DOPAMINE-GLUTAMATE INTERACTION IN THE ACTIONS OF TYPICAL ANTIPSYCHOTIC DRUGS

by

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Abstract

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Typical antipsychotic drugs (APD) are currently the most effective psychoactive agents for the treatment of schizophrenia. Studies suggest that besides their conventional action of blocking dopamine (DA) D_2 receptors, these drugs also interact with glutamatergic N-methyl-D-aspartate (NMDA) receptors. In addition, blockade of DA D_2 receptors is believed to result in DA cell depolarization block (DB) and movement disorders (catalepsy) in animals. Since it has been hypothesized that drug's antipsychotic potency may be predicted by its ability to produce DB and catalepsy, using CD rats in behavioral, microdialysis and receptor binding studies we investigated whether typical APD induce DB and catalepsy though action on the dopaminergic system, glutamatergic system, or through the interaction between the two systems. Focus of this project was on striatum (STR) and frontal cortex (FC), two brain regions implicated in the DA-glutamate interplay. Our behavioral results show that haloperidol, a potent APD and postsynaptic DA D_2 receptor blocker is a strong catalepsy inducer. Receptor binding study showed that chronic administration of this drug caused a decrease in maximal binding at the NMDA receptors in STR and FC but no significant changes in the DA D_2 receptor densities were seen in the two brain areas. In contrast, metoclopramide, another DA D_2 receptor blocker but not an APD,

within the therapeutic doses (5 mg/kg-10 mg/kg) did not produce catalepsy in experimental animals. The maximal binding parameters for DA D_2 and NMDA receptors in STR and FC after repeated administration of metoclopramide were significantly elevated as compared to haloperidol. However, when animals were pre-treated with metoclopramide (10 mg/kg) it sensitized the brain to haloperidol and enhanced catalepsy. Additionally, our receptor binding studies showed that psychotomimetic agents, PCP and ketamine that cause schizophrenia-like symptoms have several-fold higher binding affinity at NMDA receptors as compared to DA D_2 receptors, indicating that pharmacological effect of these drugs may be mainly mediated by blockade of NMDA receptors. Finally, studying the neurochemical mechanism for DA cell DB we saw a decrease in striatal DA release after chronic cocaine treatment compared to controls. In a series of follow-up experiments we compared the effect of low dose (0.5 mg/kg) haloperidol and high dose (3.0 mg/kg) haloperidol by acute injection to the chronic cocaine treated rats and to the control animals. Low dose haloperidol significantly increased straital DA release compared to respective controls, while the high dose haloperidol significantly reduced it compared to the low dose. On the other hand, high dose haloperidol drastically increased striatal DA release in chronic cocaine-treated rats compared to controls. These results suggest that the mechanism for catalepsy is based on the concurrent DA D_2 receptor antagonism and activation of glutamatergic NMDA transmission. Similarly, the mechanism for DA cell DB is mediated through blockade of dopaminergic D_2 receptors and stimulation of NMDA receptors. Thus, catalepsy as well as antipsychotic activity appears to be mediated through modifications of dopaminergic and glutamatergic transmissions.

This dissertation is dedicated to my Family.

I had your love, support, and understanding, therefore I achieved.

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LIST OF FREQUENTLY USED ABREVIATIONS

APD	antipsychotic drugs
DA	dopamine
DB	dopamine cell depolarization block
EPS	extrapyramidal side effects
FC	frontal cortex
GABA	gama-amino-butyric acid
MK-801	dizocilpine
NMDA	N-methyl-D-aspartate
NAc	nucleus accumbens
PCP	phencyclidine
SN	substantia nigra
STR	striatum

Chapter 1: Introduction

Antipsychotic drugs

Antipsychotic drugs (APD) are currently the most effective agents in the pharmacological management of schizophrenia and psychosis. These drugs were first used for treatment of schizophrenia in 1950s, marking chlorpromazine as the first used drug to alleviate stress in patients undergoing surgery (Delay et al., 1952). Following the use of chlorpromazine, the term neuroleptic was introduced to describe drugs that can reduce mental stress in all patients.

APD are classified into a) phenothiazine derivatives, b) thioxantane derivatives, c) butyrophenone derivatives, d) diphenylbutylpiperidine derivatives, e) benzamide derivatives, f) dibenzoxaziepine derivatives, and g) indole derivatives (Ebadi and Srinivasan, 1995). The classical neuroleptics, which generally cause extrapyramidal side effects (EPS) were subsequently called typical neuroleptics or first generation APD, and their counterparts that do not induce EPS are called atypical neuroelptics. The mechanism of action of APD remains to be elucidated. The high incidence of motor side effects associated with either acute or chronic administration of typical neuroleptics represents a particularly challenging problem.

The extrapyramidal movement disorders are one of the primary reasons why the patients discontinue self-medication. The spectrum of movement problems includes akathisia, dystonia, Parkinsonism, and tardive dyskinesia (Jankovic, 1995; Sachdev, 1995, 1995). First observations of neuroleptic-induced problems were made by Delay and colleagues in 1960s, while using haloperidol to treat psychosis. Eight years later they coined the term “neuroleptic malignant syndrome” as a drug-induced EPS (Delay and

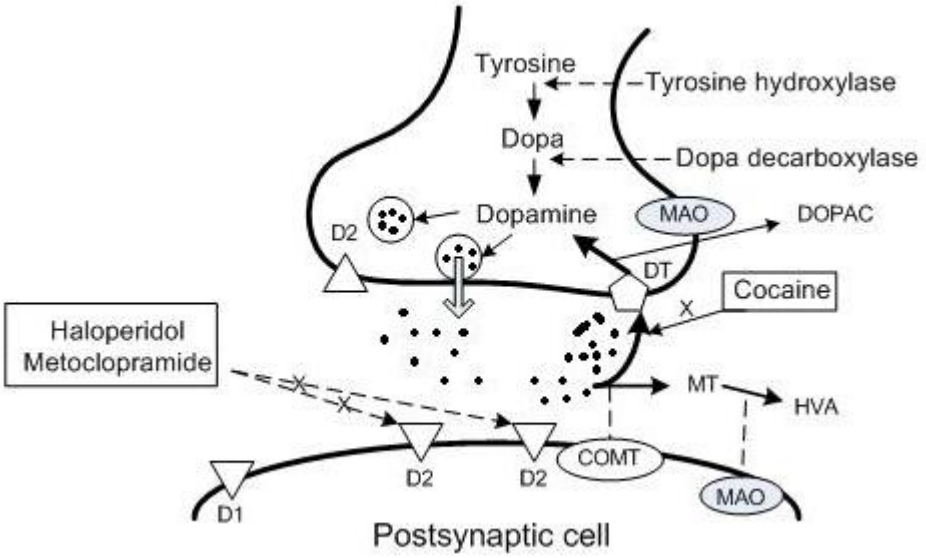
Deniker, 1968). The consequence of discontinued medication in most cases is abrupt psychotic relapse (Cassey, et al., 1995). Co-treatment with anticholinergic agents has a very limited success, mainly with some acute onset symptoms (Baldessarini, 2001). Development of a treatment for the side effects of neuroleptic drugs is severely weakened by uncertainty concerning what drug actions produce these symptoms. Based on the premises that typical neuroleptic drugs are dopamine (DA) antagonists, it has been hypothesized that stimulation of supersensitive DA D_2 receptors produces dyskinetic movements. The first observations suggesting the involvement of dopamine in neuroleptic-induced side effects was made by Henderson and Wooten (1981). Burke and colleagues confirmed this notion while studying the effects of DA depleting agents in patients with Huntington's chorea (Burke et al., 1981).

Dopaminergic and glutamatergic neurotransmitter systems

Dopaminergic synapse

DA and all other catecholamines are synthesized from the amino acid tyrosine, a product of food proteins. Synthesis of DA is a two-step enzymatic process. First, tyrosine is converted to $_L$-dihydroxyphenyl-alanine ($_L$-Dopa) by the enzyme tyrosine hydroxylase which is the rate limiting enzyme in dopamine synthesis (see Fig. 1.1). Second, $_L$-Dopa is rapidly converted to DA by aromatic ($_L$-aromatic) amino acid decarboxylase. DA is stored in nerve terminal vesicles and released into the synaptic space upon neuronal depolarization. Released DA is transported back into the neuron via DA-selective transporter. Extravesicular DA is metabolized intracellularly by the enzyme monoamine oxidase (MAO) to dihydroxyphenylacetic acid (DOPAC).

Dopaminergic synapse



Glutamatergic synapse **GABAergic synapse**

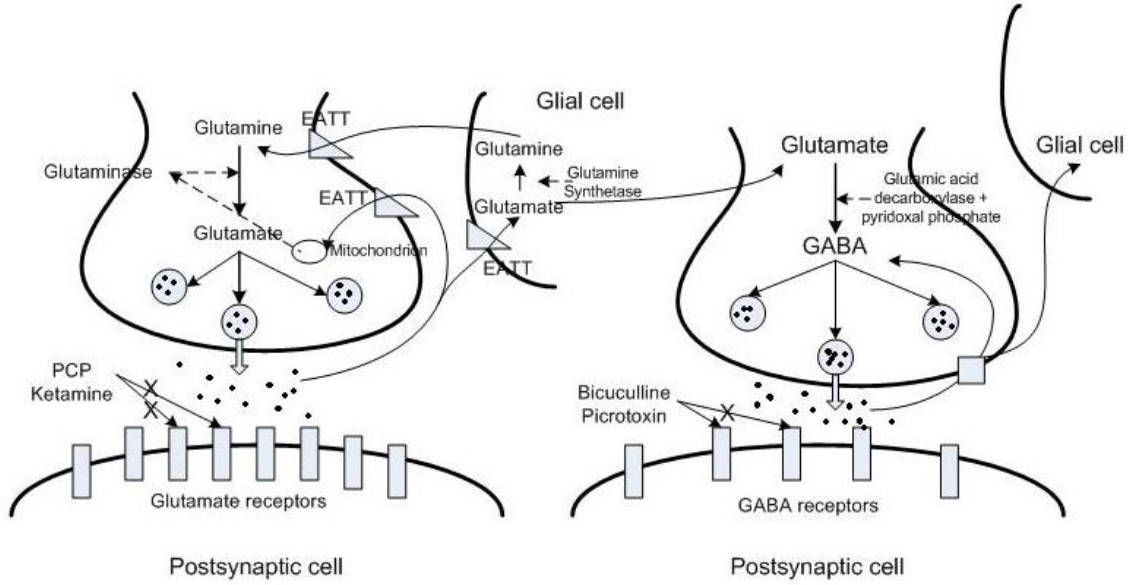


Fig. 1.1. Schematic drawing of dopaminergic, glutamatergic and GABA-ergic nerve terminals. Haloperidol, metoclopramide and cocaine were drugs used in this thesis. Abreviations: DA=dopamine, D1=D_1-like receptor, D2=D_2-like receptors, DT=dopamine transporter, EATT=Excitatory amino acid transporter.

Extracellular DA is degraded by catechol-O-methyl transferase (COMT) to 3-methoxytyramine (3-MT), which is then degraded into homovanillic acid (HVA). In the

rat brain DOPAC is the major DA metabolite and together with HVA are found in high concentrations. In the human brain HVA is the predominant product and only small amounts of DOPAC are produced (Cooper et al., 2003).

Based on their axonal projections, the DA neurons are arranged into four subsystems: the mesolimbic, the mesocortical, the nigrostriatal, and the tuberoinfundibular dopaminergic system (Fig. 1.2).

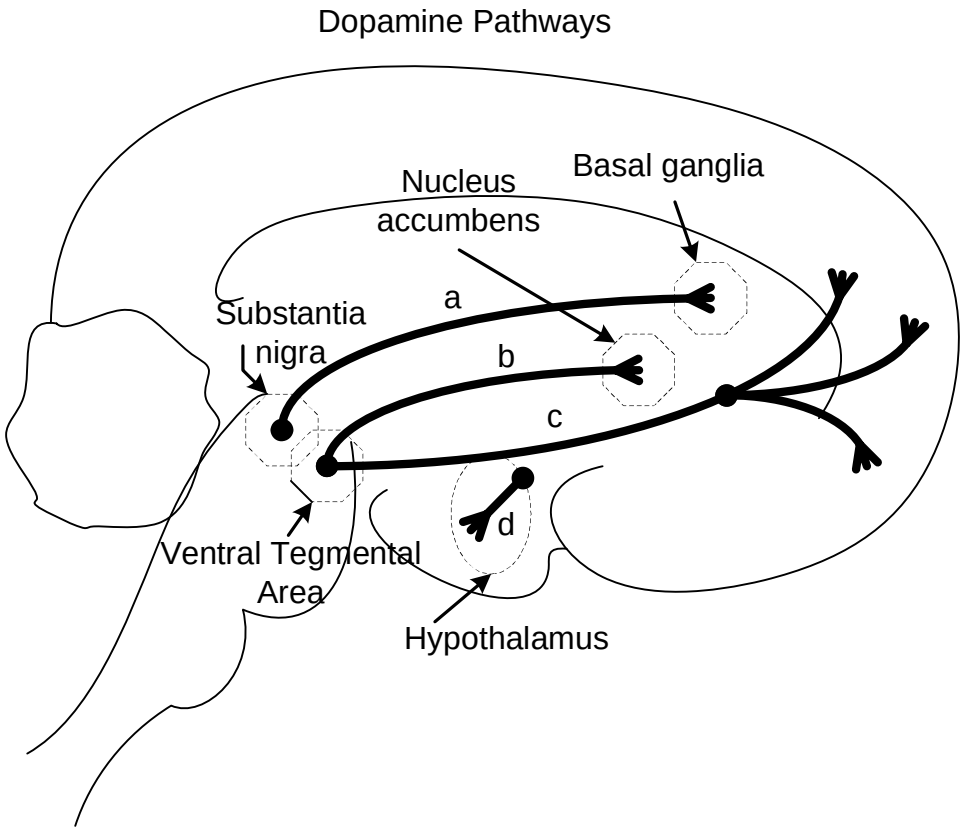


Fig. 1.2. Dopaminergic pathways in the brain. Abbreviations: (a) the nigrostriatal DA pathway; (b) the mesolimbic DA pathway; (c) the mesocortical DA pathway; (d) the tuberoinfundibular DA pathway. (Based on Stahl et al., 2002).

The *mesolimbic* DA neurons originate in the ventral tegmental area (VTA) of the brain stem and project to axon terminals in the limbic areas of the brain such as the nucleus accumbens (NAc), parts of the amygdala and hippocampus, and cingulate and entorhinal

cortices. This pathway plays role in emotional behaviors including auditory hallucinations, delusions, and thought disorders (Stahl, 2002). For many years it was believed that hyperactivity of mesolimbic DA pathway causes or enhances the positive symptoms of schizophrenia or psychosis. Neuroleptics act to suppress psychosis mainly by blocking mesolimbic DA D_2 receptors.

Very similar to the mesolimbic is the *mesocortical* DA pathway, which projects to the neocortex and prefrontal cortex. Because frontal cortex is involved in cognitive and social skills, planning and other executive functions many believe that deficit of DA in mesocortical projection areas may be responsible for the negative symptoms of schizophrenia (Goldman-Rakic and Selemon, 1997; Stahl, 2002). In addition, it is probably in this projection pathway that the long term glutamatergic overstimulation leads to excitotoxicity of the DA neurons (see appendix). The *nigrostriatal* DA pathway originates in substantia nigra (SN) and terminates in the basal ganglia or striatum (STR). This DA pathway is part of the extrapyramidal nervous system involved in motor control. DA deficiencies in this system result in movement disorders that resemble symptoms of Parkinson's disease. Typical neuroleptics which block DA D_2 receptors produce very similar symptoms. Finally, the *tuberoinfundibular* DA pathway projects from the hypothalamus to the anterior pituitary gland and controls the levels of several hormones such as prolactin and growth hormone. These neurons are normally active and inhibit release of these hormones.

Based on their pharmacological and biochemical properties DA receptors are divided into two families. The D_1 receptor family (includes D_1 and D_5 receptor subtypes) stimulates the formation of cyclic adenosine 3', 5'-monophosphate (cAMP), a

second messenger important for intracellular signal transduction. The D_2 receptor family (includes D_2, D_3 and D_4 receptor subtypes) inhibits the formation of cAMP. All DA receptor subtypes belong to the super-family of G-protein coupled transmembrane proteins. Both D_1 and D_2 families are found postsynaptically (Fig. 1), while presynaptic sites predominantly belong to the D_2 subtypes which are also known as autoreceptors (Jaber et al., 1996). Although they mainly block postsynaptic DA D_2 receptors, potent DA antagonists, such as haloperidol can also block these autoreceptors. As a result, a compensatory increase in firing rate of dopaminergic neurons is reported as well as increase of DA synthesis and release through so called feed-back activation loop (Westerink and de Vries, 1989; Cooper et al., 2003).

Quantitative autoradiographic studies determined that most abundant receptors receiving dopaminergic innervations are the DA D_1 receptors, found in the ST, NAc, SN, thalamus, hypothalamus, and cerebral cortex (Boyson et al., 1986; Camps et al., 1990). DA D_5 receptors are mainly restricted to thalamus, hypothalamus, and hippocampal areas (Meador-Woodruff et al., 1992). DA D_3 receptors are localized in the areas associated with the limbic system and partly in STR and the VTA (Levesque et al., 1992). DA D4 receptors are least frequent of all dopaminergic receptor subtypes and are mainly located in the cortex and limbic areas (Van Tol et al., 1991). Finally, DA D_2 receptors are found mainly in those areas where DA D_1 receptors are located (Creese et al., 1977). On the whole, DA D_2 receptors are generally expressed in the motor control areas, while DA D_3 and D_4 receptors are expressed in the areas involved with emotion and cognition. In addition, several functional DA receptor generalizations exist: 1) activation of D_1, D_2, and D_5 receptors causes behavioral stimulation; 2) D_2 autoreceptors have inhibitory

functions; 3) D_3 receptors have behaviorally inhibitory roles at the postsynaptic level; and 4) the function of D_4 receptors is not clear (Van Tol and Seeman, 1995).

Glutamatergic synapse

Glutamate is one of the most abundant excitatory amino acid transmitters in the brain. It is thought to be involved in higher mental functions such as cognition, learning and memory. Glutamate binds to two types of receptors to exert its excitatory action: ionotropic and metabotropic (Fig. 1.3).

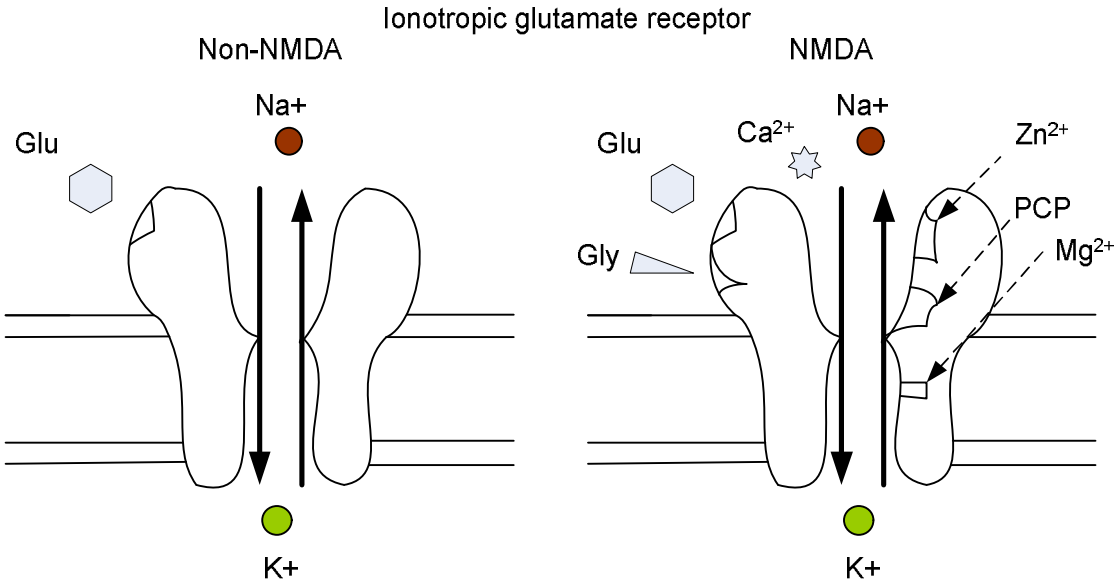


Fig. 1.3. Schematic drawing of the ionotropic excitatory amino acid receptors. (Based on Kandel et al., 1991).

Ionotropic receptors are multi-subunit transmembrane proteins that form ion channel pores and include N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA) and kainate receptors. Metabotropic receptors are indirectly linked with ion channels. For reasons of applicability, only ionotropic receptors will be discussed here. AMPA and kainate receptors are nonselective and permeable to

Na^+ and K^+, but mostly impermeable to Ca^{2+}, though AMPA can become Ca^{2+} permeable when they are devoid of the GluR-2 subunit (Sommer et al., 1991). The AMPA receptor-mediated currents exhibit very fast kinetics (including excitation and desensitization) and are widely distributed in the brain, with high density in the hippocampus and olfactory tubercle (Petralia et al., 1992; Seeburg, 1993). The NMDA receptors have slower kinetics compared to the AMPA receptors but have significant permeability to Ca^{2+} ions. The NMDA-mediated Ca^{2+} influx is physiologically a very important co-process of glutamate activation. Excessive NMDA receptor activation and Ca^{2+} influx can cause neurodegeneration (Olney and Sharpe, 1969; Choi, 1988; Appendix). However, NMDA activation is restricted and requires glycine (as co-agonist) and removal of voltage dependent Mg^{2+} block at the resting membrane potential. The AMPA receptors often depolarize the membrane to remove this block.

Since glutamate is implicated in cognitive skills and memory, antagonists of the glutamatergic NMDA receptor impair learning and memory, while agonists improve it (Francis et al., 1993; Francis, 2003). Previous studies showed that patients who use ketamine or phencyclidine (PCP), drugs that block NMDA receptors, exhibit symptoms that closely match those in schizophrenia (i.e. apathy, loss of self recognition, disorganization, inability to concentrate or think abstractly, and in higher doses catatonic stupor, ataxia and rigidity) (Luby et al., 1959). These patients have positive symptoms, they have negative symptoms and they have cognitive deficits, much what is seen in schizophrenia. For example, NMDA receptors are involved in the formation of memories but not in the retention of old memories; similarly, patients with schizophrenia have trouble forming but not retaining memories (Javitt et al., 1987). This suggests that

perhaps a deficiency in the NMDA system is involved and provides the basis for the glutamate hypofunctionig hypothesis of schizophrenia.

To rule out the possibility that these symptoms are caused by the factors of drug abuse rather than the drug itself, a low dose of ketamine was administered to healthy subjects in 1992 and similar results were achieved (Krystal et al., 1994). This group also pointed out that, administration of PCP/ketamine is not the same as having schizophrenia since these drugs mainly produce this effect by blocking NMDA glutamate receptors, and schizophrenia is very likely to involve other neurotransmitter systems including DA. Evidence also exists for the involvement of cholinergic system (Freedman et al., 1995; Court et al., 1999; Marutle et al., 2001), serotonergic system (Gonzalez-Maeso, et al., 2008) and γ-aminobutyric acid (GABA) system (Lewis, 1995, 2000; Lewis et al., 1999; Volk and Lewis, 2002; Tamminga et al., 2004; Lewis and Moghaddam, 2006) in schizophrenia, but all with indiscrete implications.

Antipsychotic drugs and movement disorders

Movement problems that are similar or identical to those caused by APD, including dystonias, dyskinesias and akathesias, are associated with Parkinson's disease, Huntington's disease and other dysfunctions of the basal ganglia. These disorders are commonly referred to as the EPS. The term extrapyramidal system was first introduced by Samuel Alexander Wilson in 1912 in describing one of the neurological disorders (now Wilson's disease). The extrapyramidal system contains putamen, globus pallidus (GPal), SN, and subthalamic nuclei (ST). These structures are masses of gray matter that are also known as the basal ganglia. The basal ganglia play an important role in the

control of movement and other motor behaviors. The interconnecting pathways include those connecting motor cortex, brain stem, basal ganglia, and spinal cord (Fig. 1.4).

