheters were also flushed with 0.2ml Timentin antibiotic (ticarcillin; GlaxoSmithKline, Research Triangle Park, NC). If catheters exhibited a resistance to flow or if an animal's responding for cocaine dropped lower than 50% of baseline average (two consecutive sessions) the catheter was tested for patency by injecting 0.05 ml Brevital (methohexital sodium; Monarch Pharmaceuticals Inc,

Bristol, TN), a fast acting barbiturate, through the catheter. Loss of muscle tone within 5 sec indicated a functional catheter.

Self-administration procedure

Following five days of recovery from surgery animals began cocaine self-administration training in1-hr daily sessions. A counterbalanced tether was attached to the animal's head-shield by way of an alligator clip. An IV infusion line (PE20; Becton Dickinson; Sparks, MD) was attached to the external catheter tip exiting between the scapulae. Infusion lines were connected to a 20 cc syringe filled with cocaine solution that was placed inside of an infusion pump (Med-Associates; Georgia, VA). Pumps were located on top of the sound-attenuating box, which housed the self-administration operant boxes. Active lever presses, on an FR1 schedule of reinforcement, resulted in a 120 μl infusion of cocaine (0.75mg/kg per infusion) over a four sec time period. Immediately following a lever press (resulting in cocaine delivery) the active bar retracted and a 20 second timeout was initiated during which cocaine was not available. A timeout procedure was necessary to prevent stimulant overdosing in drug naive animals. Simultaneous with the retraction of the active lever a stimulus light positioned over the lever was activated. The secondary reinforcer (stimulus light) was used to facilitate the transition from food reward to cocaine reward. Animals routinely acquired cocaine self-administration within ten days of initial exposure. Acquisition criterion was a minimum of five infusions of cocaine with ±15% variability in daily 1-hr sessions for three consecutive days. Once animals had reached the acquisition and stability requirements they were transitioned to 6-hr daily access to self-administer cocaine.

Microinjection of MEC

Animals that exhibited a doubling of drug intake in their first hour of LgA were classified as having escalated their cocaine intake (Ahmed & Koob, 1999; Hansen & Mark, 2007). When escalated cocaine intake stabilized, defined as ±15% variation over three days animals were habituated to the microinjection handling procedure by being gently restrained in a towel wrap with only their heads exposed. During habituation, stylets were removed and replaced and animals were then placed in their chambers. Following the habituation sessions, each animal was given a sham injection which consisted of bilateral microinjectors being lowered into the guide shafts but no drug was delivered. The microinjection protocol was designed using a pseudo latin-square design such that each animal experienced multiple microinjections of different doses of MEC; however, the order of MEC dose differed between animals. Furthermore, not all animals received all doses of MEC due to early termination from the experiment because of catheter failure. Immediately prior to intra-nucleus drug or vehicle administration animals were gently restrained and microinjectors were lowered through the guide shafts to the targeted brain-site. Drug was delivered at a rate of 3.3 nL per second for 30 seconds for a total volume of 0.1μL per side. MEC was dissolved in artificial cerebrospinal fluid (aCSF) consisting of (in mM: 120 NaCl, 4.8 KCl, 2.5 CaCl2, 1.2 MgCl2, 25 NaHCO3, 1.2 KH2PO4, and 10 glucose; the solution was pH adjusted to 7.3). Following injection microinjectors were left in place for an additional 60 seconds to allow for drug diffusion. Injectors were removed and stylets were immediately replaced into the guide shafts and animals were placed into self-administration chambers. The injection procedure took approximately two minutes to complete. Animals always received a minimum of two days between

microinjections. Doses of mecamylamine for microinjection were based upon results of pilot experiments in this laboratory and the results of reported by Nadal and colleagues (1998).

Histology

Rats were euthanized with sodium pentobarbital and perfused with saline followed by 10% formalin in phosphate buffered saline (PBS). Brains were removed and stored for a minimum of 48 hr in 20% sucrose/4% formalin followed by 24 hr immersion in 30% sucrose/PBS before being sliced at –20°C using a Cryostat (50 µm sections). Brain slices were mounted and stained with Thionin Blue in order to identify injector and probe tracks. Data from animals with injector tracks outside of the target area or with unconfirmed injector tracks were excluded from analysis. See figure 3.1 for a diagram of injector placements inside and outside of the target areas.

Statistics

Both active and inactive lever presses as well as hourly cocaine infusions were recorded daily for all animals. To analyze the effect of MEC on cocaine self-administration, a mixed-factor ANOVA compared responses on the active lever between MEC doses across time (MEC x Time) in both the first-hour of six-hour sessions and in a second statistic the total cocaine consumption over six-hour sessions comparing MEC across time. Data were normalized to each animal's baseline, which was defined as the average of two consecutive days immediately prior to the MEC microinjection.

Results

Experiment 1: *Escalation of cocaine self-administration and microinjection of MEC into the NAc.*

Rats in the first experiment acquired stable cocaine self-administration on a daily 1-hr access paradigm followed by transition to daily 6-hr access to self-administer cocaine (n = 22). To compare cocaine self-administration in 1-hr versus 6-hr access sessions, we analyzed the number of infusions rats self-administered in the first hr of all sessions. One-way ANOVA revealed that when rats had 6-hr per day to self-administer cocaine, they significantly increased the number of cocaine infusions in the first hour of daily sessions by the sixth day of extended access ($F_{9, 40} = 6.116$, $P<0.001$; see table 3.1). A one-way ANOVA between MEC groups cocaine consumption on the day of the microinjection was performed on data collected. First hour cocaine consumption was analyzed and in a second one-way ANOVA statistic total 6 h cocaine intake was analyzed. Statistical analysis indicated that no statistical differences exist between groups in either the first hour or the total six-hour drug consumption; $P >0.05$ (figure 3.2A, B).

Experiment 2: *Escalation of cocaine self-administration and microinjection of MEC into the VTA.*

Animals in the second experiment significantly increased their 1st hour cocaine consumption once given access to self-administer cocaine during six-hour daily sessions ($F_{9, 103} = 2.410$, $P<0.05$); Bonferroni *post-hoc* tests indicated animals were consuming significantly more cocaine by the eighth day of extended-access compared to the first day

of extended-access ($P<0.05$; see figure 3.3). A one-way ANOVA was conducted on first hour cocaine intake. A second one-way ANOVA was performed on total six-hour cocaine consumption between MEC groups on the day of MEC microinjection. In the first-hour of long-access a significant difference was detected ($F_{3, 32} = 3.72$; $P < 0.05$). Bonferroni *post-hoc* analysis determined 60μg MEC was significantly different from aCSF microinjection (figure 3.3A). Further analysis indicated that the total six-hour cocaine intake remained statistically significant difference between groups ($F_{3, 31} = 3.6$; $P < 0.05$). Bonferroni *post-hoc* test once again detected a significant difference between the highest doses of MEC tested—60 μg/ side—and cocaine consumption by control aCSF microinjection (figure 3.3B). A mixed-factor ANOVA was performed comparing cocaine consumption. Significant differences were detected within groups ($F_{2, 91} = 6.1$; $P<0.001$) and between groups ($F_{3, 91} = 5.5$; $P <0.001$). However, no significant interaction of treatment phase X MEC dose was detected. Bonferroni *post-hoc* analysis indicated that 60 μg MEC was significantly different from aCSF (figure 3.4A). A second two-way ANOVA was carried out comparing total 6hr cocaine consumption. Significant between-group differences were detected ($F_{3, 87} = 5.3$; $P<0.01$); however, within-group differences were no longer present and no significant interaction of Phase X [MEC] was detected. Bonferroni *post-hoc* tests confirmed that animals with the 60 μg MEC still showed significant depression in cocaine consumption on the day of microinjection as compared to their aCSF counterpart controls (figure 3.4B). No significant differences were detected on inactive lever presses (figure 3.4C).

Data in figure 3.5 represents absolute value of cocaine consumption per hour on days of MEC microinjection. One-way ANOVA between hours in each MEC grouping did not detect any significant differences ($P > 0.05$).

Discussion

The extended-access model of cocaine self-administration is a robust phenomenon that lends itself well to the study of drug dependence in general and the loss of control aspect of drug consumption in particular (Ahmed & Koob, 1998; 1999; Allen et al, 2007; Hansen & Mark, 2007). In these experiments we have shown that Sprague-Dawley rats with an extended period of time in which to self-administer cocaine will significantly increase their drug consumption before reaching a stable but escalated pattern of intake. Furthermore, we have demonstrated that nAChRs within the VTA play an important role in the maintenance of escalated cocaine intake that is observed as a function of extended access. Once animals had achieved stable patterns of cocaine intake following extended-access time to the drug, microinjections of the nicotinic receptor antagonist, mecamylamine, into the VTA, but not the NAc, significantly decreased cocaine self-administration during the initial time period in which MEC was active. However, this effect was not long-lasting nor was it cumulative: the days following microinjection of MEC were not significantly different from days preceding MEC microinjection in the VTA. In sum, this study provides evidence for a modulatory role of nicotinic receptors in the VTA; however not in the NAc, for cocaine self-administration under the escalation paradigm.

In relation to results reported in Chapter two, data presented here are in general agreement, in as much as the administration of MEC in the VTA impacts the amount of cocaine an animal will consume. In our earlier study (see Chapter 2) animals receiving systemic MEC did not increase their cocaine intake while MEC was present over the course of 5 days. In this chapter, however, animals decreased their drug intake from escalated levels down to baseline-like levels in the first hour of their daily 6 h session. The total of intake over 6 h was also significantly depressed for animals receiving the highest dose of MEC. The disparity in method of MEC administration may account for differences in the self-administration profile. The single bolus microinjection of MEC at the beginning of this experiment immediately depressed responding for cocaine. Data analysis on all hours of the experiment (hours 2-6) indicated that cocaine consumption increased gradually over the six-hour period, although no significant differences exist when comparing the within-groups between-hours cocaine consumption (see figure 3.5). In Chapter 2 animals had an 18 hour period without MEC or cocaine, which allowed for both drugs to be metabolized. Thus MEC should have been completely removed from their system by the following day's cocaine self-administration. This would explain the dramatic increase in cocaine consumption in fig 2.1 vs. data presented here which shows only a moderate increase in cocaine self-administration in the first hour of cocaine access (figure 3.5).

