ACUTE & SUBCHRONIC NMDA RECEPTOR BLOCKADE ALTERS NICOTINE-EVOKED DOPAMINE RELEASE

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

ACUTE & SUBCHRONIC NMDA RECEPTOR BLOCKADE ALTERS NICOTINE-EVOKED DOPAMINE RELEASE

Presented by Kelli R. Rodvelt

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LIST OF ABBREVIATIONS

Phencyclidine – PCP
N-Methyl-D-Aspartate – NMDA
Dopamine – DA
Prepulse Inhibition – PPI
Prefrontal Cortex – PFC
Gamma-Aminobutyric Acid – GABA
Nucleus Accumbens – NAc
Ventral Tegmental Area – VTA
Mesocortical – MC
Mesolimbic – ML
Glutamate – GLU
Ventral Striatum – VST
Pedunculopontine Tegmentum – PPT
Conditioned Taste Aversion – CTA
Latent Inhibition – LI
Nicotinic Acetylcholine Receptors – nAChRs
Dopamine Transporter – DAT
Human Embryonic Kidney - HEK

ABSTRACT

Blockade of ionotropic glutamate receptors can induce changes in central dopamine and glutamate circuits, which model the symptoms of schizophrenia. Nicotine evokes dopamine release through activation of nicotinic acetylcholine receptors, and human research indicates that nicotine improves negative and cognitive symptoms of schizophrenia. The objective was to determine the effect of the glutamate receptor antagonist, ketamine, on the function of nicotinic receptors that mediate dopamine release. Ketamine did not have intrinsic activity to evoke dopamine release from rat striatal or prefrontal cortical slices. Acute NMDA receptor blockade augmented the effect of nicotine to evoke dopamine release. To model progression of schizophrenia, rats received injections of either a high or low dose of ketamine or vehicle for 30 days and then nicotine-evoked dopamine release was measured. Subchronic NMDA receptor blockade altered the effect of nicotine to evoke dopamine release. Overall, these data indicate that nicotinic receptor function is altered in this model of schizophrenia, and support a role for nicotinic receptors in schizophrenia treatment.

INTRODUCTION

Schizophrenia is a chronic, severe neuropsychiatric disorder that has been studied for decades. However, scientists are still baffled by the underlying mechanisms of this devastating disorder. Schizophrenia affects cognition and behavior and is characterized by a combination of positive and negative symptoms and cognitive dysfunction. Positive symptoms include hallucinations, delusions, as well as thought and movement disorders ("Schizophrenia", 2006). Negative symptoms include social withdrawal, flattened affect, and decreased motivation and reward function ("Schizophrenia", 2006). Cognitive dysfunction includes deficits in attention, learning, memory, and executive function ("Schizophrenia", 2006). Scientists have developed preclinical methods to study behavioral changes and underlying mechanisms associated with positive and negative symptoms and cognitive dysfunction of schizophrenia.

Pharmacological Animal Models of Schizophrenia

Animal models of schizophrenia have been developed in order to study cause, progression, and treatment of this disorder. Most of these models are based on neurotransmitter manipulation, namely pharmacological models of schizophrenia. Animals exhibit changes in behavior associated with positive and negative symptoms and cognitive dysfunction of schizophrenia when treated with drugs like ketamine and phencyclidine (PCP), noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonists or indirect dopamine (DA) receptor agonists, like amphetamine. Behavioral changes associated with positive

symptoms in animals include hyperactivity and stereotypy. Changes in behavior associated with negative symptoms in animals include social withdrawal and decreased reward function. Behavioral changes associated with cognitive dysfunction in animals include working memory and learning impairments. Pharmacological models of schizophrenia have primarily been based upon two major neurochemical theories of schizophrenia, the DA and NMDA hypotheses of schizophrenia.

The Dopamine Hypothesis

The DA hypothesis of schizophrenia proposes that dysfunction of dopaminergic neurotransmission is the underlying cause of the symptoms of the disorder (A. Carlsson, 1988; Marcotte, Pearson, & Srivastava, 2001). More specifically, an excess in subcortical DA activity in response to amphetamine administration results in a transient emergence or exacerbation of positive symptoms of schizophrenia in patients diagnosed with schizophrenia (Abi-Dargham et al., 1998; Laruelle, 1998). The development of the DA hypothesis of schizophrenia was based on two major observations (1) stimulant abuse (amphetamine and cocaine) enhances dopaminergic neurotransmission which resembles positive symptoms of schizophrenia and (2) antipsychotic medications block D_2 DA receptors which improves positive symptoms of schizophrenia (Tsai & Coyle, 2002). Hence, altered DA neurotransmission causes behavioral and neurochemical changes associated with positive symptoms of schizophrenia. The production of positive symptoms is caused by hyperactivity of mesolimbic and nigrostriatal dopaminergic neurons (A. Carlsson, 1995; Seeman, 1987). The

mesolimbic DA pathway has been implicated in motivation and reinforcement, and the nigrostriatal DA pathway has been implicated in motor function. Psychostimulants such as amphetamine (Flagstad et al., 2004; Nishijima et al., 1996) and methylphenidate (Volkow, Wang, Fowler, & Ding, 2005; Volkow et al., 1999) increase extracellular DA release and produce psychotic symptoms (Angrist & Gershon, 1970; Morland, 2000). Hyperactivity (Peleg-Raibstein, Sydekum, Russig, & Feldon, 2006; Segal & Kuczenski, 1997) and stereotypy (Segal & Kuczenski, 1997), behavioral changes associated with positive symptoms of schizophrenia, are observed in rats administered escalating doses of amphetamine. Furthermore, hyperactivity and stereotypy are more profound with repeated psychostimulant abuse, like cocaine (Caster, Walker, & Kuhn, 2005). Prepulse inhibition (PPI) is an operational measure of sensorimotor gating and is impaired in schizophrenia (Braff & Geyer, 1990). In animal models of schizophrenia, the inhibition of the response to the startle reflex is measured, which is cause by a preceding weak stimulus. Rats treated with DA agonists (Swerdlow et al., 2000) and amphetamine (Peleg-Raibstein, Sydekum, Russig, & Feldon, 2006) exhibit disrupted PPI. However, as discussed below, the hyperactivity of dopaminergic neurons, by itself, cannot account for negative symptoms and cognitive dysfunction in schizophrenia (A. Carlsson, 1988).

Negative symptoms and cognitive dysfunction are thought to be derived from hypoactivity of mesocortical dopaminergic neurons in prefrontal cortex (PFC) (Dworkin & Opler, 1992). Later advances confirmed that a hypodopaminergic state in cortical regions plays a contributory role to the

pathophysiology underlying schizophrenia. However, these claims were based on frontal lobe ablations and injuries in primates and humans, respectively (Davis, Kahn, Ko, & Davidson, 1991). Moreover, administration of amphetamine (Daniel et al., 1991) and apomorphine (Daniel, Berman, & Weinberger, 1989), a DA agonist, increase prefrontal DA activity and improve negative symptoms and cognitive dysfunction (Daniel et al., 1991) indicating that hyperdopaminergia does not induce all symptoms associated with schizophrenia. Thus, pharmacological models of schizophrenia based on the DA hypothesis lack integration of positive and negative symptoms and cognitive dysfunction in order to produce a sufficient model of schizophrenia.

The N-methyl-D-aspartate Hypothesis

The NMDA hypothesis of schizophrenia proposes that antagonism of NMDA receptors may be the underlying cause of symptoms of the disorder. Hence, altered glutamate neurotransmission causes behavioral and neurochemical changes associated with symptoms of schizophrenia. More specifically, glutamatergic hypofunction in cortical areas induces dopaminergic hyperfunction in subcortical areas (A. Carlsson, Waters, & Carlsson, 1999; M. Carlsson & Svensson, 1990; Olney & Farber, 1995a). PCP and ketamine, belong to a group of arylcyclohexylamines (Ellison, 1995), which were developed as dissociative anesthetics in the late 1950s (Collins, Gorospe, & Rovenstine, 1960) and have neurotoxic effects at high doses (Olney et al., 1991). Patients began to exhibit adverse side effects after administration of subanesthetic doses of PCP and ketamine, which were characterized as resembling positive and negative

symptoms and cognitive dysfunction of schizophrenia (Ellison, 1995; Javitt, 1987; Javitt & Zukin, 1991). Luby and colleagues first proposed a model of schizophrenia based upon the psychotomimetic effects of PCP (Luby, Gottlieb, Cohen, Rosenbaum, & Domino, 1962). Hence, researchers began using noncompetitive NMDA receptor antagonists as a means to model the pathophysiology of schizophrenia more comprehensively.

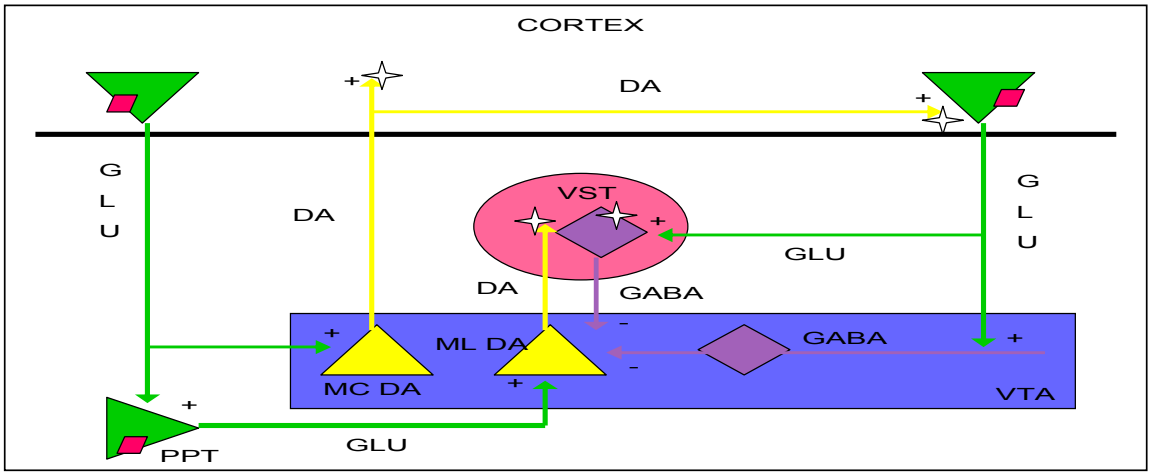
NMDA receptor antagonists, PCP, ketamine, and dizocilpine (MK-801), induce a psychotomimetic state resembling schizophrenia including positive and negative symptoms and cognitive dysfunction (Imre, Fokkema, Den Boer, & Ter Horst, 2006; Javitt & Zukin, 1991; Jentsch, Tran, Le, Youngren, & Roth, 1997; Marcotte, Pearson, & Srivastava, 2001; Sams-Dodd, 1998). Continuous infusion of amphetamine alone or PCP alone dose-dependently induces stereotyped behavior and hyperactivity associated with positive symptoms of schizophrenia in rats, but only PCP induces social withdrawal associated with negative symptoms of schizophrenia in rats (Sams-Dodd, 1998). PCP also impairs performance in learning and memory tasks associated with cognitive dysfunction of schizophrenia in rats (Campbell et al., 2004; Handelmann, Contreras, & O'Donohue, 1987; Kesner, Dakis, & Bolland, 1993). Specifically, Jentsch and colleagues observed impaired performance in rodents after subchronic PCP treatment during working memory tasks (Jentsch, Tran, Le, Youngren, & Roth, 1997); whereas, amphetamine treatment improved cognitive dysfunction (Rasmussen, Overgaard, Hildebrandt-Eriksen, & Boysen, 2006). Taken together, NMDA receptor antagonists induce positive and negative symptoms and

cognitive dysfunction associated with schizophrenia in rats, but DA agonists can only induce positive symptoms associated with schizophrenia in rats. Therefore, pharmacological models of schizophrenia based on the NMDA hypothesis are sufficient to produce positive and negative symptoms and cognitive dysfunction of schizophrenia.

Glutamatergic Facilitation of Dopaminergic Neurotransmission

Recently, integration of the two hypotheses, the DA hyperfunction and NMDA hypofunction hypotheses of schizophrenia has been heavily implicated in the pathophysiology underlying schizophrenia. Carlsson and colleagues proposed a model in which cortical glutamatergic projections modulate DA activity of mesolimbic and mesocortical DA pathways (A. Carlsson, Waters, & Carlsson, 1999) (Figure 1). NMDA receptor antagonists bind primarily to the PCP binding site on the NMDA receptor, which inhibits glutamate neurotransmission (Javitt & Zukin, 1991). Blockade of NMDA receptors, located on glutamatergic neurons, by NMDA receptor antagonists decreases DA release from mesocortical DA neurons. It also has differential effects on mesolimbic DA neurons as mesolimbic DA neurons are innervated polysynaptically by glutamatergic and gamma-aminobutyric acid (GABA)-ergic interneurons (A. Carlsson, Waters, & Carlsson, 1999). Thus, NMDA receptor antagonists inhibit GABAergic interneurons' inhibitory tone on mesolimbic DA neurons, which increases stimulant-induced DA release in the striatum (Balla, Koneru, Smiley, Sershen, & Javitt, 2001; Balla, Sershen, Serra, Koneru, & Javitt, 2003; Javitt et al., 2004).

Figure 1. Cortical-Subcortical Neurocircuitry Top. Normal Neurocircuitry: Cortical glutamatergic projections to subcortical DA neurons facilitate DA activity directly via MC DA neurons. Also DA activity is facilitated via indirect glutamatergic projections to subcortical ML DA neurons through the pedunculopontine tegmentum. Cortical glutamatergic projections to subcortical GABA interneurons inhibit DA activity in subcortical areas. Middle. Disrupted Neurocircuitry: NMDA receptor antagonists block glutamate transmission, causing dysregulation of the circuit subcortically. Decreased glutamate release results in hypoactivity of MC DA neurons, and decreased inhibitory tone of GABA interneurons on ML DA neurons, which results in hyperactivity of ML DA neurons. Bottom. Schizophrenia Neurocircuitry: Dysregulation of cortical and subcortical neurocircuitry results in decreased DA release in cortical areas via MC DA neurons, and increased DA release in subcortical areas via ML DA neurons. DA = Dopamine, GLU = Glutamate, GABA = γ-aminobutyric acid, VST = Ventral striatum, VTA = Ventral tegmental area, PPT = Pedunculopontine tegmentum, MC DA = Mesocortical DA neurons, ML DA = Mesolimbic DA neurons. Upside down and sideways triangles = Glutamate neurons, Right-side up triangles = DA neurons, diamonds = GABA interneurons, parallelograms = NMDA receptors, 4-pointed stars = nicotinic acetylcholine receptors (nAChRs). Adapted from (A. Carlsson, Waters, & Carlsson, 1999; Laruelle, Kegeles, & Abi-Dargham, 2003).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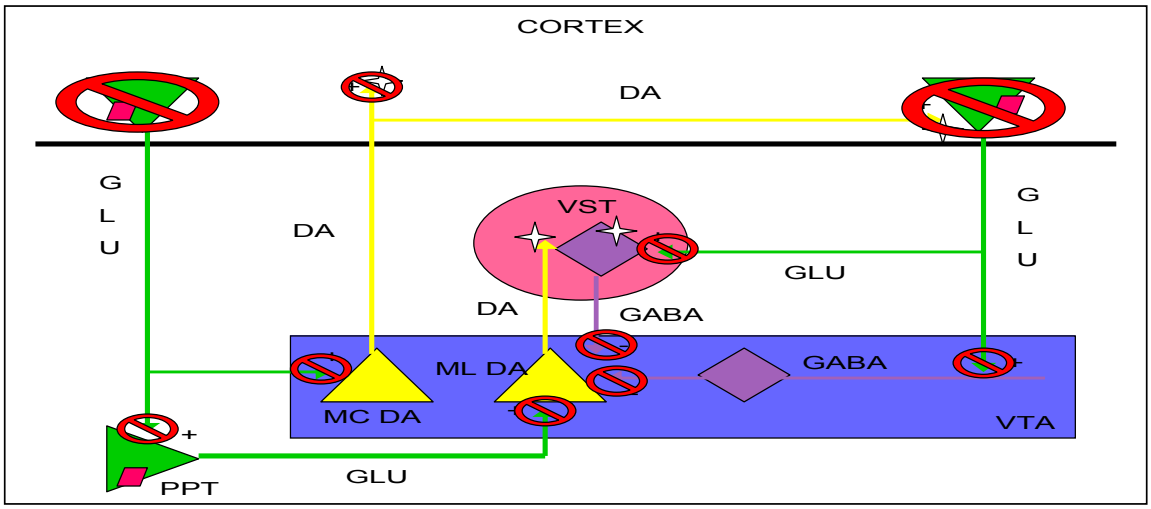
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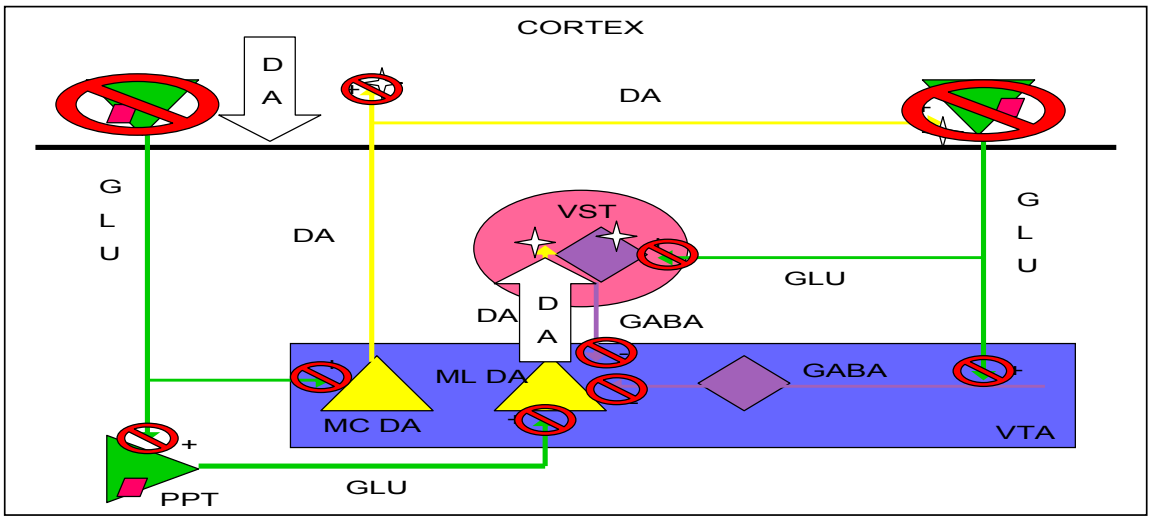
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Futhermore, an increase in DA release in nucleus accumbens (NAc) and a decrease in DA release in PFC results from blockade of glutamatergic transmission from ventral tegmental area (VTA) (Takahata & Moghaddam, 2000). Taken together, all of these findings support the model proposed by Carlsson and colleagues, where blockade of NMDA receptors induces decreased DA release in cortical regions and increased DA release in subcortical regions (A. Carlsson, Waters, & Carlsson, 1999).