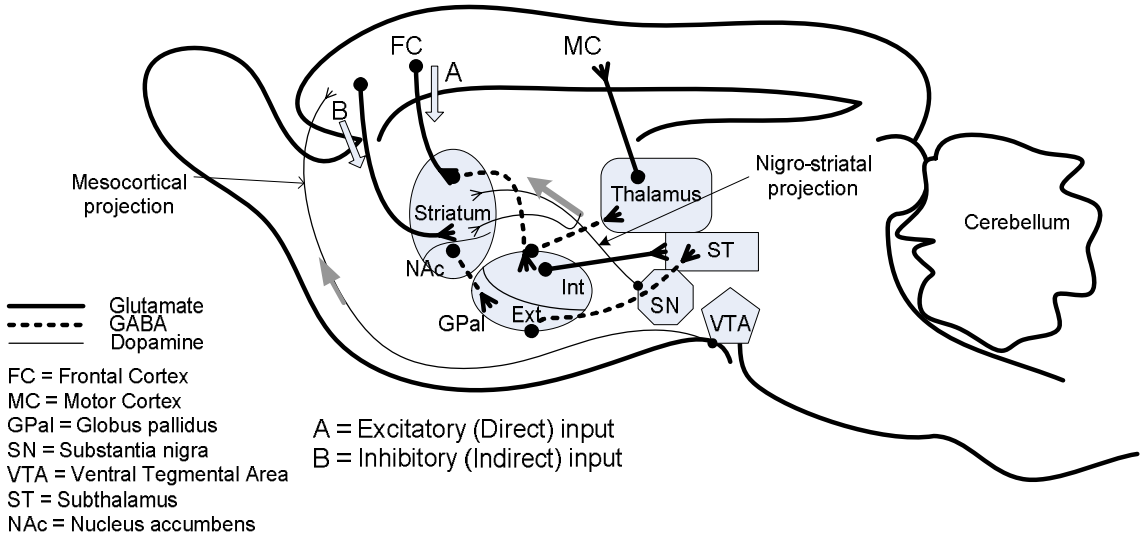


Fig. 1.4. Simplified schematic of the circuitry of the nigrostriatal and mesocortical dopamine systems in the rat brain highlighting the major inputs to the striatum and globus pallidus (GPal) (Based on Kauer and Malenka, 2007).

Each of the cortical areas (associated areas included) project to one of the telencephalic nuclei. These nuclei all have similar properties and include the caudate nucleus, putamen, and NAc, together they make the striatum (STR) (Young and Penney, 1984; Graybiel, 1990). In addition, the striatal nuclei also get input from dopaminergic neurons in midbrain and the thalamus. Normal basal ganglionic functioning depends on complex interplay between a variety of transmitter systems including DA, acetylcholine, GABA, enkephalin, substance P, somatostatin, and glutamate. However, recent conceptualization of the basal ganglia motor processing have stressed that disturbances in any of these systems must ultimately be manifest in disrupted regulation of glutamate (Albin et al., 1989; Sachdev, 1995). Among all of these interactions, those between DA and glutamate

seem to be pivotal in terms of motor functions (Albin et al., 1989; Carlsson and Carlsson, 1990).

In this thesis, two brain areas have been studied: the striatum (STR) and the frontal cortex (FC). Fig. 1.4 shows main projections pathways between the FC and the STR. These structures are interconnected to form two parallel but antagonistic circuits known as the direct and indirect basal ganglia pathways. Both pathways project back to the FC after a relay in the thalamus. Both pathways use a process known as "disinhibition" to produce their effects. Essentially, there are two basal ganglia pathways: the excitatory and inhibitory (Fig. 1.4). In the excitatory pathway the excitatory input from the cerebral cortex projects to the STR (caudate and putamen). Through disinhibition, activated GABA (inhibitory) neurons project to and inhibit additional GABA-ergic neurons in the internal segment of the globus pallidus (GPal). From here, GABA neurons project to the thalamus. Because their input to the thalamus is disinhibited, the thalamic input excites the cortex. The net effect of the disinhibition in the excitatory pathway results in an increased level of cortical excitation. In the inhibitory pathway, excitatory input from the cerebral cortex also projects to striatal neurons. These GABA-ergic neurons in the STR project and inhibit additional GABA neuons in the external segment of GPal. From here, GABA neurons project to subthalamus (ST). Through disinhibition, the ST excites inhibitory GABA neurons in the internal segment of GPal, which inhibits the thalamus. The net effect of the disinhibition in the inhibitory pathway results in a decreased level of cortical excitation (Joel and Weiner, 2000; Kauer and Malenka, 2007). In addition to GABA, dopaminergic neurons in the substantia nigra (SN) project to the STR. The effect of dopamine excites the excitatory pathway,

increasing cortical excitation. DA excites the excitatory pathway through DA D_1 receptors and inhibits the inhibitory pathway through DA D_2 receptors. Numerous physiological studies have shown that this anatomical arrangement has functional consequences: glutamatergic transmission is modulated by dopaminergic tone (Svensson et al., 1992; Romanides et al., 1999). Relevant to the context of this thesis, it has been argued that some movement disorders originating in dopaminergic dysfunction such as Parkinsonism can be ameliorated by compensatory drug action on glutamatergic transmission (Giuffra et al., 1993; Starr, 1998).

Observations from several laboratories suggest that behavioral outcome of neuroleptic-enhanced glutamatergic activity are motor difficulties. Problems in movement initiation and reaction time as well as catalepsy induced by neuroleptic drugs can be blocked by co-administration of the NMDA antagonist MK-801 (Elliott et al., 1990; Moore et al., 1993; Kaur et al., 1997). The hypothesis that these movement disorders are caused by enhancement of glutamatergic transmission in the basal ganglia is supported by the observation that vacuous jaw movements, seen after withdrawal from chronic haloperidol treatment in rats, occurs simultaneously with elevated levels of glutamate in the striatum (Steinpreis et al., 1997). Catalepsy in animals is an analogue of neuroleptic-induced Parkinsonism in humans. Catalepsy is mainly produced by very potent, typical APD (D_2 blockers) such as haloperidol (Ellenbroek et al., 1985; Boulay et al., 2000). Within therapeutic doses it can not be caused by non-antipsychotic D_2 blockers such as metocloprmaide, which can only sensitize the brain to haloperidol-induced catalepsy (Agovic et al., 2008). As mentioned earlier, administration of typical antipsychotic drugs can induce catalepsy in animals and Parkinsonian-like symptoms in

schizophrenic patients. In addition, prolonged (chronic) treatment with these drugs can result in overactivity of spefic muscle groups leading to a disorder known as tardive dyskinesia (Ebadi and Srinivasan, 1995). The dopaminergic neurons in SN are believed to be fundamentally involved in the regulation of movement (Grace and Bunney, 1986). Acute administration of neuoleptics increase firing rate of dopaminergic cells (Bunney et al., 1973; Bunney and Grace, 1978; Lidsky and Banerjee, 1993) while chronic administration decreased dopamine neuron firing in SN and VTA (Bunney and Grace, 1978; Chiodo and Bunney, 1983; Grace et al., 1997; Boye et al., 2000). This blockade of dopaminergic transmission after chronic administration of neuroleptics is known as depolarization block (DB), and is believed to be important a part of the neuroleptic pharmacological mechanism. Furthermore, cocaine (DA reuptake blocker) administered acutely causes diverse behaviors, including panic attacks, anxiety disorders, and paranoid psychosis (McDougle et al., 1994), while repeated cocaine exposure produces a behavioral phenomenon known as cocaine sensitization, which includes behavioral responses such as increased locomotion, head bobbing, and repetitive motion (Kalivas and Duffy, 1993). Consequently, the DA hypothesis of schizophrenia accounts for an imbalance of the dopaminergic system; hyperstimulation of subcortical DA D_2 receptors result in positive symptoms and understimulation of cortical DA D_1 receptors results in negative symptoms and cognitive deficits (Svensson, 1992, 2000). However, such a disregulation of DA neurotransmission associated with administration of typical APD and cocaine can also be caused by alterations in NMDA and glutamatergic functions (Itzhak and Stein, 1992; Svensson, 2000) entailing an important DA-glutamate interplay.

Hypothesis

Recent studies consistently show that typical APD mediate their action through DA D_2 receptor antagonism (Kapur and Mamo, 2003) and that chronic treatment with these drugs causes reduction in DA cell firing in brain regions such as SN and VTA (Bunney and Grace, 1978; Chiodo and Bunney, 1983; Grace et al., 1997; Boye et al., 2000). This inactivation of dopaminergic transmission through DA D_2 blockade along with activation of glutamatergic transmission is hypothesized to be the mechanism for the therapeutic action of typical antipsychotic drugs as well as the mechanism for APD-induced EPS in humans and catalepsy in animals. Previous tests of this hypothesis provided limited information about the role the dopaminergic and gutamatergic neurotransmitter systems have in the actions of neuroleptics. The focus of this project is to clarify changes in the dopaminergic and glutamatergic neurotransmitter circuits due to the actions of typical APD, which would contribute to the development of mechanisms for catalepsy and DA cell DB.

Chapter 2. Interactions of phencyclidine and ketamine with N-Methyl-D-Aspartate and dopamine D_2 receptors

Abstract

It has been claimed that conventional psychotomimetic agents such as phencyclidine (PCP) and ketamine bind to DA D_2 receptors with apparent affinities that are comparable to that for the N-methyl-D-aspartate (NMDA) receptors. This raises the possibility that schizophrenia-like symptoms induced by PCP and ketamine may be mediated by their interactions with DA D_2 receptors instead of NMDA receptors. Therefore, we decided to compare specific binding of PCP and ketamine to DA D_2 and NMDA receptors in the rat cortical membrane preparations that were extensively washed to remove all endogenous amino acids. Our results show that PCP has about 2 to 3 times higher binding affinity at NMDA receptors compared to ketamine, in the presence of either 30 μM glycine or 100 μM glutamate. Also, up to 1 mM concentrations, PCP and ketamine failed to cause 50% displacement of [^{3}H]spiroperidol binding to DA D_2 receptors in rat cortical membrane preparations in the presence or absence of 120 mM NaCl. Our results are consistent with the idea that schizophrenia-like symptoms caused by PCP and ketamine may be mediated by the blockade of the NMDA receptors without any significant modification of DA D_2 receptor activities.

2.1 Introduction

Previous investigations of individuals suffering from psychosis and of the mechanism of action of antipsychotic drugs, suggest the hyperactivity of brain dopaminergic systems may contribute to symptoms of schizophrenia (Davis et al., 1991;

Seeman, 1992; Moore et al., 1999). Over the past two decades, however, this model has been challenged by the PCP model of schizophrenia (Javitt and Zukin, 1991; Lahti et al., 1995; Coyle, 1996; Lidsky and Banerjee, 1996). The PCP model is based on observation that potent, noncompetitive antagonists of the NMDA receptors such as PCP and ketamine induce positive, negative, and cognitive schizophrenia-like symptoms in healthy individuals and increase symptoms in patients with the disease. Also, typical as well as atypical APD such as haloperidol and clozapine have been shown to be partial agonists at the NMDA receptors (Banerjee et al., 1995; Arvanov et al., 1997; Lidsky et al., 1997). Such observations are consistent with the demonstration that agents that potentiate NMDA receptor-mediated transmission decrease persistent negative and cognitive symptoms of schizophrenia (Javitt et al., 1994; Tsai et al., 1998; Javitt, 2002). A few years ago, Kapur and Seeman reported that the binding affinities of ketamine and PCP for DA D_2 and NMDA receptor subtypes were similar. They suggested that psychotomimetic effects of these drugs may not be mediated through blockade of the NMDA receptors (Kapur and Seeman, 2002). Further, they claimed to have found partial agonistic property in PCP and ketamine for DA D_2 receptors and proposed that psychosis, as induced by PCP and ketamine, may be caused by a perturbation of the dopaminergic transmission. The binding affinities of PCP and ketamine for high-affinity state DA D_2 and NMDA receptors in the striatal homogenates were reported to be within 0.5 μM to 37 μM (Kapur and Seeman, 2002). Surprisingly, the apparent affinity of ketamine was found to be 4 times higher than that of PCP at the NMDA receptors. More surprisingly, they have reported that PCP binds to human cloned DA D_2 receptors with an affinity that was 1,000 times or more different from that found in the rat striatal homogenates

(Seeman, 2004; Seeman et al., 2005). Also, dizocilpine (MK-801) was reported to have about 5 times higher affinity for the dopamine D_2 compared to NMDA receptors (Seeman et al., 2005). Finally, their claim that PCP and ketamine are partial or full dopamine D_2 receptor agonists (Kapur and Seeman, 2002; Seeman and Lasaga, 2005) has been challenged by several investigators (Sveningsson et al., 2003, 2004; Jordan et al., 2006). If PCP and ketamine are not agonists at the DA D_2 receptors, then according to the observations by Seeman and his associates, these two drugs must be antagonists at dopamine D_2 receptors. Such pharmacological properties should make PCP and ketamine antipsychotic drugs rather than psychotomimetic agents.

These conflicting observations led us to investigate binding of PCP and ketamine to DA D_2 and NMDA receptor subtypes in rat cortical membrane preparations. Our results indicate that PCP and ketamine bind with high affinity to the NMDA receptors with limited specific binding to DA D_2 receptors. Although binding affinity of PCP to the NMDA receptors was found to be two to three times higher than that of ketamine, neither PCP nor ketamine at 1 mM concentrations were able to displace 50% of [^{3}H]spiroperidol specific binding to DA D_2 receptors in rat cortical membrane preparations (in the presence or absence of 120 mM sodium chloride (NaCl)). Our results are consistent with the suggestion that behavioral effects of PCP and ketamine may be mediated through the blockade of NMDA receptors without major alterations in the DA D_2 receptor activity.

2.2 Materials and Methods

2.2.1 Drugs

PCP, ketmaine, Unlabeled MK-801, butalcamol, Tris base, L-glycine and monochloroacetic acid were purchased from Sigma/RBI (Natick, MA). EDTA and hepes were purchased from Fisher Scientific (Fair Lawn, NJ). [^{3}H]MK-801 and [^{3}H]spiroperidol were purchased from PerkinElmer Life and Analytical Sciences Division (Boston, MA).

2.2.2 Receptor binding of [^{3}H]MK-801 to NMDA glutamate receptor and [^{3}H]spiroperidol to dopamine D_2 receptor

NMDA and DA D_2 receptor binding in frontal cortex was assessed by displacement of [^{3}H]MK-801 by different concentrations of nonradioactive MK-801 in the presence of 30 µM glycine or displacement of [^{3}H]spiroperidol by different concentrations of butaclamol as previously described (Benerjee et al., 1995; Lidsky et al., 1995). Frozen rat brains were purchased from Pel-Freez Biological, Rogers, AR and stored at -80°C until used. On the day of experiment the tissue was thawed and washed to reduce residual levels of neurotransmitters. The tissue was homogenized with Brinkman Polytron PT-20 homogenizer in 20 volumes of ice-cold 0.05 M Tris buffer with EDTA and hepes (pH 7.4 at 25ºC) and centrifuged at 49,000g for 20 min. The supernatant was discarded and the pellet was re-homogenized in the same buffer and centrifuged as before. The last procedure was repeated twice and the resulting membrane pellet was frozen 1 more day at -80ºC. Receptor binding assay to NMDA receptors was carried out by incubating for 45 min at 25ºC, 800 µl of crude fractions containing (0.8 mg of tissue) with radioligand [^{3}H]MK-801 (about 100,000 cpm) and 30 µM glycine or 100 µM Glutamate, with/without 120 mM NaCl. Similarly, specific binding to dopamine D_2 receptors was conducted with 0.8 mg of tissue incubated at 25ºC in the presence of

[^{3}H]spiroperidol (about 70,000 cpm) with/without 120 mM NaCl for 45 min. To determine the nonspecific radioactivity, a parallel set of samples were incubated with large excess of 100 μM MK-801 or 100 μM of butaclamol. Specific binding is defined as the difference between total binding and nonspecific radioactivity. Incubation was terminated with rapid filtration through GF/B glass fiber filters. The filters were rinsed twice with 4ml ice-cold Tris buffer to remove unbound radioactive ligand. They were then dried and placed in vials containing 3 ml of scintillation fluid and counted by scintillation spectroscopy at 60% efficiency. The receptor binding was conducted to develop a dicplacement curve of [^{3}H]MK-801 to the NMDA receptor and [^{3}H]spiroperidol to DA D_2 receptor. The AccuFit Competitive One-Site (Beckman) program was used to compute the Scatchard analysis to estimate the maximal number of binding sites. The program is based on the principle of nonlinear least-square regression analysis to solve the equation describing the binding of labeled ligand to receptor proteins. Results were confirmed with GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA.

2.2.3 Statistical analysis

Data were presented as mean ± S.E.M. Two-tailed Student's *t* test was used to compare particular responses between two groups. Three or more groups were analyzed with one-way analysis of variance (ANOVA) followed by Tukey's multicomparison test. The threshold for mean significance was $P<0.05$. All statistical tests were performed using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA.

2.3 Results

2.3.1 Displacement of specific binding of [^{3}H]MK-801 to NMDA receptors in cortical membranes by PCP and ketamine

Specific binding of [^{3}H]MK-801 to NMDA receptors in rat cortical membrane preparations and its displacement by different concentrations of PCP and ketamine in the presence of 30 μM glycine is shown in Fig.2.1. The binding affinity constants of PCP and ketamine to NMDA receptors were found to be 5.15 nM and 9.65 nM respectively (Table 1). The affinity constants of PCP and ketamine in the presence of 100 μM glutamate (Fig. 2.2) were found to be 3.30 nM and 8.46 nM respectively (Table 1). Interestingly, the binding affinity of PCP to NMDA receptors was higher in the presence of 30 μM glycine compared to 100 μM glutamate. In sharp contrast, ketamine showed higher binding affinity to NMDA receptors in the presence of glutamate compared to glycine. Currently there are no known reasons for these differences. The maximal number of binding sites measured in the presence of 100 μM glutamate or 30 μM glycine with either PCP or ketamine varied between 1.64 pmol/mg to 1.74 pmol/mg protein. These numbers were not statistically different (Table1). The affinity constants of PCP to NMDA receptors observed in this study are similar to those previously reported by Klamer and his associates (Klamer et al., 2005). These results, however, are diametrically opposite to the observations reported by Kapur and Seeman who found that the binding affinity of ketamine to NMDA receptors in striatal homogenates was four times higher than that of PCP (Kapur and Seeman, 2002). Our results show the binding affinity of PCP at the NMDA receptors in cortical membranes was about two to three times higher than that of ketamine. These differences may be related to the differences in

the areas of brain researched by these two groups of investigators.

2.3.2 Displacement of specific binding of [^{3}H]spiroperidol to dopamine D_2 receptors in cortical membranes by PCP and ketamine

Specific binding of [^{3}H]spiroperidol to DA D_2 receptors in rat cortical membrane preparations and its displacement by different concentrations of PCP and ketamine without NaCl is shown in the Fig.2.3. PCP was unable to displace significant amount of specific binding of [^{3}H]spiroperidol to DA D_2 receptors in rat cortical membranes until the concentration of the displacing drug was increased to 1 mM. Ketamine displaced roughly 39% of [^{3}H]spiroperidol specific binding to the DA D_2 receptors at 1 nM concentration. Surprisingly, higher concentrations of ketamine failed to displace additional specific binding of [^{3}H]spiroperidol in the cortical membrane preparations. Neither PCP nor ketamine were able to displace 50% or of the specific binding to DA D_2 receptors even with as high a concentration as 1 mM. Therefore, we are unable to calculate the affinity constants for PCP and ketamine to DA D_2 receptors in cortical preparations. In the presence of 120 mM NaCl, displacement of [^{3}H]spiroperidol binding to DA D_2 receptors by PCP and ketamine was similar to that without NaCl (Fig. 2.4). The displacement curve of PCP in the presence of NaCl was almost identical to that observed without NaCl. The displacement curve of ketamine in the presence of NaCl, however, showed slightly greater displacement of [^{3}H]spiroperidol specific binding to DA D_2 receptors than that seen without NaCl (Fig. 2.3 and 2.4). Nevertheless, up to 1 mM concentrations of either ketamine or PCP, these drugs were unable to displace 50 % of specific binding to DA D_2 receptors in the presence or absence of 120 mM NaCl. Thus, our results support the possibility that psychotomimetic effects of PCP and

ketamine may not be mediated, in part, through an interaction with the DA D_2 receptors. This is in agreement with recent clinical studies (Kegeles et al., 2000; Aalto et al., 2002; Kegeles et al., 2002; Aalto et al., 2005).

2.4 Discussion

PCP and ketamine have been reported to induce acute, reversible, psychosis-like symptoms in man that has been attributed to the blockade of NMDA receptors (Luby et al., 1959; Javitt and Zukin, 1991). Since typical antipsychotic drugs exhibit DA D_2 receptor antagonism and partial agonistic activity at NMDA receptors (Banerjee et al., 1995; Arvanov et al., 1997; Lidsky et al., 1997), several hypotheses have been proposed suggesting disturbances in the glutamate-dopamine interactions may play a prominent role in the neurobiology of schizophrenia (Olney et al., 1995; Carlsson et al., 1999). Recent studies by Seeman and his associates (Kapur and Seeman, 2002; Seeman et al., 2005; Seeman, 2004; Seeman and Lasaga, 2005) indicate that PCP and ketamine cause psychosis by partial agonistic or full agonistic activity at the DA D_2 receptors. They reported the affinity constants of PCP and ketamine at the NMDA receptors to be 2 µM and 0.5 µM respectively (Kapur and Seeman, 2002). Such observations are surprising as PCP is more potent a psychotomimetic agent than ketamine (Abraham et al., 1996; Rao et al., 1989; Couper et al., 2004). And if schizophrenia is induced by these agents due to inhibition of the glutamatergic transmission through NMDA receptors, then PCP would be expected to be more effective antagonist of the NMDA receptors. Therefore, we decided to study the interactions of PCP and ketamine with the NMDA receptors. Our results show that PCP has about 2 to 3 times higher apparent affinity at NMDA receptors

in rat cortical membrane preparations compared to ketamine either with 30 μM glycine or 100 μM glutamate (Fig. 2.1 and 2.2; Table 1). Why are our results opposite to that of Kapur and Seeman (Kapur and Seeman, 2002)? There are several problems with the experimental procedure adopted by them (Kapur and Seeman, 2002) in the measurement of specific binding to NMDA receptors. First, the NMDA receptor channel is closed in the absence of co-agonists glutamate and glycine and the ionophore opens up in the presence of co-agonists to allow the binding of [^{3}H]MK-801 to its site within the channel (Reynolds et al.,1987; Wond et al., 1987). Therefore, neuronal tissue must be washed extensively to eliminate or reduce residual levels of glutamate and glycine and a known, sufficient amount of one or both amino acids must be added to open channel and to expose [^{3}H]MK-801 binding sites in the NMDA receptor to the radioactive ligand in a reproducible manner (Banerjee et al., 1995; Reynolds et al., 1987; Wong et al., 1987). They did not wash the striatal tissue and whole homogenates were used in their binding studies (Kapur and Seeman, 2002). This diluted the endogenous levels of glutamate and/or glycine that may have been insufficient to properly open the NMDA ionophore to allow [^{3}H]MK-801 binding to its specific sites at NMDA receptors. Second, they added 4mM magnesium chloride in their homogenates. In the presence of magnesium, there would be a blockade of NMDA ionophores that would prevent binding of [^{3}H]MK-801 to its specific sites in NMDA receptors (Nowak et al., 1984; Lidsky and Banerjee, 1996). Thus, even if the NMDA channels were inconsistently and/or only partially open with the variable endogenous glutamate and glycine, addition of magnesium chloride would completely shut off NMDA ionophores blocking any specific binding of [^{3}H]MK-801 to the NMDA receptors. Probably, they measured binding of [^{3}H]MK-801 to a site

displaced by dextrorphan that was not on the NMDA receptor. Therefore, it is not surprising that the results obtained by this group in their [^{3}H]MK-801 binding studies (Kapur and Seeman, 2002) are inconsistent with our observations.