The broader literature suggests that nicotinic receptors within the meso-accumbens pathway are relatively important in psychostimulant reward. For example Berlanga and colleagues (2002) have provided evidence of cholinergic interneurons within the NAc

being activated as a function of cocaine's ability to activate the dopaminergic system. Kempsill & Pratt (2000) demonstrated that systemic mecamylamine was sufficient for blocking the motor sensitization effects of the stimulant nicotine. Schoffelmeer further demonstrated the importance of nicotinic receptors and their role in the development of sensitization to cocaine and methamphetamine (Schoffelmeer et al, 2002). Previous work from our lab indicates that systemic blockade of nicotinic receptors prevents the increase in cocaine intake that is observed when rats are allowed a greater time period in which to self-administer cocaine (Hansen & Mark, 2007).

Mecamylamine exerts its antagonistic effects primarily at the $\alpha 2\beta 4$ and $\alpha 4\beta 4$ receptor subtype with >80% efficacy and to a lesser extent at the $\alpha 2\beta 2$, $\alpha 4\beta 2$ and $\alpha 7$ subtypes with roughly 50% efficacy (Chavez-Noriega et al, 1997). It has also been reported that MEC *in vitro* will transiently antagonize rat NMDA receptors (O'Dell and Christensen 1988). Although high doses of systemic administration of mecamylamine can affect blood pressure and subsequently motor activation there is no indication that microinjection of this drug into the brain regions tested had any behavioral motor effects (Champtiaux et al, 2006). In fact, non-contingently administered systemic mecamylamine has been shown to reduce the responding for cocaine but not food under ShA conditions (Levin et al, 2000). Furthermore, Nadal and colleagues (1998) have shown that microinjection of MEC into the NAc, at doses similar to ours, significantly decreased responding for ethanol, but not sucrose treated water, in rats. Data from this report indicates that MEC microinjected into the NAc had no effect on self-administration of the psychostimulant cocaine. Both of these studies provide evidence

that MEC, at the doses reported here, does not significantly retard the animal's motor ability; rather, at the doses we tested MEC appears to affect motivation for drug consumption. However, we did not directly measure the animal's locomotion following microinjection of MEC.

The findings of our data, with regards to the actions of nAChR antagonism in the NAc and VTA are in general agreement with the work of others. Champtiaux and colleagues found that antagonism of nicotinic receptors in the NAc did not block sensitization to systemic cocaine injections; however, nicotinic receptor antagonism with dihydro-beta-erythroidine (DHβE) in the VTA was sufficient for blocking behavioral/ motor sensitization to cocaine (Champtiaux et al, 2006). This may, in part, be explained by the results of Hildebrand and Svensson, which demonstrated that systemic nicotine increases dopamine efflux in the NAc. Site-specific antagonism of nicotinic receptors in the VTA, but not in the NAc, has the ability to significantly decrease the presence of extracellular accumbal dopamine elicited by systemic administration of nicotine (Hildebrand and Svensson, 2000). Interestingly, Zanetti et al, (2007), reported that 100mM mecamylamine delivered via reverse dialysis into the VTA had no effect on accumbal dopamine output following an acute IP administration of cocaine; however, DHβE and methyllycaconitine (MLA) in the VTA were shown to decrease NAc dopamine overflow following acute IP cocaine in the same experiment. Although these data contrast the work presented here, our experiment addressed a different question, and as such different methods were employed. While we did not measure DA in the NAc directly, others have found the extended-access model of cocaine self-administration and subsequent

escalation of drug intake is not strictly a function of DA overflow in the accumbal field (Ahmed et al, 2003). However, it should be noted that cocaine-reinforced responses to DA antagonists does change as a function of extended access in rats (Ahmed et al, 2004). Furthermore, we used a concentration of MEC greatly in excess of that reported by Zanetti and colleagues. Our dose of MEC may indeed have resulted in a reduction of NAc DA. However, our current findings, as well as our previous work (Hansen & Mark 2007), suggests that MEC does not completely halt cocaine self-administration. Rather, in our hands mecamylamine's net effect is a reduction, but not cessation, of cocaine self-administration. However, more work on these experiments is needed. Other nicotinic antagonists should also be employed in order to determine that the nAChR are in fact necessary for the reduction of cocaine intake seen in LgA animals. Also, future work to should employ site-specific microinjections within the VTA using antagonists more specific to the various nicotinic subtypes e.g., DHβE and MLA, and combinations of each in order to determine which receptors may be exerting the greatest influence.

Others have shown that drug history and exposure can affect DA overflow in the accumbens and that MEC sensitive receptors in the VTA can attenuate systemic nicotine-induced VTA activation and consequent accumbal DA release (Nisell et al, 1994). Also, motivated drug intake has been shown to exhibit disparities in neurotransmitter release—specifically Ach—over animals receiving drug passively (Mark et al, 1999). These disparities may not be an uncommon phenomenon: different protocols for drug abuse research have widely divergent effects down stream (Stefanski et al, 2004). This may explain our results, using MEC in the VTA, in contrast to the data provided by Zanetti

and colleagues. Future studies should address the role of glutamate in the maintenance of escalated cocaine intake. Nevertheless, the data presented here indicates that antagonism of nicotinic receptors in the VTA with mecamylamine transiently reduce elevated levels of cocaine consumption in rats with extended daily access.

Table 3.1 Mean daily values of cocaine self-administration

Group Mean (+/-) STD	Baseline 1-hr access	Days (1st hour of 6 hour access)									
		1	2	3	4	5	6	7	8	9	10
NAc group	7.6 (1.1.)	7.3 (3.3)	8.7 (3.4)	9.8 (4.0)	11.8 (4.5)	12.5 (4.2)	14.0 (4.2)*	13.7 (3.9)*	14.5 (3.6)	14 (3.7)*	13.5 (3.0)*
VTA group	7.5 (3.7)	9.1 (4.8)	9.1 (4.8)	10 (3.1)	10.6 (3.6)	12.4 (4.1)	11.8 (5.3)	15.3 (4.4)	15.3 (6.0)*	15.2 (3.9)*	14.2 (4.0)*

Table 3.1: Data represents the 1st hour of 6hr access. Numbers represent the mean absolute value of cocaine infusions +/- (STD). Asterisks denote significant difference from baseline (defined as the average 2 days of short 1 hr access prior to day 1 of long-access).

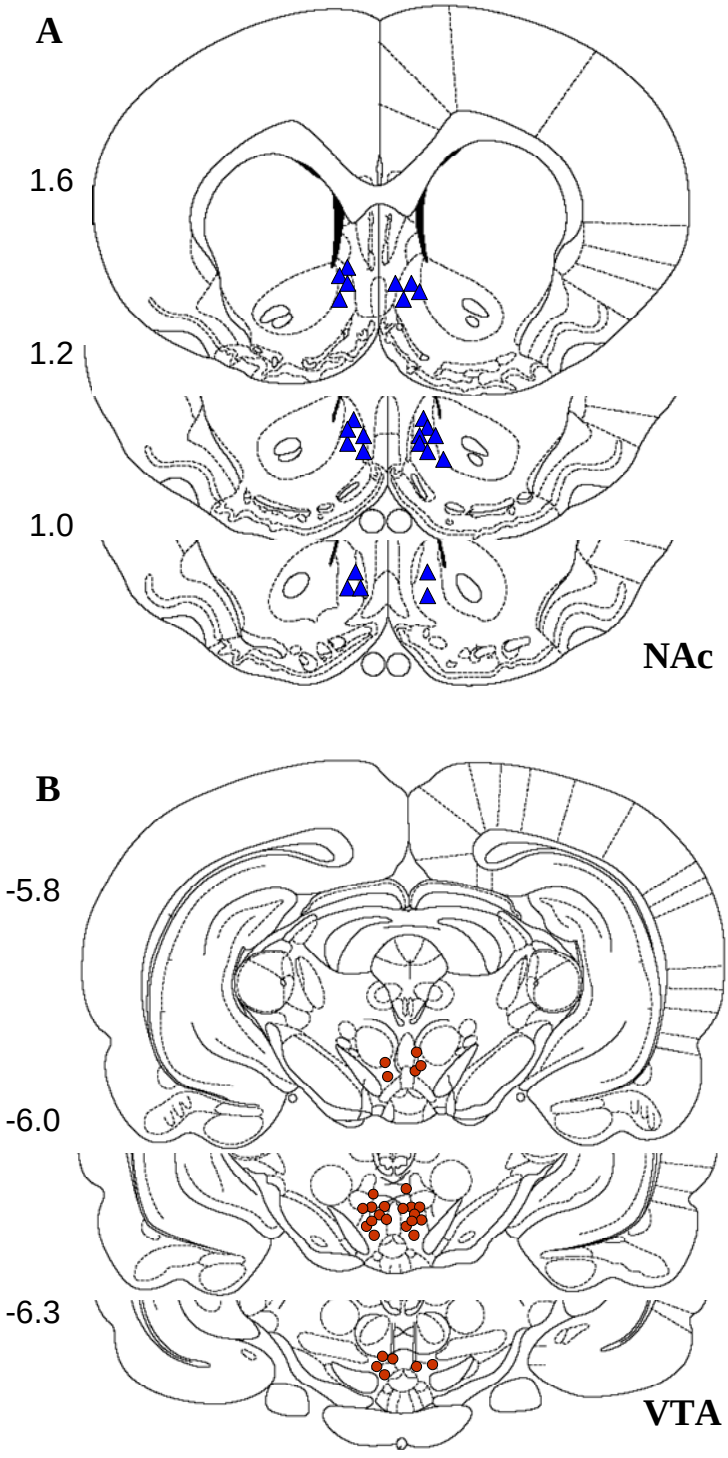
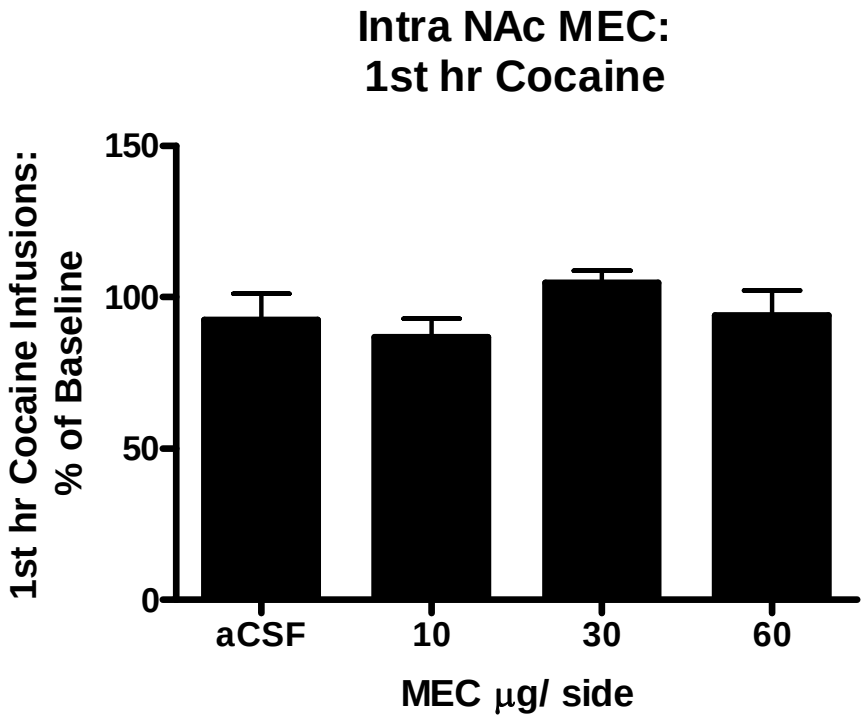