However, NMDA receptor blockade is also sufficient to account for hyperactivity of dopaminergic neurotransmission in PFC as it induces a state of hyper-responsivity to stimulants (Balla, Koneru, Smiley, Sershen, & Javitt, 2001; Balla, Sershen, Serra, Koneru, & Javitt, 2003; Javitt et al., 2004). More specifically, an increase in DA release in PFC may be caused by an initial increase in endogenous excitatory amino acid release, such as glutamate and aspartate (Bustos et al., 1992; Liu & Moghaddam, 1995), which in turn increases DA release by stimulating non-NMDA excitatory amino acid receptors (Jedema & Moghddam, 1996; Moghaddam, Adams, Verma, & Daly, 1997). Ketamine injected acutely, dose-dependently increases DA and glutamate release in PFC and striatum (Moghaddam, Adams, Verma, & Daly, 1997). Subchronic injection of ketamine increases basal DA levels in PFC; however, subsequent ketamine challenge to animals pre-treated subchronically with ketamine blocks ketamine-induced extracellular DA release measured by in vivo microdialysis (Lindefors, Barati, & O'Connor, 1997). This evidence contradicts the model proposed by Carlsson and colleagues as it suggests that hyperactivity of DA neurons in

cortical and subcortical regions induces neurochemical changes underlying schizophrenia (A. Carlsson, Waters, & Carlsson, 1999). Thus, cortical glutamatergic neurotransmission modulating cortical and subcortical dopaminergic activity is important for understanding the pathophysiology underlying schizophrenia.

Effects of NMDA Antagonists in Behavioral Experiments

Acute and repeated administrations of PCP and ketamine have been used as pharmacological animal models of schizophrenia. Acute administration of subanesthetic doses of PCP and ketamine induce hyperlocomotion (Adams & Moghaddam, 1998; Imre, Fokkema, Den Boer, & Ter Horst, 2006; Irifune, Shimizu, Nomoto, & Fukuda, 1995; Mandryk, Fidecka, Poleszak, & Malec, 2005; McCullough & Salamone, 1992; Sams-Dodd, 1995; Wilson et al., 2005) and stereotypy (Adams & Moghaddam, 1998; Sams-Dodd, 1995, , 1996, , 1997) associated with positive symptoms of schizophrenia. Social withdrawal associated with negative symptoms of schizophrenia is induced by acute administrations of subanesthetic doses of PCP (Sams-Dodd, 1995, , 1996, , 1997). Prior exposure to PCP decreases reward function as measured by voluntary sucrose consumption associated with negative symptoms of schizophrenia (Turgeon & Hoge, 2003). Cognitive deficits associated with cognitive dysfunction of schizophrenia are induced by acute administration of PCP and ketamine. Acute administration of subanesthetic doses of PCP and ketamine impairs performance in working memory tasks assessed by spatial delayed alternation tasks (Adams & Moghaddam, 1998; Imre, Fokkema, Den

Boer, & Ter Horst, 2006; Moghaddam, Adams, Verma, & Daly, 1997) and by spatial reference/working memory tasks (He et al., 2006). Acute ketamine and PCP injection also produces significant learning deficits as measured by conditioned taste aversion (CTA) and latent inhibition (LI) tasks. Ketamine blocks CTA (Aguado, del Valle, & Perez, 1997; Aguado, San Antonio, Perez, del Valle, & Gomez, 1994; Mickley et al., 1998) and disrupts LI (Gallo, Bielavska, Roldan, & Bures, 1998); however, PCP also enhances LI when administered prior to conditioning trials (Klamer et al., 2005; Palsson et al., 2005). Therefore, acute administration of NMDA receptor antagonists is sufficient to induce behavioral changes associated with positive and negative symptoms and cognitive dysfunction of schizophrenia; however, these behavioral changes are short-lived as they are most likely induced by direct drug effects (Jentsch, Taylor, & Roth, 1998).

The effects of repeated exposure are generally more persistent and resemble the psychopathology of schizophrenia more closely than the effects of acute exposure (Jentsch & Roth, 1999). The effects of repeated exposure are due to changes in the neurocircuitry induced by the drug rather than direct drug effects (Jentsch, Taylor, & Roth, 1998). Repeated administration of subanesthetic doses of PCP and ketamine induce hyperlocomotion (Leccese, Marquis, Mattia, & Moreton, 1986; Sams-Dodd, 1995, , 1998) and stereotypy (Leccese, Marquis, Mattia, & Moreton, 1986; Sams-Dodd, 1995, , 1998, , 1999) associated with positive symptoms of schizophrenia. Subchronic administration of subanesthetic doses of PCP enhances amphetamine- and stress-induced

hyperlocomotion (Balla, Koneru, Smiley, Sershen, & Javitt, 2001; Balla, Sershen, Serra, Koneru, & Javitt, 2003; Jentsch, Taylor, & Roth, 1998). Social withdrawal associated with negative symptoms of schizophrenia is induced by repeated administration of subanesthetic doses of PCP (Sams-Dodd, 1995, , 1996, , 1998, , 1999; Tanaka et al., 2003) and MK-801 (Tanaka et al., 2003). Cognitive deficits associated with schizophrenia are induced by repeated administration of subanesthetic doses of PCP and ketamine. Repeated administration of subanesthetic doses of ketamine impairs performance in working memory tasks assessed by spatial delayed alternation tasks (Jentsch, Tran, Le, Youngren, & Roth, 1997) and produces learning effects as measured by LI (Becker et al., 2003). Together these findings show that subanesthetic doses of NMDA receptor antagonists administered repeatedly induce changes in behavior associated with positive and negative symptoms and cognitive dysfunction of schizophrenia. However, anesthetic doses of ketamine elicit little psychotomimetic effects (White, Ham, Way, & Trevor, 1980; White, Way, & Trevor, 1982) indicating that anesthetic doses of ketamine are not sufficient to induce symptoms associated with schizophrenia. Pharmacological animal models of schizophrenia which use acute and repeated administration of subanesthetic doses of NMDA receptor antagonists produce similar behavioral changes associated with positive and negative symptoms and cognitive dysfunction of schizophrenia. However, repeated administration of subanesthetic doses of NMDA receptor antagonists is a more valid pharmacological animal model of schizophrenia than acute administration of subanesthetic doses of NMDA receptor antagonists.

Effects of NMDA Antagonists in Neurochemical Experiments

Positive and negative symptoms and cognitive dysfunction of schizophrenia have been associated with changes in dopaminergic and glutamatergic neurotransmission. Amphetamine (Flagstad et al., 2004; Nishijima et al., 1996) and methylphenidate (Volkow, Wang, Fowler, & Ding, 2005; Volkow et al., 1999) increase extracellular DA release and produce positive symptoms associated with schizophrenia (Angrist & Gershon, 1970; Morland, 2000). It has been postulated that PFC dopaminergic hyperactivity is an underlying cause of the cognitive dysfunction associated with schizophrenia (Balla, Sershen, Serra, Koneru, & Javitt, 2003). Administration of subanesthetic doses of NMDA receptor antagonists, ketamine and MK-801, increase DA release in PFC and to a lesser extent in striatum after acute injection (Verma & Moghaddam, 1996). Injection of subanesthetic doses of PCP and ketamine also increases DA and glutamate efflux in PFC, NAc and striatum as measured by in vivo microdialysis (Adams & Moghaddam, 1998; Lindefors, Barati, & O'Connor, 1997; Moghaddam, Adams, Verma, & Daly, 1997; Nishijima et al., 1996). Blockade of D_2 DA receptors potentiates PCP-induced DA release in striatum (Yonezawa, Kuroki, Tashiro, Hondo, & Uchimura, 1995). Superfusion of PCP in vitro increases (> 1 μM) and decreases (0.1 μM) DA release in striatal slices (Ohmori, Koyama, Nakamura, Wang, & Yamashita, 1992). However, neurochemical effects of acute administration of NMDA receptor antagonists may reflect direct drug effects (Jentsch, Taylor, & Roth, 1998). Thus, acute administration of NMDA receptor antagonists induce neurochemical changes associated with positive and negative

symptoms and cognitive dysfunction of schizophrenia, but does not produce a valid pharmacological animal model of schizophrenia.

Pharmacological animal models of schizophrenia, which use repeated exposure, produce different neurochemical effects compared to those that use acute exposure. Microdialysis procedures measuring the effects of subanesthetic doses of PCP administered subchronically found no change in basal striatal, PFC and NAc DA levels; however, amphetamine challenge increases striatal, PFC and NAc DA release markedly, which suggests that NMDA receptor antagonists induce a state of hyper-responsivity to stimulants (Balla, Koneru, Smiley, Sershen, & Javitt, 2001; Balla, Sershen, Serra, Koneru, & Javitt, 2003; Javitt et al., 2004). NMDA receptor antagonists decrease striatal ^{11}C-raclopride (D_2 dopamine receptor-selective radioligand) binding (Breier et al., 1998; Tsukada et al., 2000), reflecting enhanced striatal synaptic DA concentrations (Breier et al., 1998) and, in contrast, no significant changes in DA concentrations in striatal extracellular fluid (Tsukada et al., 2000). However, animals pre-treated with subanesthetic doses of ketamine once daily for 7 days showed increases in basal DA levels, but attenuation of ketamine-induced increase in extracellular DA release in PFC after subsequent ketamine challenge (Lindefors, Barati, & O'Connor, 1997). However, cortical dopaminergic hypofunction has also been proposed to cause impairments in cognitive functions and negative symptoms of schizophrenia (Davis, Kahn, Ko, & Davidson, 1991; Weinberger, 1987; Williams & Goldman-Rakic, 1995). Animals injected subchronically with subanesthetic doses of PCP show a reduction in DA utilization in PFC, but not NAc 3 weeks

after the final injection, hence, reduced dopaminergic activity of mesocortical DA projections (Jentsch, Taylor, & Roth, 1998). Moreover, studies where dopaminergic and glutamatergic deficits in neurotransmission are observed after withdrawal from NMDA receptor antagonists show that behavioral and neurochemical effects are due to drug-induced neurobiological changes rather than direct drug effects (Jentsch, Taylor, & Roth, 1998). Repeated administration of ketamine or PCP antagonizes NMDA receptors, which blocks glutamate release (Javitt & Zukin, 1991). NMDA receptor hypofunction decreases DA release from mesocortical DA neurons. A reduction of mesocortical DA neuron function increases DA release from mesolimbic DA neurons (A. Carlsson, Waters, & Carlsson, 1999). This results in a decrease in DA in cortical regions, which is associated with negative symptoms and cognitive dysfunction of schizophrenia, and an increase in DA in subcortical regions, which is associated with positive symptoms of schizophrenia (Figure 1). It is important to note that subanesthetic doses of NMDA receptor antagonists are preferred because anesthetic doses of ketamine elicit little psychotomimetic effects (White, Ham, Way, & Trevor, 1980; White, Way, & Trevor, 1982) indicating that anesthetic doses of ketamine are not sufficient to induce symptoms associated with schizophrenia. Taken together, these findings reveal controversy among researchers, but, more importantly, provide evidence that neurochemical effects after withdrawal from repeated exposure to subanesthetic doses of NMDA receptor antagonists induce neurobiological changes. Thus, repeated

administration of subanesthetic doses of NMDA receptor antagonists provides a more valid pharmacological animal model of schizophrenia.

Nicotine and Schizophrenia

Nicotine, the active ingredient in tobacco, induces release of many neurotransmitters in brain (i.e., DA, GABA, glutamate, serotonin and acetylcholine) (Wonnacott, 1997). The primary site of action for nicotine is nicotinic acetylcholine receptors (nAChRs), a ligand-gated ion channel composed of five subunits (Sargent, 1993). nAChRs are located on terminal ends of dopaminergic neurons and GABAergic interneurons (Freedman, Wetmore, Stromberg, Leonard, & Olson, 1993; Wooltorton, Pidoplichko, Broide, & Dani, 2003) in brain, primarily hippocampus, basal ganglia and cortex (J. A. Court, Martin-Ruiz, Graham, & Perry, 2000). The functional properties of nAChRs are defined by their subunit composition. nAChRs are formed by heteromeric or homomeric assembly of α- and β-subunits. Nicotine-evoked [^{3}H]DA release in striatum is well established, more specifically involving α3β2* and α3β4* nAChRs (Miller, Sumithran, & Dwoskin, 2002) and also a mixture of different nAChR subtypes containing α4, α5, α6, β2 and β3 subunits (Champtiaux et al., 2003; Salminen et al., 2004; Sharples et al., 2000). Nicotine evokes DA release in a concentration-dependent manner (Cohen, Perrault, Voltz, Steinberg, & Soubrie, 2002; Grilli, Parodi, Raiteri, & Marchi, 2005; Nisell, Nomikos, & Svensson, 1994; Rapier, Lunt, & Wonnacott, 1988; Sacaan, Dunlop, & Lloyd, 1995; Sharples et al., 2000) via a direct mechanism by binding to nAChRs and opening ion channels. An open nAChR channel allows sodium, potassium and calcium ions

to move across the cell membrane (Mansvelder, van Aerde, Couey, & Brussaard, 2006), thus changing the resting membrane potential, and stimulating exocytosis of vesicles containing DA.

Preliminary experiments from our laboratory determined the potency and efficacy of nicotine to evoke DA release from rat striatal slices. Consistent with previous findings, nicotine evoked DA release in a concentration-dependent manner from rat striatal slices (unpublished findings) (Figure 2). The superfusion assay used in these experiments measures [^{3}H]DA overflow from each sample, which represents the amount of DA released from each slice. Regarding total [^{3}H] overflow, a significant main effect of Nicotine Concentration ($P<0.05$) was found. Post hoc tests revealed that total [^{3}H] overflow was greater in the presence of 10 - 100 μM nicotine than in the absence of nicotine. Fractional [^{3}H]DA release is the amount of [^{3}H]DA collected from each sample. Regarding fractional [^{3}H]DA release, significant main effects of Nicotine Concentration ($P<0.05$) and Time ($P<0.05$), and a significant Nicotine Concentration x Time interaction ($P<0.05$) were found (Figure 2 insert). Nicotine produced a time- and concentration-dependent increase in fractional [^{3}H]DA release. The EC_{50} value was calculated via nonlinear regression. EC_{50} values indicate the concentration that evokes 50% of the maximum DA release. Hence, the nicotine concentration required to evoke 50% of the maximum DA release was 42.8 μM.

Nicotine may also influence dopaminergic transmission via an indirect mechanism (i.e., not acting directly on nAChRs located on DA neurons). Dopaminergic afferents from the mesencephalic areas, such as VTA, synapse

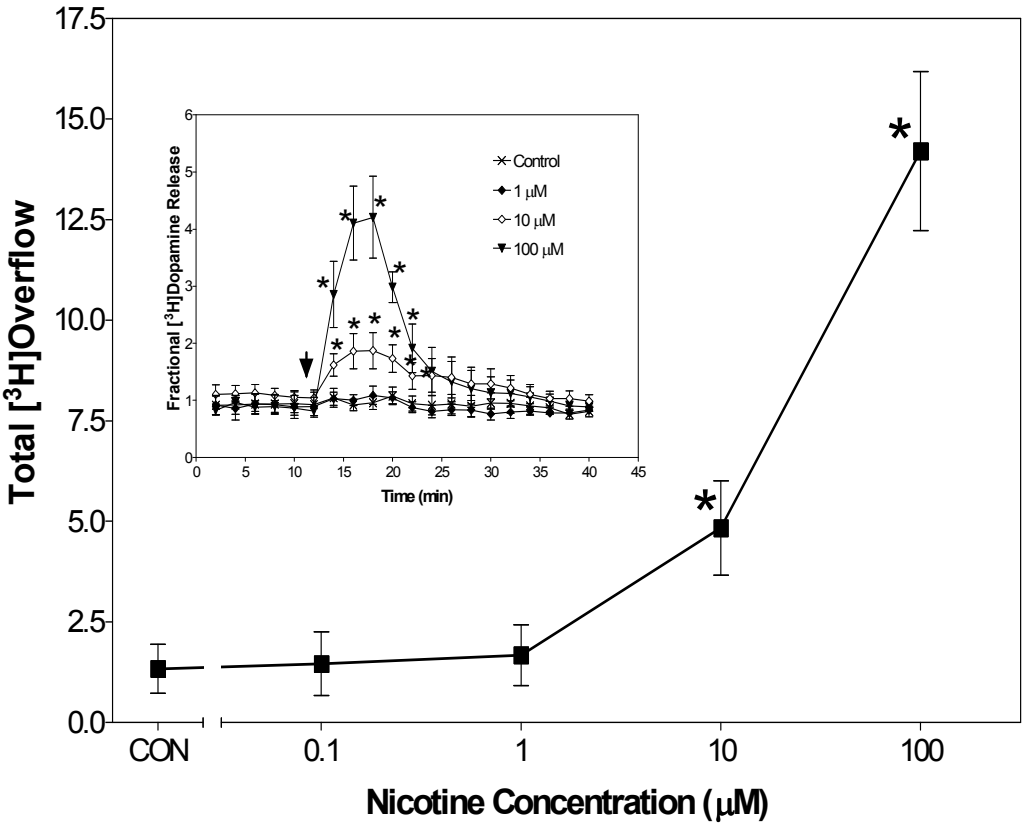

Figure 2. Nicotine produced a concentration-dependent increase in total [^{3}H] overflow from rat striatal slices preloaded with [^{3}H]DA. Data are expressed as the mean (± S.E.M.) total [^{3}H] overflow after the addition of nicotine to buffer. Inset shows the time course of nicotine-evoked [^{3}H]DA release. Data are expressed as the mean (± S.E.M.) fractional release. Asterisks designate a significant ($P<0.05$) difference from the control condition (superfusion in the absence of nicotine) and the arrow designates the addition of nicotine to buffer. *CON, control condition.* (n = 9 rats)

with the GABAergic interneurons in PFC (Li, Park, Kim, & Kim, 2004). Nicotine significantly enhances glutamate and GABA release in hippocampus (Kanno et al., 2005) and stimulates GABAergic synaptic transmission in NAc after amphetamine sensitization (de Rover et al., 2004). As stated above, increases in DA release in PFC may be caused by an initial increase in endogenous excitatory amino acid release, such as glutamate and aspartate, (Bustos et al.,

1992; Liu & Moghaddam, 1995) which in turn increases DA release by stimulating non-NMDA excitatory amino acid receptors (Jedema & Moghddam, 1996; Moghaddam, Adams, Verma, & Daly, 1997). Thus, nicotine causes DA release via a direct and/or indirect mechanism in cortical and subcortical regions.