Kapur and Seeman reported that PCP and ketamine are partial agonists of DA D_2 receptors in striatal homogenate preparations (Kapur and Seeman, 2002). Subsequently, Seeman and Lasaga reported agonistic activity of PCP at DA D_2 receptors in rat cultured anterior pituitary cells (Seeman and Lasaga, 2005). Several investigators have been unable to find DA D_2 receptor agonistic or antagonistic activities in PCP and ketamine. For example, Jordan and associates investigated the dopamine stimulated guanosine triphosphate binding and calcium mobilization in the Chinese hamster ovary cells that expressed human DA D_2 receptors (Jordan et al., 2006). Also, they conducted dopamine activated guanosine triphosphate binding to rat striatal membranes. Jordan and co-workers reported that these dopamine-mediated activities are neither induced nor blocked by PCP, ketamine or MK-801, suggesting that NMDA receptor antagonists may not cause psychosis through an interaction with the DA D_2 receptors. Similarly, Svenningsson and associates reported that psychotomimetic effects of *D*-amphetamine, *D*-lysergic acid diethylamide, and PCP are mediated through changes in the levels of phosphorylation in DARPP-32. They concluded that *D*-amphetamine-induced psychosis occurs only via activation of DA D_1 and/or D_2 receptors. *D*-lysergic acid diethylamide as well as PCP may induce psychosis via dopamine-independent mechanisms that lead to changes in DARPP-32 phosphorylation (Svenningsson et al., 2003, 2004). Finally, three PET/SPECT DA D_2 receptor imaging studies failed to show a direct effect of ketamine on DA D_2 receptors in different areas of the human brain (Kegeles et al., 2000, 2002; Aalto

et al., 2002). Also, Aalto et al. (Aalto et al., 2002) reported that dopaminergic response caused by ketamine in general acts as a compensatory mechanism that tends to attenuate the development of psychotic symptoms. Our receptor binding studies are in agreement with the suggestion that PCP and ketamine bind poorly to the DA D_2 receptors in the rat cortical membrane preparations (Figure 2.3 and 2.4). There are several differences in our experimental procedures compared with Kapur and Seeman (Kapur and Seeman, 2002). We used membrane preparations from rat cortex while they used striatal whole homogenates. Further, they used [^{3}H]raclopride and we used [^{3}H]spiroperidol for receptor binding assays. Nevertheless, such procedural differences should not result in remarkable differences in the results of these studies.

In conclusion, our studies show that PCP and ketamine bind poorly to DA D_2 receptors in the rat cortical membranes, which suggests that it is unlikely that psychotic effects are mediated through interactions with DA D_2 receptors. PCP and ketamine bind to the NMDA receptors in nM range, and PCP was found to have an apparent affinity to NMDA receptors several folds higher than that of ketamine. Thus, our results are consistent with the proposal that PCP and ketamine-induced psychosis may be mediated through interactions with the NMDA receptors.

2.5 Figures and Tables.

Table 2.1. Changes in radioligand binding values in the prefrontal cortex tissues treated with PCP and ketamine under specific conditions.

Treatment	**Mean ± S.E.M.**		
	***K_i* (nM)**	***B_{max}*(pmol/mg wet-weight)**	***n***
PCP with Glycine	5.15 ± 0.168	1.73 ± 0.271	14
Ketamine with Glycine	9.65 ± 0.164	1.74 ± 0.264	14
PCP with Glutamate	3.30 ± 0.245	1.64 ± 0.276	14
Ketamine with Glutamate	8.46 ± 0.162	1.74 ± 0.290	14

Average K_i and B_{max} values ± SEM for the competition binding of [^{3}H]MK-801 and [^{3}H]spiroperidol determined in cortical tissues treated with PCP or ketamine in the presence of 30 μM glycine or 100 μM glutamate.

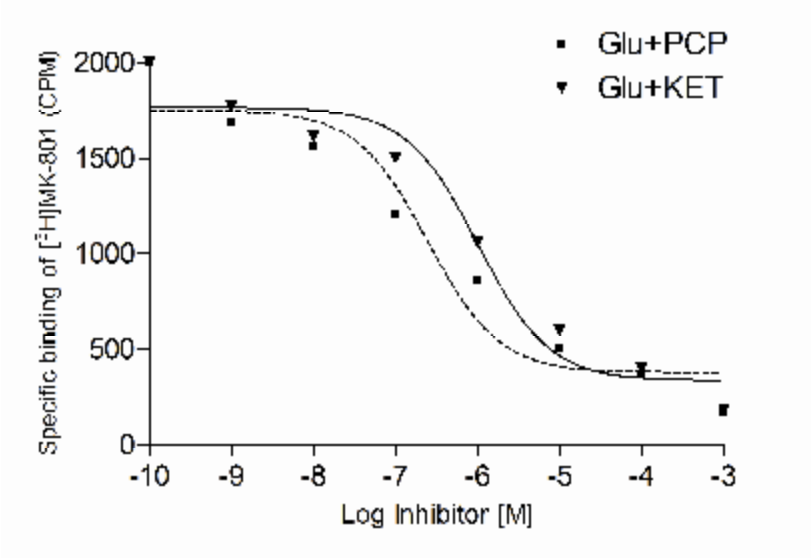


Fig. 2.1 Specific binding of [^{3}H]MK-801 to NMDA receptors in rat cortical membranes and its displacement with different concentrations of PCP and Ketamine in the presence of of 30 μM glycine. For PCP treated tissue B_{max} was 1.73 pmol/mg and the K_i was 5.15 nM and for the Ketamine treated tissue, the B_{max} was 1.74 pmol/mg and the K_i was 9.65 nM.

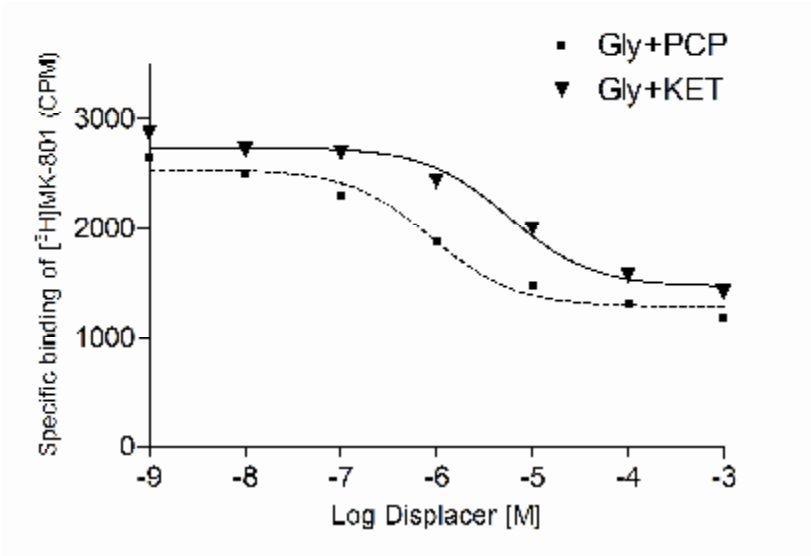


Fig. 2.2 Specific binding of [^{3}H]MK-801 to NMDA receptors in rat cortical membranes and its displacement with different concentrations of PCP and Ketamine in the presence of 100 μM glutamate. For PCP treated tissue B_{max} was 1.64 pmol/mg and the K_i was 3.30 nM and for the Ketamine treated tissue, the B_{max} was 1.74 pmol/mg and the K_i was 8.46 nM.

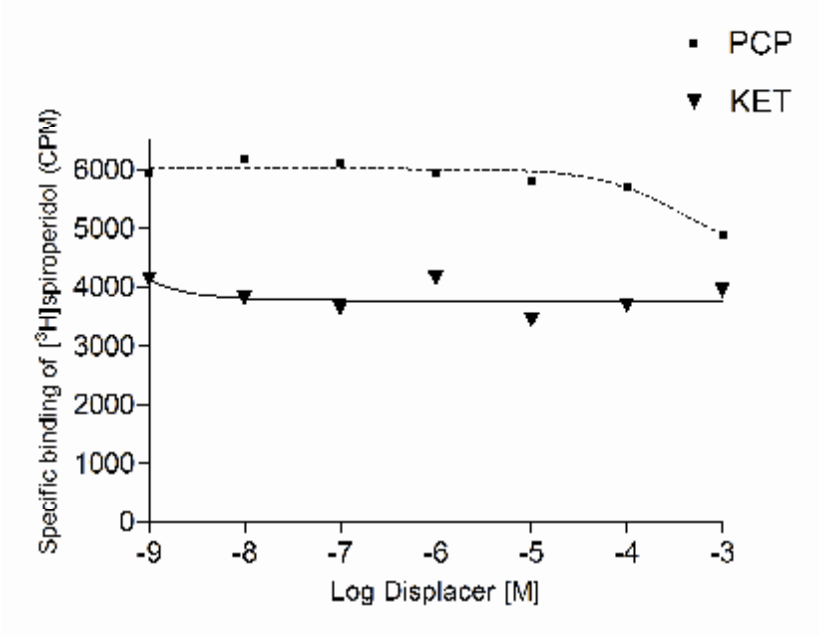


Fig. 2.3 Specific binding of [^{3}H]Spiroperidone to D_2 receptors in rat cortical membranes and its displacement by different concentrations of PCP and ketamine in the absence of NaCl. Only at high concentration [1mM] displacement of specific binding by PCP was observed. Ketamine displaced about 39% of specific binding to the D_2 receptors at 1nM.

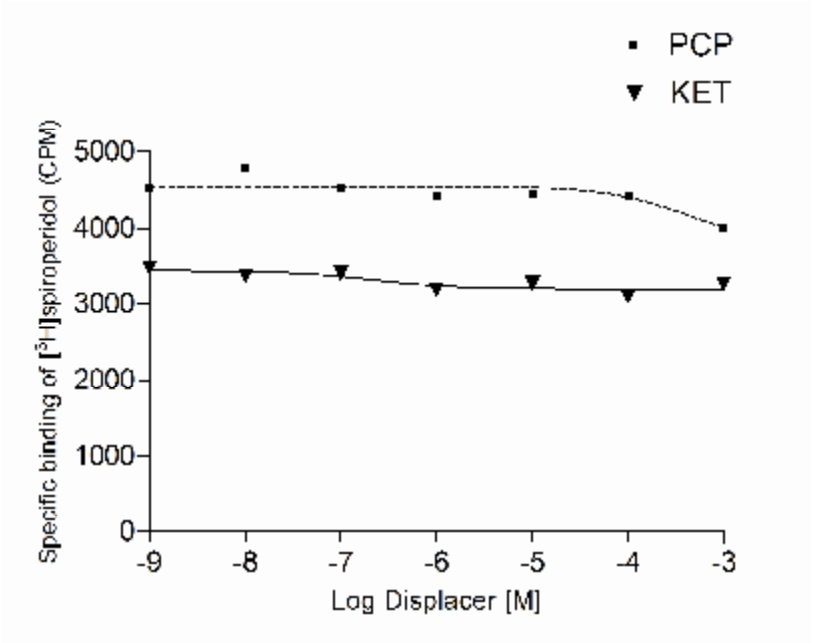


Fig. 2.4 Specific binding of [^{3}H]Spiroperidone to D_2 receptors in rat cortical membranes and its displacement by different concentrations of PCP and ketamine in the presence of 120 mM NaCl. These results are similar to those seen in Fig. 3. Neither drug was able to displace 50% specific binding to the D_2 receptors.

Chapter 3. Chronic cocaine-induced depolarization block: neurochemical mechanism and its possible role in drug dependence

Abstract

Drugs useful in the management of psychosis must be administered repeatedly for three weeks or more to achieve therapeutic efficacy. Electrophysiological experiments show that acute administration of APD enhances dopaminergic transmission and following chronic treatment, DA neuronal cell firing is suppressed. This chronic effect of the APD has been reported to occur as a result of the development of depolarization block (DB). DB has been proposed to be the neurobiological basis for the therapeutic actions of this class of drugs. The neurobiological mechanism for the development of DB remains to be elucidated. DA cell firing is regulated by multiple mechanisms including the autoreceptors, which are located on the presynaptic DA neurons. Also, blockade of DA reuptake by drugs may modify dopaminergic transmission. Cocaine, which has no value in the management of psychosis, is a DA uptake inhibitor. Therefore, we investigated by microdialysis if chronic cocaine treatment would induce DB similar to that produced by chronic APD treatment. Withdrawal following chronic cocaine treatment in rats, showed a significant and marked reduction in striatal basal DA efflux as compared to saline treated controls, perhaps due to the reported increase in the number of DA D_2 receptors. Comparison of DA efflux by different acute doses of haloperidol in control and chronic cocaine treated animals provided results that are consistent with our proposed model for cocaine-elicited DB that may be related to up-regulation of DA D_2 receptors, down-

regulation of GABA transmission, and up-regulation of NMDA-mediated glutamatergic transmission in the striatum.

Introduction

There are several theories on the neurochemical basis for the actions of typical APD's in the treatment of schizophrenia. A major one is related to blockade of DA D_2 receptors by this class of drugs (Kapur and Mamo, 2003). Interestingly, however, schizophrenic patients often require weeks of treatment to attain therapeutic benefit (Johnstone, et al., 1978) and acute effects of APD may be different from chronic effects (Grace et al., 1997). Previous studies have reported that chronic treatment with APD causes reduction of DA cell firing in SN and VTA (Bunney and Grace, 1978; Chiodo and Bunney, 1983; Grace et al., 1997; Boye et al., 2000). This phenomenon of blocking/inactivating doapminergic transmission is attributed to DB, a mechanism originally proposed to explain the therapeutic effects of the APD as well as their EPS (Bunney and Grace, 1978). Previous findings show that DB is caused by chronic but not acute adminitration of APD (Boye et al., 2000). Furthermore, the therapeutic delay of APD correlates nicely with the delayed onset of DB in VTA (Grace et al., 1997).

The main neurobiological effects of cocaine are attributed to inhibition of DA reuptake by the DA transporter in brain dopaminergic neurons (Ushijima et al., 1995). In humans, acute and chronic cocaine causes diverse behavioral changes, including panic attack, anxiety, and paranoid psychosis (McDougle, et al., 1994). In rodents, cocaine exposure produces a behavioral phenomenon known as cocaine sensitization, which includes behavioral responses such as increased locomotion, head bobbing, and repetitive

motion (Kalivas and Duffy, 1993). These behaviors are believed to be mediated via brain dopaminergic systems. Interestingly, chronic cocaine or its withdrawal does not change striatal DA transporter binding affinity or density (Peris et al., 1990; Pilotte et al., 1994). Striatal release of endogenous DA by cocaine is elevated seven days or more after drug withdrawal (King, et al., 1993) and striatal DA D_1 receptor number is decreased 7 and 14 days after cocaine withdrawal (Kleven et al., 1990; Neisewander et al., 1994). Striatal DA D_2 receptor number is initially increased immediately after withdrawal (Goeders and Kuhar, 1987), but no difference in DA D_2 receptor number was observed after 7 or 14 days after cocaine withdrawal (Kleven et al., 1990; Neisewander et al., 1994).

Even with the myriad of scientific reports gaining insights into the effect of acute and chronic administration of typical APD treatment, the mechanism of DB is still not fully understood. Since typical APD and cocaine increase DA in the extracellular fluid by different mechanisms, namely, either blockade of DA D_2 receptors or inhibition of DA uptake mechanism, respectively, we wondered if chronic cocaine treatment would cause DB. Thus, the purpose of this study was to investigate the neurochemical mechanism for the development of DA cell DB in chronic cocaine treated rats. Reduction of basal DA release in haloperidol-induced DB has been reported to occur in striatum (Moore et al., 1998). Therefore, we consider this to be a neurochemical representation of electro-physiologically demonstrated DB. We compared the levels of striatal DA in the extracellular fluid by microdialysis in control rats and in rats following chronic cocaine treatment and withdrawal. Previous studies in our laboratory have shown that a low dose of haloperidol increases striatal dopaminergic neuronal firing and suppresses glutamatergic transmission while at a low dose of haloperidol plus SN stimulation

paradoxically inhibited DA cellular activity. The latter was noted to be a response to activation of striatal GABA neurons (Lidsky and Banerjee, 1993). Therefore, we investigated the effects of different doses of haloperidol on extracellular DA concentration in striata of control rats and chronic cocaine treated rats after drug withdrawal. Instead of a combination of SN stimulation plus the low dose haloperidol administration as used in the previous study (Lidsky and Banerjee, 1993), in the current investigation we replaced it with the high dose of haloperidol to over-stimulate DA cell activity. Although a low dose of haloperidol significantly increased striatal DA efflux, a high dose significantly suppressed release of DA in striatum of the control group. Our results show that DB may occur in striatum following withdrawal of chronic cocaine treatment and may involve up-regulation of DA autoreceptors, diminution in GABA transmission and enhancement of glutamatergic transmission. Since cocaine is not an APD, the data suggests that DB is not sufficient to explain the therapeutic efficacy of drugs useful in the management of psychosis. Nevertheless, development of DB following withdrawal of chronic cocaine treatment may play a role in drug craving.

Materials and Methods

Drug treatment

Six male CD rats (100-350 g) obtained from Charles River Laboratories, Inc. (Wilmington, MA), housed two per cage with the female partner (female rats were used for a different assay) and allowed to acclimatize in a temperature-controlled environment were used in this study. Rodent laboratory chow was provided at libitum. These animals were administered cocaine orally for 21 days, utilizing their water bottles (100

mg cocaine/L water) at libitum. A single pack of Sweet'N Low (1g saccharine) was added to each water bottle to neutralize possible bitter taste of cocaine. The amount of drinking water was monitored and the concentration of consumed cocaine was calculated. Another six male CD rats were housed in separate cages (two per cage) in the same facility and were provided food and water at libitum. Control rats were not exposed to any drug prior to the microdialysis assay. All animal procedures were in compliance with the Animal Welfare Act, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the City University of New York (CUNY) institutional guidelines and were approved by the CCNY Animal Care and Use Committee.

Striatal microdialysis

Prior to the surgical procedure rats were fully anesthetized with 1000 mg/kg urethane intraperitoneal (i.p.). The experiment started only after the animal did not respond to the tail and forepaw pinch test. Under full anesthesia, animals were mounted on stereotaxic frame with blunt ear bars, an incision was made in the skin over the skull, a hole was drilled in the skull for placement of the concentric microdialysis probe. A microdialysis guide cannula was surgically implanted in the striatum using these coordinates relative to bregma: AP, +0.7 mm; ML, +3.0 mm; DV -6.0 mm (Paxinos and Watson, 1982). Microdialysis probe (CMA /12; Bioanalytical Systems, West Lafayette, IN) was inserted into the guide cannulae and the probe was continuously perfused with artificial cerebrospinal fluid (125 mM NaCl, 1.18 mM Cl2Mg6H2O, 2.5 mM KCl, and 1.26 mM CaCl2; pH 7.4) at a flow rate of 2 µL/min. Samples were collected every 20 min. The first collected sample was discarded since it contained excessive amount of neurotransmitters released during tissue damage. The next 6 vials of

dialysate were baseline samples. Haloperidol (0.5 mg/kg) was injected immediately before the seventh sample collection and 4 more vials of dialysate collected. Finally, Haloperidol (3.0 mg/kg) was injected prior to the collection of the 11th vial. All collected vials were capped and immediately processed with HPLC or stored at -80°C until analyzed. Samples were analyzed using HPLC with electrochemical detection.

Measurement of DA by high pressure liquid chromatography (HPLC)

Quantification of DA in the dialysis samples or endogenous tissue extracts were performed using a bioanalytical HPLC system with electrochemical detection (BAS Bioanalytical Systems, West Lafayette, IN) consisting of a Perking Elmer series 410 pump and a BAS LC4B electrochemical detector and a Synergi 4u Hydro-RP column 150-4.60 mm (Phenomenex, Torrance, CA) and a mobile phase composed of 0.07 M monochloroacetic acid, 0.5 M sodium ethylenediaminetetraacetic acid, 1 mM sodium dodecyl sulfate, and 1.5 % acetonitrile, pH adjusted to 4.1. Twenty µL of each sample was injected onto the column for analysis. A potential on-flow cell of the electrochemical detector was set to +0.65 V versus Ag/AgCl. The integrated chromatograms were compared to standards, and analyzed using Perkin Elmer Turbochrom software. The approximate sensitivity limits of the assay with this detector settings and chromatographic separation is about 10pg for DA. The concentration of DA was quantified by comparing the height of DA in the treated sample with its height in the controls (represented as percent of controls).

Statistical analyses

Data were presented as mean ± S.E.M. Two-tailed Student's *t* test was used to compare particular responses between two groups. Three or more groups were analyzed

with one-way analysis of variance (ANOVA) followed by Tukey's multicomparison test. The threshold for mean significance was $p<0.05$. All statistical tests were calculated using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego California, USA).

Results

The effect of chronic cocaine on striatal DA efflux

Striatal DA efflux was measured by microdialysis in controls and in rats that were withdrawn 24 hrs. from a 21 day, 20 mg/kg treatment with cocaine. Chronic cocaine significantly ($*p<0.05$) reduced striatal extracellular DA as compared to controls (Fig. 1). This observation parallels previously observed effects in chronic haloperidol (Moore et al., 1998) and is consistent with reported suppression of DA cell firing in SN following perinatal cocaine exposure (Wang and Pitts, 1994).

The effect of low dose haloperidol on striatal extracellular DA in control and chronic cocaine treated rats

The effect of 0.5 mg/kg haloperidol on striatal extracellular DA was tested in control and chronic cocaine treated rats. In both chronic cocaine-treated and control animals significantly greater extracellular DA levels were obtained following acute, 0.5 mg/kg haloperidol when compared to the respective basal DA levels (Figs. 1 and 2). However, a significantly lower extracellular DA levels were observed in the chronic cocaine treated rats compared to controls after the low, acute dose of haloperidol (Fig. 2).

The effect of high dose haloperidol on striatal extracellular DA in control and chronic cocaine treated rats

In contrast to the effects of a low dose of haloperidol, a dose of 3.0 mg/kg haloperidol suppressed striatal extracellular DA when compared to basal DA concentration in the control group (Figs. 3.1,3.3 and 3.4) and significantly increased extracellular DA by more than 300% as compared to basal efflux in the chronic cocaine-treated group (Figs. 3.1, 3.3, and 3.5). After low, as well as high doses of haloperidol in the chronic cocaine-treated animals, extracellular DA was significantly higher than that of basal DA levels. The levels of extracellular DA, however, following low and high doses of haloperidol were not significantly different in the chronic cocaine-treated group (Fig. 3.5).

Discussion

Previous investigations have provided limited information on how chronic administration of APD causes DB in DA neurons. Most of those investigations on DB employed electrophysiological techniques and extracellular DA was not measured. Thus, perhaps the neurochemical mechanism by which the APD elicit neuronal inactivation is not conclusive. Our present findings are based on the measurements of extracellular DA by microdialysis after chronic cocaine treatment. Development of DB following chronic haloperidol treatment has been demonstrated by using microdialysis (Moore et al., 1998). Basal DA (efflux) in the extracellular fluid was diminished in the striatum after chronic haloperidol treatment presumably due to DB. Similarly, we found that after chronic cocaine treatment there was a significant reduction in striatal extracellular DA compared

to saline-treated controls (Fig. 3.1). This observation is in agreement with reported reduction of DA cell firing in the SN after withdrawal from perinatal cocaine treatment (Wang and Pitts, 1994). It is our hypothesis that the suppression of extracellular basal DA is due to cocaine-induced DB that results in a reduction in DA release from DA neurons. Moreover, we propose that this effect is caused by up-regulation of DA autoreceptors in response to chronic cocaine or haloperidol. Goeders and Kuhar (1987) have shown that drug withdrawal after chronic cocaine treatment increases the number of striatal DA D_2 receptors, possibly including the autoreceptors. The up-regulation of DA autoreceptors would be expected to result in DB and to exhibit a reduction in basal DA efflux.

In another series of experiments we compared the effect of an intraperitoneal injection of low dose haloperidol (0.5 mg/kg) in chronic cocaine and control rats. In both groups of animals we saw a significantly greater DA release following acute administration of the low dose of haloperidol (Fig. 3.2) compared to the respective basal levels (Fig. 3.1). Acute administration of low dose haloperidol appears to interact with presynaptic DA D_2 receptors to stimulate DA release (Lidsky and Banerjee, 1993). Since chronic cocaine treatment is expected to up-regulate presynaptic DA receptors (autoreceptors), one would expect greater release of DA in response to haloperidol in the cocaine treated group. Surprisingly, we found significant less induced DA efflux in the chronic cocaine-treated group as compared to controls following acute, low dose haloperidol treatment (Fig. 3.2). We believe that this paradoxical effect may be due to a preferential interaction of haloperidol with postsynaptic DA D_2 receptors rather than with DA autoreceptors in the chronic cocaine-treated group.