Figure 3.1

Figure 3.1: Slice sections from A) the nucleus accumbens (blue triangles) and B) the ventral tegmental area (red circles) indicate microinjector tracks. Animals with tracks lying outside the target regions or with microinjector tracks that could not be confirmed were excluded from analysis.


Intra NAc MEC:
1st hr Cocaine
1st hr Cocaine Infusions:
% of Baseline
150
100
50
0
aCSF
10
30
60
MEC μg/ side

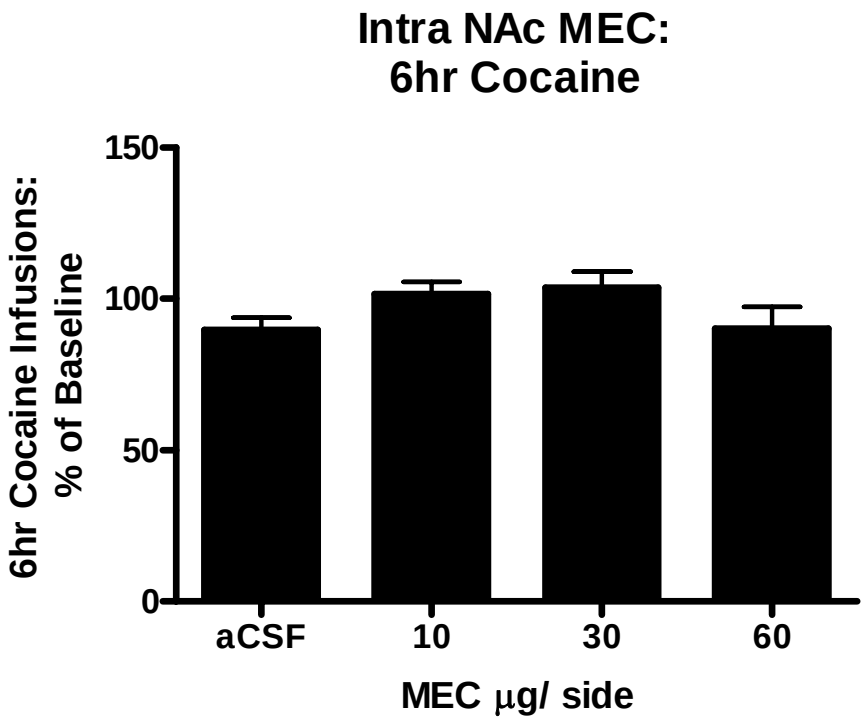
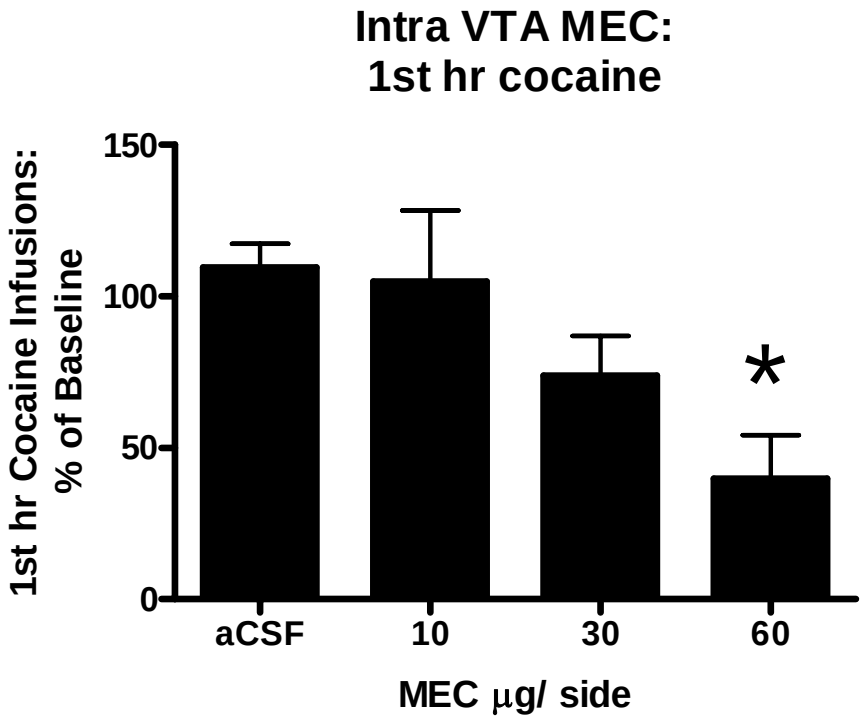

Intra NAc MEC:
6hr Cocaine
6hr Cocaine Infusions:
% of Baseline
150
100
50
0
aCSF
10
30
60
MEC μg/ side

Figure 3.2: Cocaine consumption following intra-NAc microinjection of MEC. One-way ANOVA carried out between groups indicated no significant difference was detected between groups (aCSF, n = 6; 10μg, n = 5; 30 μg, n = 5; 60 μg, n = 6) in either the first hour of LgA or during the entire six-hour period; $P>0.05$.


Intra VTA MEC:
1st hr cocaine
1st hr Cocaine Infusions:
% of Baseline
150
100
50
0
*
aCSF
10
30
60
MEC μg/ side

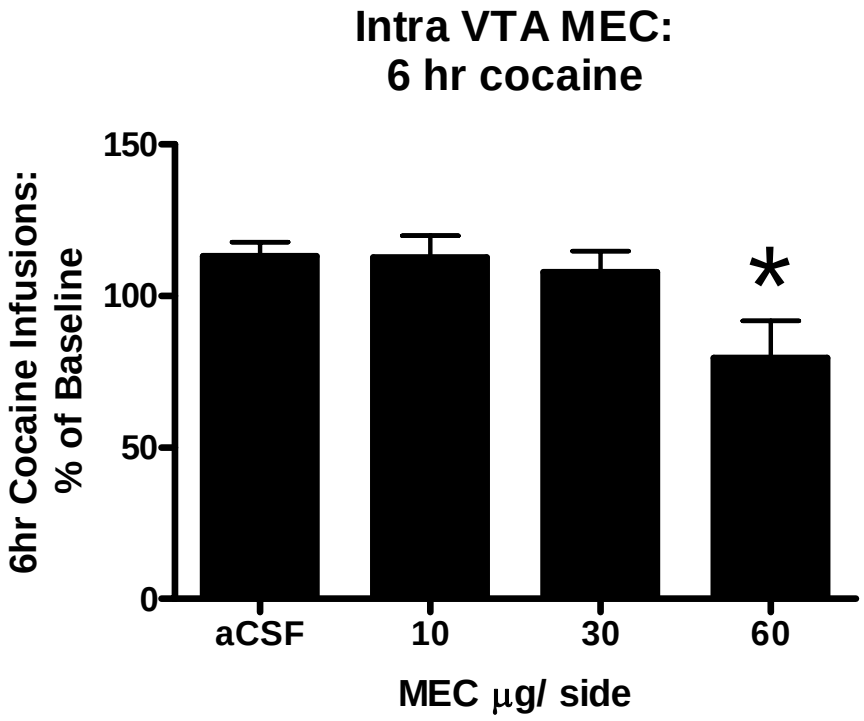

Intra VTA MEC:
6 hr cocaine
6hr Cocaine Infusions:
% of Baseline
150
100
50
0
*
aCSF
10
30
60
MEC μg/ side

Figure 3.3: Cocaine consumption following intra-VTA microinjection of MEC. One-way ANOVA carried out between groups indicated a significant difference between aCSF and 60 μg MEC groups (aCSF, n = 11; 10μg, n = 8; 30 μg, n = 9; 60 μg, n = 6) in both the first hour of LgA or during the entire six-hour period; $P>0.05$. * indicates $P<0.05$