People who suffer from schizophrenia are two to four times more likely to smoke cigarettes than people who do not have schizophrenia (Sacco, Bannon, & George, 2004). People diagnosed with schizophrenia typically use cigarettes as a therapeutic agent for negative symptoms and cognitive dysfunction of schizophrenia (Kumari & Postma, 2005; Leonard et al., 2001; Levin, 2002). Additionally, people with schizophrenia who also smoke extract more nicotine from cigarettes as compared to smokers without schizophrenia (Olincy, Young, & Freedman, 1997). As stated above, cortical dopaminergic hypofunction is postulated to cause negative symptoms and cognitive dysfunction of schizophrenia (Davis, Kahn, Ko, & Davidson, 1991; Weinberger, 1987; Williams & Goldman-Rakic, 1995), and the ability of nicotine to increase DA release in the PFC may temporarily improve these symptoms (Dalack, Healy, & Meador-Woodruff, 1998). Jentsch and colleagues showed that subchronic injection of PCP decreased PFC DA utilization and had no effect on striatal and NAc DA utilization after 24 hours to 3 weeks withdrawal, which suggests a reduction of mesocortical DA neuron activity (Jentsch, Taylor, & Roth, 1998; Jentsch, Tran, Le, Youngren, & Roth, 1997). Olney and Farber (1995a, b) observed neurotoxic effects induced by NMDA receptor antagonist administration (Olney & Farber, 1995a, , 1995b), which may cause a reduction in glutamatergic and GABAergic

axon terminals in the brain (Mirnics, Middleton, Marquez, Lewis, & Levitt, 2000), which then results in a decrease in DA release. People diagnosed with schizophrenia have a reduction in the number of high (α4β2)- and low (α7)-affinity nAChRs that are expressed in the brain (Breese et al., 2000; J. Court et al., 1999; Freedman, Hall, Adler, & Leonard, 1995; Leonard et al., 2000; Mexal et al., 2005) and smokers lack up-regulation of their high-affinity nAChRs as compared to smokers without schizophrenia (Breese et al., 2000), which may also contribute to decreases in DA release. Activation of α4β2* and α7* nAChRs occurs at low (100 - 250 nM) nicotine concentrations, but α4β2* desensitize more than α7* nAChRs. Furthermore, at higher (250 nM) nicotine concentrations, α4β2* nAChRs are completely desensitized within minutes, but α7* nAChRs remain available for activation (Mansvelder & McGehee, 2002; Wooltorton, Pidoplichko, Broide, & Dani, 2003). The targeting of low-affinity nAChRs could explain why people with schizophrenia smoke more heavily and extract more nicotine from cigarettes (Kumari & Postma, 2005). Together, these findings indicate that it takes higher concentrations of nicotine to produce effects in people who have schizophrenia.

The model proposed by Carlsson and colleagues suggests that mesocortical DA neurons in VTA are directly innervated by cortical glutamatergic neurons and NMDA receptor blockade inhibits glutamate from facilitating DA release in PFC via mesocortical DA neurons (A. Carlsson, Waters, & Carlsson, 1999)(Carlsson et al., 1999) (Figure 1). Ketamine (IC_{50} = 2.8 μM) and MK-801 (IC_{50} = 15 μM) inhibit nAChR-mediated current (Buisson & Bertrand, 1998;

Furuya et al., 1999), which suggests that NMDA receptor antagonists are also acting as nAChR antagonists (Kumari & Postma, 2005; Pereira et al., 2002). However, the effect of ketamine treatment on nicotine-evoked DA release has not been determined. Nicotine exposure may be sufficient to overcome this inhibition in order to increase DA release in PFC by targeting nAChRs located on DA neurons as they may be more sensitive to nicotine after exposure to ketamine. Thus, it is important to identify the effect of ketamine exposure on nAChRs and nicotine pharmacology.

Behavioral studies have shown that acute and repeated administration of nicotine or selective nicotinic agonists improve performance on working memory tasks assessed by radial arm maze (Levin, 2002; Levin, Bettegowda, Blosser, & Gordon, 1999; Levin & Christopher, 2003; Levin, Kaplan, & Boardman, 1997; Levin, Rose, & Abood, 1995). While acute administration of nonspecific nicotinic antagonist, mecamylamine, into VTA, substantia nigra, and ventral hippocampus impairs performance on working memory tasks assessed by radial arm maze (Kim & Levin, 1996; Levin, Briggs, Christopher, & Auman, 1994), which indicates that the effects of nicotine on memory are mediated by nAChRs. Schizophrenic smokers who abstain for ten weeks show greater working memory impairments compared to non-smoking controls (George et al., 2002). Thus, nicotine may improve cognitive deficits associated with cognitive dysfunction of schizophrenia by increasing DA release in the PFC.

Summary

Schizophrenia is a complex, debilitating disorder, of which we do not fully understand the pathophysiology underlying development, progression, and treatment of the disease. Initially, researchers formulated the DA hypothesis of schizophrenia to account for positive symptoms of schizophrenia caused by a hyperdopaminergic state of the mesolimbic DA neurons. Subsequently, the DA hypothesis was modified to include cortical hypodopaminergia as it was found to be associated with negative symptoms and cognitive dysfunction of schizophrenia. Subcortical increases in DA release are induced by DA agonists, but cortical decreases in DA release cannot be induced by DA agonists. However, the development of the NMDA hypothesis of schizophrenia has revealed unique mechanisms by which NMDA receptor antagonists induce positive and negative symptoms and cognitive dysfunction of schizophrenia. Further research has integrated these two hypotheses in order to provide a more adequate explanation of the pathophysiology and neurocircuitry dysregulation underlying schizophrenia. Thus, the interaction of glutamate and DA neurotransmission is important for understanding the underlying mechanisms of schizophrenia.

Pharmacological animal models of schizophrenia were developed to study cause, progression, and treatment of the disorder and are based on neurotransmitter manipulation. As indicated above, pharmacological animal models of schizophrenia using amphetamine or other DA agonist administration are limited to inducing behavioral and neurochemical changes associated with

positive symptoms of schizophrenia. However, pharmacological animal models of schizophrenia using ketamine or other NMDA antagonists are more adequate as they induce behavioral and neurochemical changes associated with positive and negative symptoms and cognitive dysfunction of schizophrenia. Thus, a pharmacological animal model of schizophrenia using NMDA antagonist exposure will be used to induce behavioral and neurochemical changes associated with positive and negative symptoms and cognitive dysfunction of schizophrenia in this study.

Cigarette smoking appears to be much higher among people diagnosed with schizophrenia than in the general population. This high prevalence of smoking among people with schizophrenia leads us to believe that there is a common underlying neurobiology as nicotine temporarily improves negative symptoms and cognitive dysfunction of schizophrenia. The ability of nicotine to induce neurotransmitter release, particularly DA, has been heavily implicated as the underlying mechanism to improve social withdrawal, decreased reward function, learning, memory, and attention. Thus, it is important to determine the role of nAChRs and nicotine pharmacology in the underlying mechanisms and treatment of schizophrenia.

EXPERIMENTAL DESIGN AND PROPOSAL

The purpose of this study is to compare acute and subchronic pharmacological animal models of schizophrenia and to determine the effect of nicotine pharmacology. The long-term objective is to determine the role of nAChRs and nicotine pharmacology in the underlying mechanism and treatment of schizophrenia.

The purpose of Experiment 1 was to determine the concentration-response profile for ketamine, a noncompetitive NMDA receptor antagonist, to evoke DA release from rat striatal and PFC slices. As discussed above, PCP and ketamine exposure in vivo (Adams & Moghaddam, 1998; Lindefors, Barati, & O'Connor, 1997; Moghaddam, Adams, Verma, & Daly, 1997; Nishijima et al., 1996; Verma & Moghaddam, 1996) and in vitro (Dwoskin, Jewell, Buxton, & Carney, 1992; Ohmori, Koyama, Nakamura, Wang, & Yamashita, 1992) increases DA release in striatum and PFC. This is consistent with the hypothesis that hyperactivity of DA neurons in cortical and subcortical regions is an underlying mechanism for positive and negative symptoms and cognitive dysfunction of schizophrenia (Abi-Dargham et al., 1998; Balla, Sershen, Serra, Koneru, & Javitt, 2003; Laruelle, 1998). However, this is inconsistent with the hypothesis proposed by Carlsson and colleagues in which cortical hypodopaminergia and subcortical hyperdopaminergia are the underlying mechanisms for positive and negative symptoms and cognitive dysfunction of schizophrenia (A. Carlsson, Waters, & Carlsson, 1999). Thus, it is important to

determine if ketamine has intrinsic activity and at what concentrations to evoke DA release from rat striatal and PFC slices.

Ketamine and PCP have similar pharmacological effects on NMDA receptors (Ellison, 1995), however, they differ in potency (Olney, Labruyere, & Price, 1989). PCP is a more potent NMDA receptor antagonist than ketamine. Although ketamine may be a less potent NMDA receptor antagonist, it does not induce a “weaker” or “less complete” model of schizophrenia (Becker et al., 2003). As such, equimolar concentrations of ketamine, as compared to PCP, may not increase DA release in striatum and PFC. However, high concentrations of ketamine may be sufficient to increase DA release in striatum and PFC, although not pharmacologically relevant. Thus, the hypothesis is that ketamine will not have intrinsic activity to evoke [^{3}H] overflow from rat striatal and PFC slices at pharmacologically relevant concentrations.

In experiment 1, striata and PFC were dissected from rats and sliced. Rat striatal and PFC slices preloaded with [^{3}H]DA were superfused with buffer for a baseline. Slices were then superfused with a range of ketamine concentrations (0.1 - 300 μM). Anesthetic doses of ketamine interfere with the function of dopamine reuptake sites (Nishimura & Sato, 1999) causing increases in DA in the synapse. Ketamine inhibits the dopamine transporter (DAT), as expressed in human embryonic kidney (HEK) cells (K_i = 66.8 μM) (Nishimura & Sato, 1999). This indicates that the effects of subanesthetic concentrations of ketamine are not due to DAT blockade, but rather NMDA receptor blockade (K_i = 3.1 μM) (Oye, Paulsen, & Maurset, 1992; Seeman, Ko, & Tallerico, 2005). Additionally,

anesthetic doses of ketamine elicit little psychotomimetic effects (White, Ham, Way, & Trevor, 1980; White, Way, & Trevor, 1982) indicating that anesthetic doses of ketamine are not sufficient to induce symptoms associated with schizophrenia. Rat brain concentrations of ketamine during anesthesia are approximately 100 μM (Livingston & Waterman, 1978), so concentrations less than 100 μM are considered to be subanesthetic. Subsequently, slices were superfused with buffer not containing ketamine for a second baseline. The concentration-response profile for ketamine was determined in every rat. When collection was complete, radioactivity was measured from slices and superfusate.

The purpose of Experiment 2 was to determine the effect of ketamine on nicotine pharmacology using an acute pharmacological animal model of schizophrenia. Acute exposure to NMDA receptor antagonists has been developed as a pharmacological animal model of schizophrenia. As discussed above, acute exposure to ketamine and PCP induces changes in behavior associated with positive (Adams & Moghaddam, 1998; Imre, Fokkema, Den Boer, & Ter Horst, 2006; Sams-Dodd, 1995, , 1996, , 1997) and negative (Sams-Dodd, 1995, , 1996, , 1997) symptoms and cognitive dysfunction (Adams & Moghaddam, 1998; Imre, Fokkema, Den Boer, & Ter Horst, 2006; Moghaddam, Adams, Verma, & Daly, 1997) of schizophrenia. Acute exposure to ketamine and PCP also induces neurochemical changes associated with positive and negative symptoms and cognitive dysfunction of schizophrenia (Adams & Moghaddam, 1998; Lindefors, Barati, & O'Connor, 1997; Moghaddam, Adams, Verma, & Daly, 1997; Nishijima et al., 1996; Verma & Moghaddam, 1996). However, these

behavioral and neurochemical changes may be due to direct drug effects rather than drug-induced neurobiological changes in neurocircuitry (Jentsch, Taylor, & Roth, 1998). As previously discussed, people diagnosed with schizophrenia are more likely to smoke cigarettes (Sacco, Bannon, & George, 2004), and extract more nicotine from cigarettes as compared to nonmentally ill smokers (Olincy, Young, & Freedman, 1997). Schizophrenic smokers use cigarettes as a therapeutic agent for negative symptoms and cognitive dysfunction of schizophrenia (Kumari & Postma, 2005; Leonard et al., 2001; Levin, 2002) and the ability of nicotine to increase DA release in the PFC may temporarily improve these symptoms (Dalack, Healy, & Meador-Woodruff, 1998). In this study, we modeled schizophrenia by ketamine superfusion through rat brain slices. This is a method to model acute ketamine injection. Subsequently, we added nicotine in order to determine the role of nAChRs and nicotine pharmacology in an acute animal model of schizophrenia. The effect of NMDA receptor blockade, by ketamine, on nicotine-evoked DA release has not been determined. The [^{3}H] overflow assay is used to probe the nAChRs that mediate DA release. The hypothesis is that NMDA receptor blockade, by ketamine, will augment nicotine-evoked [^{3}H] overflow in striatal and PFC slices.

Striata and PFC were dissected from rats and sliced. Rat striatal and PFC slices preloaded with [^{3}H]DA were superfused with buffer and ketamine (0 – 10 μM) for a baseline. These ketamine concentrations were selected based on results from experiment 1. Subanesthetic ketamine concentrations (0 – 10 μM) did not have intrinsic activity to evoke DA release from rat striatal and PFC slices.

These concentrations are also below rat brain concentrations of ketamine (100 μM) during anesthesia (Livingston & Waterman, 1978) and the K_i value (66.8 μM) at which ketamine inhibits DAT, as expressed in HEK cells (Nishimura & Sato, 1999), indicating that ketamine is most likely producing its effects due to NMDA receptor blockade (K_i = 3.1 μM) (Oye, Paulsen, & Maurset, 1992; Seeman, Ko, & Tallerico, 2005). Slices were then superfused with nicotine (1 - 100 μM). The nicotine concentrations were chosen based on studies in which nicotine induced DA release from striatal neurons in a concentration-dependent manner (Rapier, Lunt, & Wonnacott, 1988; Sharples et al., 2000) and are inhibited by nAChR antagonists (Wilkins, Haubner, Ayers, Crooks, & Dwoskin, 2002). Subsequently, slices were superfused with buffer not containing nicotine for a second baseline. The concentration-response profile for nicotine was determined in every rat. When collection was complete, radioactivity was measured from slices and superfusate.

The purpose of Experiment 3 was to determine the effect of subchronic ketamine exposure on nicotine pharmacology using a pharmacological animal model of schizophrenia. Male Sprague-Dawley rats were injected (IP) daily with 30 or 50 mg/kg of ketamine for 30 days. These doses of ketamine were chosen because they are sufficient to induce behavioral and neurochemical changes associated with positive and negative symptoms and cognitive dysfunction of schizophrenia in animals (Becker et al., 2003; Imre, Fokkema, Den Boer, & Ter Horst, 2006; Lannes, Micheletti, Warter, Kempf, & Di Scala, 1991; Leccese, Marquis, Mattia, & Moreton, 1986). The duration of treatment was adapted from

several studies that showed ketamine administered subchronically (5 - 90 days consecutively) (Becker et al., 2003; Lannes, Micheletti, Warter, Kempf, & Di Scala, 1991; Leccese, Marquis, Mattia, & Moreton, 1986; Nelson, Burk, Bruno, & Sarter, 2002) induced behavioral and neurochemical changes associated with positive and negative symptoms and cognitive dysfunction of schizophrenia in animals. Animals representing the control group were injected (IP) with saline at corresponding times. Behavioral changes associated with positive symptoms of schizophrenia were assessed by a locomotor activity assay the day of and 1 day after the final injection of ketamine or saline.

Ketamine exposure has been used as a model of schizophrenia as it induces behavioral and neurochemical changes associated with positive and negative symptoms and cognitive dysfunction of schizophrenia (Becker et al., 2003; Imre, Fokkema, Den Boer, & Ter Horst, 2006; Lannes, Micheletti, Warter, Kempf, & Di Scala, 1991; Leccese, Marquis, Mattia, & Moreton, 1986; Mandryk, Fidecka, Poleszak, & Malec, 2005; Miyamoto, Leipzig, Lieberman, & Duncan, 2000; Moghaddam, Adams, Verma, & Daly, 1997; Nelson, Burk, Bruno, & Sarter, 2002). The effect of subchronic ketamine injection on locomotor activity was examined to ensure that subchronic ketamine exposure induced behavioral changes associated with positive symptoms of schizophrenia. NMDA receptor antagonists have been shown to induce behavioral changes associated with positive symptoms of schizophrenia in rodents including hyperlocomotion (Adams & Moghaddam, 1998; Hunt, Kessal, & Garcia, 2005; Imre, Fokkema, Den Boer, & Ter Horst, 2006; Irifune, Shimizu, Nomoto, & Fukuda, 1995;

Mandryk, Fidecka, Poleszak, & Malec, 2005; McCullough & Salamone, 1992; Sams-Dodd, 1998; Wilson et al., 2005). Rats were put into an automated locomotor activity monitor for 60 min to determine if behavioral changes associated with positive symptoms of schizophrenia had been induced. The hypothesis is that subchronic exposure to ketamine will induce hyperlocomotion.

Experiment 3 also determined the effect of subchronic ketamine exposure on the potency and efficacy of nicotine to evoke [^{3}H]DA release from preloaded rat brain slices. As discussed above, people diagnosed with schizophrenia are more likely to smoke cigarettes (Sacco, Bannon, & George, 2004), and extract more nicotine from cigarettes as compared to nonmentally ill smokers (Olincy, Young, & Freedman, 1997). Schizophrenic smokers use cigarettes as a therapeutic agent for negative symptoms and cognitive dysfunction of schizophrenia (Kumari & Postma, 2005; Leonard et al., 2001; Levin, 2002) and the ability of nicotine to increase DA release in PFC may temporarily improve these symptoms (Dalack, Healy, & Meador-Woodruff, 1998). In this study, we modeled schizophrenia by repeated ketamine injections, which blocked NMDA receptors. This is a subchronic animal model of schizophrenia. Subsequently, we superfused nicotine through rat brain slices in order to determine the role of nAChRs and nicotine pharmacology in a subchronic animal model of schizophrenia. The effect of subchronic ketamine treatment on nicotine-evoked DA release has not been determined. The [^{3}H] overflow assay is used to probe the nAChRs that mediate DA release. The hypothesis is that subchronic

ketamine administration will augment the effect of nicotine-evoked [^{3}H] overflow in striatal and PFC slices.