Lastly, a high dose of haloperidol (3.0 mg/kg) significantly suppressed the release of DA when compared to the effect of low dose haloperidol or to basal efflux in the control group (Figs. 3.1-3.3). In a previous publication, we have shown that acute administration of low dose haloperidol (0.5 mg/kg) increased firing of DA neurons in striatum and a combination of same dose of haloperidol and stimulation of SN suppressed DA cell firing rate due to the negative feedback response mediated by activation of the inhibitory striatal GABA-ergic transmission, which is caused by supra-maximal release of DA in response to administration of haloperidol plus stimulation of SN (Lidsky and Banerjee, 1993). We conducted similar investigation in the current study except we replaced haloperidol treatment plus SN stimulation with the administration of high dose of haloperidol. We believe that the same explanation is applicable to the present results on the effect of haloperidol on DA efflux as assessed by microdialysis. Interestingly, in the chronic cocaine-treated animals, high dose haloperidol increased DA release by more than 300% as compared to basal conditions (Figs. 3.1 and 3.5). This observation is opposite to that seen in the control animals. Why do cocaine treated rats respond differently from the control group? There may be two explanations for this observation. First, there is evidence that chronic cocaine induces sub-sensitivity of GABA neurons in the striatum (Liu et al., 2005). Also, repeated cocaine treatment diminishes GABA release in the striatum (Jung et al., 1999). Therefore, in chronic cocaine-treated rats the feedback response, mediated through GABA neurons, will be suppressed and it would fail to diminish DA release. Second, our results suggest that in chronic cocaine-treated rats, haloperidol may preferentially interact with the postsynaptic DA D_2 receptors (Figs. 3.2 and 3.5). Blockade of postsynaptic DA D_2 receptors would prevent activation of the

feedback mechanism mediated by GABA neurons. Thus, inability of GABA neurons to suppress DA release in chronic cocaine-treated animals mediated by two different mechanisms would facilitate an increase in DA release following a high dose of haloperidol in the cocaine-treated group.

Finally, previous investigators have reported increased glutamatergic transmission after repeated cocaine exposure (Pierce et al., 1996; Boudreau et al., 2005, 2007). Also, Itzhak and Stein (1992) have shown the up-regulation of glutamate activated NMDA receptors following chronic cocaine treatment. Thus, it appears that three inter-related or independent factors contribute to the development of DB. These are up-regulation of DA autoreceptors, suppression of a GABA-mediated inhibitory pathway and the enhancement of glutamatergic transmission.

Grace and his associates (1997) have proposed that "the therapeutic efficacy of APD in humans correlates with their ability to induce DB of mesolimbic DA neurons, whereas their potential to produce extrapyramidal side effects correlates with their propensity for inducing DB in the nigrostriatal DA system." Although we do not know if chronic cocaine treatment would induce DB of mesolimbic DA neurons, our results show DB in the nigrostriatal DA system may occur following repeated cocaine exposure. Nevertheless, cocaine is neither an effective antipsychotic nor does it induce EPS. Then, what is the consequence for the development of DB in the actions of APD? It may well be that development of DB is an important component in the therapeutic actions APD. This effect, however, by itself does not appear to be sufficient to predict the therapeutic action of this class of drugs. We and others have reported that typical as well as atypical APD are partial agonists at glutamatergic NMDA receptors (Banerjee et al., 1995; Lidsky

et al., 1997; Arvanov et al., 1997). Thus, APD may activate glutamatergic transmission by two mechanisms, namely, partial agonistic activity and establishment of DB. In contrast, cocaine has not been reported to be a partial agonist at NMDA receptors and therefore is ineffective as an antipsychotic drug. Furthermore, this dual activation of glutamatergic transmission by APD may help this class of drugs to exhibit their therapeutic efficacy as well as EPS. Typical APD may induce EPS by neurodegeneration reported to occur following chronic treatment with haloperidol in animals (Lidsky et al., 1995) and humans (Liberman et al., 2005). In contrast, after chronic cocaine treatment there are no changes in the DA transporter binding affinity or number (Peris et al., 1990; Pilotte et al., 1994) suggesting that there is no neurodegeneration. Furthermore, there are no known EPS following repeated cocaine administration. Thus, development of DB appears to be needed for the therapeutic actions of APD, but this by itself is not sufficient for their clinical value.

In conclusion, drug dependence occurs due to activation of DA-mediated reward system. Since withdrawal after chronic cocaine-induced DB decreases spontaneous DA cell firing (Fig. 3.1 and Wang and Pitts, 1994), development of DB may contribute, in part, to drug seeking behavior seen following cocaine abuse.

3.5 Figures.

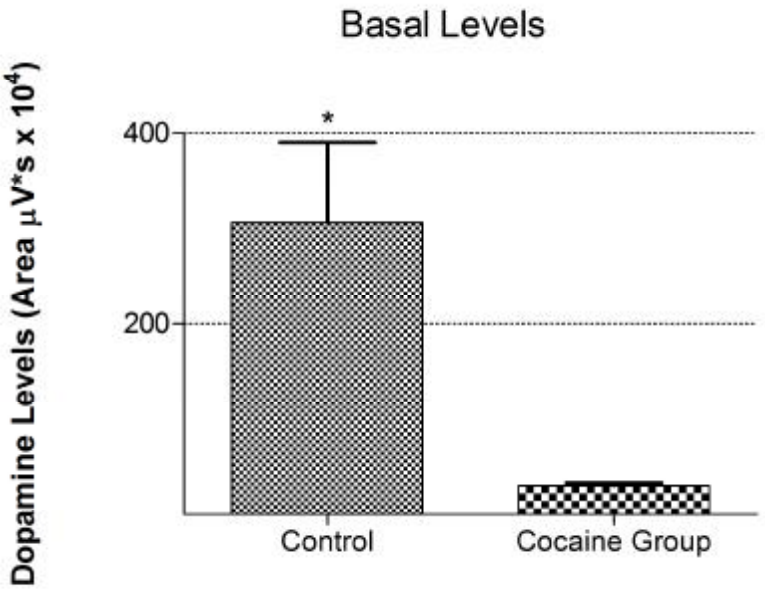


Fig. 3.1. Effect of chronic cocaine treatment on basal DA levels in the striatum. Rats were treated with oral cocaine (in drinking water) at 20 mg/kg with 1 packet Sweet'N Low for 21 days and cocaine was measured and microdialysis experiments were conducted 24 hrs. after the last exposure to cocaine. Control animals were allowed to drink water with same amount of Sweet'N Low and no cocaine added as in the other group. 40-minutes after initiating microdialysis, samples were collected and analyzed for DA by HPLC. Significant reduction in extracellular basal level of DA was found after chronic cocaine treatment compared to control rats. Two-tailed *t*-test: $t=3.26$ df=4; $*p<0.05$. Error bars, S.E.M.; $n=6$.

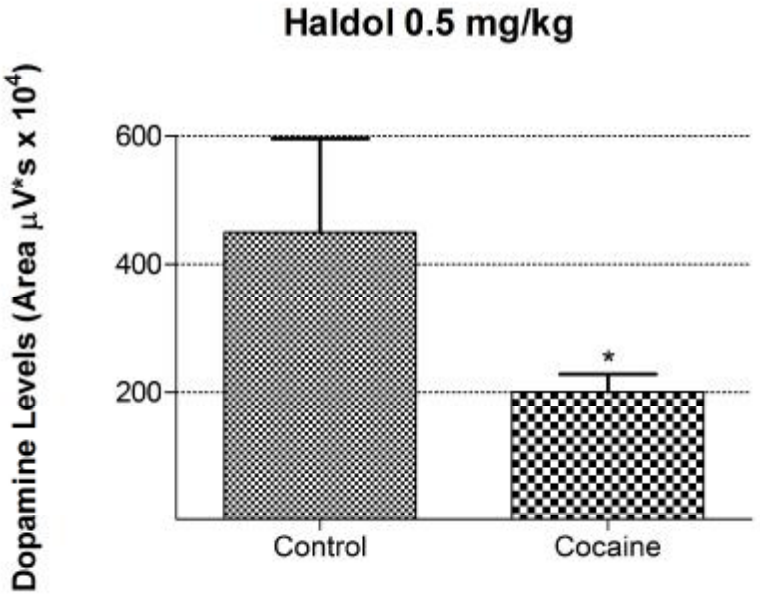


Fig. 3.2. Effect of low dose (0.5 mg/kg) haloperidol on DA release in control and chronic cocaine-treated rats. After the collection of baseline dialysis samples, 0.5 mg/kg haloperidol was injected intraperitoneally and extracellular DA levels in the dialysate were measured by HPLC. A dose of 0.5 mg/kg haloperidol significantly increased the release of DA in control as compared to the chronic cocaine treated group. Two-tailed *t*-test: $t=2.46$ df=4; $*p<0.05$; Error bars, S.E.M.; $n=6$. Also, when compared to the respective extracellular basal DA levels in Fig. 1 (comparison graph not shown), a substantial and significant increase in extracellular DA levels were found in the cocaine-treated groups (two-tailed t-test: t=6.06 df=4; Error bars, S.E.M. **p<0.01). In contrast, in the two control groups 0.5 mg/kg haloperidol substantially increased DA release that did not achieve statistical significance (two-tailed t-test: t=0.84 df=4; p>0.05).

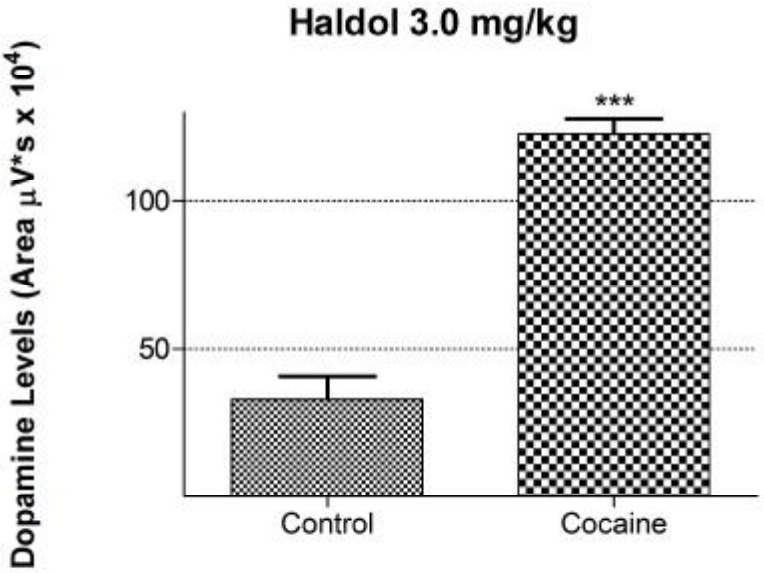


Fig. 3.3. Effect of high dose (3.0 mg/kg) haloperidol on DA release in control and chronic cocaine-treated rats. A similar protocol to that described in the legend to Fig. 2 was followed. A significant reduction in striatal DA release were found after the injection of 3.0 mg/kg haloperidol in the control group as compared to cocaine-treated rats (two-tailed *t*-test: $t=3.22$ df=4; ***$p<0.001$; Error bars, S.E.M.; $n=6$). In contrast, in the chronic cocaine-treated group, 3.0 mg/kg haloperidol significantly increased DA release when compared to extracellular basal levels of drug treated group (Fig. 1) (two-tailed t-test: t=16.01 df=4; ***p<0.001). Similarly, 3.0 mg/kg haloperidol-treated group was compared with the control group in Fig.1; the difference was statistically significant (two-tailed t-test: t=3.32 df=4; *p<0.05).

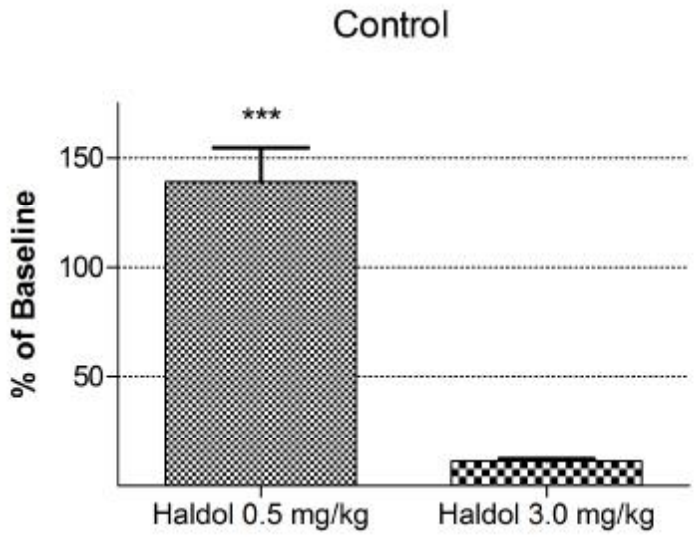


Fig. 3.4. Comparison between the effects of low dose (0.5 mg/kg) and high dose (3.0 mg/kg) haloperidol on DA release in control rats. Haloperidol-induced increases in DA release were calculated as % of basal levels. A markedly greater DA release was noted following the injection of a low dose haloperidol compared to the effect of the high dose (3.0 mg/kg) haloperidol. Two-tailed *t*-test: $t=8.17$ df=4; ***$p<0.001$; $n=6$. Error bars, S.E.M.; $n=6$.

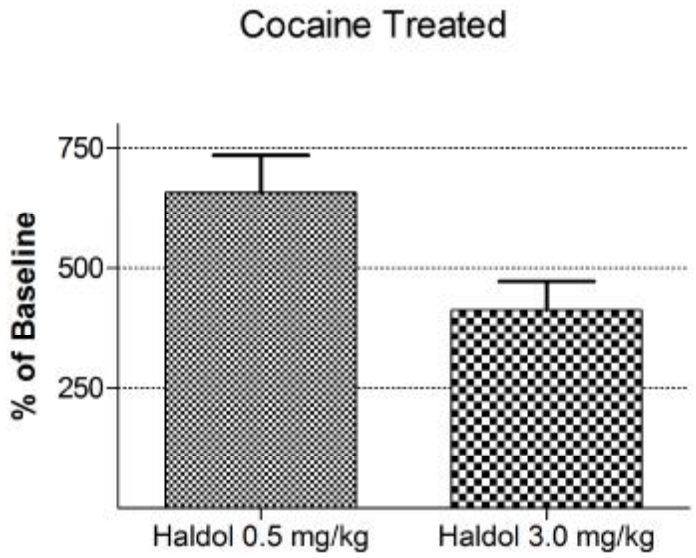


Fig. 3.5. Comparison between the effects of low dose (0.5 mg/kg) and high dose (3.0 mg/kg) haloperidol on DA release in chronic cocaine-treated rats. Haloperidol-induced increases in DA release were calculated as % of basal levels. A noticeably high release of striatal DA is seen following the injection of high dose haloperidol compared to the basal release in Fig. 1 or to the controls in Fig. 3 (two-tailed *t*-test: t=16.01 df=4; ***p<0.001; Error bars, S.E.M; n=6. However, there was no significant difference between the low and high dose haloperidol injections in terms of striatal DA release in the cocaine-treated group (two-tailed *t*-test: t=2.50 df=4; p<0.05; Error bars, S.E.M.; n=6).

Chapter 4. Mechanisms for metoclopramide-mediated sensitization and haloperidol-induced catalepsy in rats

Abstract

Typical antipsychotics such as the DA D_2 receptor antagonist, haloperidol are known to cause movement disorders or catalepsy in experimental animals. Catalepsy is believed to result from blockade of DA D_2 receptors. In this study two drugs that differ in antipsychotic potency but are similar in blocking DA D_2 receptors were used to investigate the mechanism for catalepsy and its sensitization. Metoclopramide is a strong postsynaptic DA D_2 receptor blocker with no antipsychotic potency. At low doses of 5 or 10 mg/kg given subcutaneously (s.c.), metoclopramide did not produce catalepsy or movement disturbance for seven days after drug treatment. Also metoclopramide at 10 mg/kg given for five days, failed to induce catalepsy. Haloperidol, another potent DA D_2 receptor blocker at 0.5 mg/kg (s.c.) rapidly produced catalepsy and suppressed movement one hour after a single dose of the drug. Chronic as well as acute treatment with metoclopramide caused sensitization of haloperidol-induced catalepsy. Neurochemical analyses revealed significant DA D_2 receptor up-regulation in both frontal cortex and striatum of rats chronically treated with metoclopramide. However, no changes in DA D_2 receptor numbers were noted in these areas after chronic treatment with low doses of haloperidol. Significant increases in NMDA receptor numbers were observed in both frontal cortex and striatum of metoclopramide treated animals, while haloperidol elicited significant decreases in NMDA receptor numbers in both brain areas. These observations plus previous reports have led us to propose a model for catalepsy and its sensitization. According to this model the increase in NMDA receptors by metoclopramide sensitizes

the brain to haloperidol-induced catalepsy. Thus, catalepsy appears to be elicited by simultaneous activation of glutamatergic NMDA and DA D_1 receptors as well as a blockade of DA D_2 receptors.

4.1 Introduction

Previous reports show that chronic administration of typical neuroleptics produces catalepsy (Barnes et al., 1990), develops tolerance (Ezrin-Waters and Seeman, 1977) or sensitizes catalepsy (Antelman et al., 1986) in animals. Mechanisms for the development of catalepsy, tolerance, and its sensitization following repeated treatment with haloperidol remain to be elucidated. Alterations of several neurotransmitter functions have been implicated in the appearance of catalepsy in experimental animals. Haloperidol causes catalepsy because of the blockade of DA D_2 receptors in the striatum (Boulay et al., 2000; Ellenbroek et al., 1985). Similarly, DA D_1 receptor antagonist, SCH23390 produces catalepsy that was prevented by DA D_2 receptor agonist (Meller et al., 1985; Morelli and Di Chiara, 1985; Ogren and Fuxe, 1988). Also, a combination of DA D_1 and D_2 receptor antagonists showed synergistic effect in development of catalepsy (Wanibuchi and Usuda, 1990). Neurotransmitter systems other than dopamine may also play a role in developing catalepsy. For example, cholinergic agonists enhance catalepsy induced by DA D_1 or D_2 receptor antagonists (Ushijima et al., 1997). The mechanisms for such augmentation of catalepsy by cholinergic agonists have been reported to be different. While DA D_1 receptor blockade enhances catalepsy through activation of muscarinic M_1 receptor subtype, catalepsy augmentation caused by DA D_2 receptor antagonism is due to stimulation of muscarinic M_2 receptor subtype (Ushijima et al.,

1997). Also, because NMDA receptor blocker MK-801 prevents catalepsy in rats caused by either DA D_1 or D_2 receptor antagonists (Elliot et al., 1990; Moore et al., 1993), excitatory amino acids such as glutamate may play a role in mediating catalepsy via DA D_2 or D_1 receptor antagonists.

We have been studying how interactions between dopaminergic and glutamatergic systems affect the development of neuroleptic-induced catalepsy. Studies in our laboratory have shown that G_{M1} ganglioside and taurine attenuated chronic haloperidol-induced increase in DA D_2 receptor density, catalepsy, and a decrease in endogenous striatal levels of dopamine as well as tyrosine hydroxylase activity (Lidsky et al., 1994, 1995). In the present study we investigated changes in dopaminergic and glutamatergic functions, which may contribute to the development of catalepsy or its sensitization following acute or chronic administration of haloperidol or metoclopramide. Although, both drugs are antagonist of DA D_2 receptors, only haloperidol is valuable in treating psychosis; metoclopramide is restricted to its use as an antiemetic agent (Baldessarini and Tarazi, 2001). Besides blocking DA D_2 receptors, haloperidol acts as a partial agonist at glutamatergic NMDA receptors (Banerjee et al., 1995). Metoclopramide shows no agonistic activity at the NMDA receptors, but in high doses blocks NMDA receptors (Lidsky et al., 1997). Our results suggest that low doses of metoclopramide do not cause catalepsy, but do enhance haloperidol-induced catalepsy. While sensitization to haloperidol-induced catalepsy requires activation of NMDA receptor activity in the striatum and/or frontal cortex, catalepsy itself is seen only when there is an increase in glutamatergic transmission along with augmentation of DA D_1 receptor mediated transmission and blockade of DA D_2 receptors. Increased glutamatergic transmission via

the NMDA receptor system may occur either by an increase in NMDA receptor density or by a partial agonistic effect that may be elicited by low doses of haloperidol.

4.2 Materials and methods

4.2.1 Drugs

Haloperidol, metoclopramide HCl, MK-801, butaclamol, Tris base, $_{L}$-glycine and monochloroacetic acid were purchased from Sigma/RBI (Natick, MA). EDTA and Hepes were purchased from Fisher Scientific (Fair Lawn, NJ). [^{3}H]MK-801 and [^{3}H]spiroperidol were purchased from PerkinElmer Life and Analytical Sciences Division (Boston, MA).

4.2.2 Animals

Male CD rats (100-350g) obtained from Charles River Laboratories, Inc. (Wilmington, MA), housed two per cage and allowed to acclimatize in a temperature-controlled environment were used in this study. Rodent laboratory chow and tap water were provided ad libitum. For behavioral assay rats received weekly subcutaneous injections of either haloperidol 0.5 mg/kg (b/w) once a week for three weeks, or a single administration of metoclopramide HCl, 5mg/kg and 10 mg/kg (b/w). For neurochemical and additional behavioral analyses animals were injected daily for 5 days with haloperidol 0.5 mg/kg (b/w) or metoclopramide HCl 10 mg/kg (b/w). Both drugs were dissolved in dimethylsulfoxide (DMSO). All animal procedures were in compliance with the Animal Welfare Act, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the City University of New York (CUNY) institutional guidelines and were approved by the CCNY Animal Care and Use Committee.

4.2.3 Catalepsy analysis

Behavioral testing was conducted 60 minutes after the injection of the test drugs. Catalepsy was evaluated using standard bar test (Barnes, 1990; Elliott, 1990). The rat's forepaws were placed on a 10 cm high horizontal bar located in a sound attenuated testing box with background white (static) noise. Catalepsy was measured for 5 minutes and each animal underwent three consecutive trials with 5-10 minutes break between the tests. An animal was considered cataleptic if both forepaws remained on the bar for at least 1 minute. Catalepsy score (immobility time in seconds) of each animal was analyzed in calculating mean scores.

4.2.4 Receptor binding of [^{3}H]MK-801 to NMDA glutamate receptor and [^{3}H]spiroperidol to dopamine D_2 receptor

NMDA and dopamine D_2 receptor binding in frontal cortex or striatum of control and experimental animals was assessed by displacement of [^{3}H]MK-801 by different concentrations of nonradioactive MK-801 in the presence of 30 μM of glycine or displacement of [^{3}H]spiroperidol by different concentrations butaclamol as previously described (Banerjee et al. 1995; Lidsky et al., 1995). Twenty-four hours after the last dose of either metoclopramide or haloperidol rats were sacrificed, brains removed, frontal cortices and striata dissected on ice and immediately frozen at -80ºC. On the day of experiment the tissue was thawed and washed to reduce residual levels of neurotransmitters. The tissue was homogenized with Brinkman Polytron PT-20 homogenizer in 20 volumes of ice-cold 0.05 M Tris buffer with EDTA and hepes (pH 7.4 at 25ºC) and centrifuged at 49,000g for 20 min. The supernatant was discarded and the pellet was rehomogenized in the same buffer and centrifuged as before. The last

procedure was repeated twice, and the resulting membrane pellet was frozen for one more day at -80ºC. Receptor binding assays were carried out by incubating 800 μl of crude fractions containing (0.8 mg of tissue) with radioligands [^{3}H]MK-801 (about 100,000 cpm) with 30 μM glycine or [^{3}H]spiroperidol (about 70,000 cpm) for 45 min at 25ºC. To determine nonspecific binding, a parallel set of samples was incubated with large excess, 100 μM of MK-801 or 100 μM of butaclamol. Specific binding was defined as the difference between total binding and nonspecific radioactivity. Incubations were terminated by rapid filtration through GF/B glass fiber filters, followed by 2 rinses with 4ml ice-cold Tris buffer to remove unbound radioactive ligand. Filters were then dried and placed in vials containing 3 ml of scintillation fluid and counted by scintillation spectroscopy at 60% efficiency. Binding parameters were calculated from displacement curves of [^{3}H]MK-801 to the NMDA receptor and [^{3}H]spiroperidol to dopamine D_2 receptor by conducting the binding assays with eight different concentrations of unlabeled MK-801 in presence of 30 μM glycine or eight different concentrations of unlabeled butaclamol, respectively. Scatchard analysis and the maximal number of binding sites were computed with Beckman's AccuFit competitive two-site program. The program is based on the principle of nonlinear least-square regression analysis to solve the equation describing the binding of labeled ligand to receptor proteins. Results were confirmed with GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA.