Figure 3.4

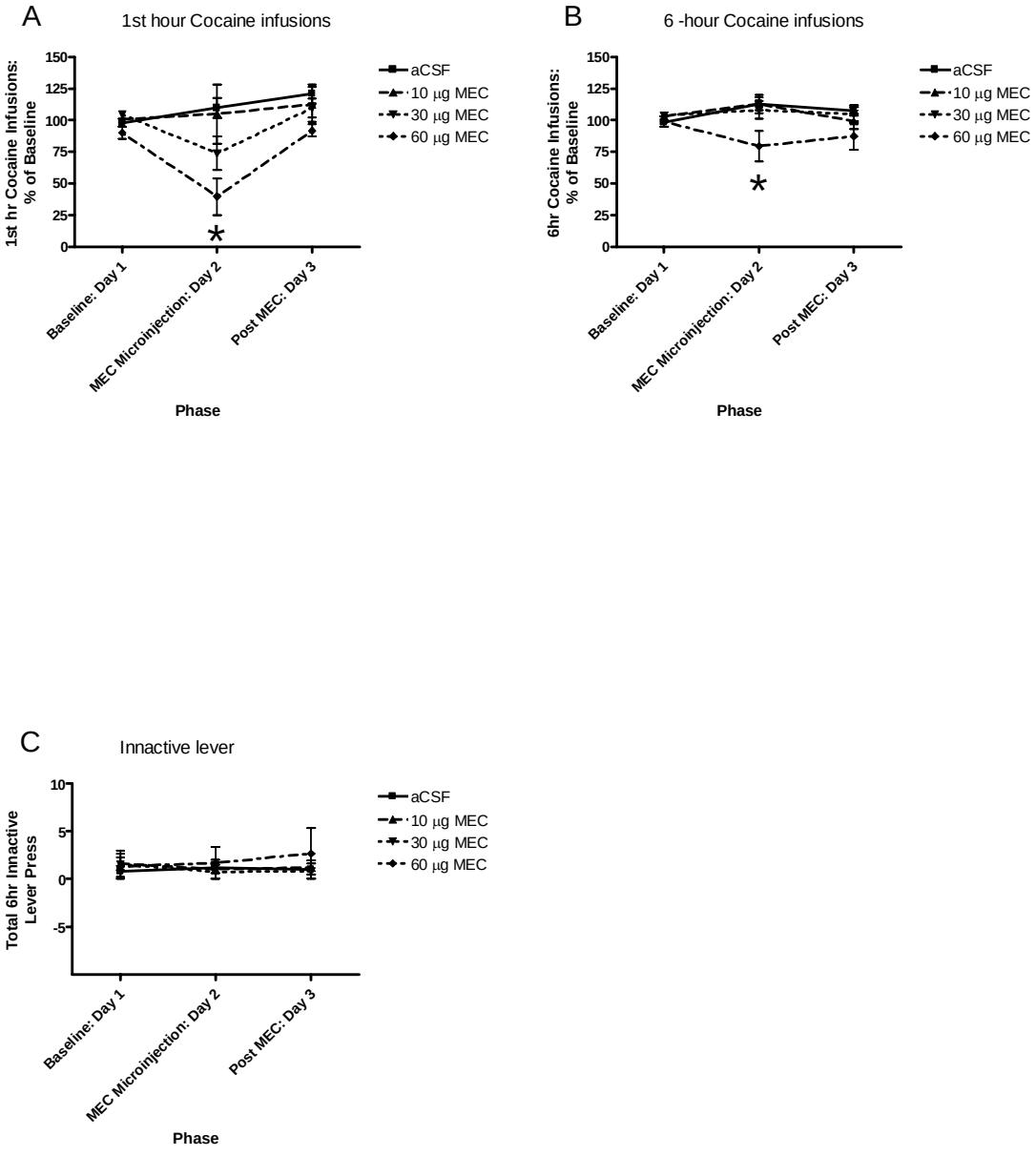

A
1st hour Cocaine infusions
1st hr Cocaine Infusions: % of Baseline
150
125
100
75
50
25
0
*
Baseline: Day 1
MEC Microinjection: Day 2
Post MEC: Day 3
Phase
aCSF
10 μg MEC
30 μg MEC
60 μg MEC
B
6 -hour Cocaine infusions
6hr Cocaine Infusions: % of Baseline
150
125
100
75
50
25
0
*
Baseline: Day 1
MEC Microinjection: Day 2
Post MEC: Day 3
Phase
aCSF
10 μg MEC
30 μg MEC
60 μg MEC
C
Innactive lever
Total 6hr Innactive Lever Press
10
5
0
-5
Baseline: Day 1
MEC Microinjection: Day 2
Post MEC: Day 3
Phase
aCSF
10 μg MEC
30 μg MEC
60 μg MEC

Figure 3.4: All animals experienced long-access protocol to significantly increase cocaine intake (see intro text and table 3.1). Intra-VTA microinjections of MEC resulted in A) two-way ANOVA indicated 60 μg/side dose of MEC significantly decreased responding for cocaine between groups; $P<0.001$ during the 1^{st} of six-hour access and within groups; $P< 0.01$. B) This significant difference was maintained between groups over the course of the six-hour experimental session; $P<0.01$. All values normalized to a percentage of individual cocaine responding. C) Absolute value of inactive lever presses reported for the total six-hour period. No significant differences were detected at any time

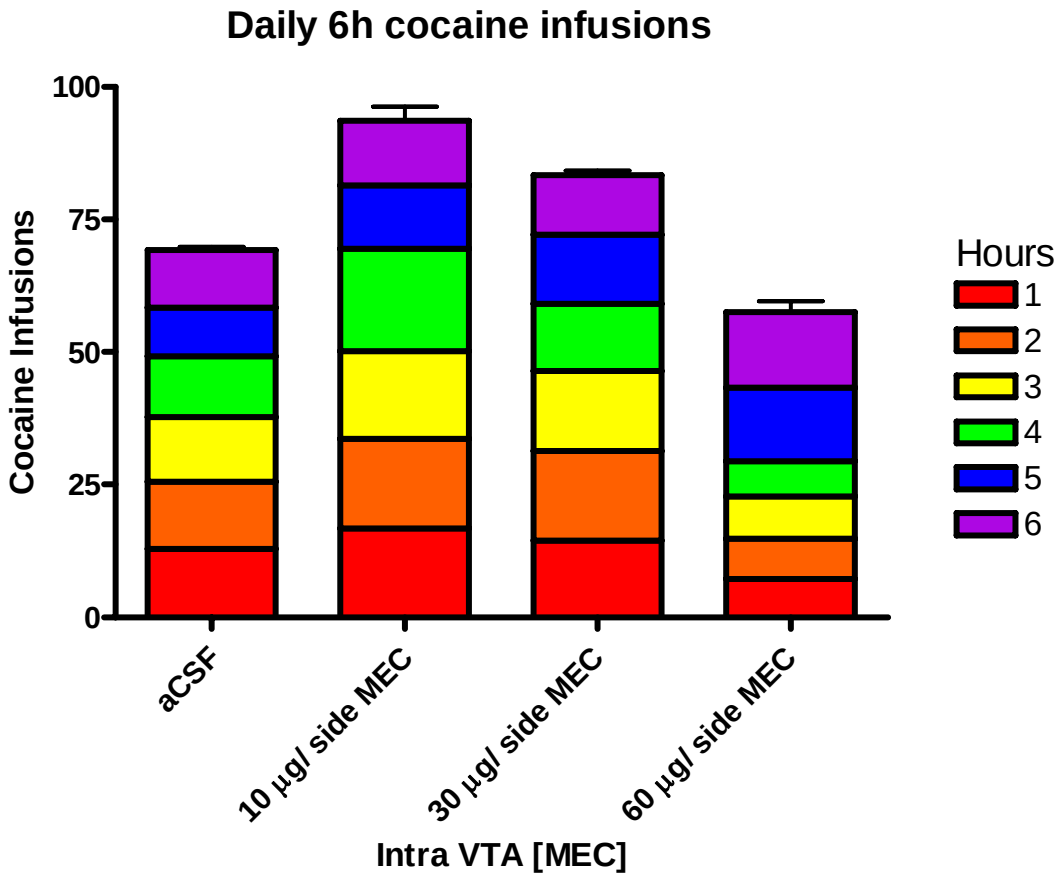


Figure 3.5: Data represents the absolute value of cocaine consumption on day of intra VTA MEC microinjection partitioned by each hour of six-hour access. aCSF n = 11; 10µg MEC, n = 8; 30µg MEC, n = 9; 60 µg MEC, n = 6.

CHAPTER 4

Nicotinic acetylcholine receptors in the ventral tegmental area attenuate the somatodendritic dopamine release following systemically administered cocaine

Introduction

Research into dopamine's (DA) influence in reward and motivation has traditionally been confined to dopaminergic terminals and their associated receptors within the nucleus accumbens (NAc). Evidence indicates that the dopamine cells within the ventral tegmental area (VTA) also release a significant amount of DA neurotransmitter (Aghajanian & Bunney, 1977; Llinas et al, 1984; Sesack et al, 1994) in an exocytotic fashion (Fortin et al, 2006; John & Jones, 2006). This intra-VTA DA has been shown to be a physiologically relevant signal (Ranaldi & Wise, 2001). Acting in a negative-feedback like fashion, intra-VTA DA release activates the inhibitory DA D2 autoreceptors located on the cell's dendrites as well as acting at presynaptic DA D1 receptors on the GABA cell, resulting in an attenuation of excitation of the DA cell (Martin & Waszczak, 1994; Chen & Pan, 2000). Ultimately, somatodendritic release of DA within the VTA serves to decrease DA cell firing rate that results in decreased somatodendritic release as well as decreased DA release in the terminal fields. Evidence suggests that intra-VTA DA may be taken as an indicator of DA cell activation (Kalivas & Duffy, 1991; Beckstead et al, 2007), which results in the DA release at the terminal fields such as the amygdala and the NAc.

Acetylcholine and dopamine interact at several sites within the mesolimbic pathway. It is therefore no surprise that nicotine (the prototypical agonist of the nicotinic acetylcholine receptor) and cocaine (an indirect agonist of the D1 and D2 receptors) are commonly co-abused drugs. Nicotinic acetylcholine receptors (nAChRs) are ubiquitous throughout the mesolimbic reward circuit. ACh from the pontine nuclei: the lateral dorsal tegmentum (LDTg) and the pedunculopontine nucleus (PPTg) activate both metabotropic muscarinic ACh receptors (mAChRs) and ionotropic nicotinic ACh receptors (nAChRs). Although mAChR have been shown to be fundamentally important for activating DA cells in the VTA following a reward stimulus, the nicotinic receptor also activates the DA cell, but to a lesser extent. In the VTA nAChRs are both pre-and postsynaptically located (Jones et al, 2004; Marshall et al, 1997; Wonnacot 1997). Data from several neurochemical experiments indicate that cocaine can affect cholinergic activity (Imperato et al, 1992; Mark et al, 1999). Conversely, the nAChRs are activated in cocaine reward processing (Berlanga et al, 2003) and have been shown to be important for developing escalated drug intake under long-access conditions (Hansen & Mark, 2007). Furthermore, nicotinic AChRs in the VTA modulate DA release in the NAc following systemic cocaine.

Based upon previous research indicating the importance of nAChRs localized within VTA as important to DA signaling we hypothesized that these receptors would modulate intra-VTA dopamine signaling in the presence of an indirect DA agonist i.e. cocaine.