Experiment 3 began with 30 days of 30 or 50 mg/kg ketamine or saline injections to rats and locomotor activity assessment the day of and 1 day after the final ketamine or saline injection. A DA release assay was used in order to determine the effect of subchronic ketamine exposure on the potency and efficacy of nicotine to evoke [^{3}H]DA release from preloaded rat brain slices. Striata and PFC were dissected and sliced. Rat striatal and PFC slices preloaded with [^{3}H]DA were superfused with buffer for a baseline and then superfused with nicotine. Subsequently, slices were superfused with buffer not containing nicotine for a second baseline. The concentration-response profile for nicotine was determined in every rat. When collection was complete, radioactivity was measured from slices and superfusate.

EXPERIMENT 1: KETAMINE-EVOKED [^{3}H] OVERFLOW

The purpose of Experiment 1 was to determine the concentration-response profile for ketamine, a noncompetitive NMDA receptor antagonist, to evoke DA release from rat striatal and PFC slices. Rat striatal and PFC slices preloaded with [^{3}H]DA were superfused with buffer and then superfused with ketamine (0.1 - 300 μM) to determine the intrinsic effect of ketamine to evoke [^{3}H] overflow.

Materials and Methods

Subjects. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Missouri. The subjects were male Sprague-Dawley rats (Harlan, Indianapolis, IN; 175-200 g upon arrival to the laboratory) that were housed 2 per cage with ad libitum access to tap water and standard rat chow. The colony was maintained under a 12-hr/12-hr light/dark cycle and the experiments were conducted during the light phase of the cycle.

Procedure. Rats were euthanized via rapid decapitation. Striata and PFC were dissected and sliced (750 μm and 1000 μm, respectively) using a Stoelting (Wood Dale IL) tissue chopper. Slices were incubated in oxygenated buffer (in mM, 108 NaCl, 25 $NaHCO_3$, 11.1 glucose, 4.7 KCl, 1.3 $CaCl_2$, 1.2 $MgSO_4$, 1.0 Na_2HPO_4, 0.11 ascorbic acid, 0.001 EDTA) in a metabolic shaker at 37˚C for 30 min. Slices were transferred to fresh buffer, [^{3}H]DA (0.1 μM) was added, and slices were incubated for an additional 30 min. Striatal slices were transferred to 6 of 12 reaction chambers in an automated superfusion system (Suprafusion

2500, Brandel, Gaithersburg MD). PFC slices were transferred to the remaining 6 of 12 reaction chambers. Each reaction chamber (0.2 ml) was bound by glass microfiber filters (GF/B, Whatman, Madistone England). Pargyline (10 μM) was included in buffer to inhibit monoamine oxidase (Westerink & Kikkert, 1986), which inhibits dopamine metabolization. Slices were superfused with buffer at a rate of 0.75 ml/min (Figure 3). After 60 min, sample collection commenced at a rate of 1 sample/3 min. After the collection of 3 baseline samples, ketamine (0.1 - 300 μM) was added to buffer, and superfusion continued for 6 min (Figure 3). One chamber was superfused with buffer alone for both striatal and PFC slices and represented a control condition. Subsequently, all slices were superfused with buffer that did not contain ketamine for 15 min (Figure 3). When collection was complete, slices and filters were removed from the reaction chambers and incubated with tissue solubilizer (0.25 ml/sample). Radioactivity in superfusate samples and slices/filters was measured by liquid scintillation spectroscopy (LS 6500 Scintillation counter, Beckman-Coulter, Fullerton CA; counting efficiency ≈ 60%).

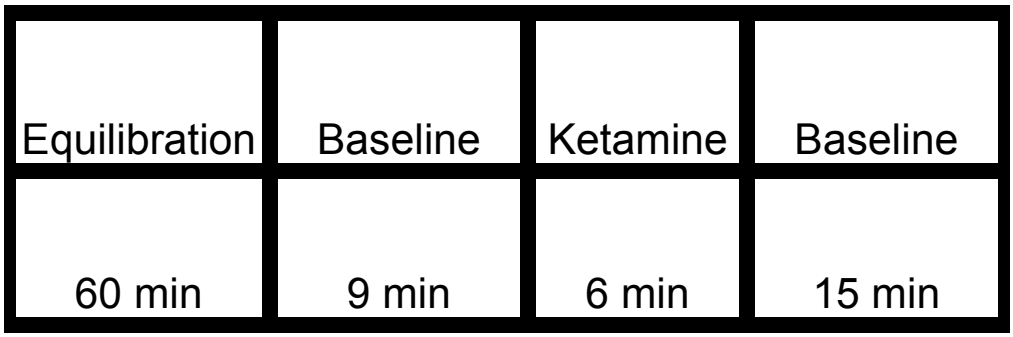

Figure 3. Experiment 1 [^{3}H] Overflow Assay. Slices were superfused with buffer for 60 min in order for the system to equilibrate and additional 9 min in order to determine a baseline. Ketamine was then added to buffer and superfusion continued for 6 min. Subsequently, slices were superfused with buffer for 15 min in order to determine a second baseline.

Drugs and chemicals. Pargyline hydrochloride, (±)-Ketamine, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Company (St. Louis MO). Alpha-D(+)-glucose, NaH_2PO_4, and L(+)-ascorbic acid were purchased from Acros Organics (Geel, Belgium). Radiolabeled dopamine (dihydroxyphenylethylamine 3,4-[7-^{3}H], specific activity = 34.8 - 60.0 Ci/mmol) was purchased from PerkinElmer Life Sciences (Boston MA). Scintigest tissue solubilizer and all of the other chemicals were purchased from Fisher Scientific (Fairlawn NJ).

Data analysis. Fractional release was calculated by dividing the [^{3}H] collected in each 3-min sample by the total [^{3}H] present in the tissue at the time of sample collection. Total [^{3}H] overflow was calculated by subtracting the overflow in the presence of drug from average basal overflow. Total [^{3}H] overflow data were analyzed via 1-way repeated measures ANOVA (RM-ANOVA) with Ketamine Concentration as a within-subject factor. Fractional [^{3}H] release was analyzed via 2-way RM-ANOVA with Ketamine Concentration and Time as within-subjects factors. For all analyses, a significance level of $P < 0.05$ was established a priori and paired t-tests were performed as post hoc comparisons using Bonferroni adjustment when appropriate. All data are expressed as the mean, plus or minus the standard error of the mean. Data from striatal and PFC slices were analyzed separately.

Results

Ketamine did not evoke [^{3}H]DA release in striatum or PFC. A concentration-response profile for ketamine (0.1 - 300 μM) was determined in

Experiment 1. Ketamine did not significantly evoke [^{3}H] overflow from either striatal or PFC slices (Table 1). The main effect of Ketamine Concentration was not significant for the measure of total [^{3}H] overflow from striatal [$F(5,25)=2.20$, $P=0.09$] and PFC [$F(5,25)=1.08$, $P=0.40$] slices.

Regarding fractional [^{3}H]DA release from striatal slices, the main effects of Ketamine Concentration [$F(5,25)=1.28$, $P=0.30$] and Time [$F(9,45)=0.40$, $P=0.93$] were not significant. However, a significant Ketamine Concentration x Time interaction [$F(45,225)=2.77$, $P<0.05$] was found. Post hoc analyses did not reveal any significant differences in fractional release among ketamine concentrations and control groups.

Ketamine Does Not Evoke [^{3}H] Overflow						
	Ketamine Concentration (μM)					
	Control	0.1	1.0	10	100	300
Striatum	0.35 ± 0.31	0.82 ± 0.52	1.39 ± 0.91	0.59 ± 0.34	0.74 ± 0.49	3.18 ± 2.0
Prefrontal Cortex	0.22 ± 0.09	0.14 ± 0.13	0.01 ± 0.01	0.07 ± 0.06	0.05 ± 0.03	0.11 ± 0.07

Table 1. Ketamine (0.1 – 300 μM) did not significantly evoke [^{3}H] overflow from either striatal or PFC slices. Data represent mean (±S.E.M.) total [^{3}H] overflow. Control values represent superfusion in the absence of ketamine. (n = 6 rats)

Regarding fractional [^{3}H]DA release from PFC slices, the main effects of Ketamine Concentration [$F(5,25)=2.77$, $P<0.05$] and Time [$F(9,45)=7.48$, $P<0.05$] were significant. However, the Ketamine Concentration x Time interaction [$F(45,225)=1.18$, $P=0.22$] was not significant. Thus, ketamine did not have

intrinsic activity to significantly evoke [^{3}H] overflow from either striatal or PFC slices.

Discussion

Ketamine did not significantly evoke [^{3}H] overflow from either striatal or PFC slices. The subanesthetic concentrations of ketamine (0.1 – 10 μM) consistently did not evoke DA release from either striatal or PFC slices (Table 1). However, there was a trend for the anesthetic concentrations of ketamine (100 - 300 μM) to evoke DA release from striatal and PFC slices (Table 1).

There is significant evidence to suggest that NMDA receptor antagonists, like ketamine and PCP, increase DA release in striatum and PFC (Lindefors, Barati, & O'Connor, 1997; Moghaddam, Adams, Verma, & Daly, 1997; Verma & Moghaddam, 1996). However, those studies were done in vivo, which means that the cortical-subcortical neurocircuitry was intact. It is possible that DA release was being facilitated by cortical-subcortical connectivity. In contrast, rats injected with a subanesthetic dose of ketamine (15 mg/kg) showed no alteration in striatal DA release as measured by reverse phase chromatography (Lannes, Micheletti, Warter, Kempf, & Di Scala, 1991). However, if there is an effect of ketamine, it is much less compared to our preliminary findings on nicotine-evoked DA release (Figure 2). Ketamine is also less potent than PCP and MK-801 (Olney, Labruyere, & Price, 1989), so it would take a higher ketamine concentration to evoke DA release as compared to PCP or MK-801. In the present study, in vitro slice superfusion assay was used, which means that cortical-subcortical neurocircuitry was not intact. This could be one reason that

our findings are inconsistent with others. Additionally, several PET/SPECT studies in man have reported no effect of subanesthetic doses of ketamine on DA release in striatum (Aalto et al., 2002; Kegeles et al., 2000). Furthermore, NMDA receptor blockade with MK-801 reduces the ability of D_1 receptor stimulation to modulate firing rates of dopaminergic cells in the substantia nigra (Huang, Bergstrom, Ruskin, & Walters, 1998). This indicates that NMDA receptor blockade leads to DA receptor hypoactivity, a finding that is in line with the results of the present study.

Ketamine has also been implicated as a DA agonist. Seeman and colleagues reported that ketamine has higher affinity for D_2 DA receptors (K_i = 55 nM) than for NMDA receptors (K_i = 3100 nM) (Seeman, Ko, & Tallerico, 2005). In PFC, D_2 DA receptors are localized on GABA interneurons and stimulation of these DA receptors results in GABA-mediated inhibition of pyramidal cells (Retaux, Besson, & Penit-Soria, 1991). Taken together, this indicates that activation of PFC DA receptors by ketamine facilitates inhibition of pyramidal cells by GABA and results in overall DA hypoactivity.

The aim of the present study was to determine the concentration-response profile for ketamine to evoke DA release from rat striatal and PFC slices. The results indicate that subanesthetic concentrations of ketamine do not have intrinsic activity to evoke DA release; however, there appears to be a trend for anesthetic concentrations of ketamine to evoke DA release (Table 1). The fact that this trend was not statistically significant is most likely a product of using an ANOVA. We may be able to detect this trend by adding more rats to the study in

order to increase the power. The effects of ketamine on DA release in striatum and PFC is controversial in the literature. It was important to determine the effects of ketamine on DA release in our assay in order to investigate the effects of subanesthetic ketamine concentrations on nicotine-evoked DA release in Experiment 2.

EXPERIMENT 2: EFFECT OF NMDA RECEPTOR BLOCKADE, BY KETAMINE, ON NICOTINE-EVOKED [^{3}H] OVERFLOW

The purpose of Experiment 2 was to determine the effect of NMDA receptor blockade, by ketamine, on nicotine pharmacology. Ketamine superfusion through brain slices models an acute ketamine injection. This is an acute animal model of schizophrenia. Rat striatal and PFC slices preloaded with [^{3}H]DA were superfused with buffer containing ketamine (0, 1, or 10 μM) and then nicotine (1 - 100 μM) was added to determine the interaction between ketamine and nicotine in the [^{3}H] overflow assay.

Materials and Methods

Subjects. All animal and colony procedures and maintenance of the animals were similar to those described for Experiment 1.

Procedure. Rat brain slices and buffer were prepared as described in Experiment 1 with the exception that ketamine (0, 1, or 10 μM) was also included in buffer. Slices were superfused with buffer at a rate of 0.75 ml/min (Figure 4). After 60 min, sample collection commenced at a rate of 1 sample/3 min. After the collection of 3 baseline samples, nicotine (1 - 100 μM) was added to buffer, and superfusion continued for 6 min (Figure 4). The nicotine concentrations were selected from preliminary experiments in our laboratory that determined the concentration-response profile for nicotine (Figure 2). The ketamine concentrations were selected based on results from experiment 1. Subanesthetic ketamine concentrations (0 – 10 μM) did not have intrinsic activity to evoke DA

release from rat striatal and PFC slices. Furthermore, these concentrations are also below rat brain concentrations of ketamine (100 μM) during anesthesia (Livingston & Waterman, 1978), which elicits little psychotomimetic effects (White, Ham, Way, & Trevor, 1980; White, Way, & Trevor, 1982). One chamber was superfused with buffer alone for both striatal and PFC slices and represented a control condition. Subsequently, all slices were superfused with buffer that did not contain nicotine for 15 min (Figure 4). When collection was complete, slices and filters were removed from the reaction chambers and incubated with tissue solubilizer (0.25 ml/sample).

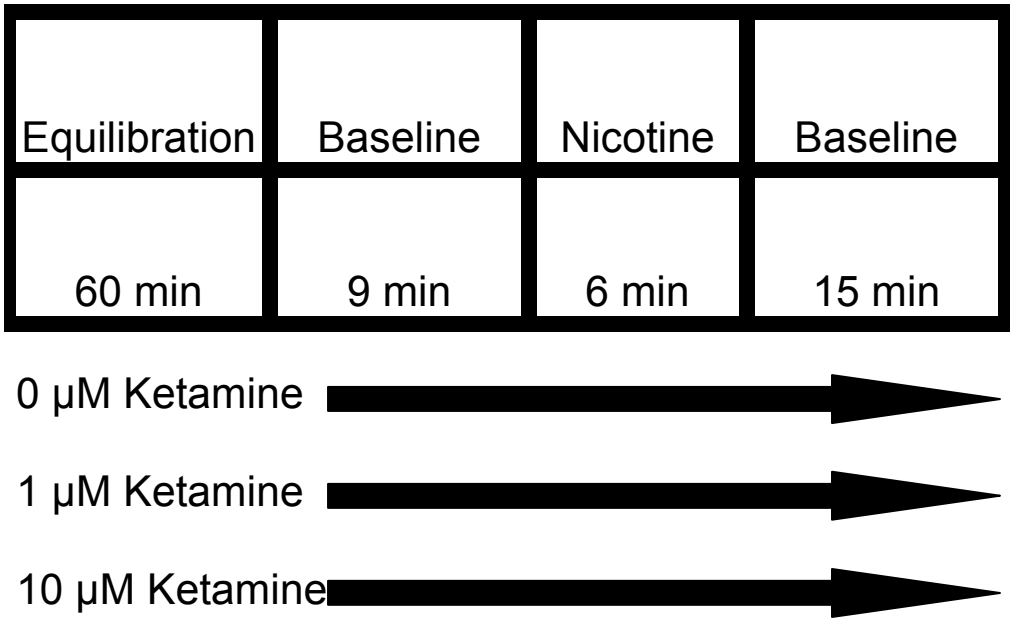

Figure 4. Experiment 2 [^{3}H] Overflow Assay. Slices were superfused with buffer for 60 min in order for the system to equilibrate and additional 9 min in order to determine a baseline. Nicotine was then added to buffer and superfusion continued for 6 min. Subsequently, slices were superfused with buffer for 15 min in order to determine a second baseline.

Drugs and chemicals. (-)-nicotine ditartrate was purchased from Sigma Chemical Company (St. Louis MO). All other drugs and chemicals acquired as described earlier.

Data analysis. Fractional release for each superfusate sample was calculated by dividing the [^{3}H] collected in each 3-min sample by the total [^{3}H] present in the tissue at the time of sample collection. Basal [^{3}H] overflow was calculated from the average of fractional release in the three samples just before addition of nicotine. For the 6 min period of superfusion with nicotine alone, the fractional samples greater than baseline were summed and basal [^{3}H] overflow was subtracted to determine total [^{3}H] overflow. Initially, the data for each ketamine concentration were analyzed separately by the following analyses. Total [^{3}H] overflow data were analyzed via 1-way RM-ANOVA with Nicotine Concentration as a within-subject factor. Fractional [^{3}H] release was analyzed via 2-way RM-ANOVA with Nicotine Concentration and Time as within-subjects factors. Comparisons among ketamine concentrations were analyzed by the following analyses. Total [^{3}H] overflow data were analyzed via 2-way RM-ANOVA with Nicotine Concentration as a within-subject factor and Ketamine Concentration as a between-subjects factor. Fractional [^{3}H] release was analyzed via 3-way RM-ANOVA with Nicotine Concentration and Time as within-subjects factors and Ketamine Concentration as a between-subjects factor. For all analyses, a significance level of $P < 0.05$ was established a priori and paired t-tests were performed as post hoc comparisons using Bonferroni adjustment when appropriate. Data from striatal and PFC slices were analyzed separately.

EC_{25}, EC_{50}, and EC_{75} values for striatal and PFC slices were calculated via nonlinear regression for each rat and analyzed via Kruskal-Wallis test. Kruskal-Wallis post hoc tests were performed when appropriate.