4.2.5 Statistical analysis

Data were presented as mean ± S.E.M. Two-tailed Student's *t* test was used to compare particular responses between two groups. Three or more groups were analyzed

with one-way analysis of variance (ANOVA) followed by Tukey's multicomparison test. The threshold for mean significance was $P<0.05$. All statistical tests were performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego California USA).

4.3 Results

4.3.1 Behavioral testing

Seven days after a single dose of 0.5 mg/kg haloperidol animals showed moderate but significant degree of catalepsy compared with control animals that received an equal volume of solvent (Fig. 4.1). The intensity of catalepsy was significantly enhanced at weeks 2 and 3 following weekly administration of the same dose of haloperidol. These results are similar to our previously reported observation on cataleptic effects of haloperidol (Lidsky et al. 1994, 1995). In sharp contrast, a week after a single injection of 5 mg/kg or 10 mg/kg of metoclopramide no catalepsy was observed. No other behavior differences were noted in the drug treated groups when compared to control group (Fig. 4.1). Interestingly, when rats pretreated with 5 or 10 mg/kg of metoclopramide were given 0.5 mg/kg of haloperidol seven days later, animals exhibited significantly higher degree of catalepsy compared to solvent plus haloperidol treated rats (Fig. 4.1). Further, weekly administration of the same dose of haloperidol showed significant enhancement of catalepsy in the metoclopramide-pretreated groups compared to solvent plus haloperidol group at weeks two and three. The degree of catalepsy was significantly greater in animals receiving 10 mg/kg metoclopramide compared to those receiving 5 mg/kg metoclopramide at each of the three experimental time points (Fig. 4.1). Thus,

metoclopramide was found to be effective in sensitizing haloperidol-induced catalepsy. Haloperidol and metoclopramide are DA D_2 receptor antagonists (Kebabian and Cote, 1981; Seeman, 1980). However, while low doses of haloperidol induced catalepsy the low doses of metoclopramide failed to do so. Nonetheless, metoclopramide was found to sensitize the cataleptic response mediated by haloperidol. Also, a high dose of metoclopramide (50 mg/kg) injected daily for 5 days exhibited the same level of catalepsy as did haloperidol (0.5 mg/kg) after three weeks of treatment (data not shown).

We wondered if chronic treatment with a low dose of metoclopramide would cause catalepsy. Therefore, we carried out experiments with 12 rats. Six rats received 10 mg/kg of metoclopramide a day for 5 days and the other six rats received equal volume of solvent. None of the metoclopramide treated rats showed any sign of catalepsy even 48 hours after the termination of drug treatment. Subsequent administration of 0.5 mg/kg of haloperidol following 5 doses of metoclopramide treatment exhibited sensitization of haloperidol-induced catalepsy. Thus, the results of acute vs. chronic treatments with metoclopramide were similar with regard to developing sensitization of haloperidol-mediated catalepsy. These observations are different from the effects of haloperidol as repeated treatment with daily injections of 0.5 mg/kg haloperidol were previously shown to elicit gradual increase in the intensity of catalepsy (Lidsky et al., 1994, 1995).

4.3.2 Effects of chronic drug administration on striatal and frontal cortical dopamine D_2 receptor densities

In order to determine whether changes in DA D_2 receptor numbers contribute to the development of either catalepsy or its sensitization following chronic treatments with either haloperidol or metoclopramide, we measured [^{3}H]spiroperidol binding in striatal

and frontal cortical membrane preparations taken from control and drug treated animals. The maximal number of DA D_2 receptors was significantly increased in striata of rats treated with metoclopramide compared to the control group (Fig.4.2A and Table 4.1). Similarly, there was a significant increase in DA D_2 receptor density in the frontal cortex of the metoclopramide group (Fig. 4.3A and Table 4.1). On the other hand, the maximal number of DA D_2 receptors in striatal as well as frontal cortical membrane preparations obtained from rats receiving haloperidol, showed no significant changes compared to control group (Figs. 4.2B, 4.3B and Table 4.1). Previously, we have shown that daily administration of haloperidol for 21 days significantly increased maximal number of DA D2 receptors compared to control (Lidsky et al., 1994, 1995). Therefore, in the present study the increase in DA D_2 receptor density failed to achieve statistical significance because of a shorter duration of haloperidol treatment.

4.3.3 Effects of chronic drug administration on striatal and frontal cortical NMDA receptor concentrations

Previous studies in our laboratory have shown that agents that alter glutamatergic and/or GABA-ergic transmissions decrease haloperidol-induced catalepsy (Lidsky et al., 1994, 1995). Metoclopramide has been shown to be a weak antagonist at the NMDA receptor (Lidsky et al., 1997). Repeated administration of metoclopramide might, therefore be expected to up-regulate the NMDA receptors. On the other hand, haloperidol has been reported to be a partial agonist at the NMDA receptor (Arvanov et al., 1997; Banerjee et al., 1995; Lidsky et al., 1997). Thus, at low doses haloperidol functions as an agonist at the NMDA receptor and may be expected to down-regulate these receptors. In addition, haloperidol was shown to release glutamate (Yamamoto and Davy, 1992;

Yamamoto and Cooperman, 1994), an action that may contribute in the subsensitization of NMDA receptor function. Therefore, we decided to investigate changes in NMDA receptor concentrations following chronic haloperidol or metoclopramide treatments. Repeated administration of metoclopramide was found to significantly increase maximal NMDA receptor binding sites in striatal as well as prefrontal cortical membranes compared to control preparations (Figs. 4.4A, 4.5A and Table 4.1). In sharp contrast a significant decrease in the maximal number of NMDA receptor-binding sites was found in striatal and cortical membranes prepared from haloperidol treated rats (Figs. 4.4B, 4.5B and Table 4.1).

4.4 Discussion

The results of the present study show that low doses of metoclopramide (5 or 10 mg/kg) administered acutely or chronically did not produce catalepsy in experimental animals (Fig. 4.1). This is in sharp contrast to the effects of haloperidol that consistently induced catalepsy even at 0.5 mg/kg doses that gradually increased in intensity with repeated administration of the same dose of the drug (Fig. 4.1). This observation is in agreement with previous reports from our laboratory as well as from other laboratories (Barnes et al., 1990; Lidsky et al., 1994, 1995). The effect of metoclopramide is controversial. Previous investigators have reported that either this agent elicits catalepsy or does not following acute or chronic administrations. A careful analysis of previous results indicates that the effect of metoclopramide on development of catalepsy may be dose dependent. In general, low doses of metoclopramide such as 2 mg/kg failed to cause catalepsy in rats (Chinen and Frussa-Filho, 1999; Imamura et al., 1988), while higher

doses, such as 20 mg/kg consistently induced catalepsy (Ahtee, 1975; Hassan et al., 1986; Stanley et al., 1980). Despite the inability of low doses of metoclopramide to cause catalepsy, it induced sensitization to haloperidol-mediated catalepsy (Fig. 4.1). This observation is in agreement with a previous report by Imamura and associates (1988).

To understand the mechanism by which metoclopramide elicits sensitization to haloperidol-induced catalepsy, we assessed maximal DA D_2 and NMDA receptor binding in striatum and frontal cortex following chronic drug treatments (Figs. 4.2-4.5 and Table 4.1). The maximal binding parameters of DA D_2 as well as NMDA receptors were elevated in striatum and frontal cortex after repeated (5 doses) metoclopramide treatment. On the other hand, repeated haloperidol treatment showed a significant decrease in maximal binding at the NMDA receptors in striatum and frontal cortex but no significant changes in the DA D_2 receptor densities in the two brain areas (Figs. 4.2-4.5 and Table 4.1). How do these receptor number changes explain the mechanism by which sensitization develops in catalepsy? Previous investigators have reported that DA D_1 as well as DA D_2 receptor antagonists may induce catalepsy (Boulay et al., 2000; Undie and Friedman, 1988). Interestingly, DA D_1 and D_2 receptor antagonists-mediated catalepsy was prevented by the relatively selective DA D_2 receptor agonist, apomorphine (Meller et al., 1985; Morelli and Di Chiara, 1985; Ushijima et al., 1997). Therefore, activation of dopaminergic transmission reverses catalepsy induced by either DA D_1 or D_2 receptor antagonists. Apomorphine is a presynaptic DA D_2 receptor agonist at low doses while at high doses it activates postsynaptic DA D_2 receptors (Yamada and Furukawa, 1980). While low doses of apomorphine may cause or augment haloperidol induced catalepsy (Balsara et al., 1982; Ushijima et al., 1997), high doses block catalepsy mediated by

either DA D_1 or D_2 receptor antagonists. Thus, a low dose of apomorphine, which activates presynaptic DA D_2 receptors and decreases release of dopamine, facilitates the development of catalepsy mediated by haloperidol. Similarly, other means of suppressing dopamine release such as the administration of the GABA agonist, muscimol, along with either DA D_1 (SCH 23390) or D_2 (metoclopramide) receptor antagonists further potentiate the catalepsy induced by the respective antipsychotics (Asin and Bednarez, 1990). Such observations are consistent with the idea that catalepsy may require blockade of dopaminergic D_2 transmission and activation of transmission via the DA D_1 receptor subtype along with changes in other neurotransmitter functions.

The role of DA D_1 receptor in developing catalepsy remains to be elucidated. Several investigators reported conflicting results in the changes in DA D_1 receptor densities following chronic treatment with DA D_1 receptor antagonists. While some investigators reported an increase in the maximal number DA D_1 receptor binding sites (Jenner et al., 1985; Porceddu et al., 1986; See et al., 1990) others reported no change in the density of DA D_1 receptor number in the striatum (Lappalainen et al., 1990; Murugaiah et al., 1984) following chronic administration of different types of DA D_1 receptor antagonists. Despite such differences, several investigators have found an increase in DA D_1 receptor stimulated adenylate cyclase in the striatum (Jenner et al., 1985; Murugaiah et al., 1984) following chronic treatment with DA D_1 receptor antagonists. Therefore, it is our hypothesis that chronic treatment with DA D_1 receptor antagonists would lead to enhanced dopamine-stimulated adenylate cyclase that contributes to the development of catalepsy. This hypothesis would suggest that DA D_1 receptor stimulation would itself cause or enhance haloperidol-induced catalepsy.

Although, Ushijima and associates (1997) did not study the effects of DA D_1 receptor agonist SKF38393 alone on catalepsy, they showed that 1mg/kg of SKF38393 protected the catalepsy caused by 0.3 mg/kg of haloperidol by about 25 to 30%. This enhancement of catalepsy did not, however, reach statistical significance. It may be related to dosages of the drugs, the intervals between the treatments with the two drugs, or the time period between assessment of catalepsy and haloperidol treatment.

Besides the dopaminergic system, changes in glutamatergic transmission may play a critical role in the development of catalepsy. Previous investigators have reported that NMDA receptor antagonists prevented catalepsy induced by DA D_1 and D_2 receptor blockers (Elliot et al., 1990; Moore et al., 1993). Similarly, we have reported that G_{M1} ganglioside or taurine prevented haloperidol-mediated catalepsy (Lidsky et al., 1994, 1995). Repeated treatment with 10 mg/kg of metoclopramide increased the maximal binding sites of NMDA receptors the striatum as well as frontal cortex (Fig. 4.4 and 4.5). Despite such increases in NMDA receptor densities, acute or chronic treatments with 10-mg/kg metoclopramide failed to cause catalepsy (Fig. 4.1). This may be related to the fact that acute or chronic treatments with metoclopramide may not result in significant up-regulation of NMDA receptors to induce catalepsy. The mechanism by which haloperidol mediates catalepsy appears to be different. Although haloperidol down-regulated NMDA receptors in striatum and frontal cortex (Fig. 4.4 and 4.5), it functions as an agonist at the NMDA receptors at low doses (Arvanov et al., 1997; Banerjee et al., 1995) and stimulates release of glutamate (Yamamoto and Cooperman, 1994). Therefore, haloperidol may cause catalepsy by blocking the DA D_2 receptors, stimulating NMDA receptors, and leaving DA D_1 receptor function unaltered. Based upon such observations,

we propose that catalepsy may be induced by the blockade of DA D_2 receptors and activations of DA D_1 and NMDA receptor systems and/or by increased interactions between these receptor systems. There is evidence that glutamatergic transmission mediated by the NMDA system may be enhanced through the activation of DA D_1 receptors (Cepeda and Levine, 1998; Maguire and Werblin, 1994). Thus, interactions between NMDA and DA D_1 receptor systems may play a critical role in the development of catalepsy. Our hypothesis, however, does not explain how acute administration of DA D_1 receptor antagonists induces catalepsy. Additional work is needed to elucidate the mechanism of action for this acute effect of DA D_1 receptor antagonists.

Acute as well as chronic treatments with low doses of metoclopramide induced sensitization of haloperidol-mediated catalepsy (Fig.4.1). Also, chronic treatment with 10 mg/kg of metoclopramide caused augmentation of NMDA and DA D_2 receptors in the striatum as well as frontal cortex (Figs.4.2-4.5; Table 4.1). It appears that increase in DA D_2 receptor numbers does not contribute to sensitization of catalepsy by metoclopramide as haloperidol is mandatory in the development of catalepsy and it is a potent blocker of all DA D_2 receptors. In contrast, enhancement of NMDA receptors by chronic treatment with low doses of metoclopramide is likely to cause sensitization of haloperidol-induced catalepsy. Interestingly, metoclopramide at the daily doses of 50 mg/kg produces catalepsy by itself. We believe that this is caused by the large augmentation in NMDA receptor density that is by itself sufficient to induce catalepsy. On the other hand, haloperidol down-regulates NMDA receptors (Figs. 4.4 and 4.5; Table 4.1) but is still able to cause catalepsy. This is likely mediated by other actions of haloperidol. This drug is a partial agonist at NMDA receptors (Arvanov et al., 1997; Banerjee et al., 1995)

and stimulates release of glutamate (Yamamoto and Cooperman, 1994). Thus, it is our hypothesis that metoclopramide causes sensitization of haloperidol-induced catalepsy by increasing glutamatergic transmission via NMDA receptors. Moreover, haloperidol produces catalepsy when administered by blocking DA D_2 receptors, stimulating NMDA receptors and leaving DA D_1 receptor function unaltered.

In conclusion, results of the present study has helped us to develop a model for metoclopramide mediated sensitization and haloperidol-induced catalepsy. According to the proposed model, metoclopramide causes sensitization by augmenting the density of NMDA receptors. Haloperidol induces catalepsy by increasing glutamatergic transmission via NMDA system, blocking DAD_2 receptors, and leaving DA D_1 receptors function unaltered. Induction of catalepsy has been utilized in the evaluation of pharmacological agents for identifying their potential value as antipsychotic drugs (Seeman, 1980). No previous investigator has defined a neurochemical rationale for use of the induction of catalepsy in the development of antipsychotic drugs. An analysis of the literature and our present results provide a neurochemical basis for catalepsy. Catalepsy appears to result from inactivation of DA D_2 receptor function and simultaneous enhancement of NMDA receptor mediated transmissions and interaction with DA D_1 receptors. Interestingly, typical as well as atypical antipsychotic drug effects may be mediated through their partial agonistic activity at the NMDA receptor system (Lidsky and Banerjee, 1996). In addition, typical antipsychotic drugs have been shown to exhibit a close correlation between therapeutic potency and their apparent affinity in blocking DA D_2 receptor function (Seeman, 1992). Thus, catalepsy as well as antipsychotic activity appears to be mediated through modifications of dopaminergic and

glutamatergic transmissions.

4.5 Figures and Tables.

Table 4.1 Changes in radioligand binding values in the striatum and frontal cortex (FC) of chronically treated rats with metoclopramide and haloperidol.

Radioligand	**Brain Area**	**Treatment Groups**	***Bmax* (mol/mg wet weight x 10^{14}) Mean ± S.E.M.**	***n***	***P***
[^{3}H-spiroperidol]	Striatum	Control	2.01 ± 0.005	6	
		Metoclopramide	4.08 ± 0.023	6	$P<0.05$
		Haloperidol	2.07 ± 0.315	6	$P>0.05$
	FC	Control	3.03 ± 0.024	6	
		Metoclopramide	3.70 ± 0.073	6	$P<0.05$
		Haloperidol	3.08 ± 0.030	6	$P>0.05$
[^{3}H-MK 801]	Striatum	Control	4.17 ± 0.033	6	
		Metoclopramide	4.91 ± 0.008	6	$P<0.05$
		Haloperidol	3.39 ± 0.037	6	$P<0.05$
	FC	Control	1.29 ± 0.087	6	
		Metoclopramide	1.47 ± 0.040	6	$P<0.05$
		Haloperidol	1.02 ± 0.028	6	$P<0.05$

B_{max} is the maximal density (number) of receptor sites. Metoclopramide increased dopamine D_2 receptor numbers in both brain areas while haloperidol kept these numbers unchanged. NMDA receptors were up-regulated by metoclopramide in both areas whereas haloperidol caused their down-regulation.

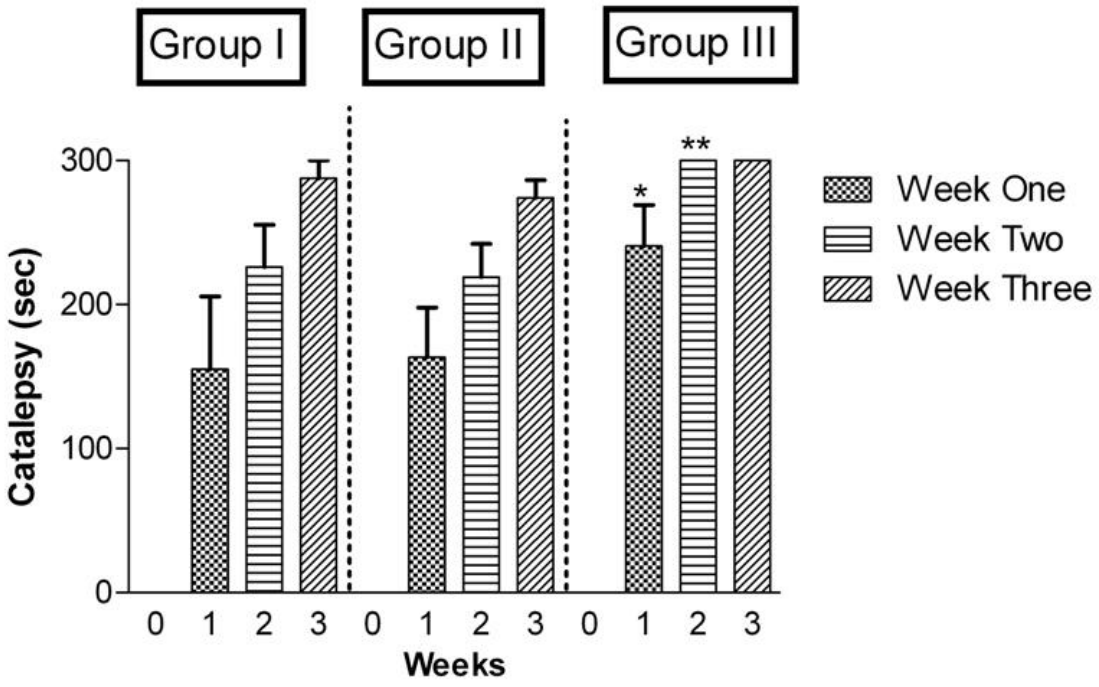


Fig. 4.1 Behavioral assessment of catalepsy. Twenty-four rats were divided into three groups with eight animals in each group. The first group received solvent and served as control. Groups 2 and 3 received 5 or 10 mg/kg of metoclopramide, respectively. A week later all 24 rats received 0.5 mg/kg haloperidol (I). Administration of 0.5 mg/kg haloperidol was repeated in weeks two (II) and three (III). The data are expressed as mean ± S.E.M. Statistical differences were evaluated using One-way ANOVA followed by Tukey test for multiple comparisons. **P* < 0.05; ***P*< 0.01 when compared with respective value in comparison group.

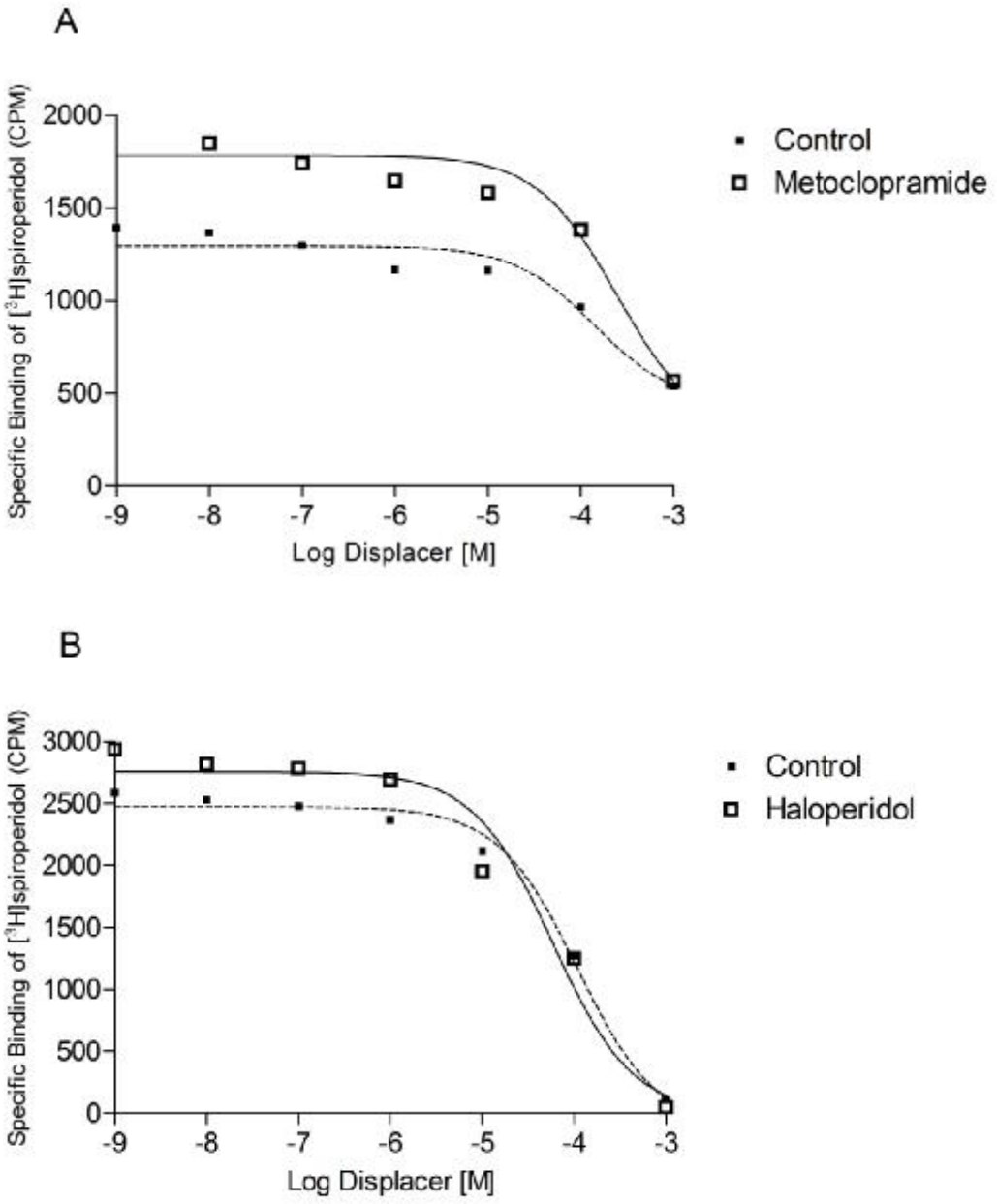


Fig. 4.2 Displacement of [^{3}H]spiroperidol binding by butaclamol in striatum of control vs. chronic metoclopramide (**A**) and haloperidol (**B**) treated rats. Rats received metoclopramide (10 mg/kg s.c.) or haloperidol (0.5 mg/kg s.c.) daily for 5 days and were sacrificed 48 h after the last injection.