Methods

Animals

Fifteen male Sprague-Dawley albino rats weighing 300g at the time of the experiment were obtained from Charles River Laboratory (Willmington, MA). Upon arrival all animals were allowed at least five days acclimation before being used for experiments. Rats were pair housed until the time of surgery, and then were single housed. Home cages were made of clear plastic (28×28×18 cm) covered with filter tops in a temperature-controlled environment (22°C) on a 12-h light/dark cycle (lights on at 06:00). Rodent chow food (LabDiet; Richmond, IN) and water were available *ad libitum* in the home cage. Animal experimental procedures complied with the guidelines set forth in the "Guidelines for the Care and Use of Mammals in Neuroscience Research" (National Research Council of the National Academies 2003) and were approved by the Oregon Health & Science University's Institutional Animal Care and Use Committee.

Microdialysis probe construction

Microdialysis probes were constructed in house. Detailed descriptions of probe design have been previously published (Mark, et al, 1991). Briefly, probes were constructed with silica glass tubing (37 µm i.d.; Polymicro Tech Inc.) nested inside a 26 ga. stainless steel tube with a microdialysis tip of cellulose tubing (0.2 mm o.d; 6000 MW cutoff; Spectrum Med. Co.) sealed at the end with epoxy cement. Tip lengths were 1.0 mm to restrict sampling to terminals within the VTA. Probes were perfused with a buffered perfusion medium at a flow rate of 1.0 µl/min throughout the experiment.

Drugs

Cocaine HCl, was supplied by the National Institute on Drug Abuse drug supply program (RTI International, Research Triangle Park, NC), and was dissolved in physiological saline (0.9%) and pH adjusted to 6.0 with 1M NaOH. Mecamylamine was obtained from Sigma (St. Louis, MO).

Surgery

Animals were sedated with anesthetic mixture (55.5 mg/cc ketamine, 5.5 mg/cc xylazine and 1.1 mg/cc acepromazine) given in a volume of 0.15cc IP. Sub-cutaneous analgesic (carprofen) was also given prior to surgery. Under anesthetic sedation rats were placed in the stereotactic instrument and heads were leveled in the XY and Z planes. All coordinates are reported relative to Bregma: A/P -6.0; L/M- +/-0.6; D- -4.7. Bilateral guide shafts were sterotaxically lowered into position and cemented in place with cranioplastic acrylic. Animals were also outfitted with aluminum head shields in order to protect guide shafts and serve as an attachment point for a counter balanced spring alligator clip. 26g obdurators were placed in guide shafts following surgery in order to keep the shaft clean and unobstructed.

Microdiaylsis

Microdialysis chambers consisted of standard operant training boxes with all operant stimuli removed. Boxes measured (30×24×29 cm) and were housed inside of light- and sound-attenuating boxes (Med Associates, St Albans, VT). 18hr prior to microdialysis experiments animals were briefly sedated with isoflurane and a microdialysis probe was

unilaterally lowered into the guide shaft and secured in place with a drop of cranio-plastic cement. Animals were gently wrapped in a towel when they recovered from the brief anesthesia and while waiting for the acrylic to cure. This procedure usually required 10 min. Microdialysis probes extended 5 mm beyond the guide shaft to reach the VTA. Flow and recovery of dialysate Ringer was visually determined before placing the rat in the microdialysis chamber. Following 18hrs recovery, pump speed was increased to 8nL/s and allowed to equilibrate for 1hr. Probes were constantly perfused with a buffered perfusion medium at a flow rate of 1.0 μl/min. To facilitate DA detection from the small (1 mm) sampling area of the VTA, sample vials used to collect dialysate were treated with 2 μl EDTA to prevent DA degradation. Probes were implanted at least 22 hr before each experiment to allow for recovery and stabilization of neurotransmitters. Measurement of DA in dialysates was detected by high-performance liquid chromatography with electrochemical detection (HPLC-ECD). Full descriptions of these procedures are published (Keys and Mark, 1998; Mark, et al, 1999; Rada, et al, 2000). Dialysis samples were collected every 15 min for a minimum of 1 hr or until stable baselines were obtained wherein peak heights did not vary by more than 15% between samples. Samples were injected directly into an HPLC system for immediate analysis.

Experimental treatment

Once DA levels reached stable equilibrium animals were treated with either systemic cocaine (20mg/kg), reverse dialysis MEC (100 μM for fifteen min prior to sample collection, MEC was administered via 15cm loop of PE tubing filled with 100 μM MEC attached to main microdialysis line) or a combination of cocaine and MEC in a repeated

measures design. Animals experienced systemic cocaine only once in order to avoid confounding issues with sensitization that may occur upon repeated treatment. IP injections of saline were used as a control for cocaine injection. Detected DA levels remain stable throughout the day in non-active rats. Relative amount of baseline dopamine recovered is our dependent variable; therefore, manipulations that disrupt stable DA levels (as detected by HPLC) will be considered independent variables. Independent variables that may affect recovered DA will be the introduction of IP saline, IP cocaine, intra-VTA MEC and intra-VTA MEC concomitantly with IP cocaine. Each animal will only experience IP cocaine once in order to avoid difficulties in data interpretation that may arise due to drug sensitization.

Histology

Rats were euthanized with sodium pentobarbital and perfused with saline followed by 10% formalin in phosphate buffered saline (PBS). Brains were removed and stored for a minimum of 48 hr in 20% sucrose/4% formalin followed by 24 hr immersion in 30% sucrose/PBS before being sliced at –20°C using a Cryostat (50 µm sections). Brain slices were mounted and stained with Thionin Blue in order to identify injector and probe tracks. Data from animals with injector tracks outside of the target area or with unconfirmed injector tracks were excluded from analysis (see figure 4.1).

Statistics

Absolute recovery of DA varies considerably between animals. Therefore, all data has been normalized and reported as a percentage of baseline DA response (defined as two

data points prior to experimental manipulation). A mixed-factor ANOVA was performed on data comparing Drug Treatment X Time (5 time points) and a one-way ANOVA carried out on DA recovery during manipulation time point.

Results

Data from one animal were removed from analysis because dopamine peaks could not be confirmed. A mixed-factor ANOVA calculating Treatment x Time indicated that treatment with IP cocaine significantly increased recovered DA ($F_{3, 77} = 3.23$; $P < 0.05$). Bonferroni *post-hoc* analysis indicated that treatment with cocaine significantly altered DA recovery as compared to baseline. Conversely, treatment with MEC prior to IP cocaine did not significantly alter DA levels as compared with baseline treatment. Treatment with MEC alone did not significantly alter DA levels in the VTA. IP injections of saline did not significantly increase recovered DA from the VTA.

Discussion

The results presented in this manuscript indicate that nAChRs in the VTA exert a measure of control over cocaine-stimulated DA neurons. Basal activity of the dopamine neuron within the VTA releases DA in a somatodendritic fashion. A single systemic injection of cocaine significantly increased the presence of intra-VTA DA. The nicotinic receptor antagonist MEC did not affect basal DA levels. However, MEC significantly attenuated the extracellular DA response in the VTA to systemic cocaine. The ability of MEC to block the exaggerated DA output in the VTA was confined to cocaine-elicited activation and did not significantly decrease intra-VTA DA levels alone.

The ability of MEC to inhibit somato-dendritic release of DA in the VTA could work by a combination of mechanisms. Nicotinic receptors have been localized to the dendrites and soma of the DA cell (Woolterton et al, 2003; Wonnacott, 1997). Mecamylamine, acting at these receptors may prevent the release of somatodendritic DA by attenuating the overall excitability of the DA cell; somato-dendritic release of DA within the VTA is thought to be a function of DA cell excitation (Kalivas & Duffy, 1991). While it is possible that MEC is inhibiting the DA cell activation directly it seems an unlikely mechanism of action. It has been shown that nicotinic receptors are not directly responsible for cellular activation, despite being fast acting excitatory receptors. The preponderance of evidence suggests that nAChRs are primarily presynaptically located on GLU (Pidoplichko et al, 2004; Dani et al, 2006) and GABA terminals with in the VTA. Therefore, their activation likely enhances excitatory GLU signal from the prefrontal cortex. Conversely, inhibition of the nAChRs on the GLU terminal would result in less GLU release in the presence of a stimulus.

Nicotinic receptors exist in a wide variety of subtypes. The alpha-beta configuration is the most common. Experimental evidence indicates that nAChRs with the β2 subunit are most important for DA cell activation. However, the homopentameric α7 subtype of nAChR is located on the GLU terminals and is actively responsible for the preponderance of nAChRs excitation resulting in GLU release (Pidoplichko et al, 2004; Wooltorton et al, 2003). Mecamylamine antagonizes a broad spectrum of nicotinic receptors. Although MEC has shown to be of greatest efficacy at the α6β4 sub-variety of receptors, it is still

considerably effective at antagonizing the β2 and the α7 receptors (Papke et al, 2001; Young et al, 2001). Because of this non-specificity it remains to be tested as to which receptor subtypes are the most important for limiting the extracellular DA response in the VTA as a function of systemic cocaine. In particular future studies should test the compound methyllycaconatine (MLA) which antagonizes the α7 nicotinic receptors specifically. Also, the β2 subunit specific antagonist dihydro-beta-eurothrodine (DHβE) should be tested as well. Experiments of this nature may provide insight into the relative importance of each nicotinic receptor subtype and their location i.e. pre or post synaptic (see figure 1.2, pg 23 of this thesis).

In this experiment we investigated the pharmacological properties of MEC and cocaine on somatodendritic DA recovery. Cocaine was passively administered to the rat and recovered DA levels increased. However, active motivated drug intake may affect DA levels in an entirely different manner. Future work would be well served to employ a cocaine self-administration component to the microdialysis procedure, given that motivated drug can significantly alter underlying neurochemical interactions when compared to passive drug intake (Mark et al, 1999; Stefanski et al, 2002; Jacobs et al, 2003). In a similar vein, data reported in this experiment is from drug naive animals. Other researchers have shown that animals with a prior drug experience may exhibit different relative levels of neurotransmitter in response to drugs compared to animals without prior drug experience (Morgan et al, 2005). Therefore, future work in this area should employ animals with a previous drug experience. Disparity in techniques may provide insightful data into neurobiological changes that occur as a function of drug use

Our work presented here is in agreement with the wider body of evidence suggesting a primarily modulatory role for the nicotinic acetylcholine receptor and reward related processing relative to dopamine cell activation (Vizi et al, 1999; Mameli-Engvall et al, 2006). However, the data presented indicates that MEC can modulate extracellular VTA DA levels following systemic cocaine injections while leaving basal intra VTA DA levels unchanged. This data gives further insight into the role of the nicotinic receptor and its involvement in the stimulation and modulation of the mesolimbic DA system.