Results

Nicotine evokes [^{3}H]DA release in striatum and PFC. Nicotine evoked [^{3}H] overflow in a concentration-dependent manner from both striatal and PFC slices. A significant main effect of Nicotine Concentration was found for the measure of total [^{3}H] overflow from striatal [$F(4,28)=12.37$, $P<0.05$] and PFC [$F(4,28)=20.23$, $P<0.05$] slices. Post hoc analyses revealed greater [^{3}H] overflow with 10 -100 μM nicotine than for control (0 μM nicotine) from striatal slices (Figure 5). The threshold nicotine concentration was 10 μM in striatum. Post hoc analyses also revealed greater [^{3}H] overflow with 30 – 100 μM nicotine than for control from PFC slices (Figure 6). The threshold nicotine concentration was 30 μM in PFC.

Nicotine evoked DA release in a time- and concentration-dependent manner from striatal slices. Regarding fractional [^{3}H]DA release from striatal slices, the main effect of Nicotine Concentration [$F(4,28)=9.53$, $P<0.05$] and Time [$F(9,63)=3.63$, $P<0.05$] was significant. A significant Nicotine Concentration x Time interaction [$F(36,252)=16.16$, $P<0.05$] was also found. Post hoc analyses revealed that fractional release was greater for 100 μM nicotine than for control (0 μM nicotine) at the 12, 15, 18, and 21 min time points. Fractional release was greater for 30 μM nicotine than for control at the 15, 18, and 21 min time points. Fractional release was greater for 10 μM nicotine than for control at the 18 and

21 min time points. Thus, nicotine had intrinsic activity to evoke [^{3}H] overflow from striatal slices.

For PFC slices, nicotine evoked DA release in a time- and concentration-dependent manner. Regarding fractional [^{3}H]DA release from PFC slices, the main effects of Nicotine Concentration [$F(4,28)=3.69$, $P<0.05$] and Time [$F(9,63)=5.56$, $P<0.05$] were significant. A significant Nicotine Concentration x Time interaction [$F(36,252)=19.34$, $P<0.05$] was also found. Post hoc analyses revealed that fractional release was greater for 100 μM nicotine than for control (0 μM nicotine) at the 12, 15, 18, 21, and 24 min time points. Fractional release was greater for 10 μM nicotine than for control at the 15 min time point. Thus, nicotine had intrinsic activity to evoke [^{3}H] overflow from PFC slices.

Ketamine augments the effect of nicotine to evoke [^{3}H]DA release in striatum and PFC. Ketamine (1 – 10 μM) altered nicotine-evoked [^{3}H] overflow from both striatal and PFC slices. A significant main effect of Nicotine Concentration was found for the measure of total [^{3}H] overflow from striatal [$F(4,80)=65.36$, $P<0.05$] and PFC [$F(4,80)=72.85$, $P<0.05$] slices. A significant main effect of Ketamine Concentration was also found for the measure of total [^{3}H] overflow from striatal [$F(2,20)=4.93$, $P<0.05$] and PFC [$F(2,20)=5.90$, $P<0.05$] slices. A significant Nicotine Concentration x Ketamine Concentration interaction was found for the measure of total [^{3}H] overflow from striatal [$F(8,80)=3.15$, $P<0.05$] and PFC [$F(8,80)=3.16$, $P<0.05$] slices. Post hoc analyses revealed greater total [^{3}H] overflow with 30 - 100 μM nicotine for the 1 μM ketamine group than for the 0 μM ketamine group from striatal slices (Figure

5). Post hoc analyses also revealed greater total [^{3}H] overflow with 100 μM nicotine for the 10 μM ketamine group than for the 0 μM ketamine group from PFC slices (Figure 6).

Ketamine increased nicotine-evoked DA release in a time- and concentration-dependent manner from striatal slices. Regarding fractional [^{3}H]DA release from striatal slices, the main effects of Nicotine Concentration [$F(4,80)=45.34$, $P<0.05$] and Time [$F(9,180)=47.99$, $P<0.05$] were significant. However, the main effect of Ketamine Concentration was only marginally significant [$F(2,20)=3.39$, $P=0.054$]. Significant Nicotine Concentration x Ketamine Concentration [$F(8,80)=2.26$, $P<0.05$], Time x Ketamine Concentration [$F(18,180)=3.40$, $P<0.05$], and Nicotine Concentration x Time [$F(36,720)=55.03$, $P<0.05$] interactions were also found. A significant Nicotine Concentration x Time x Ketamine Concentration [$F(72,720)=3.22$, $P<0.05$] interaction was also found. Post hoc analyses revealed that fractional release was greater with 100 μM nicotine for the 1 μM ketamine group than for the 0 μM ketamine group at the 18, 21, 24, and 27 min time points (Figure 7 Top). Fractional release was greater with 30 μM nicotine for the 1 μM ketamine group than for the 0 μM ketamine group at the 15, 18, 21, 24, and 27 min time points (Figure 7 Bottom). Fractional release was greater with 100 μM nicotine for the 10 μM ketamine group than for the 0 μM ketamine group at the 21, 24, and 27 min time points (Figure 7 Top). Fractional release was greater with 10 μM nicotine for the 10 μM ketamine group than for the 0 μM ketamine group at the 24 min time point. Thus, ketamine (1 -10

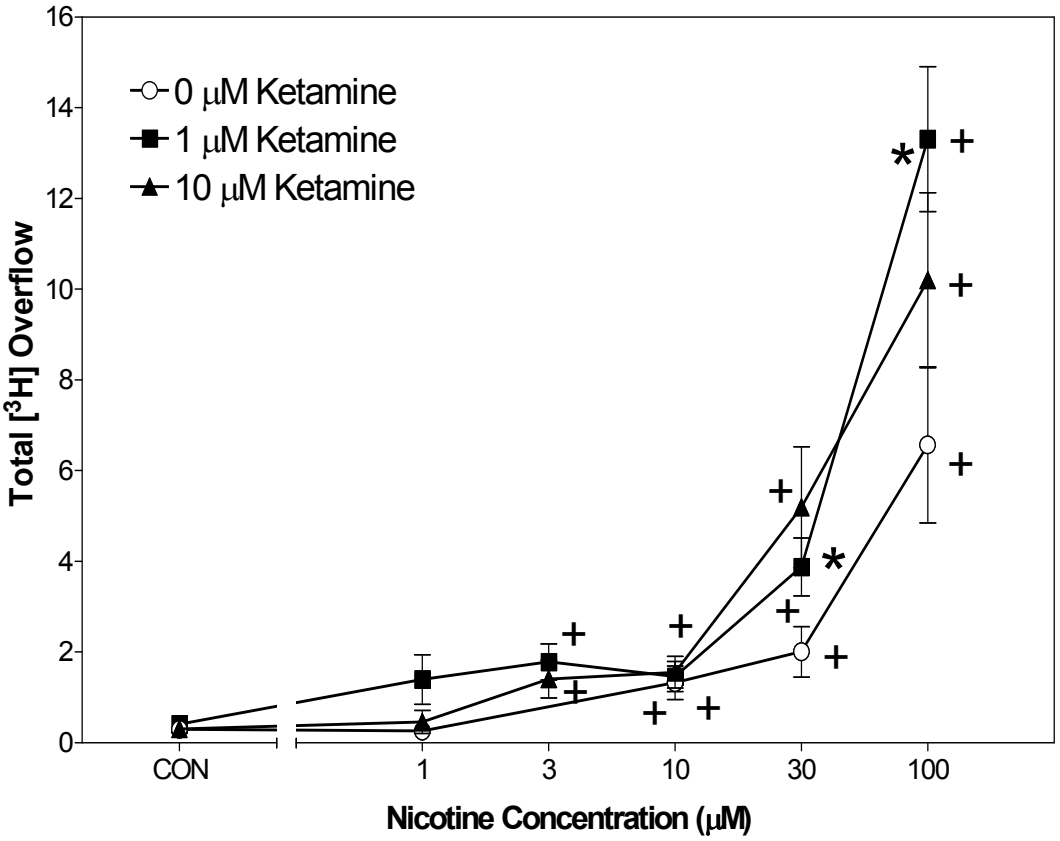

Figure 5. Ketamine (1 – 10 μM) augmented the effect of nicotine to increase total [^{3}H] overflow from rat striatal slices preloaded with [^{3}H]DA in a concentration-dependent manner. Data are expressed as the mean (± S.E.M.) total [^{3}H] overflow after the addition of nicotine to buffer containing ketamine (0, 1, or 10 μM). Asterisks designate a significant ($P<0.05$) difference from 0 μM Ketamine (superfusion in the absence of ketamine). Plus signs designate a significant ($P<0.05$) difference from control condition within each ketamine group. *CON, control condition.* (n = 7 - 8 rats/group)

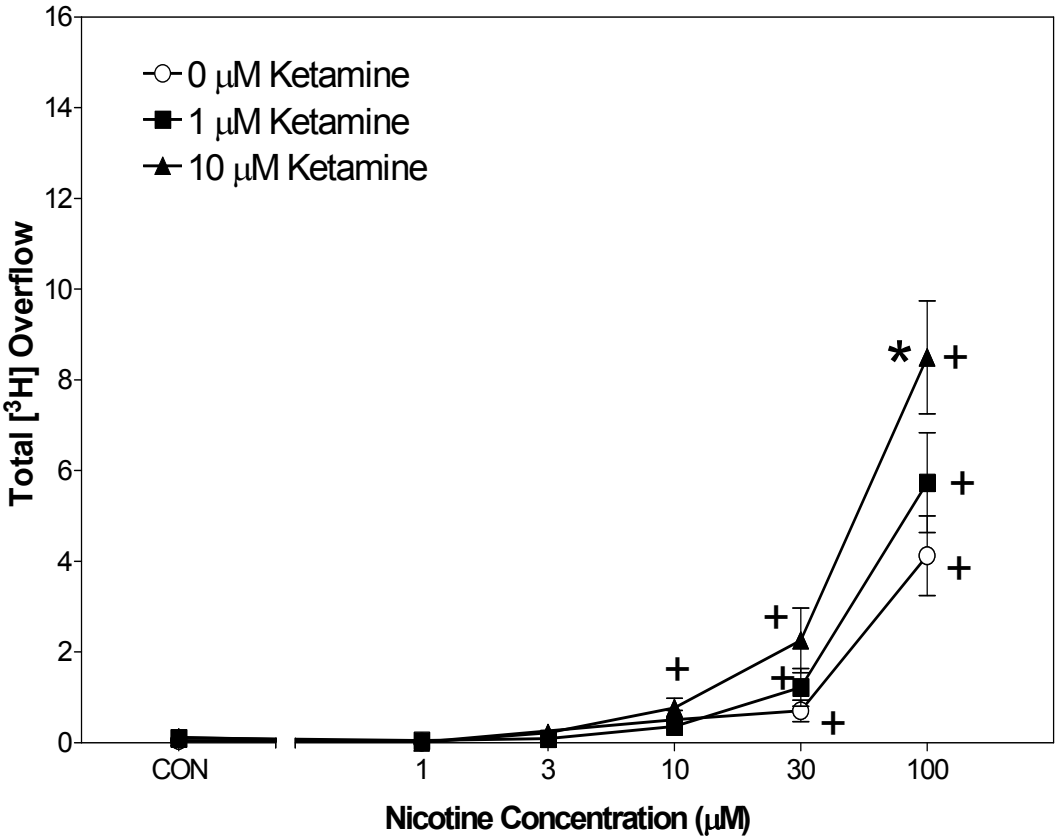

Figure 6. Ketamine (1 – 10 μM) augmented the effect of nicotine to increase total [^{3}H] overflow from rat PFC slices preloaded with [^{3}H]DA in a concentration-dependent manner. Data are expressed as the mean (± S.E.M.) total [^{3}H] overflow after the addition of nicotine to buffer containing ketamine (0, 1, or 10 μM). Asterisks designate a significant ($P<0.05$) difference from 0 μM Ketamine (superfusion in the absence of ketamine). Plus signs designate a significant ($P<0.05$) difference from control condition within each ketamine group. *CON, control condition.* (n = 7 - 8 rats/group)

μM) augmented nicotine-evoked [^{3}H] overflow from striatal slices relative to the 0 μM ketamine group.

For PFC slices, ketamine (1 - 10 μM) increased nicotine-evoked DA release in a time- and concentration-dependent manner. Regarding fractional [^{3}H]DA release from PFC slices, the main effects of Nicotine Concentration [$F(4,76)=30.59$, $P<0.05$], Ketamine Concentration [$F(2,19)=4.44$, $P<0.05$], and Time [$F(9,171)=28.96$, $P<0.05$] were significant. Significant Time x Ketamine Concentration [$F(18,171)=3.15$, $P<0.05$] and Nicotine Concentration x Time [$F(36,684)=42.67$, $P<0.05$] interactions were also found. However, the Nicotine Concentration x Ketamine Concentration interaction [$F(8,76)=1.61$, $P=0.136$] was not significant. A significant Nicotine Concentration x Time x Ketamine Concentration [$F(72,684)=3.01$, $P<0.05$] interaction was found. Post hoc analyses revealed that fractional release was greater with 100 μM nicotine for the 1 μM ketamine group than for the 0 μM ketamine group at the 24, 27, and 30 min time points (Figure 8 Top). Fractional release was greater with 30 μM nicotine for the 1 μM ketamine group than for the 0 μM ketamine group at the 21, 24, and 27 min time points (Figure 8 Bottom). Fractional release was greater with 100 μM nicotine for the 10 μM ketamine group than for the 0 μM ketamine group at the 18, 21, 24, 27, and 30 min time points (Figure 8 Top). Fractional release was greater with 30 μM nicotine for the 10 μM ketamine group than for the 0 μM ketamine group at the 21 and 24 min time points (Figure 8 Bottom). Thus, ketamine (1 -10 μM) augmented nicotine-evoked [^{3}H] overflow from PFC slices relative to the 0 μM ketamine group.

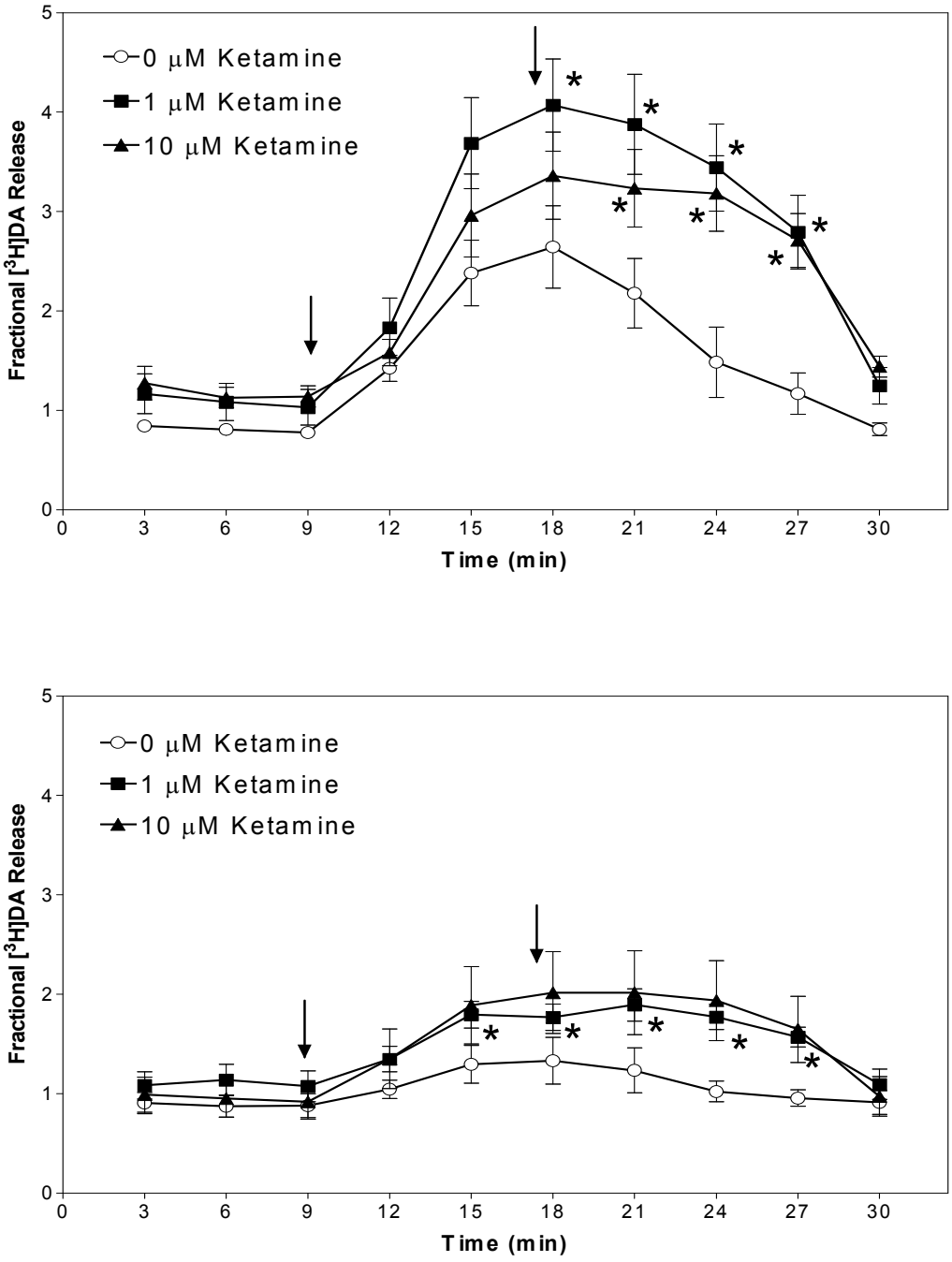

Figure 7. Ketamine (1 – 10 μM) augmented the effect of nicotine to increase fractional [^{3}H]DA release from rat striatal slices preloaded with [^{3}H]DA in a time- and concentration-dependent manner. Top. Time course of nicotine (100 μM)-evoked [^{3}H]DA release. Bottom. Time course of nicotine (30 μM)-evoked [^{3}H]DA release. Data are expressed as the mean (± S.E.M.) fractional release. Asterisks designate a significant ($P<0.05$) difference from 0 μM Ketamine (superfusion in the absence of ketamine) and the arrows designate the addition and removal of nicotine to ketamine buffer. *CON, control condition.* (n = 7 - 8 rats/group)