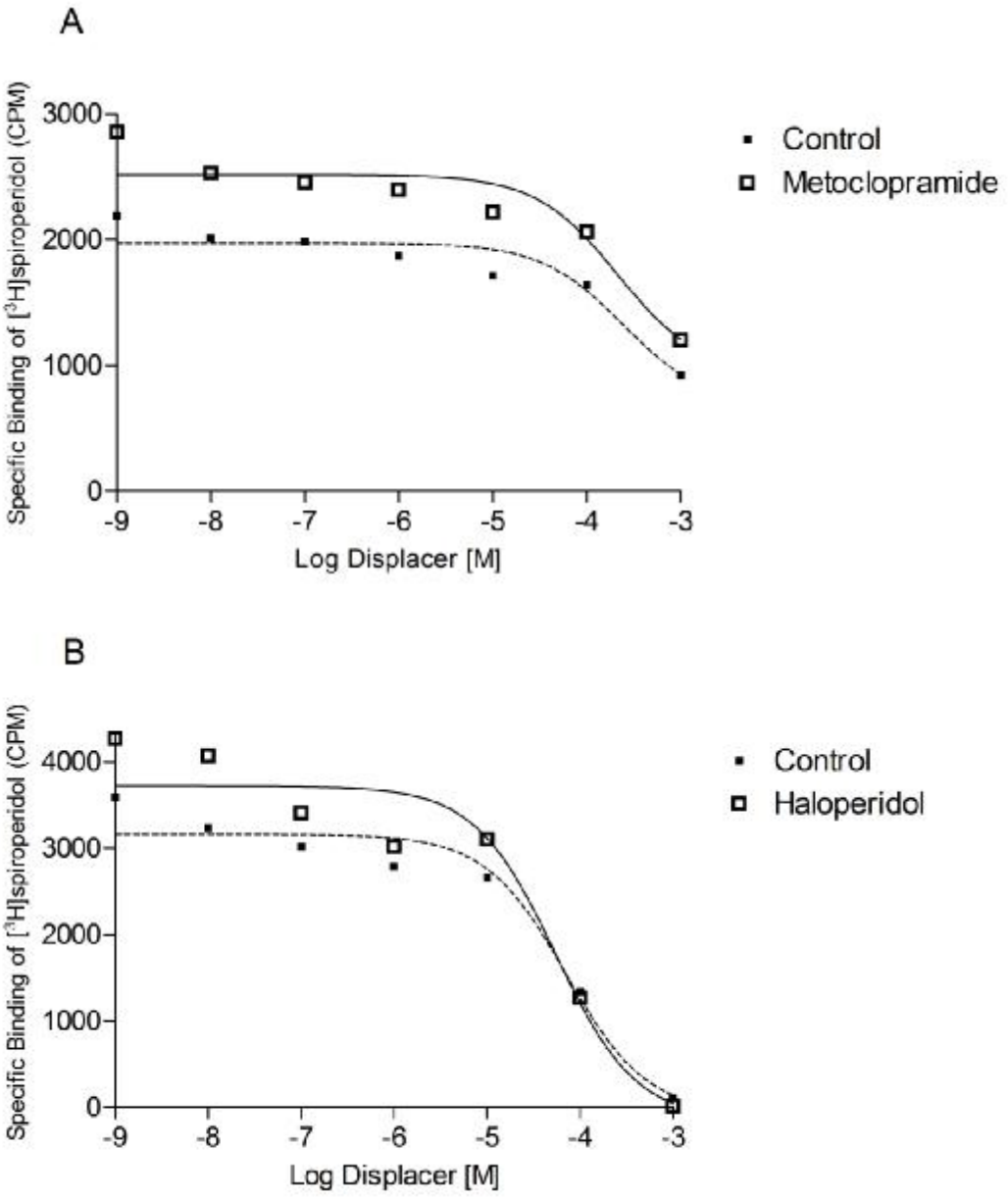


Fig. 4.3 Displacement of [^{3}H]spiroperidol binding by butaclamol in frontal cortex of control vs. chronic metoclopramide (**A**) and haloperidol (**B**) treated rats. Rats received metoclopramide (10 mg/kg s.c.) or haloperidol (0.5 mg/kg s.c.) daily for 5 days and were sacrificed 48 h after the last injection.

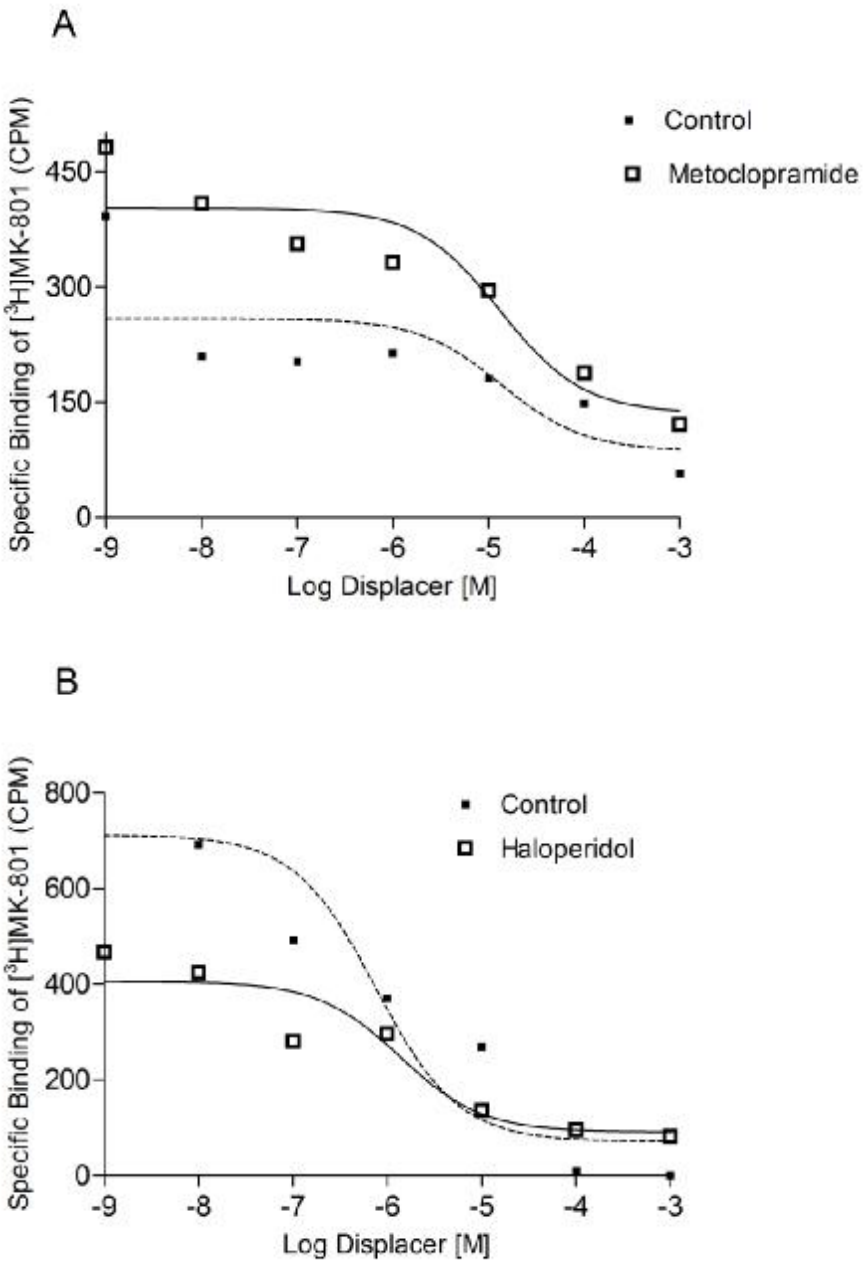


Fig. 4.4 Displacement of [^{3}H]MK 801 binding by non-radioactive MK-801 in striatum of control vs. chronic metoclopramide (**A**) and haloperidol (**B**) treated rats. Rats received metoclopramide (10 mg/kg s.c.) or haloperidol (0.5 mg/kg s.c.) daily for 5 days and were sacrificed 48 h after the last injection.

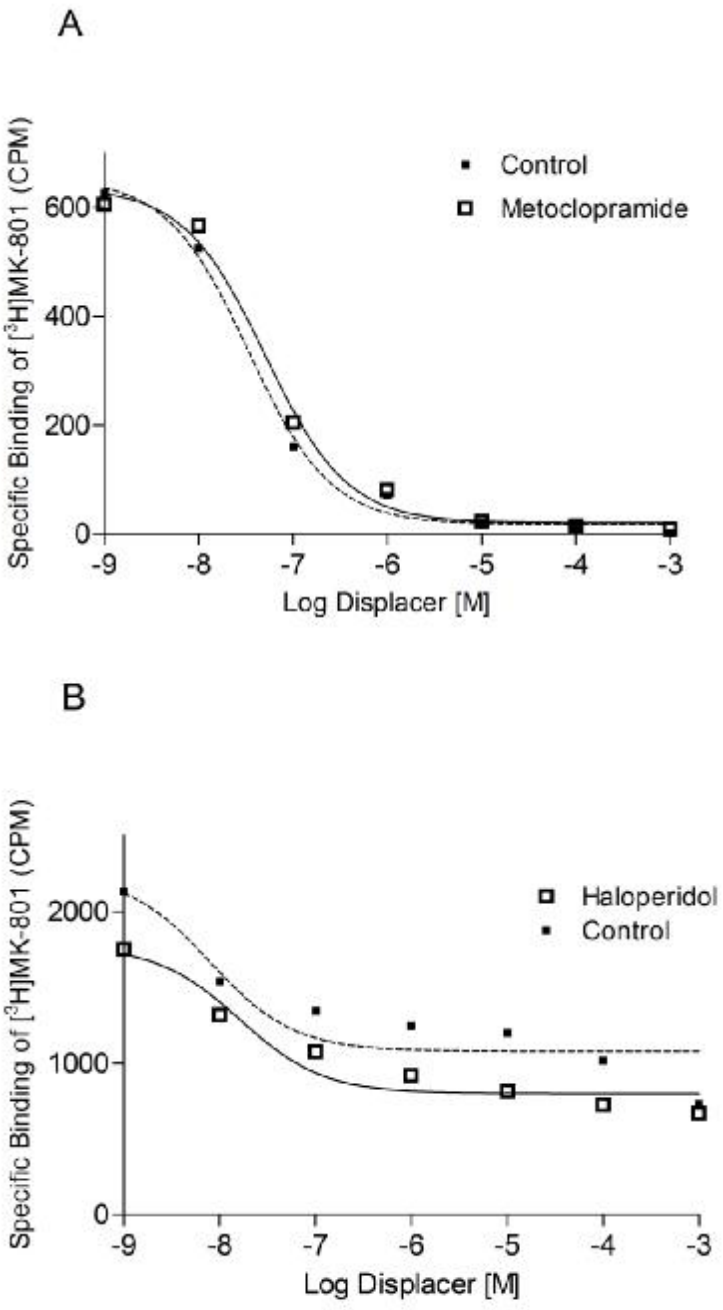


Fig. 4.5 Displacement of [^{3}H]MK-801 binding by non-radioactive MK-801 in frontal cortex of control vs. chronic metoclopramide (**A**) and haloperidol (**B**) treated rats. Rats received metoclopramide (10 mg/kg s.c.) or haloperidol (0.5 mg/kg s.c.) daily for 5 days and were sacrificed 48 h after the last injection.

Chapter 5: Conclusion

In conclusion, results of the investigation presented herein have helped us to better understand the neurochemical mechanism of typical APD. The most interesting feature is the similarity between the mechanisms for catalepsy and the DB. According to our hypothesis, catalepsy is induced by DA D_2 receptor blockade and activation of glutamatergic NMDA transmission. Similarly, the mechanism of action of typical APD involves blockade of DA D_2 receptors and activation of glutamatergic NMDA transmission due to DB following 3 weeks of treatment. Subsequent administration of typical APD would further enhance glutamatergic transmission, through the partial agonistic activity at the NMDA and blockade of DA D_2 receptors. Thus, it is no wonder that pharmaceutical companies use the ability of chemical agents to produce catalepsy as a criterion in the development of antipsychotic drugs. Previous studies have proposed that thereapeutic potential of APD in humans parallels their ability to induce DB of mesolimbic DA neurons, while their inclination to produce EPS (or catalepsy in animals) correlates with their capacity to induce DB in the nigrostriatal DA pathway (Grace et al., 1997). It appears that development of DB may be an important component needed in the expression of therapeutic actions of APD. This effect, however, by itself is not sufficient for the therapeutic action of this class of drugs. Our laboratory and others have reported that typical as well as atypical antipsychotics are partial agonists on the glutamatergic NMDA receptors (Banerjee et al., 1995; Lidsky et al., 1997; Arvanov et al., 1997). Subsequently APD may activate glutamatergic transmission by two mechanisms, namely, partial agonistic activity and establishment of DB. Although we do not know if chronic cocaine treatment would induce DB of mesolimbic dopamine neurons, our results show

that DB in the nigrostriatal dopamine system may occur following repeated cocaine exposure. However, cocaine has not been reported to be a partial agonist on the NMDA receptors and it is neither an effective antipsychotic nor can it induce EPS, therefore cocaine is ineffective in the treatment of psychosis. Nevertheless, cocaine-induced DB may play an important role in drug craving. In summary, this dissertation has shown the following:

1. That the psychotic actions of PCP and ketamine are mediated entirely through blockade of NMDA receptors with no direct effects on DA D_2 receptors;
2. The plausible neurochemical mechanisms for DA cell DB and catalepsy;
3. The similarities in neurochemical mechanisms between DA cell DB and catalepsy; and
4. That cocaine-induced DA cell DB may contribute to cocaine craving.

Appendix. Cocaine challenge enhances release of neuroprotective amino acid taurine in the striatum of chronic cocaine treated rats: a microdialysis study

Yablonsky-Alter, E., **Agovic, M**., Gashi, E., Lidsky, T., Friedman, E., Banerjee, S. (2009) Brain Res. Bull. (In press).

Abstract

Drug addiction is a serious public health problem. There is increasing evidence on the involvement of augmented glutamatergic transmission in cocaine-induced addiction and neurotoxicity. We investigated effects of acute or chronic cocaine administration and cocaine challenge following chronic cocaine exposure on the release of excitotoxic glutamate and neuroprotective taurine in the rat striatum by microdialysis. Cocaine challenge, following withdrawal after repeated cocaine exposure markedly increased the release of glutamate, which may cause neurotoxicity. Simultaneously, cocaine challenge after withdrawal also significantly increased the release of taurine, which counteracts glutamate-mediated excitotoxicity and possibly cell death. Thus, the mammalian brain has an endogenous self-protective mechanism against cocaine-mediated neurotoxicity and potentially addiction.

1. Introduction

Cocaine inhibits high-affinity neurotransmitter uptake at the presynaptic nerve terminals to increase synaptic levels of dopamine, norepinephrine and serotonin [1]. This increase of synaptic dopamine may cause neurotoxicity [2,3]. At least two different mechanisms have been proposed for the development of dopamine-related neurotoxicity:

1) dopamine produces a free radical that may induce cell toxicity [2, 3] and 2) dopamine reduces glutamate transport at its presynaptic sites to increase synaptic levels of this amino acid [4] and augments glutamate transmission by activating dopamine D_1 receptors in different areas of the brain [5, 6, 7]. Increase in glutamatergic transmission mediated by the activation of N-methyl dextro-aspartate (NMDA) receptors has been shown to cause excitotoxicity and neuro-degeneration [8]. Others and we have reported protection against different psychotropic drug-induced neurotoxicity that may be achieved by prior or simultaneous administration of various pharmacological agents. For example, repeated treatment of rats with haloperidol induced neuronal damage that is ameliorated by prior administration of either G_{M1} ganglioside [9] or the endogenous amino acid, taurine [10]. Similarly, chronic gestational cocaine exposure causes neurotoxicity that could be prevented by co-administration of clozapine [11]. To our knowledge, there is no information if chronic cocaine would enhance release of the endogenous protective agent taurine that may oppose the over activation of glutamatergic system. Here we show that cocaine challenge following repeated cocaine treatment increased synaptic levels of the neuroprotective amino acid taurine that oppose the excessive excitatory actions of the glutamatergic system in the rat brain [10, 12]. Thus, mammalian brain has an auto-protective mechanism to counter excitotoxicity and taurine or its synthetic derivative may be useful in the management and treatment of cocaine addiction and its neurotoxic effect.

2. Materials and Methods

The extracellular levels of amino acids were measured by microdialysis followed by high-pressure-liquid-chromatography (HPLC) in striata of male CD rats after acute

and chronic administrations of cocaine. Rats were anesthetized with 60 mg/kg intraperitoneal pentobarbital and a microdialysis guide cannula was surgically implanted in the striatum using these coordinates: AP, +0.7 mm; ML, +2 .7 mm; DV -6.0 mm [13]. Use of pentobarbital as an anesthetic agent has been shown to decrease dopamine release in striatum and nucleus acumbens [14, 15]. Studies in our laboratory have shown that cortico-striatal glutamatergic neuronal firing rates are similar with either pentobarbital or urethane anesthesia [16]. Furthermore, the synaptic level of glutamate in the striatum under pentobarbital anesthesia was found to be similar as compared to the levels seen in the nucleus acumbens of un-anesthetized rats (Fig. 1) and [17]. Microdialysis probes (CMA /12; Bioanalytical Systems, West Lafayette, IN) were inserted into the guide cannulae and the probes were continuously perfused with artificial cerebrospinal fluid at a flow rate of 2 μl/min, and samples were collected every 10 min via a refrigerated fraction collector. Samples were analyzed using HPLC with electrochemical detection. Twenty-four CD rats were divided into four groups consisting of a control group, acute cocaine treated group, chronic cocaine treated group, and chronic cocaine group challenged with a single dose of cocaine. Acute cocaine treated rats received 10 mg/kg of intraperitoneal cocaine and the control group received equal volume of saline 30 min before microdialysis sample collection. Chronic cocaine treated group received 10 mg/kg of intraperitoneal cocaine six days each week for three weeks and were anesthetized for microdialysis 24 hours after the last dose of cocaine. In the cocaine challenge group, previously chronic cocaine treated rats (24 hours after the last dose of chronic cocaine treatment) received a single dose of 10 mg/kg intraperitoneal cocaine 30 min before microdialysis sample collection.

Measurement of amino acids by HPLC: A derivatation solution was prepared by dissolving 27mg o-phthal-adehyde (OPA) in 1ml methanol (MeOH) to which 5µl beta-mercapto-ethanol and 9ml of 0.1M Na-tetraborate was added. Two mobile phases were used (pH 5.1). Mobile phase A: 20% MeOH and 80% 1M di-sodium hydrogen phosphate. Mobile phase B: 80% MeOH and 20% 1M di-sodium hydrogen phosphate. Gradient solution was: (1) linear addition of B to A over 4 min for a final ration 25% A to 75%B (v/v); (2) step-wise change to 100% mobile phase A for 8 min. The OPA solution (10 µl) was mixed and reacted for 2 min with 20 µl standards or samples and 20 µl of the solution was injected onto a column (ODS, 3x100 mm). Samples were quantified with three external standards (1, 10, and 100 pmol). Goodness of fit for all standards was 0.97-0.99. Typical retention times were: Glu 3.2 min; Gln 3.8 min; Gly 5.9 min; Tau 9.4 min. The lower limit of sensitivity was at least 1 pmol/sample.

All animal procedures were in compliance with the Animal Welfare Act, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the City University of New York (CUNY) institutional guidelines and were approved by the CCNY Animal Care and Use Committee.

3. Results

The effect of acute cocaine treatment on extracellular levels of glutamate and taurine are shown in Figure 1. Although, there were small decreases in extracellular striatal efflux of glutamate and taurine in the acute cocaine treated rats compared to control group, these differences did not reach statistical significance. This observation regarding extracellular concentrations of glutamate following acute cocaine

administration is in agreement with Miguenes et al. who reported similar results in the nucleus accumbens after 1 mg/kg intravenous cocaine treatment [17]. Other investigators found higher concentrations of extracellular glutamate in the nucleus accumbens after a relatively high dose of 30 mg/kg intraperitoneal cocaine treatment [18]. Previously, a dose of 15 mg/kg of cocaine was shown to release somatodendritic dopamine that stimulated dopamine D_1 receptors that in turn increased release of glutamate in the ventral tegmental area [19]. In contrast, it appears that lower doses of cocaine do not increase the levels of dopamine to a sufficient degree to activate release of glutamate to the synaptic cleft in the striatum (Fig. 1).

Our microdialysis experiments show that basal extracellular release of striatal glutamate in rats receiving chronic cocaine decreased significantly compared to the control group (Fig. 2). Interestingly, in the chronic cocaine treated group, the extracellular efflux of taurine increased by about 38% compared to the control group; but this difference did not reach statistical significance (Fig. 2). The mechanism for the reduction of extracellular glutamate following repeated cocaine administration may be due to a decrease in the activity of cysteine/glutamate exchange that was reported in the nucleus accumbens following long-term neuroadaptation induced by chronic cocaine exposure [20].

When our chronically cocaine treated group was challenged by an intraperitoneal administration of 10 mg/kg cocaine, a significant increase in extracellular concentrations of both glutamate and taurine were noted (Fig. 3). There are two possible mechanisms that may be involved in the dramatic increase of the extracellular release of glutamate after cocaine challenge following chronic cocaine treatment. First, repeated cocaine

exposure may increase the number of dopamine D_1 receptors that could enhance glutamate release [19]. Such observation would suggest that chronic cocaine might, at least in part, cause neurotoxicity mediated by the release of glutamate. Second, repeated cocaine administration may desensitize group II metabotropic glutamate receptors in several brain areas [21, 22] and subsequently enhance glutamate release following cocaine challenge after chronic cocaine treatment and withdrawal (Fig. 3). The mechanism for the release of taurine following cocaine challenge is not known. Excessive exposure to another neurotoxin (i.e. ammonia) may increase mRNA levels of taurine transporter [29]. This, however, does not explain how cocaine challenge increases extracellular taurine release.

4. Discussion

Sulfur-containing amino acids, such as taurine and homocysteine exhibit opposite effects in neuronal cells. The former is a neuroprotective agent [12] while the latter is an independent risk factor for cognitive dysfunction [30]. There are no previous reports on the release of endogenous taurine or homocysteine in the extracellular fluid of striatum after acute or chronic cocaine treatment. There are some reports on effect of treatment with various drugs on the release or levels of taurine. Acute administration of D-cycloserine has been shown to increase extracellular concentration of D-serine without significant effect on taurine in the medial frontal cortex [31]. Moreover, repeated morphine exposure significantly reduced the endogenous level of hippocampal taurine [32]. Taurine has been shown to be a neuroprotective agent in numerous investigations and several mechanisms for its neuroprotective effects have been proposed. Most of the

studies have suggested that the taurine-induced neuroprotective effect may be mediated by its antagonism of the glutamate-induced excitotoxicity. For example, it has been proposed that taurine may reduce glutamate-induced cell death by augmentation of mitotochondrial function and the regulation of intracellular (cytoplasmic and intramitochondrial) calcium homeostasis [12]. In another study, Chen et al. [33] showed that taurine exerts its neuroprotective effect by a reduction of glutamate-induced elevation of intracellular Ca^{2+} by inhibiting the reverse mode of Na^{+}/ Ca^{2+} exchange in cultured neurons. Also, taurine was reported to inhibit glutamate-mediated calcium influx through L-, P/Q-, N- type voltage gated calcium channels and NMDA receptor calcium channel in whole brain primary neuronal cell cultures obtained from rat embryos [34]. Since taurine was reported to hyperpolarize neurons in the cerebellum [35] and hippocampus [36] by activating chloride channel, it appears likely that taurine may inhibit glutamate-induced neuronal depolarization through its action on opening the chloride channels. Taurine was also shown to decrease D-[^{3}H]aspartate (a non-metabolized analog of glutamate) release from mouse corticostriatal slices by the activation of a chloride channel that is insensitive to regulation by GABA and/or strychnine-sensitive glycine receptors [23]. Moreover, acamprosate (calcium acetylhomotaurine), a synthetic analog of taurine has been reported to reduce NMDA receptor activation either through partial agonistic activity at the spermidine site or through its action at the metabotropic glutamate receptors [24]. Thus, multitude of mechanisms may be involved in the diminution of glutamate-induced excitotoxicity by taurine making it a good neuroprotective agent.