Figure 4.1

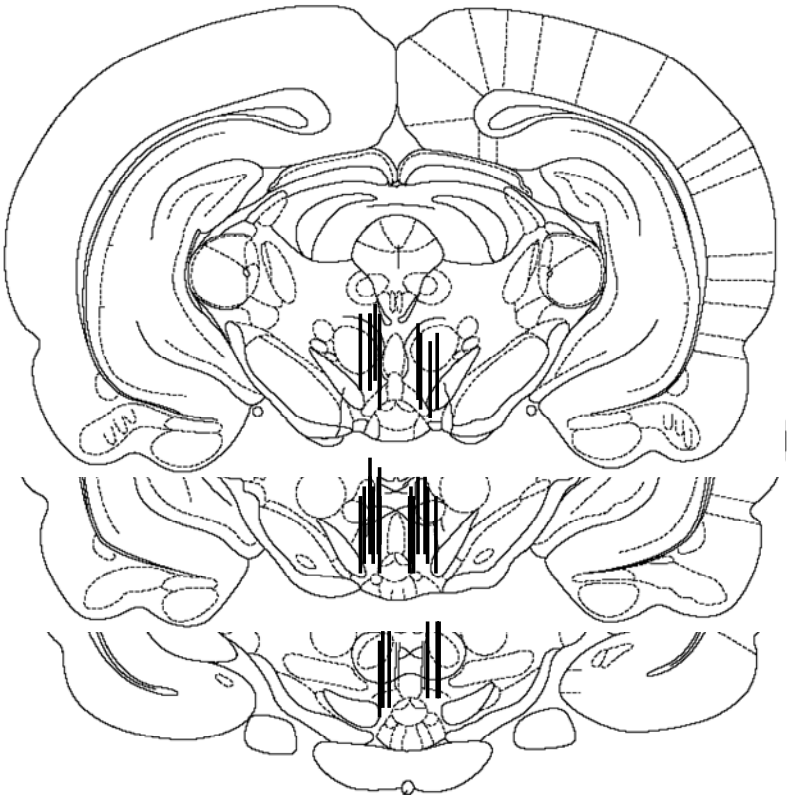


Figure 4.1: Cross section of rat brain. Represents VTA: -5.8 (top), -6.0 (middle), -6.3 (bottom).

Figure 4.2

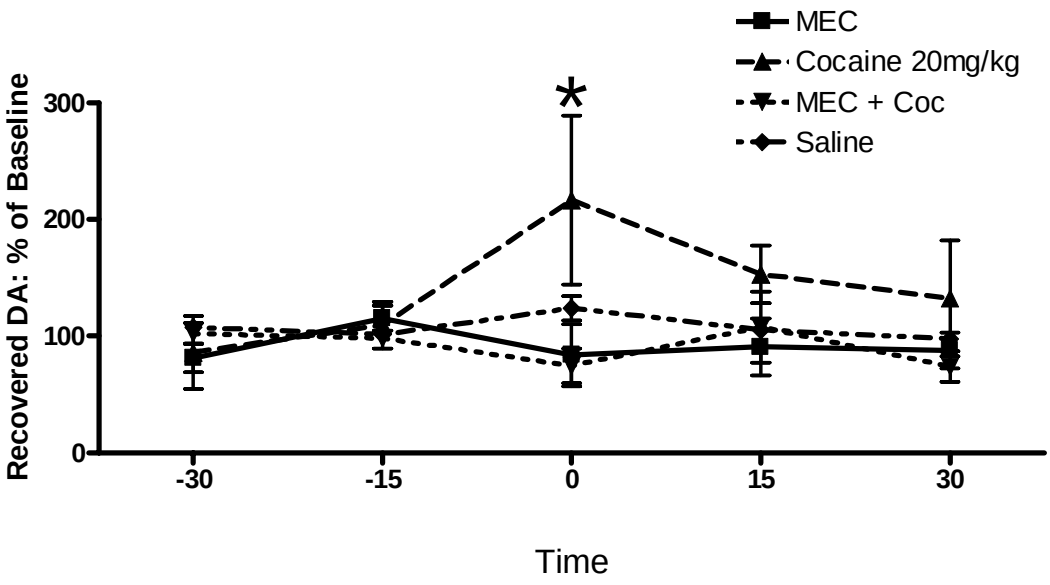


Figure 4.2: Dopamine recovery from intra-VTA microdialysis. All data normalized to percentage of individual baselines. All samples taken at fifteen min intervals, baseline samples were two samples immediately prior to drug treatment. Mixed-factor ANOVA revealed a significant main effect. Bonferroni *post-hoc* test revealed cocaine treatment significantly different from MEC and MEC+Coc treatment; $P < 0.01$. No significant differences were detected between MEC and MEC + Coc conditions and no significant differences were observed between Coc and Saline. Solid line with squares represents relative DA levels during 15min intra-VTA 100 μM MEC (n = 6). Dashed line with triangle represents relative DA levels before during and after IP injection of 20mg/ kg cocaine (n = 7) Solid diamond with semi-dashed line represent saline (n = 4). Dotted line, inverted triangle represent 15min of 100μM MEC in the presence of 20mg/ kg IP injection of cocaine (n = 6).

CHAPTER 5: GENERAL DISCUSSION

5.1 SUMMARY OF EXPERIMENTAL RESULTS

The overarching goal of this research was to elucidate the role of nicotinic acetylcholine receptors in the production and maintenance of drug dependence; specifically, the consumption of greater amounts of drug over long periods of time. These specific traits of drug dependence were modeled using the extended-access paradigm in which animals were allowed longer period of time (6hr) in which to self-administration cocaine.

Chapter two demonstrated that systemic antagonism of the nAChRs with the nicotinic antagonist mecamylamine, prevented animals from increasing their cocaine intake—despite being subjected to conditions that would otherwise promote escalated drug consumption. However, MEC did not preclude cocaine consumption altogether. Rather, animals continued self-administering cocaine, but, neither significantly increased nor decreased their daily drug intake. This effect was observed only while MEC was present; once MEC was removed from the experiment animals increased their cocaine intake in a fashion similar to control animals. In total, it appears that MEC sensitive nAChRs are important for the putative neurobiological shift necessary for the production of escalated cocaine intake.

Data reported in chapter three demonstrated that nAChRs located within the mesolimbic pathway are important for the maintenance of elevated levels cocaine intake following procedures which elicit escalated drug intake. Once animals had reached significantly

elevated responding for cocaine under the 6 h protocol, they were given microinjections of the nAChR antagonist MEC. Site specific administration of MEC into the NAc produced no change in either their first hour cocaine intake or their total 6 h cocaine consumption. However, microinjections of MEC into the VTA produced a significant decrease in both their first hour as well as their total six-hour daily cocaine consumption. The responding for cocaine was not completely abolished, however. Therefore, nAChRs in the VTA, but not in the NAc are influential in maintaining consumption of greater amounts of cocaine under the LgA paradigm.

The data reported in chapter four provide evidence for a possible mechanism by which nAChRs in the VTA may be exerting their effect on cocaine stimulation within the VTA. The experiment performed in Chapter four is distinct from Chapters two and three in that no self-administration behavior was required of the animals. Despite this fact data from this experiment provides useful insight into the actions of MEC in the VTA. However, future work should employ both self-administration behavior as well as LgA conditions. Information from such experiments would then be compared to results reported in Chapter four. It may well be the case that motivated cocaine intake offers a different neurochemical profile in the VTA then was observed with this data. However, in this chapter systemic administration of cocaine elicited an extracellular DA response in the VTA, which was taken as evidence of cocaine's activating effect on DA neurons within the VTA. The nicotinic receptor antagonist MEC administered via reverse dialysis into the VTA alone had no effect on tonic levels of DA activity from this nucleus. However, MEC blocked the exaggerated extracellular DA response seen in the VTA following a

systemic cocaine injection. Thus, MEC sensitive nicotinic receptors in the VTA appear to be a source of control, in whole or in part, for the cocaine induced dramatic somato-dendritic release of DA in the VTA. However, these MEC sensitive receptors do not inhibit tonic DA cell activation. These data provide further evidence that nicotinic receptors modulate the DA signal under the influence of cocaine reward. Therefore, it can be surmised that nicotinic acetylcholine receptors within the mesolimbic reward pathway modify escalated cocaine consumption; making them necessary but perhaps not sufficient in this biological adaptation to increased drug self-administration. Why this is so will be considered in the following sections, taking into account the behavioral and neurobiological contributions to escalated drug intake.

5.1.2 TECHNICAL CONSIDERATIONS

While the data provided in this thesis offers new insights into the involvement of the nicotinic receptor and cocaine self-administration, as well as nicotinic receptors role in intra-VTA DA release in response to systemic cocaine injections, however, there remain limitations to the interpretation of this data as well. The antagonist used to target the nAChR was mecamylamine HCl. This is a broad-spectrum nicotinic receptor antagonist (Papke et al, 2002). In high concentrations it has been shown to have antagonistic properties at the glutamatergic NMDA receptor as well (O'Dell & Christiansen, 1988). However, MEC does not appear to antagonize the muscarinic ACh receptors in any meaningful fashion (De Sarno et al, 2003). In order to be more confident that nicotinic receptor inactivation was responsible for the changes in cocaine intake that was observed and that MEC was targeting only the nicotinic receptors future studies should employ

other nicotinic antagonists such as MLA, α-bungarotoxin or a new class of nicotinic antagonists derived from conotoxin (Loughnan et al, 2006). The experimental approaches would provide convergent evidence that antagonism of the nAChR was responsible for the results reported here.

In Chapter 3 microinjections of MEC into the VTA decreased cocaine self-administration. The *in* vivo concentration of MEC delivered was 3M (60 μg/ 0.1μl), much higher then used by others. This may account for the theoretical discrepancy in the results reported here vs. results reported by Zoli and workers (2007). Even so, it remains unclear why we did not see a decrease in cocaine responding following microinjections of MEC into the NAc using similar doses as Nadal and colleagues (1998). Possibly, the differences may be a function of the different mechanisms of actions of alcohol and cocaine within the CNS. In order to gain further confidence in the results reported in Chapter 3 it is necessary to add microinjection data of MEC from outside the two targeted nuclei (i.e. the NAc and the VTA). The data reported in Chapter 3 had 1 rat with with unconfirmed tracts. In the VTA three rats had injectors outside and/or straddling the boundaries of the VTA and no unconfirmed injector tracts. Although it appears that MEC microinjected into these errant sites did not affect cocaine responding, no meaningful data can be derived from this, given the small sample size.