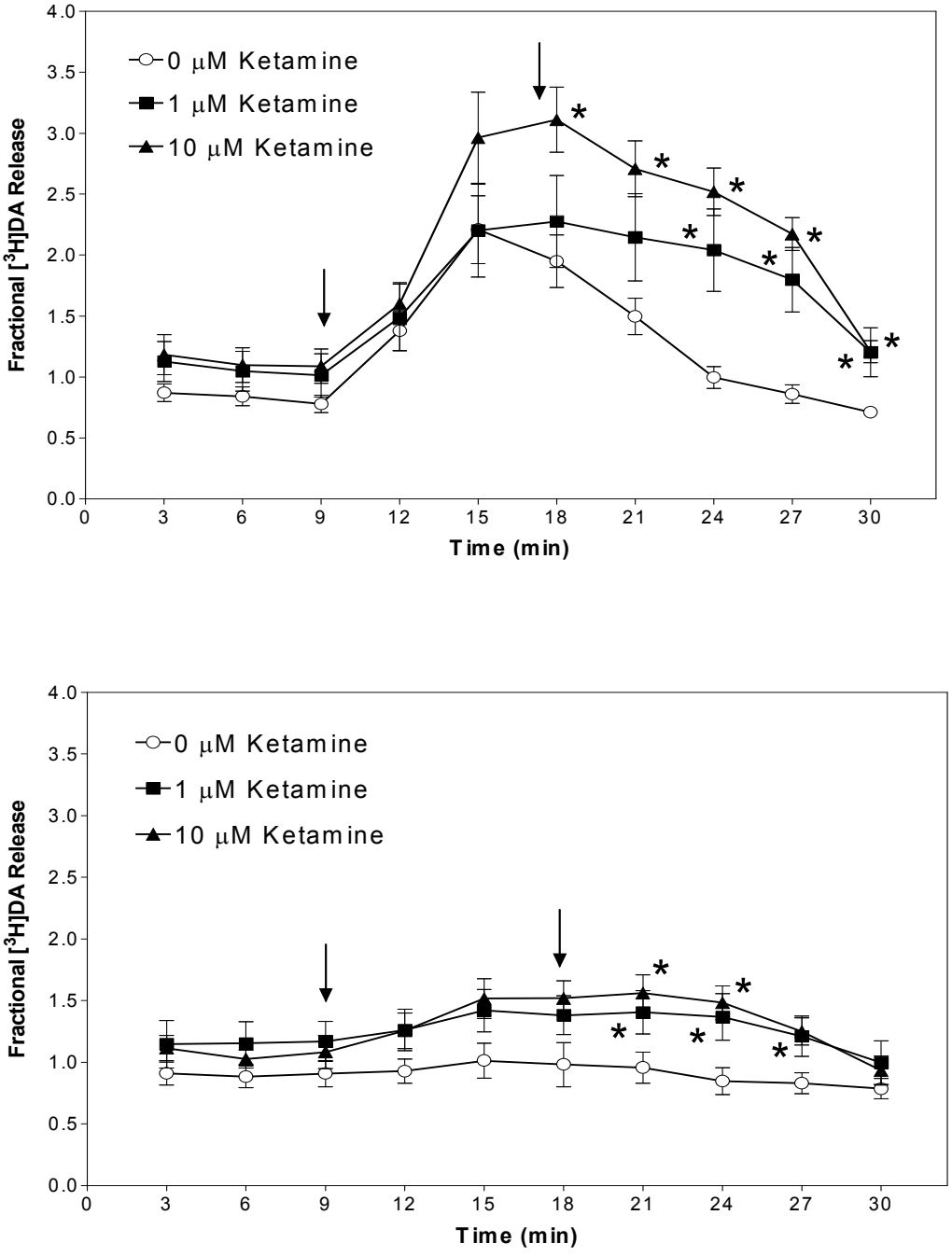

Figure 8. Ketamine (1 – 10 μM) augmented the effect of nicotine to increase fractional [^{3}H]DA release from rat PFC slices preloaded with [^{3}H]DA in a time- and concentration-dependent manner. Top. Time course of nicotine (100 μM)-evoked [^{3}H]DA release. Bottom. Time course of nicotine (30 μM)-evoked [^{3}H]DA release. Data are expressed as the mean (± S.E.M.) fractional release. Asterisks designate a significant ($P<0.05$) difference from 0 μM Ketamine (superfusion in the absence of ketamine) and the arrows designate the addition and removal of nicotine to ketamine buffer. *CON, control condition.* (n = 7 - 8 rats/group)

Ketamine (1 -10 μM) decreased the effective nicotine concentration to evoke DA release in striatum and PFC. EC_{25}, EC_{50}, and EC_{75} values for striatal and PFC slices were calculated for each rat. For striatal slices, there was a significant effect of Ketamine Concentration for EC_{25} [X^2=14.71, df=2, P<0.05], EC_{50} [X^2=15.40, df=2, P<0.05], and EC_{75} [X^2=15.23, df=2, P<0.05] values (Figure 9). Post hoc analyses revealed significant differences between 1 – 10 μM ketamine and control (0 μM ketamine) for EC_{25}, EC_{50}, and EC_{75} values. Ketamine (1 – 10 μM) decreased the nicotine concentration that evokes 25%, 50%, and 75% DA release from striatal slices. For PFC slices, there was a significant effect of Ketamine Concentration for EC_{25} [X^2=15.90, df=2, P<0.05], EC_{50} [X^2=15.06, df=2, P<0.05], and EC_{75} [X^2=15.23, df=2, P<0.05] values (Figure 10). Post hoc analyses revealed significant differences between 1 – 10 μM ketamine and control (0 μM ketamine) for EC_{25}, EC_{50}, and EC_{75} values. Ketamine (1 – 10 μM) decreased the nicotine concentration that evokes 25%, 50%, and 75% DA release from PFC slices. This indicates that ketamine augmented nicotine-evoked DA release and decreased the nicotine concentration needed to evoke DA release from striatal and PFC slices in each rat.

Discussion

Ketamine (1 – 10 μM) significantly increased nicotine-evoked [^{3}H] overflow from rat striatal and PFC slices preloaded with [^{3}H]DA. The results from the present study indicate that nicotine evokes [^{3}H]DA release in a time- and concentration-dependent manner from rat PFC and striatal slices, which is consistent with previous research (Cohen, Perrault, Voltz, Steinberg, & Soubrie,

2002; Grilli, Parodi, Raiteri, & Marchi, 2005; Nisell, Nomikos, & Svensson, 1994; Rapier, Lunt, & Wonnacott, 1988). Furthermore, ketamine increased nicotine-evoked DA release from rat striatal and PFC slices. Our finding is consistent with the finding that Ro 25-6981, an NR2B-selective NMDA receptor antagonist, administered alone increased nicotine-induced locomotor hyperactivity and nicotine-evoked DA release in NAc (Kosowski & Liljequist, 2004). Kosowski and Liljequist's findings indicate that blockade of NMDA receptors increased the reinforcing and stimulant effects of nicotine. However, this is inconsistent with previous reports that repeated MK-801 treatment inhibited nicotine-induced DA release in NAc. In these experiments, MK-801 and nicotine were administered simultaneously (Shoaib, Benwell, Akbar, Stolerman, & Balfour, 1994). Shoaib's findings indicate that repeated co-administration of MK-801 and nicotine blocked sensitization. In contrast, pretreatment with MK-801 alone enhanced locomotor activity in response to a subsequent acute administration of nicotine (Shoaib, Benwell, Akbar, Stolerman, & Balfour, 1994). Shoaib's latter finding suggests that MK-801 pretreatment may induce nAChR sensitization causing nAChRs to be easily activated by the subsequent administration of nicotine. Since subanesthetic ketamine concentrations did not evoke DA release intrinsically in Experiment 1, we can conclude that the augmentation of nicotine-evoked DA release observed is not from ketamine-induced DA release. Thus, in our acute animal model of schizophrenia, rat brain slices are more sensitive to nicotine, which suggests that ketamine may have induced nAChR sensitization.

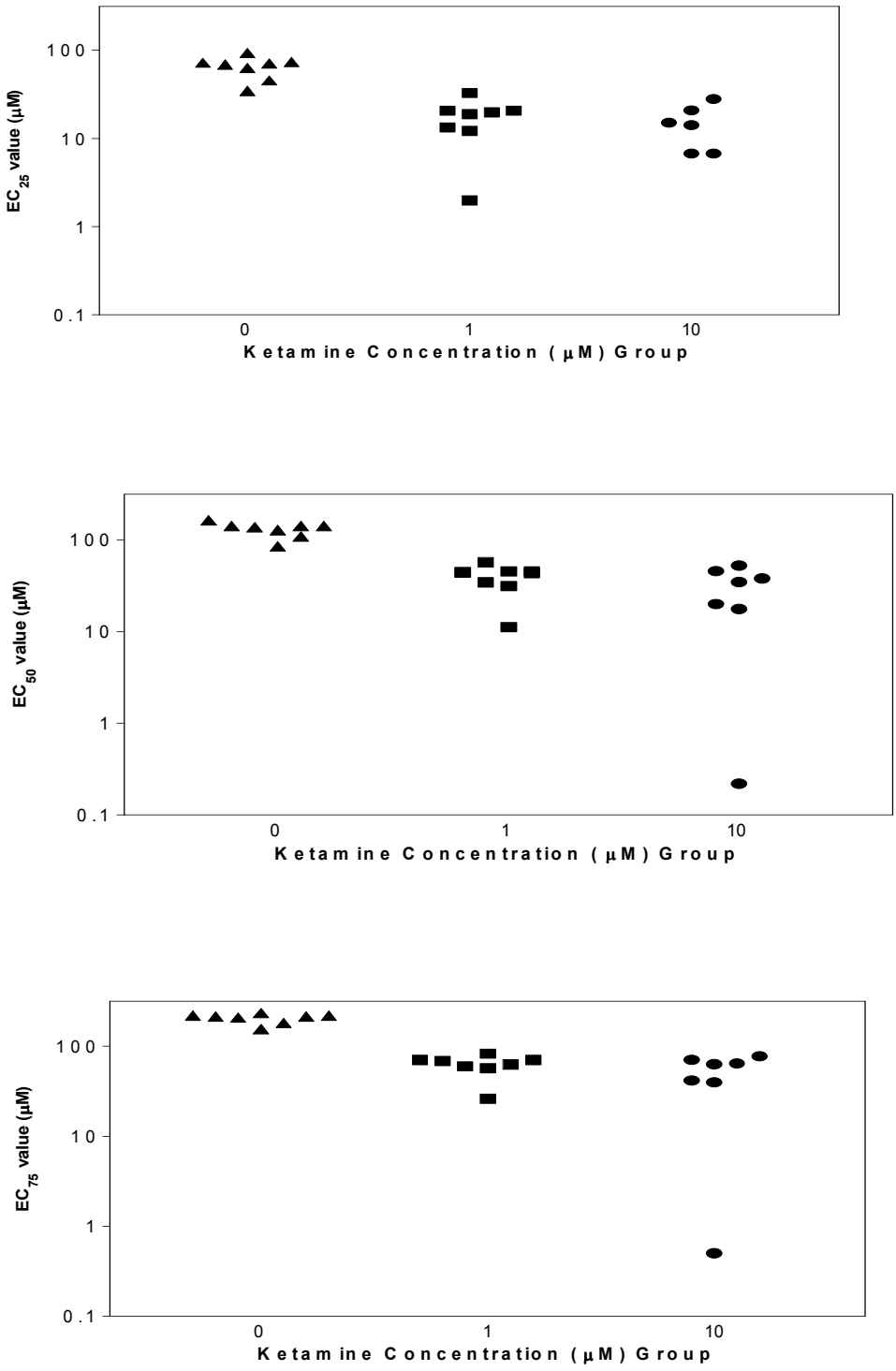

Figure 9. EC_{25}, EC_{50}, and EC_{75} values for striatal slices. Ketamine (1 - 10 µM) augmented nicotine-evoked DA release from each rat striatal slice. Top. EC_{25} values Middle. EC_{50} values Bottom. EC_{75} values. (n = 7 - 8 rats/group)

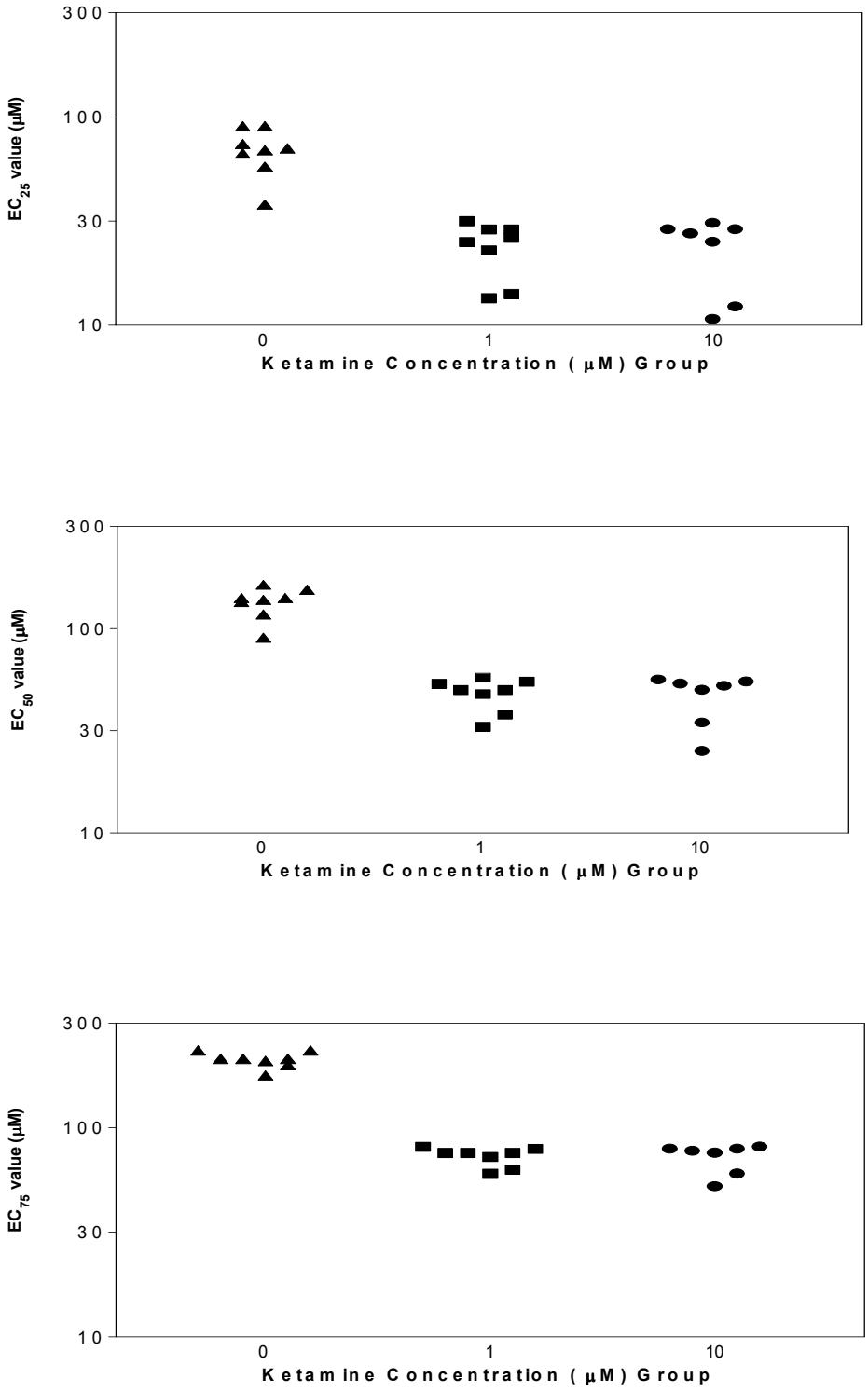

Figure 10. EC_{25}, EC_{50}, and EC_{75} values for PFC slices. Ketamine (1 - 10 µM) augmented nicotine-evoked DA release from each rat PFC slice. Top. EC_{25} values Middle. EC_{50} values Bottom. EC_{75} values. (n = 7 - 8 rats/group)

Ketamine is thought to produce its actions primarily through inhibition of NMDA receptors by binding to the PCP binding site. However, recently its selectivity for NMDA receptors has been questioned. Ketamine may be increasing nicotine-evoked DA release by activation of DA neurons. Some research groups have reported that PCP and ketamine are DA agonists (Ohmori, Koyama, Nakamura, Wang, & Yamashita, 1992), more specifically, activating D_2 DA receptors (Seeman, Ko, & Tallerico, 2005). Seeman and colleagues reported that ketamine has higher affinity for D_2 DA receptors (K_i = 55 nM) than for NMDA receptors (K_i = 3100 nM) (Seeman, Ko, & Tallerico, 2005). This suggests that ketamine could be augmenting nicotine-evoked DA release by activating DA receptors. However, we did not observe DA release from striatal and PFC slices in Experiment 1, which means that it is unlikely that ketamine was working as a DA agonist.

There is also evidence to suggest that ketamine is a DAT inhibitor (Nishimura & Sato, 1999), which would cause an increase in extracellular DA levels. However, we do not believe that the ability of ketamine to increase nicotine-evoked DA release is due to its effects on DAT as the K_i value is 66.8 μM (Nishimura & Sato, 1999), and our ketamine concentrations are much lower.

It has been widely reported that ketamine is a potent nAChR antagonist (Coates & Flood, 2001; Flood & Krasowski, 2000) with IC_{50} = 2.8 μM (Furuya et al., 1999), however, this is not in line with our findings. We would expect ketamine to attenuate nicotine-evoked DA release if it were working as a nAChR

antagonist. Instead ketamine may have induced sensitization of nAChRs allowing them to become easily activated by the addition of nicotine.

Ketamine has also been reported to inhibit voltage-gated calcium channels in rat PC-12 cells (Wong & Martin, 1993). It is unlikely that the ability of ketamine to augment nicotine-evoked DA release is due to its effects on voltage-gated calcium channels as the reported K_i value is 33 μM (Wong & Martin, 1993). Also, we would expect a decrease in DA release if ketamine were blocking calcium influx.

Ketamine produces is actions via a myriad of receptor sites and the mechanism of action by which ketamine increased nicotine-evoked DA release is unclear. However, ketamine is most likely not acting as a DA agonist, DAT inhibitor, nAChR antagonist, or calcium channel blocker in this study. Ketamine may have induced nAChR sensitization as it altered the function of nAChRs that mediate DA release in striatum and PFC.