Although, taurine and its synthetic analog acamprosate are known to be neuroprotective agents that are used in the treatment of drug addiction [24, 25, 26] no previous investigation has demonstrated spontaneous release of taurine in the mammalian brain by substances of abuse. We report spontaneous increased release of taurine following cocaine challenge after chronic, but not acute cocaine treatment in striatum (Figs. 1 and 3). The amount of taurine released following acute administration of cocaine was not significantly different from control. This may be related to the dose of the drug or the duration between drug administration and collection of samples for microdialysis. Nevertheless, the results of our study suggest that the mammalian brain has a unique ability to counteract insult to neuronal tissue caused by glutamate in response to chronic exposure to substances of abuse by releasing taurine. Despite a substantial release of taurine following cocaine challenge (Fig. 3), chronic cocaine ingestion may cause neurotoxicity in the mammalian brain. For example pre- and post-natal exposure to cocaine has been reported to induce neurotoxicity [11]. In addition, cocaine addiction has been shown to cause dysregulation of prefrontal cortex-accumbens synaptic glutamate transmission that underlies the high motivation to seek drugs [37]. Clearly, released taurine is not sufficient to completely neutralize glutamate-induced excitotoxicity in all cases. Consequently, excessive intake of substances of abuse would lead to the development of drug addiction despite mammalian brains' effort to mitigate these adverse effects by the release of endogenous taurine. Nonetheless, high pharmacological doses of taurine and/or acamprosate have been found to be of therapeutic value in neuronal protection and in the management of cocaine, alcohol, and morphine addiction [24, 27, 28]. Future studies may determine if prior intake of

pharmacological dosages of taurine or acamprosate would be of preventive value in mitigating neurotoxicity and addictive sequel to exposure to drugs of abuse.

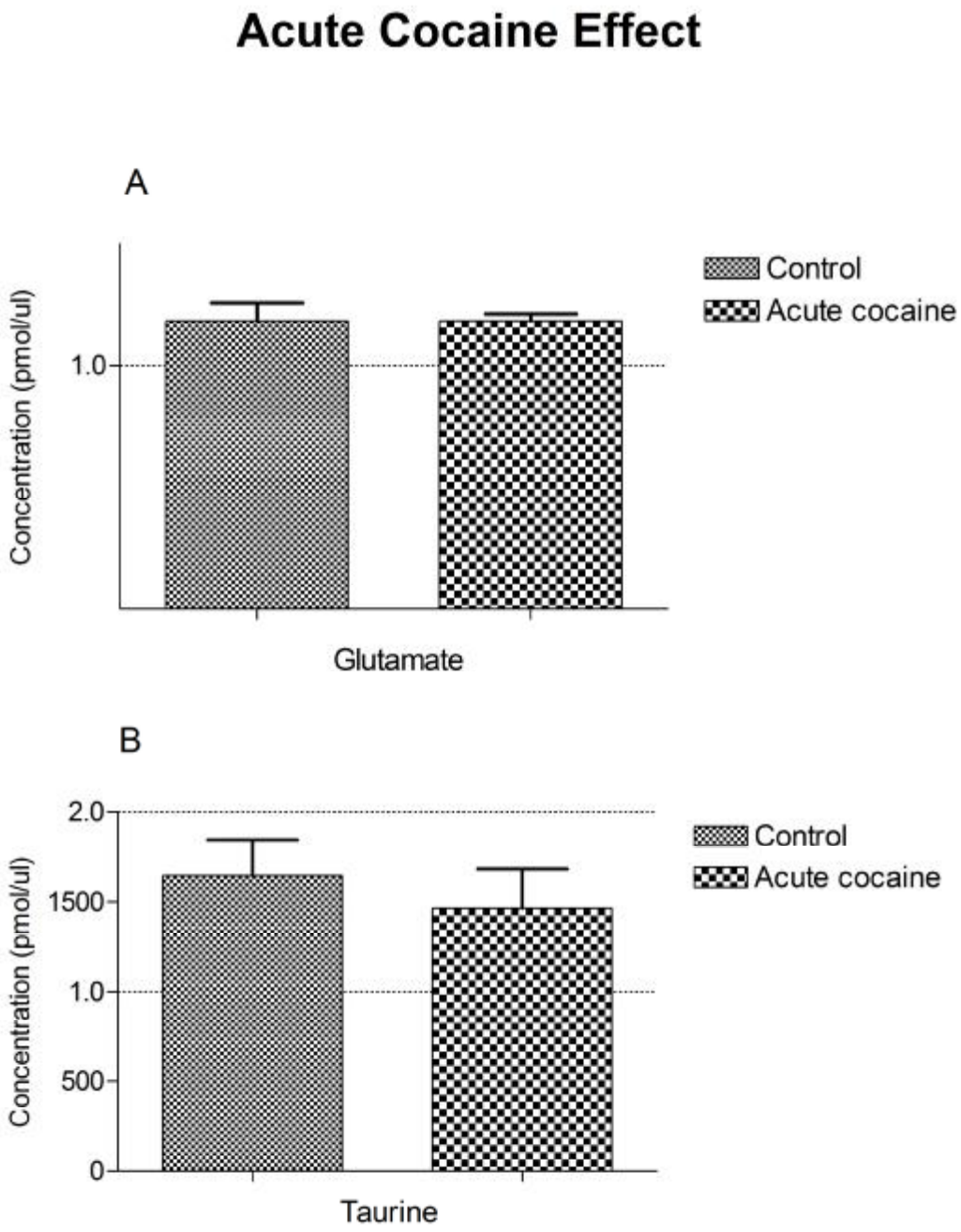


Fig. 1. Extracellular release of glutamate and taurine in striatum after acute cocaine treatment. 30-minutes before the microdialysis assay rats were injected intraperitoneally with 10mg/kg cocaine or an equal volume of saline (controls). There were no significant changes in synaptic levels of glutamate (**A**) or taurine (**B**) by the Two-tailed ***t*-test**: (**A**) t=0.004904 df=7; P>0.05; (**B**) t=0.9954 df=7; P>0.05. Error bars, s.e.m.; n=8 in **A** and **B**.

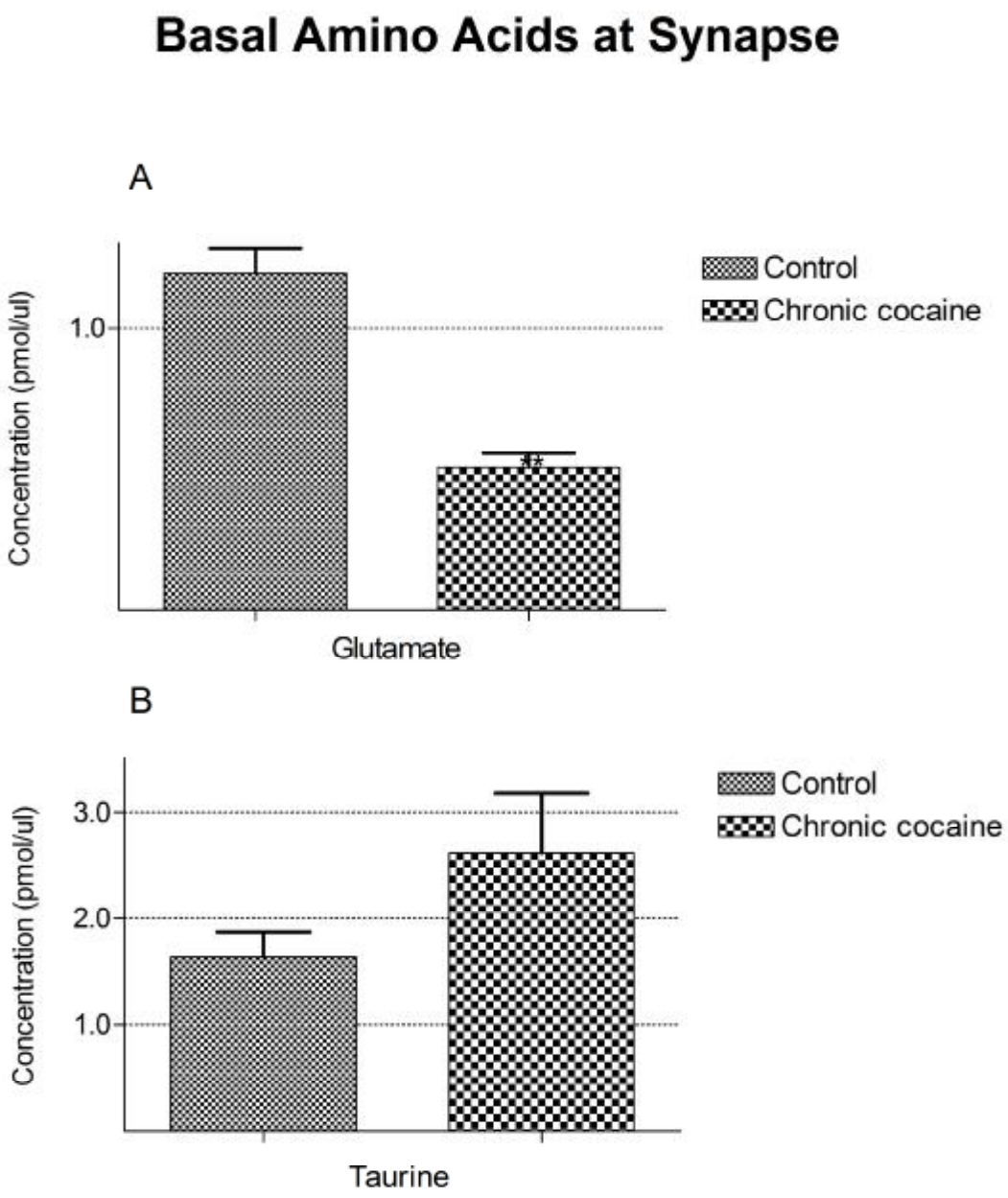


Fig. 2. Extracellular release of glutamate and taurine in striatum after chronic cocaine treatment. Two groups of rats were injected intraperitoneally with 10mg/kg cocaine or equal volume of saline (controls) six-days per week for three-weeks. Microdialysis was initiated 24-hours after last injection of cocaine. (**A**) Basal level of glutamate in the cocaine group was significantly decreased compared to controls (Two-tailed ***t*-test:** t=5.588 df=6; $^{**}P<0.05$). (**B**) Basal taurine levels were increased by 37% compared to controls, however, this change was not statistically significant (Two-tailed ***t*-test:** t=1.370 df=6; $P>0.05$). Error bars, s.e.m.; n=7 in **A** and **B**.

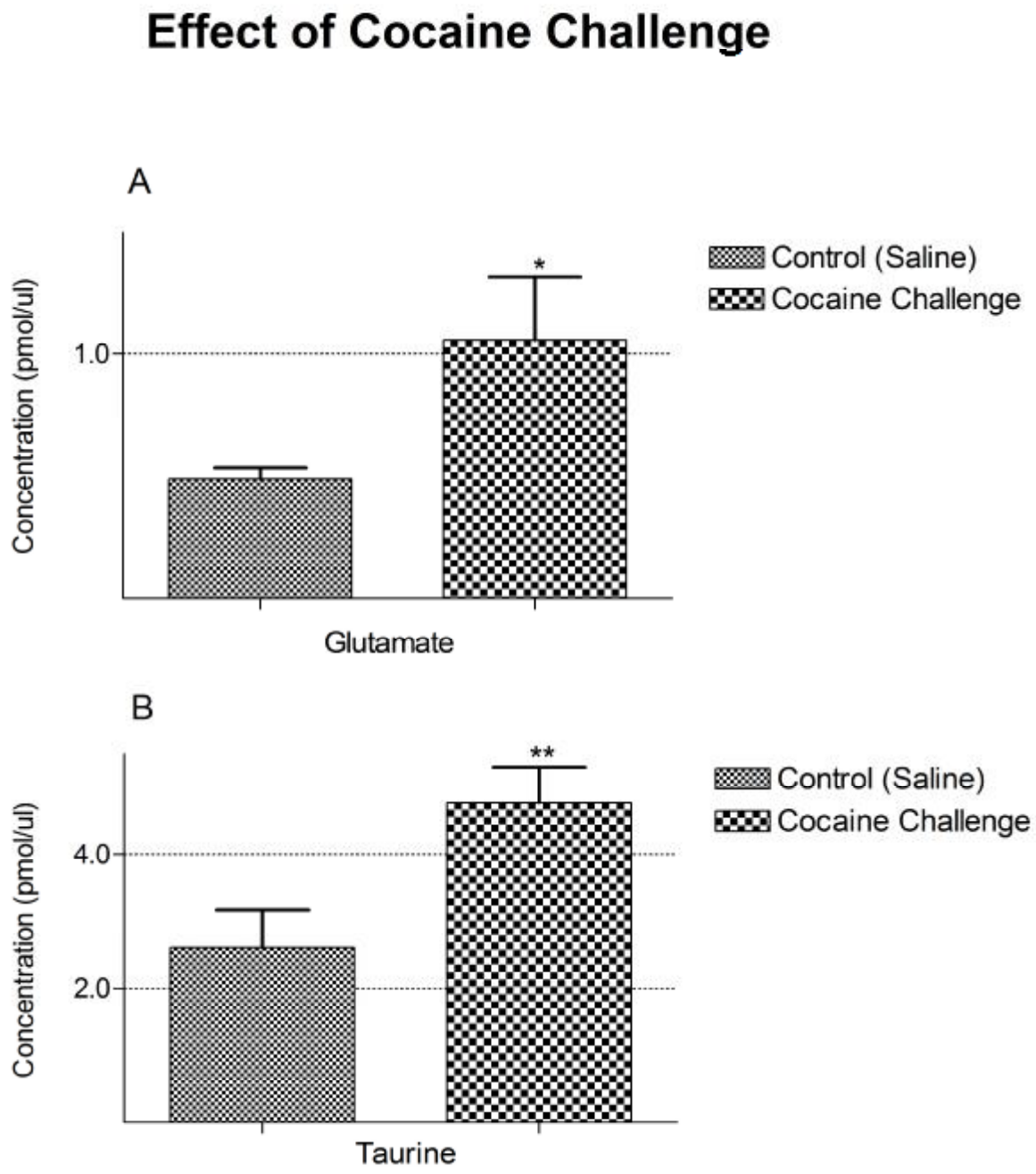


Fig. 3. Extracellular release of glutamate and taurine in striatum of chronic cocaine-treated rats measured after a cocaine "challenge". Rats received 10 mg/kg cocaine (i.p.) for six days per week for three weeks. 24 hours after the last chronic cocaine dose, rats were challenged with 10 mg/kg cocaine (i.p.) or an equal volume of saline (i.p.) 30 minutes before microdialysis. There was a significant increase in both extracellular glutamate (**A**) (Two-tailed ***t*-test:** t=2.456 df=6; *P<0.05) and taurine (**B**) (Two-tailed ***t*-test:** t=4.469 df=6; **P<0.05). Error bars, s.e.m.; n=7 in **A** and **B**.

References for Appendix

1. D.K. Pitts, J. Marwah, 1987. Neuropharmacology of cocaine: role of monoaminergic systems. Monogr. Neural Sci. 13 (1987) 34-54.

2. W. Dauer, S. Przedborski, Parkinson's disease: mechanisms and models. Neuron 39 (2003) 889-909.

3. T. Muller, H. Hefter, R. Hueber, W.H. Jost, K.L. Leenders, P. Odin, J. Schwarz, Is levodopa toxic? J. Neurol. 251 (2004) VI/44-6.

4. L. Kerkerian, N. Dusticier, A. Nieoullon, A., Modulatory effect of dopamine on high-affinity glutamate uptake in the rat striatum. J. Neurochem. 48 (1987) 1301-6.

5. C. Cepeda, M.S. Levine, Dopamine and N-methyl-D-aspartate receptor interactions in the neostriatum. Dev. Neurosci. 20 (1998) 1-18.

6. P.W. Kalivas, P. Duffy, Repeated cocaine administration alters extracellular glutamate in the ventral tegmental area. J. Neurochem. 70 (1998), 1497-502.

7. K.Y. Li, C. Xiao, M. Xiong, E. Delphin, J.H. Ye, Nanomolar propofol stimulates glutamate transmission to dopamine neurons: a possible mechanism of abuse potential? J. Pharmacol. Exp. Ther. 325 (2008) 165-74.

8. J.W. Olney, J. Labruyere, G. Wang, D.F. Wozniak, M.T. Price, M.A. Sesma,

NMDA antagonist neurotoxicity: mechanism and prevention. Science 254 (1991) 1515-1518.

9. T.I. Lidsky, J.S. Schneider, L.G. Zuck, E. Yablonsky-Alter, S.P. Banerjee, S.P., GM1 ganglioside attenuates changes in neurochemistry and behaviour caused by repeated haloperidol administration. Neurodegeneration. 3 (1994) 135-140.

10. T.I. Lidsky, J.S. Schneider, E. Yablonsky-Alter, L.G. Zuck, S.P. Banerjee, Taurine prevents haloperidol-induced changes in striatal neurochemistry and behavior. Brain Res. 686 (1995) 104-106.

11. E. Yablonsky-Alter, E. Gashi, T.I. Lidsky, H.Y. Wang, S.P. Banerjee, Clozapine protection against gestational cocaine-induced neurochemical abnormalities. J. Pharmacol. Exp. Ther. 312 (2005) 297-302.

12. A. El Idrissi, Taurine increases mitochondrial buffering of calcium: role in neuroprotection. Amino Acids. 34 (2008) 321-328.

13. G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, Academic Press, San Diego, 1986.

14. Y.U. Adachi, S. Yamada, M. Satomoto, K. Watanabe, H. Higuchi, T. Kazama, M. Doi, S. Sato, Pentobarbital inhibits L-DOPA-induced dopamine increases in the rat striatum: An in vivo microdialysis study. Brain Res. Bull. 69 (2006) 593-596.

15. M. Masuzawa, S. Nakao, E. Miyamoto, M. Yamada, K. Murao, K. Nishi, K. Shingu, Pentobarbital inhibits ketamine-induced dopamine release in the rat

nucleus accumbens: a microdialysis study. Anesth. Analg. 96 (2003) 148-152, table of contents.

16. T. I. Lidsky, S. P. Banerjee, Acute administration of haloperidol enhances dopaminergic transmission. J. Pharmacol. Exp. Ther. 265 (1993): 1193-8.

17. M. Miguens, N. Del Olmo, A. Higuera-Matas, I. Torres, C. Garcia-Lecumberri, E. Ambrosio, Glutamate and aspartate levels in the nucleus accumbens during cocaine self-administration and extinction: a time course microdialysis study. Psychopharmacology (Berl) 196 (2008) 303-13.

18. M.S. Reid, K. Jr. Hsu, S.P. Berger, Cocaine and amphetamine preferentially stimulate glutamate release in the limbic system: studies on the involvement of dopamine. Synapse 27 (1997) 95-105.

19. P.W. Kalivas, P. Duffy, D1 receptors modulate glutamate transmission in the ventral tegmental area. J. Neurosci. 15 (1995) 5379-5388.

20. D.A. Baker, H. Shen, P.W. Kalivas, Cystine/glutamate exchange serves as the source for extracellular glutamate: modifications by repeated cocaine administration. Amino Acids. 23 (2002) 161-162.

21. C.C. Huang, P.C. Yang, H.J. Lin, K.S. Hsu, Repeated cocaine administration impairs group II metabotropic glutamate receptor-mediated long-term depression in rat medial prefrontal cortex. J. Neurosci. 27 (2007) 2958-2968.

22. Z.X. Xi, S. Ramamoorthy, D.A. Baker, H. Shen, D.J. Samuvel, P.W. Kalivas,

Modulation of group II metabotropic glutamate receptor signaling by chronic cocaine. J. Pharmacol. Exp. Ther. 303 (2002) 608-615.

23. S.M. Molchanova, S.S. Oja, P. Saransaari, Inhibitory effect of taurine on veratridine-evoked D-[3H]aspartate release from murine corticostriatal slices: involvement of chloride channels and mitochondria. Brain Res. 1130 (2007) 95-102.

24. M. Heilig, M. Egli, Pharmacological treatment of alcohol dependence: target symptoms and target mechanisms. Pharmacol. Ther. 111 (2006) 855-76.

25. P.W. Kalivas, Glutamate systems in cocaine addiction. Curr. Opin. Pharmacol. 4 (2004) 23-9.

26. C.C. Lapish, J.K. Seamans, L. Judson Chandler, Glutamate-dopamine cotransmission and reward processing in addiction. Alcohol Clin. Exp. Res. 30 (2006) 1451-1465.

27. A.J. McGeehan, M.F. Olive, Attenuation of cocaine-induced reinstatement of cocaine conditioned place preference by acamprosate. Behav. Pharmacol. 17 (2006) 363-367.

28. J. Sepulveda, A. Ortega, G. Zapata, E. Contreras, Acamprosate decreases the induction of tolerance and physical dependence in morphine-treated mice. Eur. J. Pharmacol. 445 (2002) 87-91.

29. M. Belanger, T. Asashima, S. Ohtsuki, H. Yamaguchi, S. Ito, T. Terasaki,

Hyperammonemia induces transport of taurine and creatine and suppresses claudin-12 gene expression in brain capillary endothelial cells in vitro. Neurochem. Int. 50 (2007) 95-101.

30. Y. Jin, L. Brennan, Effects of homocysteine on metabolic pathways in cultured astrocytes. Neurochem. Int. 52 (2008) 1410-1415.

31. T. Fujihira, S. Kanematsu, A. Umino, N. Yamamoto, T. Nishikawa, Selective increase in the extracellular D-serine contents by D-cycloserine in the rat medial frontal cortex. Neurochem. Int. 51(2007) 233-236.

32. H. Gao, Y. Xiang, N. Sun, H. Zhu, Y. Wang, M. Liu, Y. Ma, H. Lei, Metabolic changes in rat prefrontal cortex and hippocampus induced by chronic morphine treatment studied ex vivo by high resolution 1H NMR spectroscopy. Neurochem. Int. 50 (2007) 386-394.

33. W.Q. Chen, H. Jin, M. Nguyen, J. Carr, Y.J. Lee, C.C. Hsu, M.D. Faiman, J.V. Schloss, J.Y. Wu, Role of taurine in regulation of intracellular calcium level and neuroprotective function in cultured neurons. J. Neurosci. Res. 66 (2001) 612-619.

34. H. Wu, Y. Jin, J. Wei, H. Jin, D. Sha, J.Y. Wu, Mode of action of taurine as a neuroprotector. Brain Res. 1038 (2005) 123-131.

35. K. Okamoto, H. Kimura, Y. Sakai, Taurine-induced increase of the Cl- conductance of cerebellar Purkinje cell dendrites in vitro. Brain Res. 259 (1983)

319-323.

36. N. del Olmo, J. Bustamante, R.M. del Rio, J.M. Solis, Taurine activates GABA(A) but not GABA(B) receptors in rat hippocampal CA1 area. Brain Res. 864 (2000) 298-307.

37. P. W. Kalivas, N. Volkow, J. Seamans, Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. Neuron 45 (2005): 647-650.

REFERENCES

Aalto S, Hirvonen J, Kajander J, Scheinin H, Nagren K, Vilkman H, Gustafsson L, Syvalahti E and Hietala J (2002) Ketamine does not decrease striatal dopamine D2 receptor binding in man. *Psychopharmacology (Berl)* **164**:401-406.

Aalto S, Ihalainen J, Hirvonen J, Kajander J, Scheinin H, Tanila H, Nagren K, Vilkman H, Gustafsson LL, Syvalahti E and Hietala J (2005) Cortical glutamate-dopamine interaction and ketamine-induced psychotic symptoms in man. *Psychopharmacology (Berl)* **182**:375-383.

Abraham HD, Aldridge AM and Gogia P (1996) The psychopharmacology of hallucinogens. *Neuropsychopharmacology* **14**:285-298.