The volume of liquid microinjected was small, however, no data was collected with regards to the volume of distribution in the brain. It may have been possible MEC in the concentration used—3M—diffused outside of out target area. Without evidence to the

contrary we cannot state with any certainty that MEC stayed within the nuclei of interest in the rat brain. With regards to the null data recorded in the NAc it would be prudent to include a positive control such as raclopride, which would target D2 receptors located presynaptically. This would enhance interpretation of the data by providing confirmatory evidence that the null result was indeed real and not a function of poor methodology.

5.2 LONG-ACCESS MODEL & ESCALATED DRUG INTAKE:

A well established characteristic of drug dependence is a progressive increase in drug consumption over time. This increase in drug use is often viewed as a loss-of-control over drug intake leading to chronic and often maladaptive drug consumption. Historically the loss of control aspect of drug use disorders has been attributed to a development of tolerance to the rewarding properties of the drugs, i.e. the same unit dose of the drug no longer elicits the same hedonic state. However, emerging data from the animal model discussed in chapters two and three of this thesis have encouraged researchers to rethink the argument in favor of tolerance as a way to explain escalated drug use. The evolving view of escalated drug use is more complex than can be accounted for with the explanation of tolerance.

Through the basic protocol of LgA for self-administration, many variations—sometimes overt and at other times subtle—have been reported. Several hypotheses have been proposed to account for data being developed with this model and will be discussed below. Although tolerance to the drug's pharmacological properties remains a possibility, other explanations include a sensitization to drug reward, drug reward

allostasis, an increase in the incentive salience of drug-associated stimuli, an increase in the strength of the drug as a reward relative to alternative rewards and finally compulsive behavior i.e. habit learning. These are not mutually exclusive in nature and some cases may be components or subcategories of each other. Each of these explanations will now be considered and weighed against the available data.

Tolerance to a drug's activating effects requires greater amount of drug consumption in order to achieve the same level of euphoric hedonia when the unit dose of the drug remains constant. In this view escalated drug intake, as a function of LgA, is the animal titrating the reward signal through behaviorally increasing drug intake. Some evidence for this point of view comes by the research performed by Ben-Shahar and colleagues indicating that the transition from controlled to escalated drug intake is a function of the loss of sensitization and an emerging tolerance to cocaine's physiological effects (Ben-Shahar et al, 2004; 2005). At face value this would seem so. However, other evidence suggests that this may only be a small component to loss of control over drug intake. Like tolerance, reward allostasis, contends that an ever increasing amount of drug intake is a function of a shifting reward baseline i.e. reward baseline is the threshold level of activation an animal requires in order to work for the rewarding stimulus (Koob & LeMoal, 1997). A shift in reward baseline is a result of the animal becoming less responsive to natural rewards and more focused on drug reward. This explanation of increased drug intake relies on tolerance to some degree. As an animal develops a tolerance to the drug's activating effects, greater amounts of drug are required to achieve a hedonic state. Ahmed and colleagues provided evidence for allostasis: rats that

experienced repeated withdrawals following prolonged cocaine self-administration exhibited a persistent decrease in brain reward function. This was highly correlated with escalated cocaine intake and reduced the hedonic value of cocaine in general (Ahmed et al, 2002). However, the allostasis model diverges from tolerance in that baseline reward thresholds have changed as opposed to reward activation thresholds. The allostasis model has apparent face validity in that many human drug dependent persons report continued drug use being driven by a desire to avoid withdrawal symptoms rather than a desire to obtain drug induced euphoria (Khantzian et al, 1985; Wise & Bozarth, 1987). Indeed, the allostasis model may better account for the plateau effect which follows the initial dramatic increase in cocaine intake following the transition from short to long-access drug self-administration.

Another possible explanation for escalated cocaine intake under LgA conditions is that drugs of abuse take on a greater relative reinforcing strength relative to alternative natural reinforcers. This model would account for data obtained using the two-lever choice model. This model allows an animal to choose between drugs and natural stimuli such as food, water or a receptive sexual mate. Invariably animals previously exposed to the drug will choose it over the natural reinforcer. This model appears to be an apt comparison of human drug dependent individuals as well. Given their ability to seek an array of reinforcing stimuli in the environment e.g. eating, social interaction etc, human drug dependent individuals invariably choose drugs. The reward-sensitization hypothesis is highlighted by research performed by Goldstein and Volkow, which indicated a

decreased incentive to seek non-drug rewards in drug dependent patients (Goldstein & Volkow, 2002).

Alternative explanations for the escalated drug intake model posit that it is a function of pathology of drug reward learning and subsequent habit formation. In this view behavior persists in the face of devalued reinforcer/ reward e.g. pre feeding an animal prior to an experimental session in which an operant is needed to be performed for a food reinforcer. Should the operant behavior occur, while the animal is in a state of satiety, it may be said to be under habit-control. Such a scenario could arise out of the LgA model of drug self-administration. However it is difficult to test when using psychostimulants largely due to their ability to withstand satiety. Being exposed to the long-access paradigm attenuates extinction learning and may further prime the animal for greater reinstatement to drug seeking, both of which may be taken as signs of rigid habit learning formation.

Finally, the escalated drug consumption phenomena can be accounted for by the proposed incentive-salience of drug associated stimuli model (Robinson & Berridge, 1993). This hypothesis separates the drug taking behavior into separate components: drug seeking behavior (appetitive) and drug taking behavior (consummatory). Under the incentive- salience model of escalated drug intake, drug related stimuli—both external and internal—take on a greater importance. Thus the animal becomes fixated on "wanting" but not necessarily "liking" the drug. This would account for greater amounts of drug intake. In fact, evidence indicates that animals titrate their cocaine intake to a "set level" such that by increasing the unit doses of the drug a concomitant decrease in

the number of self-administered drug reinforcers is observed (Mantsch et al, 2004). These data argue against the notion that the rewarding properties of the drug alone are responsible for the escalation of drug intake. The incentive-salience model has strong face validity as many human drug dependent patients report greater amounts of time spent in pursuit of drugs as well as stimuli associated with drugs generating cravings. Paterson and Markou have provided data that would suggest the long-access paradigm results in an increased incentive motivational value to self-administer cocaine as demonstrated by an upward shift in the dose-response-curve in LgA animals compared to ShA rats (Paterson & Markou, 2003). This increased motivation is not merely a function of sensitization to the psychomotor stimulating effects of the drug (Ahmed & Cador, 2006). In fact, increased motivation for drug seeking behavior has been shown to occur with opioids as well as stimulants (Ahmed et al, 2000). Escalated heroin administration has also been shown to be a function of increased motivation to obtain it as opposed to the rewarding pharmacology of the drug alone (Lenior et al, 2007). Such a position is further augmented by the work of Liu and colleagues, when they demonstrated that the long-access model of drug self-administration appears to be a model of motivation to consume cocaine without increasing the properties of reward strength. Rats exposed to the LgA protocol increased their rate but did not increase their break point under a progressive ratio schedule of reinforcement (Liu et al, 2005). Deroche and colleagues highlight the fact that a longer history with cocaine consumption results in an increase in intake. This escalation of drug use is paralleled by an increase in motivational properties of the drug to elicit drug seeking behavior from the animals without overt signs of tolerance to the pharmacological drug effects (Deroche-Gamonet et al, 2004).

Experimental evidence exists to support each of these disparate hypotheses. Although it remains debatable as to what theoretical underpinnings the LgA protocol is measuring, researchers do agree that the escalated drug intake model is a robust phenomenon and one which models the loss-of-control aspect observed in human drug users. As a model it possesses face and predictive validity while construct validity is still being debated. Regardless, a wealth of data has been generated using the LgA paradigm as a model of the loss of control aspect of drug dependence.

5.3 REWARD AND MOTIVATION

Drugs of abuse act upon the mesolimbic system and exert their influence by modulating either or both the rewarding aspect of the drug and the motivation to obtain it. It is within this system that the loss of control aspect of drug abuse may gain a foothold.

Dopamine remains the primary neurotransmitter in the mesolimbic circuit involved in reward and motivation. The actions of DA vis-à-vis reward and motivation are the stamping in of response habits elicited by various rewards and in attaching a motivational significance to environmental stimuli (Schmidt, 1983; Balleine, 2005; Kelley & Berridge, 2002). It is acknowledged that reward can occur in the absence of DA, e.g. dopamine-deficient mice will still engage in the consummatory aspect of reward, but these animals are unable to initiate the goal directed behavior necessary for obtaining the food (Pecina et al, 2003). However, the preponderance of evidence suggests that the motivation for reward is mediated through the DA system. Studies of this nature place the role of DA as a primary motivator with regards to appetitive behavior and reward.

Attentional arousal is linked to motivation and conveys the ability to discriminate between multiple signals in the environment and readiness for input processing. The ascending arousal pathway, particularly the cholinergic component, contributes to the attentional arousal focused on salient stimuli in the environment (Chiba et al 2005; Everitt & Robbins, 1997). Acetylcholine has been implicated in detection of behaviorally significant cues and aides in cognitive processing (Parikh et al, 2007). Acetylcholine also participates in reward aspects of psychostimulants (Mark et al, 1999; Grasing et al, 2008). Enhancement of ACh in the NAc augments the release of DA (Zhang et al, 2004) and enhancement of ACh in the VTA leads to greater release of DA in the NAc, too (Blaha et al, 1996).

The evidence that has accumulated thus far highlights the LgA model of drug self-administration as being conducive to enhancing the motivational aspect of drug consumption without, necessarily, increasing or decreasing the reward value of the drug (Liu et al, 2005; Patterson and Markou, 2003). Motivation and reward/ reinforcement models of drug abuse are particularly useful when viewed through the lens of the central nervous system and the neurobiological underpinning of drug abuse.