This aim of this study was to investigate the interaction of ketamine and nicotine on DA release from striatal and PFC slices. Nicotine induced time- and concentration-dependent increases in DA release from rat striatal and PFC slices as measured by a [^{3}H] overflow assay. Ketamine augmented the effect of nicotine to increase DA release in a concentration-dependent manner from rat striatal and PFC slices. The effect of nicotine to evoke [^{3}H]DA release has been well characterized (Cohen, Perrault, Voltz, Steinberg, & Soubrie, 2002; Grilli, Parodi, Raiteri, & Marchi, 2005; Nisell, Nomikos, & Svensson, 1994; Rapier, Lunt, & Wonnacott, 1988), however, the interaction of ketamine and nicotine on

DA release is less well characterized. Additionally, the effects of ketamine on DA release in striatum and PFC is controversial in the literature. Researchers suggest that ketamine increases (Lindefors, Barati, & O'Connor, 1997; Moghaddam, Adams, Verma, & Daly, 1997; Verma & Moghaddam, 1996) and decreases (A. Carlsson, Waters, & Carlsson, 1999; Javitt & Zukin, 1991) DA release in striatum and PFC. Few studies have investigated the effect of NMDA receptor antagonists on nicotine-evoked DA release and the results of these studies are contradictory as well (Kosowski & Liljequist, 2004; Shoaib, Benwell, Akbar, Stolerman, & Balfour, 1994). Taken together, these findings make it difficult to interpret the interaction of ketamine and nicotine on DA release. However, after considering possible targets of ketamine, our results indicate that ketamine altered the function of nAChRs that mediate DA release in striatum and PFC, perhaps by inducing nAChR sensitization.

This study was designed to investigate the role of nicotine pharmacology in an acute pharmacological animal model of schizophrenia via a slice superfusion assay in which subanesthetic ketamine concentrations were used to induce neurochemical changes associated with schizophrenia. Ketamine augmented the effect of nicotine to evoke DA release in striatum and PFC. This indicates that it requires less nicotine to activate nAChRs in order to increase DA release in the presence of ketamine than in the absence. Taken together, these findings support an overall role of nAChRs as targets for schizophrenia treatments. Furthermore, our findings support the research suggesting that

schizophrenic smokers use nicotine more frequently in order to alleviate negative symptoms and cognitive dysfunction associated with schizophrenia.

EXPERIMENT 3: EFFECT OF SUBCHRONIC KETAMINE EXPOSURE ON NICOTINE-EVOKED [^{3}H] OVERFLOW

The purpose of Experiment 3 was to determine the effects of subchronic ketamine exposure on nicotine pharmacology as a pharmacological animal model of schizophrenia. Rat striatal and PFC slices preloaded with [^{3}H]DA were superfused with buffer and then nicotine (1 - 100 μM) was added.

Materials and Methods

Animals and pretreatment exposure regimen. Prior to the start of the experiments, 27 male Sprague-Dawley rats (Harlan, Indianapolis, IN; 175-200 g upon arrival to the laboratory) were weighed and assigned randomly to one of three ketamine exposure groups (0 mg/kg ketamine (n = 9), 30 mg/kg ketamine (n = 10), and 50 mg/kg ketamine (n = 8)). All other animal and colony procedures and maintenance were similar to those as described above.

On Day 1, the pretreatment exposure regimen began. Rats were injected (IP) with either 30 or 50 mg/kg of ketamine or saline once daily for 30 days (Days 1 – 30) during the light phase of the cycle. On Days 30 & 31, behavioral changes associated with positive symptoms of schizophrenia were assessed using a locomotor activity assay. Rats were transferred from their home cage and placed in the locomotor activity monitor for 60 min after drug or vehicle injection (Figure 11).

Apparatus. Locomotor activity was monitored automatically using Med Associates' (Georgia VT) Open Field Test Environments (ENV-515), comprised

of a 16×16 horizontal grid of infrared sensors and a bank of 16 vertical sensors. Each monitor surrounded an acrylic cage (43.2×43.2×30.5 cm), and each monitor and cage was housed in a large sound-resistant cubicle (ENV-017M). Locomotor activity data was collected in 5 min intervals using Med Associates' Open Field Activity Software (SOF-811) that records the number of sensor breaks throughout the monitor and computes these data as measures of distance traveled (cm).

Procedure. After the locomotor activity assessment, rats were not administered any injections or tests and continued to receive ad libitum access to water and standard rat chow for Days 32 - 36. On Day 37, one week after the final ketamine or saline injection, rat brain slices and buffer were prepared as described in Experiment 1. Slices were superfused with buffer at a rate of 0.75 ml/min. After 60 min, sample collection commenced at a rate of 1 sample/3 min. After the collection of 3 baseline samples, nicotine (1 - 100 μM) was added to buffer, and superfusion continued for 6 min. The nicotine concentrations were selected from preliminary experiments in our lab that determined the concentration-response profile for nicotine (Figure 2). One chamber was superfused with buffer alone for both striatal and PFC slices and represented a control condition. Subsequently, all slices were superfused with buffer that did not contain nicotine for 15 min. When collection was complete, slices and filters were removed from the reaction chambers and incubated with tissue solubilizer (0.25 ml/sample).

Drugs and chemicals. (±)-Ketamine hydrochloride (Ketasthesia) was purchased from Butler Animal Health Supply (Chicago IL). All other drugs and chemicals acquired as described above.

For pretreatment exposure regimen, ketamine was diluted in 0.9% saline vehicle. Injection volume was 1.0 ml solution/kg body weight.

Data analysis. For the locomotor activity probe, distance traveled data were analyzed via 2-way RM-ANOVA with Ketamine Dose as a between-subjects factor and Time as a within-subjects factor. Fractional [^{3}H]DA release and total [^{3}H] overflow were calculated as described above. Initially, the data for each ketamine dose were analyzed separately by the following analyses. Total

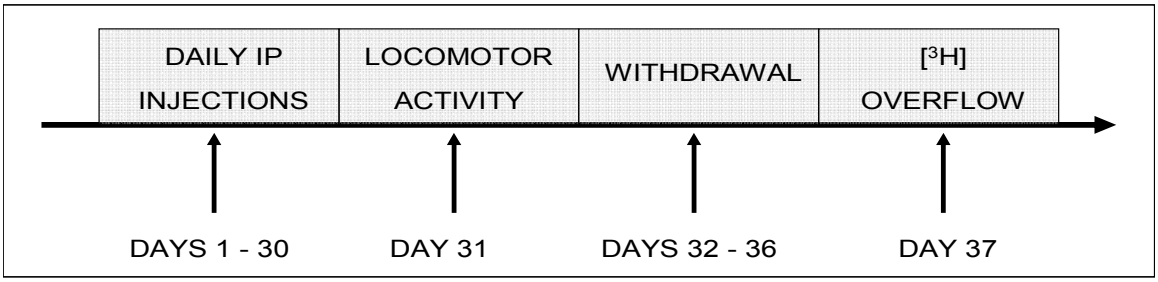

Figure 11. Experiment 3 Timeline. Rats receive injections daily for 30 days. On Day 30 & 31, locomotor activity is assessed. On Days 32 – 36, no behavioral testing or injections are given. On Day 37, [^{3}H] overflow assay is performed.

[^{3}H] overflow data were analyzed via 1-way RM-ANOVA with Nicotine Concentration as a within-subject factor. Fractional [^{3}H] release was analyzed via 2-way RM-ANOVA with Nicotine Concentration and Time as within-subjects factors. Comparisons among ketamine concentrations were analyzed by the following analyses. Total [^{3}H] overflow data were analyzed via 2-way RM-ANOVA with Nicotine Concentration as a within-subject factor and Ketamine Dose as a between-subjects factor. Fractional [^{3}H] release was analyzed via 3-way RM-ANOVA with Nicotine Concentration and Time as within-subjects factors and Ketamine Dose as a between-subjects factor. For all analyses, a significance level of $P < 0.05$ was established a priori and paired t-tests were performed as post hoc comparisons using Bonferroni adjustment when appropriate. Data from striatal and PFC slices were analyzed separately.

Results

Ketamine (30 – 50 mg/kg) induced hyperactivity. The presence of behavioral changes associated with positive symptoms of schizophrenia by repeated exposure to ketamine (0, 30, or 50 mg/kg) was assessed using a locomotor activity assay. Ketamine induced hypo- and hyperactivity in rats. Analysis of total distance traveled revealed a significant main effect of Ketamine Dose [$F(2,24)=51.61$, $P<0.05$]. Regarding the time course, a significant main effect of Time was found [$F(11,264)=13.15$, $P<0.05$] and the Ketamine Dose x Time interaction was also significant [$F(22,264)=6.37$, $P<0.05$]. Rats in the ketamine-treated groups (30 or 50 mg/kg) were more active than rats in the saline-treated group, indicating that ketamine induced hyperactivity following the

last injection (Figure 12 Top). Additionally, ketamine appeared to induce modest hypo- and hyperactivity in rats 1 day after the last injection. The main effect of Ketamine Dose [$F(2,24)=0.31$, $P=0.74$] was not significant. Regarding the time course, a significant main effect of Time was found [$F(11,264)=116.68$, $P<0.05$] and the Ketamine Dose x Time was also significant [$F(22,264)=2.16$, $P<0.05$]. One day after the last injection, ketamine appeared to induce modest hypo- and hyperactivity as rats in the ketamine-treated groups showed significant differences in activity from the rats in the saline-treated group (Figure 12 Bottom).

Nicotine evokes [^{3}H]DA release in striatum and PFC. Nicotine evoked [^{3}H] overflow in a concentration-dependent manner from both striatal and PFC slices from saline treated animals. A significant main effect of Nicotine Concentration was found for the measure of total [^{3}H] overflow from striatal [$F(5,35)=13.79$, $P<0.05$] and PFC [$F(5,35)=18.98$, $P<0.05$] slices. Post hoc analyses revealed greater [^{3}H] overflow for 10 -100 μM nicotine than for control (0 μM nicotine) from striatal slices (Figure 13). Post hoc analyses also revealed greater [^{3}H] overflow for 30 – 100 μM nicotine than for control from PFC slices (Figure 14). Regarding fractional [^{3}H]DA release from striatal slices, the main effects of Nicotine Concentration [$F(5,35)=7.34$, $P<0.05$] and Time [$F(9,63)=24.56$, $P<0.05$] were significant. A significant Nicotine Concentration x Time interaction [$F(45,315)=12.61$, $P<0.05$] was also found. Regarding fractional [^{3}H]DA release from PFC slices, the main effects of Nicotine Concentration [$F(5,35)=23.54$, $P<0.05$] and Time [$F(9,63)=16.49$, $P<0.05$] were significant. A significant

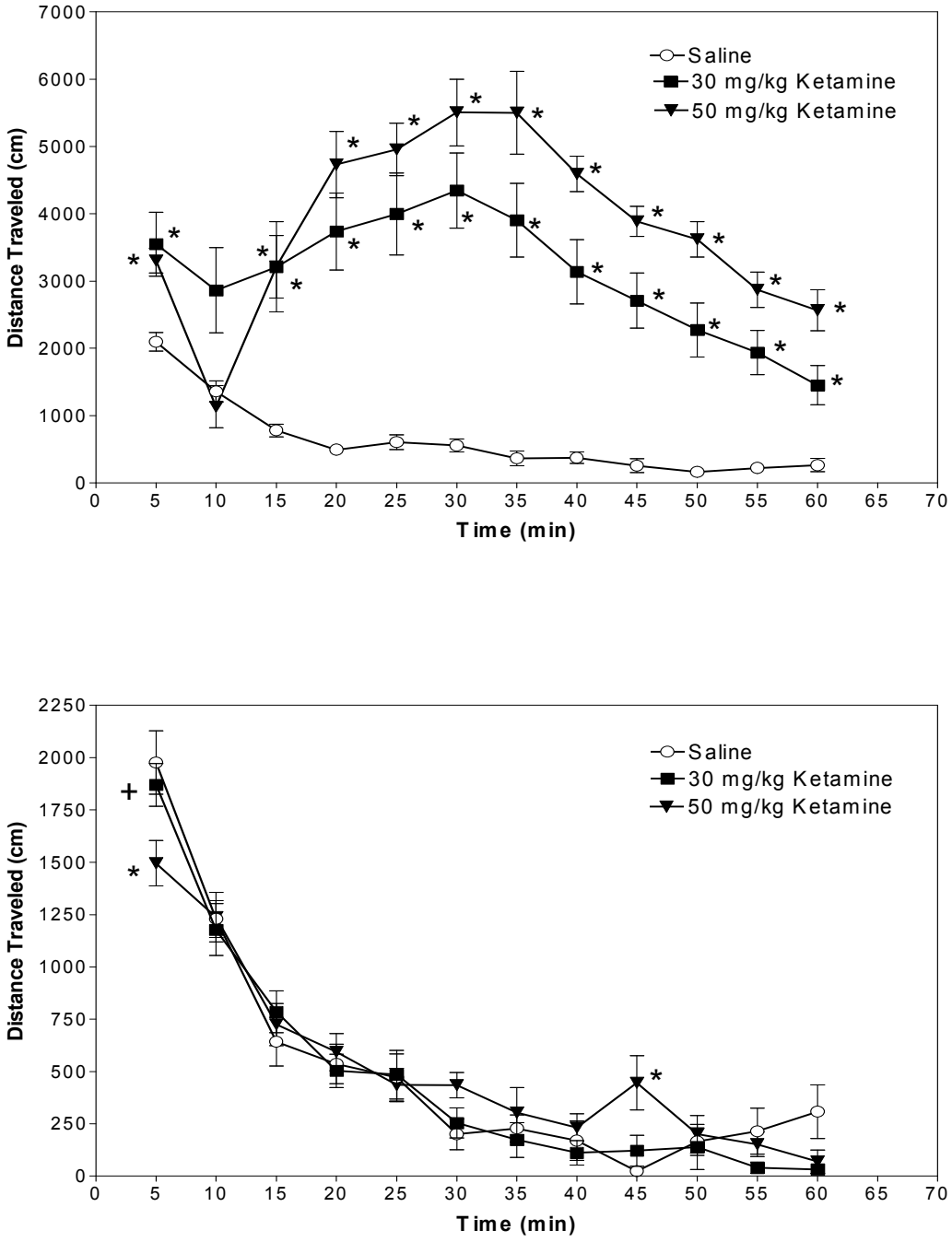

Figure 12. Locomotor Activity on Day 30 & 31. Top. Ketamine (30 – 50 mg/kg) induced hyperactivity following the last injection. Rats were given injection and put into locomotor activity monitor for 60 min. Bottom. Ketamine (30 – 50 mg/kg) induced modest hyperactivity 1 day after the last injection. Rats were put into locomotor activity monitor for 60 min. Asterisks designate significant ($P<0.05$) difference from Saline-treated group and plus signs designate significant ($P<0.05$) difference between Ketamine-treated groups. Note ordinate scale differences. (n = 8 - 10 rats/group)

Nicotine Concentration x Time interaction [F(45,315)=19.48, P<0.05] was also found. Thus, nicotine evoked [^{3}H] overflow from both striatal and PFC slices from saline treated animals, additionally, the threshold nicotine concentration for striatal and PFC slices was 10 μM and 30 μM, respectively.

Effect of ketamine (30 – 50 mg/kg) on nicotine-evoked [^{3}H]DA release in striatum and PFC. Ketamine (30 – 50 mg/kg) did not alter nicotine-evoked [^{3}H] overflow from both striatal (Figure 13) and PFC (Figure 14) slices. A significant main effect of Nicotine Concentration was found for the measure of total [^{3}H] overflow from striatal [F(5,115)=48.82, P<0.05] and PFC [F(5,110)=82.07, P<0.05] slices. However, the main effect of Ketamine Concentration was not significant for the measure of total [^{3}H] overflow from striatal [F(2,23)=1.42, P=0.26] and PFC [F(2,22)=0.37, P=0.69] slices. Also the Nicotine Concentration x Ketamine Concentration interaction was not significant for the measure of total [^{3}H] overflow from striatal [F(10,115)=1.17, P=0.32] and PFC [F(10,110)=0.20, P=0.99] slices. Thus, there were no significant differences for nicotine-evoked DA release among ketamine-treated groups. Subsequent analyses were performed to further investigate the data.

Ketamine (50 mg/kg) lowered the threshold nicotine concentration. Nicotine evoked [^{3}H] overflow in a concentration-dependent manner from both striatal and PFC slices of ketamine (50mg/kg)-treated animals. A significant main effect of Nicotine Concentration was found for the measure of total [^{3}H] overflow from striatal [F(5,35)=79.69, P<0.05] and PFC [F(5,35)=56.67, P<0.05] slices. Post hoc analyses revealed greater [^{3}H] overflow with 1 - 100 μM nicotine than

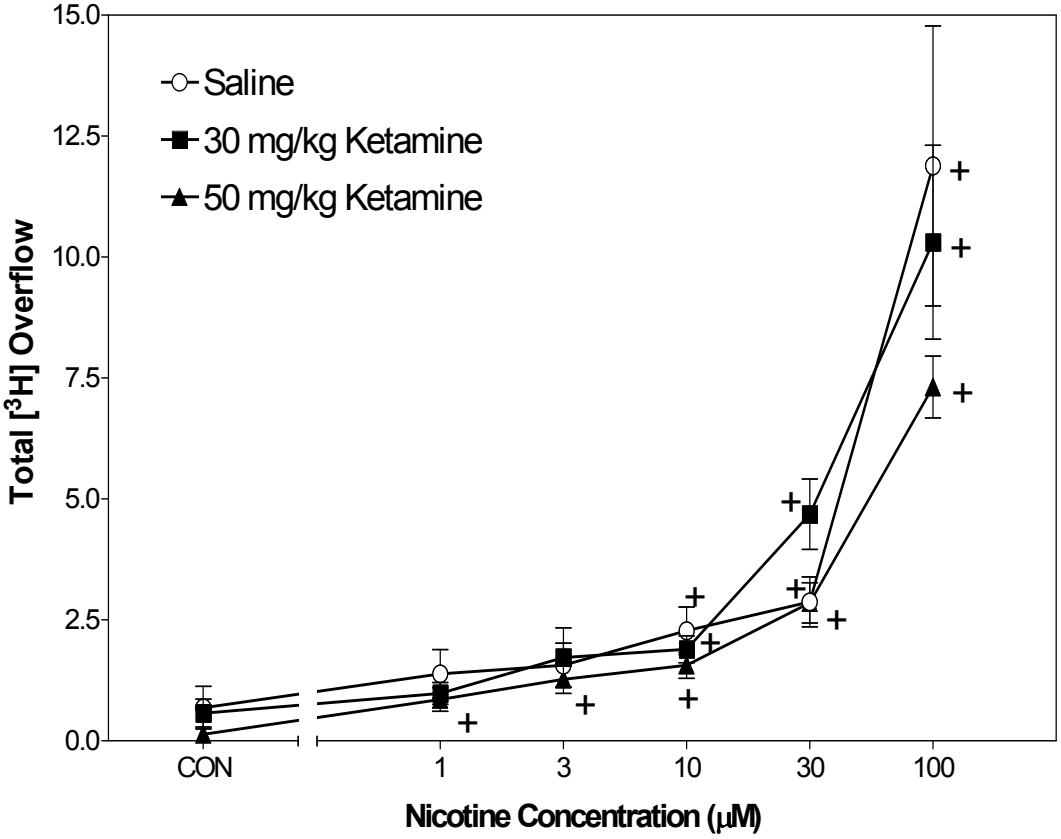

Figure 13. Ketamine (30 – 50 mg/kg) altered the effect of nicotine on total [^{3}H] overflow from rat striatal slices preloaded with [^{3}H]DA. Data are expressed as the mean (± S.E.M.) total [^{3}H] overflow after the addition of nicotine to ketamine buffer. Plus signs designate a significant ($P<0.05$) difference from control condition within each ketamine group. *CON, control condition.* (n = 8 - 10 rats/group)

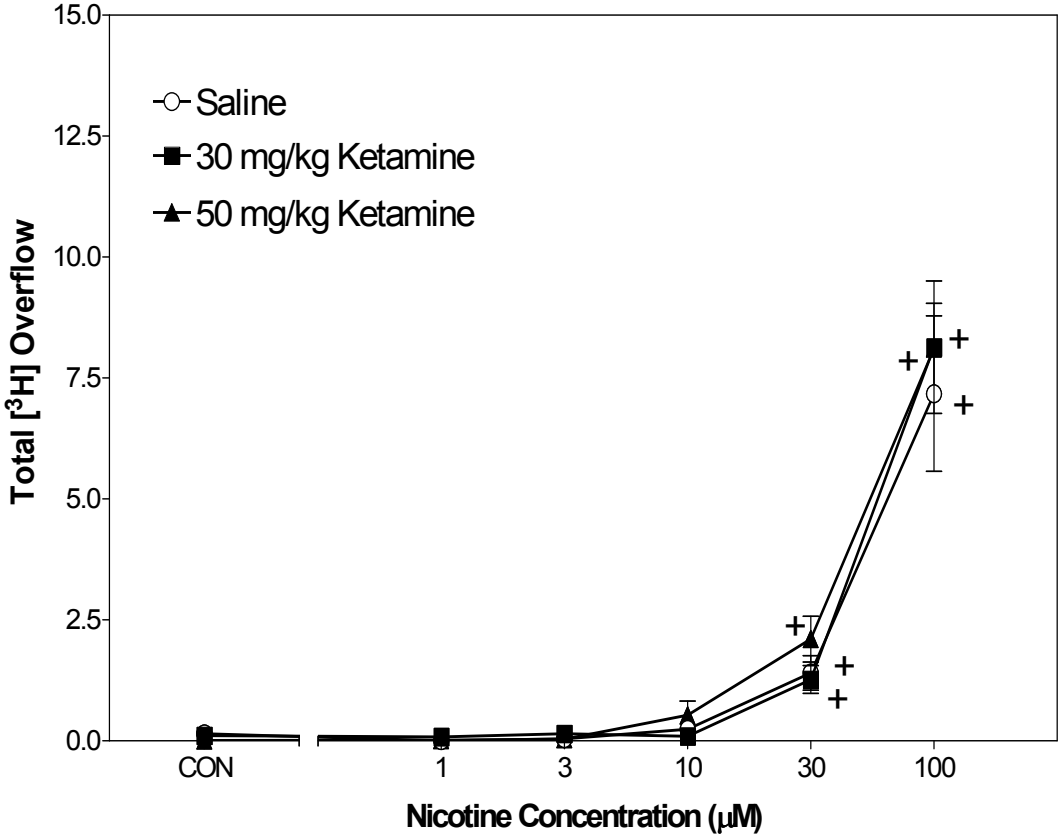

Figure 14. Ketamine (30 – 50 mg/kg) altered the effect of nicotine on total [^{3}H] overflow from rat PFC slices preloaded with [^{3}H]DA. Data are expressed as the mean (± S.E.M.) total [^{3}H] overflow after the addition of nicotine to ketamine buffer. Plus signs designate a significant ($P<0.05$) difference from control condition within each ketamine group. *CON, control condition.* (n = 8 - 10 rats/group)

for control (50 mg/kg ketamine/0 μM nicotine) from striatal slices (Figure 13). The threshold nicotine concentration for 50 mg/kg ketamine group was 1 μM and the threshold nicotine concentration for control (0 mg/kg ketamine) was 10 μM in striatum. Post hoc analyses also revealed greater [^{3}H] overflow with 30 – 100 μM nicotine than for control (50 mg/kg ketamine/0 μM nicotine) from PFC slices (Figure 14). The threshold nicotine concentration for 50 mg/kg ketamine group was 30 μM and the threshold nicotine concentration for control (0 mg/kg ketamine) was 30 μM in PFC. Thus; ketamine (50 mg/kg) lowered the threshold nicotine concentration in striatum as compared to control (0 mg/kg ketamine).

Nicotine evoked DA release in a time- and concentration-dependent manner from striatal slices of ketamine (50 mg/kg)-treated rats. Regarding fractional [^{3}H]DA release from striatal slices, the main effects of Nicotine Concentration [$F(5,35)=10.03$, $P<0.05$] and Time [$F(9,63)=44.47$, $P<0.05$] were significant. A significant Nicotine Concentration x Time interaction [$F(45,315)=31.12$, $P<0.05$] was also found. Post hoc analyses revealed that fractional release was greater for 100 μM nicotine than for control (50 mg/kg ketamine/0 μM nicotine) at the 12, 15, 18, 21, 24, 27, and 30 min time points. Fractional release was greater for 30 μM nicotine than for control at the 18, 21, 24, and 27 min time points. Fractional release was greater for 10 μM nicotine than for control at the 18, 21, and 24 min time points. Thus, nicotine evoked DA release in a time- and concentration-dependent manner from striatal slices of ketamine-treated rats.

For PFC slices, nicotine evoked DA release in a time- and concentration-dependent manner of ketamine (50 mg/kg)-treated rats. Regarding fractional [^{3}H]DA release from PFC slices, the main effects of Nicotine Concentration [$F(5,35)=23.61$, $P<0.05$] and Time [$F(9,63)=32.41$, $P<0.05$] were significant. A significant Nicotine Concentration x Time interaction [$F(45,315)=26.57$, $P<0.05$] was also found. Post hoc analyses revealed that fractional release was greater for 100 μM nicotine than for control (50 mg/kg ketamine/0 μM nicotine) at the 12, 15, 18, 21, 24, 27, and 30 min time points. Fractional release was greater for 30 μM nicotine than for control at the 15, 18, 21, 24, and 27 min time points. Thus, nicotine evoked DA release in a time- and concentration-dependent manner from PFC slices of ketamine-treated rats.

Ketamine (30 mg/kg) did not lower the threshold nicotine concentration. Nicotine evoked [^{3}H] overflow in a concentration-dependent manner from both striatal and PFC slices of ketamine (30mg/kg)-treated animals. A significant main effect of Nicotine Concentration was found for the measure of total [^{3}H] overflow from striatal [$F(5,45)=17.56$, $P<0.05$] and PFC [$F(5,40)=24.53$, $P<0.05$] slices. Post hoc analyses revealed greater [^{3}H] overflow for 10 - 100 μM nicotine than for control (30 mg/kg ketamine/0 μM nicotine) from striatal slices (Figure 13). The threshold nicotine concentration for 30 mg/kg ketamine group was 10 μM and the threshold nicotine concentration for control (0 mg/kg ketamine) was also 10 μM in striatum. Post hoc analyses also revealed greater [^{3}H] overflow with 30 – 100 μM nicotine than for control (30 mg/kg ketamine/0 μM nicotine) in PFC slices (Figure 14). The threshold nicotine concentration for 30 mg/kg ketamine group was 30

μM and the threshold nicotine concentration for control (0 mg/kg ketamine) was also 30 μM in PFC. Thus, nicotine evoked DA release in striatum and PFC of ketamine (30 mg/kg)-treated rats. However, the threshold nicotine concentration was not different from saline-treated rats.

Discussion

The results of the present study indicate that ketamine (30 – 50 mg/kg) significantly altered locomotor activity. Ketamine-treated rats were hyperactive compared to saline-treated rats on Day 30 and 31. Ketamine significantly altered nicotine-evoked [^{3}H] overflow, when administered subchronically, from rat striatal and PFC slices. Subchronic ketamine (50 mg/kg) exposure decreased the threshold nicotine concentration in striatum compared to the saline-treated rats.

The effect of subchronic ketamine administration on locomotor activity was determined in order to make sure that exposure to ketamine induces behavioral changes associated with positive symptoms of schizophrenia. Subchronic administration of subanesthetic doses of ketamine and PCP induced positive symptoms associated with schizophrenia such as hyperactivity (Leccese, Marquis, Mattia, & Moreton, 1986; Sams-Dodd, 1995, , 1996) in animals. The production of positive symptoms associated with schizophrenia is caused by hyperactivity of DA neurons in mesolimbic and nigrostriatal pathways (A. Carlsson, 1995; Seeman, 1987). In the present study, ketamine induced hypo- and hyperactivity following the last injection; however, this is most likely caused by a direct drug effect. The hypoactivity was observed at the 10 min time point in both 30 - 50 mg/kg ketamine-treated rats. Through crude observations, this can

be explained by drug-induced stereotypy. Additionally, modest hypo- and hyperactivity were observed after 24 hr ketamine withdrawal, which is most likely due to neuroadaptive changes induced by repeated ketamine exposure. Hypoactivity was observed in the 50 mg/kg ketamine-treated rats at the 5 min time point. Additionally, hypoactivity was observed in both ketamine-treated groups compared to the previous day; whereas, the saline-treated rats showed no differences in locomotor activity across Days 30 & 31. The hypoactivity observed on Day 31 supports our suggestion that ketamine-induced hyperactivity on Day 30 is due to direct-drug effects. Hence, subchronic ketamine exposure was sufficient to induce behavioral changes associated with positive symptoms of schizophrenia, which is consistent with the model proposed by Carlsson and colleagues (A. Carlsson, Waters, & Carlsson, 1999).

The effect of subchronic ketamine administration on the ability of nicotine to evoke DA release was also determined. The ability of nicotine to improve symptoms of schizophrenia (Levin, 2002; Levin, Bettegowda, Blosser, & Gordon, 1999; Levin & Christopher, 2003; Levin, Kaplan, & Boardman, 1997; Levin, Rose, & Abood, 1995) leads us to believe that there is a common underlying neurobiology between schizophrenia and nicotine addiction. The ability of nicotine to increase DA release has been implicated as having potential to improve symptoms of schizophrenia (Dalack, Healy, & Meador-Woodruff, 1998). In the present study, subchronic ketamine (50 mg/kg) exposure decreased the threshold nicotine concentration as compared to the saline-treated rats (Figure 13). This suggests that ketamine induced neurobiological changes in cortical-

subcortical neurocircuitry as the effect of nicotine to evoke DA release was intensified. Thus, subchronic ketamine exposure induced neuroadaptive changes which increased the brain's sensitivity to nicotine.

Our findings appear to support the model proposed by Carlsson and colleagues in which cortical glutamatergic projections modulate DA activity of mesolimbic and mesocortical DA pathways (Figure 1). According to this model, NMDA receptor blockade, by ketamine, inhibits glutamate neurotransmission. A decrease in glutamate inhibits mesocortical DA neurons which results in decreased DA release in cortical regions. Inhibition of glutamate neurotransmission also accounts for hyperactivity of mesolimbic DA neurons via decreased inhibitory tone of GABAergic interneurons. This results in a subcortical increase in DA release (A. Carlsson, Waters, & Carlsson, 1999). The addition of nicotine after this dysregulation of cortical-subcortical neurocircuitry would increase DA release in cortical and subcortical regions with DA release being greater in subcortical regions. Our results support this notion as DA release was greater in striatum as compared to PFC (Compare Figures 13 & 14). Thus, this suggests that subchronic ketamine administration altered the cortical-subcortical neurocircuitry and the effect of nicotine to evoke DA release.

Interestingly, subchronic ketamine (30 - 50 mg/kg) exposure did not significantly augment nicotine-evoked DA release in PFC. However; there was a trend of the low dose of ketamine toward augmentation of nicotine-evoked DA release. Nevertheless, neurotoxic effects induced by NMDA receptor antagonist administration have been observed (Olney & Farber, 1995a, , 1995b), which

cause a reduction in glutamatergic and GABAergic axon terminals in the brain (Mirnics, Middleton, Marquez, Lewis, & Levitt, 2000). Thus, a deficit in glutamatergic and GABAergic axon terminals would result in decreased DA release.

The aim of the present study was to determine the effect of subchronic ketamine on locomotor activity and the interaction of subchronic ketamine and nicotine on DA release. Ketamine (30 – 50 mg/kg) increased locomotor activity in rats compared to saline-treated rats. This indicates that ketamine induced behavioral changes associated with positive symptoms of schizophrenia, which is consistent with previous research (Leccese, Marquis, Mattia, & Moreton, 1986). Subchronic ketamine exposure altered nicotine-evoked DA release in rat striatal and PFC slices. The high dose of ketamine decreased the threshold nicotine concentration in striatum and the low dose of ketamine produced a trend toward augmented nicotine-evoked DA release. The effect of nicotine to evoke [^{3}H]DA release has been well characterized (Cohen, Perrault, Voltz, Steinberg, & Soubrie, 2002; Grilli, Parodi, Raiteri, & Marchi, 2005; Nisell, Nomikos, & Svensson, 1994; Rapier, Lunt, & Wonnacott, 1988), however, the interaction of subchronic ketamine and nicotine on DA release is less well characterized. To this author's knowledge, the effect of ketamine administered subchronically in vivo on nicotine-evoked DA release has not been investigated. Several studies indicate that ketamine is a potent nAChR antagonist (Furuya et al., 1999; Yamakura, Chavez-Noriega, & Harris, 2000); however, this is not in line with the results from the present low dose of ketamine. Furthermore, these studies were

done in vitro. Schizophrenia involves dysregulation of multiple neurotransmitter systems and cortical-subcortical neurocircuitry, and ketamine produces its actions via a myriad of receptor sites. Therefore, our model provides a comprehensive system to investigate behavioral and neurochemical changes associated with schizophrenia. Few studies have investigated the effect of NMDA receptor antagonists on nicotine-evoked DA release and the results of these studies are contradictory (Kosowski & Liljequist, 2004; Shoaib, Benwell, Akbar, Stolerman, & Balfour, 1994). Repeated co-administration of MK-801 and nicotine decreased locomotor activity and DA release in NAc indicating that MK-801 prevented sensitization to nicotine in rats (Shoaib, Benwell, Akbar, Stolerman, & Balfour, 1994). However, repeated administration of MK-801 alone increased locomotor activity in response to a subsequent nicotine injection indicating that pretreatment with MK-801 produced nAChR sensitization (Shoaib, Benwell, Akbar, Stolerman, & Balfour, 1994). Additionally, Ro 25-6981 administered alone increases nicotine-induced locomotor hyperactivity and nicotine-evoked DA release in NAc (Kosowski & Liljequist, 2004). This indicates that blockade of NMDA receptors increases the reinforcing and stimulant effects of nicotine. Thus, this supports our results and indicates that subchronic ketamine exposure may have induced nAChR sensitization which caused an increase in DA release.

SUMMARY AND CONCLUSION

Ketamine, at pharmacologically relevant concentrations, did not have intrinsic activity to evoke DA release from striatal and PFC slices. In an acute pharmacological animal model of schizophrenia, ketamine augmented nicotine-evoked DA release from striatal and PFC slices. In a subchronic pharmacological animal model of schizophrenia, ketamine altered nicotine-evoked DA release from striatal and PFC slices. Overall, these data indicate that nAChR function is altered in this model of schizophrenia, and support a role for nAChRs in schizophrenia treatment.

Acute and repeated pharmacological animal models of schizophrenia were developed to study cause, progression, and treatment of this disorder. Administration of ketamine induced behavioral and neurochemical changes associated with symptoms of schizophrenia. Acute pharmacological animal models of schizophrenia are sufficient to induce behavioral and neurochemical changes associated with symptoms of schizophrenia, but do not produce a valid model of schizophrenia as these changes are not long-lasting and are most likely due to direct drug effects (Jentsch, Taylor, & Roth, 1998). However, we can still observe valuable interactions. Generally, researchers have observed similar behavioral changes associated with symptoms of schizophrenia in repeated animal models of schizophrenia, but researchers must be careful when comparing acute and repeated models as the neurochemical changes remain controversial in the literature. Repeated pharmacological animal models of

schizophrenia are generally preferred over acute models as they induce behavioral and neurochemical changes associated with symptoms of schizophrenia that are persistent and therefore, provide a more valid model of schizophrenia. The subsequent addition of nicotine to animals treated with ketamine, acutely and subchronically, revealed an important role of nicotine in the underlying mechanism and treatment of schizophrenia. In the present study, ketamine induced hyperactivity, a behavioral change associated with positive symptoms of schizophrenia. This suggests that ketamine altered the function of the nigrostriatal pathway as the production of positive symptoms of schizophrenia is caused by hyperactivity of nigrostriatal DA neurons (A. Carlsson, 1995; Seeman, 1987). Subanesthetic concentrations/doses of ketamine altered nicotine-evoked DA release in both an acute and a subchronic animal model of schizophrenia. This indicates that NMDA receptor blockade by ketamine altered the function of nAChRs and subchronic ketamine exposure induced dysregulation of cortical-subcortical neurocircuitry. Together, these experiments provide support for use of ketamine in pharmacological animal models of schizophrenia, and the role of nAChRs in mechanisms underlying schizophrenia.

In summary, ketamine induces a valid pharmacological animal model of schizophrenia. Ketamine altered neuronal circuits in the brain which resulted in an augmented response of nicotine-evoked DA release. This supports a role for nAChRs as a potential therapeutic target for schizophrenia.

Future Studies

In the future, it would be beneficial to further investigate the role of nAChRs in an acute and a subchronic pharmacological animal model of schizophrenia in freely moving rats. In the acute pharmacological animal model of schizophrenia, in this experiment, ketamine was administered to rats in vitro. In contrast, in the subchronic pharmacological animal model of schizophrenia, ketamine was administered to rats in vivo. Infusion of NMDA receptor antagonists, acutely and subchronically, followed by nicotine directly into specific brain regions would increase validity of the model and decrease variability. Additionally, measuring other neurotransmitters, like glutamate, GABA, and serotonin, would give us an overall picture of the mechanisms underlying schizophrenia. Microdialysis would also allow us to detect decreases in neurotransmitter release. Also targeting specific nAChR subtypes with radioligands will aid in determining which nAChRs are important in schizophrenia and could assist in developing new treatments for symptoms of schizophrenia. The use of other behavioral assays, like radial arm maze, Morris water maze, and operant conditioning, would allow us to investigate other symptoms of schizophrenia.

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