5.4 NEUROBIOLOGY OF DRUG REWARD

Research in the field of drug reward has identified dopamine as the key neurotransmitter and its actions within the nucleus accumbens as being central to the study of brain reward and motivation (Wise, 2004; Di Chiara 1988). The NAc is the site of convergence for

multiple sensory and reward signals within the brain. It is within the NAc where cocaine exerts its primary rewarding and motivating effect (Carelli & Deadwyler, 1996; Peoples et al, 1997). DA cell activity is evident in three phases of the reward condition: preparatory, consummatory and post-consummatory (Schultz et al, 1993; Ljugdberg et al, 1992; McCullough & Salamone, 1992; Bayer & Glimcher 2005). As this is the case it appears that DA activity could be considered to reflect the motivational relevance of the stimuli as it is weighed against the motivational state of the animal. Evidence to corroborate this indicates that midbrain DA neurons activate with a bias in favor of appetitive rather than aversive stimuli or events (Mirenowicz & Schultz, 1996).

Acetylcholine also plays a role in natural reward and drug self-administration. Ach has a strong presence in the striatum (Butcher & Butcher 1974; Pisani, 2001; Smith et al, 2004, as well as being involved in memory (Imperato et al, 1993; Hasselmo & Fehlau, 2001) learning (Legault et al, 2006; McIntyre et al, 1998), attention (Baxter & Chiba, 1999; Robbins, 2002) and general motivation and arousal (Mesulam, 1996).

Nicotinic ACh receptors are located throughout both the mesolimbic and the nigral-striatal circuit. Drugs that activate of the nAChR will maintain self-administration behavior (Dadmarz & Vogel, 2003). However, emerging evidence indicates that nicotine self-administration is not as robust as other drugs, e.g. psychostimulants, in maintaining self-administration behavior. Although nAChRs have been localized to the cell body of the DA neuron within the VTA (Azam et al, 2002; Wooltorton et al, 2003), nicotinic receptors are primarily presynaptic at the glutamatergic and GABAergic terminals within the VTA (Wonnacot et al, 1997). In the accumbens the nAChRs have been localized to

the presynaptic DA terminals (Zoli et al, 2002; Britt & McGehee 2008). Thus it would seem that ACh acting through the nicotinic receptor exerts its influence through mass-action or volume transmission (Descarries et al, 1997). Strong cholinergic activity in the nucleus would eventually activate these presynaptic nicotinic receptors to cause a feed forward cascade of increasing activation. Under the LgA paradigm this view of nAChR as being modulatory is consistent with data indicating that activation of nAChR alone does not produce escalated nicotine intake.

DA cell burst firing: control by GLU and ACh

Dopamine cells are tonically active punctuated by temporal shifts in firing patterns i.e. rapid bursts of neurotransmission (Morris et al, 2004). The burst firing of the DA cell encodes information about salient stimuli including information regarding reward and expectation of reward (Schultz, 1998; Heien & Wightman, 2006). Burst firing has been shown to be a relevant temporal signal, largely due to the increase in NAc DA release that occurs following bursting activity from the DA cell (Schotanus & Chergui, 2008). Both in vivo and in vitro data report that GLU, particularly projections coming from the mPFC, control DA burst firing from within the VTA (Grace & Bunney, 1984; Johnson et al, 1992). However, ACh—acting through nAChRs—modulates the GLU neurochemical signal by acting at the GLU terminal; which ultimately results in changes to the DA cell tonic and bursting activation (Schilstrom et al, 2003). Experimental evidence confirms the importance of ACh in DA cell burst firing (Lodge & Grace, 2006). Indeed, it may be through these modulatory nAChRs that MEC may be acting in order to blunt the increase of cocaine intake during LgA conditions as was reported in chapters two and three of this

thesis. Furthermore, it has been shown that glutamate neurotransmission has been linked to increased cocaine intake: Kenny and colleagues experimentally demonstrated blockade of the metabotropic mGLUR5 receptor decreased cocaine consumption under LgA conditions. This was reasoned to occur as a result of decreased brain reward function (Kenny et al, 2005).

Neurobiological changes to the mesolimbic DA system appears to be a feature common to all chronically used drugs of abuse (Nestler, 1996). Several groups have attempted to ascertain what neurobiological adaptations underlie the transition from low levels of cocaine intake to escalated levels of drug intake following a period of extended access to cocaine. Others have reported increased cocaine intake over time i.e. months. The key difference is the time course. Deroche and colleagues demonstrated increased cocaine intake in rats self-administering the drug over several weeks (DeRoche et al, 1999). However, the LgA protocol seems unique in its ability to induce escalated drug intake in such a short time. It is apparent that the LgA is more than the sum of access time to self-administer cocaine. Long-access self-administration protocols induces a hypersensitivity in the CRF system of rats, which further primes the animal for increased motivation to self-administer cocaine (Specio et al, 2008). Using the LgA model of drug self-administration Ahmed and colleagues reported significant differences in gene transcription resulting in an up-regulation of gene products responsible for controlling synaptogenisis and post-synaptic proteins involved in neurotransmission. Gene transcriptional differences were seen in the NAc, amygdala, VTA, septum, prefrontal cortex and the VTA. However the majority of transcriptional change occurred in the

lateral hypothalamus, which projects GLU to the VTA and in turn receives DA from the same nuclei (Ahmed et al, 2005). This is consistent with work by Ferrario and colleagues demonstrating synaptic reorganization in the NAc following exposure to the LgA self-administration protocol (Ferrario et al, 2005). These differences may either affect, or are affected by, a change in DA neurotransmission within the NAc (Ahmed et al, 2004; Ben-Shahar et al, 2006).

5.5 CONCLUSION

It may be the case the nicotinic receptors within the VTA, acting both presynaptically and postsynaptically, are affecting reward related processing via their actions on the DA cell. Presynaptically, nicotinic receptors located on glutamate terminals enhance GLU release and therefore indirectly promote burst firing activity of the DA cell. Although it remains to be tested experimentally the development of escalated cocaine, as a function of LgA, is almost certainly being driven by burst firing of the DA cells within the VTA. DA cells have been shown to be highly entrained to fire in the presence of novel stimuli (Schultz, 1993). The LgA paradigm is also one of novelty, at least initially and data resulting from LgA is more than the sum of its parts. For example animals with short access do not increase their drug intake to the same degree LgA do, despite equivalent time exposed to cocaine (Roberts et al, 2002). The dramatic increase in cocaine self-administration occurs within 7 to 10 days of the lengthened self-administration time. Following the initial rise in cocaine consumption a plateau effect of drug consumption is observed. It is possible that these two phases of escalated cocaine intake represent distinct differences in the DA cell firing patterns. DA activity is the primary force behind greater and greater

amounts of cocaine consumption, at least initially. However, over time other systems replace DA in order of prominence. As has been previously discussed neurobiological adaptations are occurring as a function of increased DA activity, which may replace the prominence of DA activity over time. Nicotinic receptors may have dual roles in both the production and later maintenance of escalated cocaine consumption. Many others have described the LgA protocol as being a sensitization to the appetitive aspects of drug consumption rather than the consummatory aspects (Ahmed et al, 2006; Robinson & Berridge 1993). If this is the case then data presented here, with regards to the importance of the nicotinic acetylcholine receptor, and compulsive drug intake is in agreement with clinical work that demonstrates the use of nicotine in the treatment of obsessive-compulsive disorder (OCD; Lundberg et al, 2004; Pasquini et al, 2005). Although OCD is quite different than drug dependence both disorders exhibit a loss-of-control aspect of motivated behavior. It may therefore be the case the nicotinic receptors are fundamental modulators of the DA aspect of motivation.

This thesis has provided evidence that nAChRs are involved in the escalation of cocaine intake that is observed by allowing an animal an extended period of time in which to self-administer cocaine. The nAChR antagonist MEC is a non-selective nicotinic antagonist. MEC preferentially binds the non-α 7 nAChR subgroup of receptors. Research has shown that the β2 subunit is often implicated in drug self-administration (Connolly et al, 1992). Furthermore, others have demonstrated the importance of the α-7 homopentameric configuration, located presynaptically on the GLU terminals in the VTA, to be important for drug self-administration (Pidoplichko et al, 2004). Because

MEC is a broad spectrum nAChR antagonist future studies should employ the long-access followed by the antagonist microinjection protocol using more selective antagonists. The pre and post-synaptic α7 receptors could be selectively targeted with methyllycaconatine (MLA) which has shown to have preferential binding at these receptors. In order to target the α4β2 subunit containing receptors' the antagonist of dehydro-beta-eurothrodine (DHβE) should also be employed.

Future studies that systemically antagonize the nAChRs could further address the ability of MEC to blunt the escalation of cocaine intake under the long-access protocol. Work from other researchers in the field indicates that animals titrate to their "preferred" level of cocaine activation (Mantsch et al, 2001). If MEC is devaluing the motivation to self-administer cocaine then increasing the unit dose of cocaine each day through the initial five day period may provide a more concrete answer.

Other studies to address the mechanisms of action would expand on the data obtained in chapter four. Using the microdialysis procedure to sample recovered DA from the VTA; and reverse dialysis MEC during short access self-administration and long-access self administration. If VTA DA is a proxy measurement of DA cell activation then the ensuing self-administration behavior by the animals following MEC treatment should be similar to the results reported in Chapter 3. Under this same scenario ACh from the VTA should also be recovered in the same fashion as Chapter 4 and then again under these proposed future direction. Because microdialysis is such a powerful tool it would be possible to partition which nicotinic receptors may be modulating the DA signal in the

VTA following systemic cocaine injections. For example if similar results were obtained using the α7 receptor antagonist MLA then it could be argued that the observed effect of intra VTA DA increase following cocaine is being mediated by the GLU terminal; given the colocalazition of the α7 receptor. A DHβE specific antagonist giving similar results to the ones in chapter 4 would argue in favor of soma localized nicotinic receptors being responsible for intra VTA DA release. Eventually, intra VTA GLU levels should also be measured. Relative changes in this neurotransmitter within the VTA would indicate whether the pre/ post synaptic nicotinic receptors are more important with regards to MEC attenuated cocaine recovery in the VTA.

Currently there is renewed interest in the VTA ACh input from the pontine nuclei (see Maskos, 2008 for a review). The modulatory ability of the nAChR possibly makes these receptors important for altering DA cell activity. Therefore, nAChR maybe an important pharmacological target for the treatment of individuals already addicted to drugs of abuse, particularly cocaine.

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