Agovic MS, Yablonsky-Alter E, Lidsky TI and Banerjee SP (2008) Mechanisms for metoclopramide-mediated sensitization and haloperidol-induced catalepsy in rats. *Eur J Pharmacol* **587**:181-186.

Albin RL, Young AB and Penney JB (1989) The functional anatomy of basal ganglia disorders. *Trends Neurosci* **12**:366-375.

Andreasen NC (1995) Symptoms, signs, and diagnosis of schizophrenia. *Lancet* **346**:477-481.

Antelman SM, Kocan D, Edwards DJ, Knopf S, Perel JM and Stiller R (1986) Behavioral effects of a single neuroleptic treatment grow with the passage of time. *Brain Res* **385**:58-67.

Arvanov VL, Liang X, Schwartz J, Grossman S and Wang RY (1997) Clozapine and haloperidol modulate N-methyl-D-aspartate- and non-N-methyl-D-aspartate receptor-mediated neurotransmission in rat prefrontal cortical neurons in vitro. *J Pharmacol Exp Ther* **283**:226-234.

Asin KE and Bednarz LM (1990) Behavioral interactions between muscimol and compounds selective for the D1 and D2 dopamine receptor subtypes. *Neurosci Lett* **113**:286-291.

Baldessarini R and Tarazi F (2001) *Drugs and the treatment of psychiatric disorders.* McGraw-Hill Companies, New York.

Balsara JJ, Bapat TR, Gada VP and Chandorkar AG (1982) Small doses of apomorphine induce catalepsy and antagonize methamphetamine stereotypy in rats. *Psychopharmacology (Berl)* **78**:192-194.

Banerjee SP, Zuck LG, Yablonsky-Alter E and Lidsky TI (1995) Glutamate agonist

activity: implications for antipsychotic drug action and schizophrenia. *Neuroreport* **6**:2500-2504.

Barnes DE, Robinson B, Csernansky JG and Bellows EP (1990) Sensitization versus tolerance to haloperidol-induced catalepsy: multiple determinants. *Pharmacol Biochem Behav* **36**:883-887.

Boudreau AC, Reimers JM, Milovanovic M and Wolf ME (2007) Cell surface AMPA receptors in the rat nucleus accumbens increase during cocaine withdrawal but internalize after cocaine challenge in association with altered activation of mitogen-activated protein kinases. *J Neurosci* **27**:10621-10635.

Boudreau AC and Wolf ME (2005) Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. *J Neurosci* **25**:9144-9151.

Boulay D, Depoortere R, Oblin A, Sanger DJ, Schoemaker H and Perrault G (2000) Haloperidol-induced catalepsy is absent in dopamine D(2), but maintained in dopamine D(3) receptor knock-out mice. *Eur J Pharmacol* **391**:63-73.

Boye SM and Rompre PP (2000) Behavioral evidence of depolarization block of dopamine neurons after chronic treatment with haloperidol and clozapine. *J Neurosci* **20**:1229-1239.

Boyson SJ, McGonigle P and Molinoff PB (1986) Quantitative autoradiographic localization of the D1 and D2 subtypes of dopamine receptors in rat brain. *J Neurosci* **6**:3177-3188.

Bunney BS and Grace AA (1978) Acute and chronic haloperidol treatment: comparison of effects on nigral dopaminergic cell activity. *Life Sci* **23**:1715-1727.

Burke RE, Fahn S, Mayeux R, Weinberg H, Louis K and Willner JH (1981) Neuroleptic malignant syndrome caused by dopamine-depleting drugs in a patient with Huntington disease. *Neurology* **31**:1022-1025.

Camps M, Kelly PH and Palacios JM (1990) Autoradiographic localization of dopamine D 1 and D 2 receptors in the brain of several mammalian species. *J Neural Transm Gen Sect* **80**:105-127.

Carlsson A (1988) The current status of the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology* **1**:179-186.

Carlsson A and Lindqvist M (1963) Effect of Chlorpromazine or Haloperidol on Formation of 3methoxytyramine and Normetanephrine in Mouse Brain. *Acta Pharmacol Toxicol (Copenh)* **20**:140-144.

Carlsson A, Waters N and Carlsson ML (1999) Neurotransmitter interactions in schizophrenia--therapeutic implications. *Biol Psychiatry* **46**:1388-1395.

Carlsson M and Carlsson A (1990) Interactions between glutamatergic and monoaminergic systems within the basal ganglia--implications for schizophrenia and Parkinson's disease. *Trends Neurosci* **13**:272-276.

Carpenter WT, Jr. and Buchanan RW (1994) Schizophrenia. *N Engl J Med* **330**:681-690.

Cassey DE (1995) *Tardive dyskinesia*. Raven Press, New York.

Cepeda C and Levine MS (1998) Dopamine and N-methyl-D-aspartate receptor interactions in the neostriatum. *Dev Neurosci* **20**:1-18.

Chinen CC and Frussa-Filho R (1999) Conditioning to injection procedures and repeated testing increase SCH 23390-induced catalepsy in mice. *Neuropsychopharmacology* **21**:670-678.

Chiodo LA and Bunney BS (1983) Typical and atypical neuroleptics: differential effects of chronic administration on the activity of A9 and A10 midbrain dopaminergic neurons. *J Neurosci* **3**:1607-1619.

Choi DW (1988) Glutamate neurotoxicity and diseases of the nervous system. *Neuron* **1**:623-634.

Cooper J, Bloom F and Toth R (2003) *The Biochemical Basis of Neuropharmacology*. Oxford University Press, Inc., New York.

Couper F and Barry L (2004) Ketamine, in *Drugs and Human Performance Fact Sheets*.

Court J, Spurden D, Lloyd S, McKeith I, Ballard C, Cairns N, Kerwin R, Perry R and Perry E (1999) Neuronal nicotinic receptors in dementia with Lewy bodies and schizophrenia: alpha-bungarotoxin and nicotine binding in the thalamus. *J Neurochem* **73**:1590-1597.

Coyle JT (1996) The glutamatergic dysfunction hypothesis for schizophrenia. *Harv Rev Psychiatry* **3**:241-253.

Creese I, Burt DR and Snyder SH (1976) Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* **192**:481-483.

Creese I, Schneider R and Snyder SH (1977) 3H-Spiroperidol labels dopamine receptors in pituitary and brain. *Eur J Pharmacol* **46**:377-381.

Davis KL, Kahn RS, Ko G and Davidson M (1991) Dopamine in schizophrenia: a review and reconceptualization. *Am J Psychiatry* **148**:1474-1486.

Delay J, Deniker P and Harl JM (1952) [Therapeutic use in psychiatry of phenothiazine of central elective action (4560 RP).]. *Ann Med Psychol (Paris)* **110**:112-117.

Ebadi M and Srinivasan SK (1995) Pathogenesis, prevention, and treatment of neuroleptic-induced movement disorders. *Pharmacol Rev* **47**:575-604.

Ellenbroek B, Schwarz M, Sontag KH, Jaspers R and Cools A (1985) Muscular rigidity and delineation of a dopamine-specific neostriatal subregion: tonic EMG activity in rats. *Brain Res* **345**:132-140.

Elliott PJ, Close SP, Walsh DM, Hayes AG and Marriott AS (1990) Neuroleptic-induced catalepsy as a model of Parkinson's disease. II. Effect of glutamate antagonists. *J Neural Transm Park Dis Dement Sect* **2**:91-100.

Ezrin-Waters C and Seeman P (1977) Tolerance of haloperidol catalepsy. *Eur J Pharmacol* **41**:321-327.

Francis PT (2003) Glutamatergic systems in Alzheimer's disease. *Int J Geriatr Psychiatry* **18**:S15-21.

Francis PT, Sims NR, Procter AW and Bowen DM (1993) Cortical pyramidal neurone loss may cause glutamatergic hypoactivity and cognitive impairment in Alzheimer's disease: investigative and therapeutic perspectives. *J Neurochem* **60**:1589-1604.

Freedman R, Hall M, Adler LE and Leonard S (1995) Evidence in postmortem brain tissue for decreased numbers of hippocampal nicotinic receptors in schizophrenia. *Biol Psychiatry* **38**:22-33.

Giuffra ME, Sethy VH, Davis TL, Mouradian MM and Chase TN (1993) Milacemide therapy for Parkinson's disease. *Mov Disord* **8**:47-50.

Goeders NE and Kuhar MJ (1987) Chronic cocaine administration induces opposite changes in dopamine receptors in the striatum and nucleus accumbens. *Alcohol Drug Res* **7**:207-216.

Goldman-Rakic PS and Selemon LD (1997) Functional and anatomical aspects of prefrontal pathology in schizophrenia. *Schizophr Bull* **23**:437-458.

Gonzalez-Maeso J, Ang RL, Yuen T, Chan P, Weisstaub NV, Lopez-Gimenez JF, Zhou M, Okawa Y, Callado LF, Milligan G, Gingrich JA, Filizola M, Meana JJ and Sealfon SC (2008) Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature* **452**:93-97.

Grace AA and Bunney BS (1986) Induction of depolarization block in midbrain dopamine neurons by repeated administration of haloperidol: analysis using in

vivo intracellular recording. *J Pharmacol Exp Ther* **238**:1092-1100.

Grace AA, Bunney BS, Moore H and Todd CL (1997) Dopamine-cell depolarization block as a model for the therapeutic actions of antipsychotic drugs. *Trends Neurosci* **20**:31-37.

Graybiel AM (1990) Neurotransmitters and neuromodulators in the basal ganglia. *Trends Neurosci* **13**:244-254.

Harrison PJ and Weinberger DR (2005) Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. *Mol Psychiatry* **10**:40-68; image 45.

Hassan MN, Reches A, Kuhn C, Higgins D and Fahn S (1986) Pharmacologic evaluation of dopaminergic receptor blockade by metoclopramide. *Clin Neuropharmacol* **9**:71-78.

Henderson VW and Wooten GF (1981) Neuroleptic malignant syndrome: a pathogenetic role for dopamine receptor blockade? *Neurology* **31**:132-137.

Imamura Y, Harada S, Okano Y, Miyata T and Otagiri M (1988) The effect of metoclopramide on the absorption and pharmacology of chlorpromazine in the rat. *J Pharm Pharmacol* **40**:116-119.

Itzhak Y and Stein I (1992) Sensitization to the toxic effects of cocaine in mice is associated with the regulation of N-methyl-D-aspartate receptors in the cortex. *J Pharmacol Exp Ther* **262**:464-470.

Jaber M, Robinson SW, Missale C and Caron MG (1996) Dopamine receptors and brain function. *Neuropharmacology* **35**:1503-1519.

Jablensky A (1997) The 100-year epidemiology of schizophrenia. *Schizophr Res* **28**:111-125.

Jankovic J (1995) Tardive syndromes and other drug-induced movement disorders. *Clin Neuropharmacol* **18**:197-214.

Javitt DC (1987) Negative schizophrenic symptomatology and the PCP (phencyclidine) model of schizophrenia. *Hillside J Clin Psychiatry* **9**:12-35.

Javitt DC (2002) Glycine modulators in schizophrenia. *Curr Opin Investig Drugs* **3**:1067-1072.

Javitt DC and Zukin SR (1991) Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry* **148**:1301-1308.

Javitt DC, Zylberman I, Zukin SR, Heresco-Levy U and Lindenmayer JP (1994) Amelioration of negative symptoms in schizophrenia by glycine. *Am J Psychiatry* **151**:1234-1236.

Jenner P, Rupniak NM and Marsden CD (1985) Differential alteration of striatal D-1 and D-2 receptors induced by the long-term administration of haloperidol, sulpiride or clozapine to rats. *Psychopharmacology Suppl* **2**:174-181.

Joel D and Weiner I (2000) The connections of the dopaminergic system with the striatum in rats and primates: an analysis with respect to the functional and compartmental organization of the striatum. *Neuroscience* **96**:451-474.

Johnstone EC, Crow TJ, Frith CD, Carney MW and Price JS (1978) Mechanism of the antipsychotic effect in the treatment of acute schizophrenia. *Lancet* **1**:848-851.

Jordan S, Chen R, Fernalld R, Johnson J, Regardie K, Kambayashi J, Tadori Y, Kitagawa H and Kikuchi T (2006) In vitro biochemical evidence that the psychotomimetics phencyclidine, ketamine and dizocilpine (MK-801) are inactive at cloned human and rat dopamine D2 receptors. *Eur J Pharmacol* **540**:53-56.

Jung BJ, Dawson R, Jr., Sealey SA and Peris J (1999) Endogenous GABA release is reduced in the striatum of cocaine-sensitized rats. *Synapse* **34**:103-110.

Kalivas PW (1995) Interactions between dopamine and excitatory amino acids in behavioral sensitization to psychostimulants. *Drug Alcohol Depend* **37**:95-100.

Kalivas PW and Duffy P (1993) Time course of extracellular dopamine and behavioral sensitization to cocaine. I. Dopamine axon terminals. *J Neurosci* **13**:266-275.

Kalivas PW and Duffy P (1995) D1 receptors modulate glutamate transmission in the ventral tegmental area. *J Neurosci* **15**:5379-5388.

Kandel E, Shwartz J and Jessell T (1991) *Principles of neuroscience*. McGraw-Hill Companies, Inc., New York.

Kapur S and Mamo D (2003) Half a century of antipsychotics and still a central role for dopamine D2 receptors. *Prog Neuropsychopharmacol Biol Psychiatry* **27**:1081-1090.

Kapur S and Seeman P (2002) NMDA receptor antagonists ketamine and PCP have direct effects on the dopamine D(2) and serotonin 5-HT(2)receptors-implications for models of schizophrenia. *Mol Psychiatry* **7**:837-844.

Kauer JA and Malenka RC (2007) Synaptic plasticity and addiction. *Nat Rev Neurosci* **8**:844-858.

Kaur S, Ozer H and Starr M (1997) MK 801 reverses haloperidol-induced catalepsy from both striatal and extrastriatal sites in the rat brain. *Eur J Pharmacol* **332**:153-160.

Kebabian JW and Cote TE (1981) Dopaminergic receptors and cyclic AMP: A decade of progress. *Trends Pharmacol. Sci.* **2**:69-71.

Kegeles LS, Abi-Dargham A, Zea-Ponce Y, Rodenhiser-Hill J, Mann JJ, Van Heertum RL, Cooper TB, Carlsson A and Laruelle M (2000) Modulation of amphetamine-induced striatal dopamine release by ketamine in humans: implications for schizophrenia. *Biol Psychiatry* **48**:627-640.

Kegeles LS, Martinez D, Kochan LD, Hwang DR, Huang Y, Mawlawi O, Suckow RF, Van Heertum RL and Laruelle M (2002) NMDA antagonist effects on striatal dopamine release: positron emission tomography studies in humans. *Synapse* **43**:19-29.

King GR, Kuhn C and Ellinwood EH, Jr. (1993) Dopamine efflux during withdrawal from continuous or intermittent cocaine. *Psychopharmacology (Berl)* **111**:179-184.

Klamer D, Zhang J, Engel JA and Svensson L (2005) Selective interaction of nitric oxide synthase inhibition with phencyclidine: behavioural and NMDA receptor binding studies in the rat. *Behav Brain Res* **159**:95-103.

Kleven MS, Perry BD, Woolverton WL and Seiden LS (1990) Effects of repeated injections of cocaine on D1 and D2 dopamine receptors in rat brain. *Brain Res* **532**:265-270.

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MB, Jr. and Charney DS (1994) Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry* **51**:199-214.

Lahti AC, Koffel B, LaPorte D and Tamminga CA (1995) Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. *Neuropsychopharmacology* **13**:9-19.

Lappalainen J, Hietala J, Koulu M, Seppala T, Sjoholm B and Syvalahti E (1990) Chronic treatment with SCH 23390 and haloperidol: effects on dopaminergic and serotonergic mechanisms in rat brain. *J Pharmacol Exp Ther* **252**:845-852.

Levesque D, Diaz J, Pilon C, Martres MP, Giros B, Souil E, Schott D, Morgat JL, Schwartz JC and Sokoloff P (1992) Identification, characterization, and localization of the dopamine D3 receptor in rat brain using 7-[3H]hydroxy-N,N-di-n-propyl-2-aminotetralin. *Proc Natl Acad Sci U S A* **89**:8155-8159.

Lewis DA (1995) Neural circuitry of the prefrontal cortex in schizophrenia. *Arch Gen Psychiatry* **52**:269-273; discussion 277-268.

Lewis DA (2000) GABAergic local circuit neurons and prefrontal cortical dysfunction in schizophrenia. *Brain Res Brain Res Rev* **31**:270-276.

Lewis DA and Moghaddam B (2006) Cognitive dysfunction in schizophrenia: convergence of gamma-aminobutyric acid and glutamate alterations. *Arch Neurol* **63**:1372-1376.

Lewis DA, Pierri JN, Volk DW, Melchitzky DS and Woo TU (1999) Altered GABA neurotransmission and prefrontal cortical dysfunction in schizophrenia. *Biol Psychiatry* **46**:616-626.

Lidsky TI (1997) Neuropsychiatric implications of basal ganglia dysfunction. *Biol Psychiatry* **41**:383-385.

Lidsky TI and Banerjee SP (1993) Acute administration of haloperidol enhances dopaminergic transmission. *J Pharmacol Exp Ther* **265**:1193-1198.

Lidsky TI and Banerjee SP (1996) Contribution of Glutamatergic Dysfunction to Schizophrenia. *Drug News Perspectives* **9**:453-459.

Lidsky TI, Schneider JS, Yablonsky-Alter E, Zuck LG and Banerjee SP (1995) Taurine prevents haloperidol-induced changes in striatal neurochemistry and behavior. *Brain Res* **686**:104-106.

Lidsky TI, Schneider JS, Zuck LG, Yablonski-Alter E and Banerjee SP (1994) GM1 ganglioside attenuates changes in neurochemistry and behaviour caused by repeated haloperidol administration. *Neurodegeneration* **3**:135-140.

Lidsky TI, Yablonsky-Alter E, Zuck LG and Banerjee SP (1997) Antipsychotic drug effects on glutamatergic activity. *Brain Res* **764**:46-52.

Lieberman JA, Tollefson GD, Charles C, Zipursky R, Sharma T, Kahn RS, Keefe RS, Green AI, Gur RE, McEvoy J, Perkins D, Hamer RM, Gu H and Tohen M (2005) Antipsychotic drug effects on brain morphology in first-episode psychosis. *Arch Gen Psychiatry* **62**:361-370.

Liu QS, Pu L and Poo MM (2005) Repeated cocaine exposure in vivo facilitates LTP induction in midbrain dopamine neurons. *Nature* **437**:1027-1031.

Luby ED, Cohen BD, Rosenbaum G, Gottlieb JS and Kelley R (1959) Study of a new schizophrenomimetic drug; sernyl. *AMA Arch Neurol Psychiatry* **81**:363-369.

Maguire G and Werblin F (1994) Dopamine enhances a glutamate-gated ionic current in OFF bipolar cells of the tiger salamander retina. *J Neurosci* **14**:6094-6101.

Marutle A, Zhang X, Court J, Piggott M, Johnson M, Perry R, Perry E and Nordberg A (2001) Laminar distribution of nicotinic receptor subtypes in cortical regions in schizophrenia. *J Chem Neuroanat* **22**:115-126.

McDougle CJ, Black JE, Malison RT, Zimmermann RC, Kosten TR, Heninger GR and Price LH (1994) Noradrenergic dysregulation during discontinuation of cocaine use in addicts. *Arch Gen Psychiatry* **51**:713-719.

Meador-Woodruff JH, Mansour A, Grandy DK, Damask SP, Civelli O and Watson SJ, Jr. (1992) Distribution of D5 dopamine receptor mRNA in rat brain. *Neurosci Lett* **145**:209-212.

Meller E, Kuga S, Friedhoff AJ and Goldstein M (1985) Selective D2 dopamine receptor agonists prevent catalepsy induced by SCH 23390, a selective D1 antagonist. *Life Sci* **36**:1857-1864.

Meltzer HY (2002) Suicidality in schizophrenia: a review of the evidence for risk factors and treatment options. *Curr Psychiatry Rep* **4**:279-283.

Moore H, Todd CL and Grace AA (1998) Striatal extracellular dopamine levels in rats with haloperidol-induced depolarization block of substantia nigra dopamine neurons. *J Neurosci* **18**:5068-5077.

Moore H, West AR and Grace AA (1999) The regulation of forebrain dopamine transmission: relevance to the pathophysiology and psychopathology of schizophrenia. *Biol Psychiatry* **46**:40-55.

Moore NA, Blackman A, Awere S and Leander JD (1993) NMDA receptor antagonists inhibit catalepsy induced by either dopamine D1 or D2 receptor antagonists. *Eur J Pharmacol* **237**:1-7.

Morelli M and Di Chiara G (1985) Catalepsy induced by SCH 23390 in rats. *Eur J Pharmacol* **117**:179-185.

Murugaiah K, Fleminger S, Hall MD, Theodorou A, Jenner P and Marsden CD (1984) Alterations in different populations of striatal dopamine receptors produced by 18 months continuous administration of cis- or trans-flupenthixol to rats. *Neuropharmacology* **23**:599-609.

Neisewander JL, Lucki I and McGonigle P (1994) Time-dependent changes in sensitivity to apomorphine and monoamine receptors following withdrawal from continuous cocaine administration in rats. *Synapse* **16**:1-10.

Nowak L, Bregestovski P, Ascher P, Herbet A and Prochiantz A (1984) Magnesium gates

glutamate-activated channels in mouse central neurones. *Nature* **307**:462-465.

Ogren SO and Fuxe K (1988) D1- and D2-receptor antagonists induce catalepsy via different efferent striatal pathways [corrected]. *Neurosci Lett* **85**:333-338.

Olney JW and Farber NB (1995) Glutamate receptor dysfunction and schizophrenia. *Arch Gen Psychiatry* **52**:998-1007.

Olney JW and Sharpe LG (1969) Brain lesions in an infant rhesus monkey treated with monsodium glutamate. *Science* **166**:386-388.

Peris J, Boyson SJ, Cass WA, Curella P, Dwoskin LP, Larson G, Lin LH, Yasuda RP and Zahniser NR (1990) Persistence of neurochemical changes in dopamine systems after repeated cocaine administration. *J Pharmacol Exp Ther* **253**:38-44.

Petralia RS and Wenthold RJ (1992) Light and electron immunocytochemical localization of AMPA-selective glutamate receptors in the rat brain. *J Comp Neurol* **318**:329-354.

Pierce RC, Bell K, Duffy P and Kalivas PW (1996) Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. *J Neurosci* **16**:1550-1560.

Pilotte NS, Sharpe LG and Kuhar MJ (1994) Withdrawal of repeated intravenous infusions of cocaine persistently reduces binding to dopamine transporters in the nucleus accumbens of Lewis rats. *J Pharmacol Exp Ther* **269**:963-969.

Porceddu ML, Giorgi O, Ongini E, Mele S and Biggio G (1986) 3H-SCH 23390 binding sites in the rat substantia nigra: evidence for a presynaptic localization and innervation by dopamine. *Life Sci* **39**:321-328.

Purves D, Augustine G, Fitzpatrick D and al. E (2004) *Neuroscience*. Sinauer Associates, Inc., Sounderland, MA.

Rao TS, Kim HS, Lehmann J, Martin LL and Wood PL (1989) Differential effects of phencyclidine (PCP) and ketamine on mesocortical and mesostriatal dopamine release in vivo. *Life Sci* **45**:1065-1072.

Rapoport JL, Addington AM, Frangou S and Psych MR (2005) The neurodevelopmental model of schizophrenia: update 2005. *Mol Psychiatry* **10**:434-449.

Reynolds IJ, Murphy SN and Miller RJ (1987) 3H-labeled MK-801 binding to the excitatory amino acid receptor complex from rat brain is enhanced by glycine. *Proc Natl Acad Sci U S A* **84**:7744-7748.

Romanides AJ, Duffy P and Kalivas PW (1999) Glutamatergic and dopaminergic

afferents to the prefrontal cortex regulate spatial working memory in rats. *Neuroscience* **92**:97-106.

Sachdev P (1995) The epidemiology of drug-induced akathisia: Part I. Acute akathisia. *Schizophr Bull* **21**:431-449.

Sachdev P (1995) The epidemiology of drug-induced akathisia: Part II. Chronic, tardive, and withdrawal akathisias. *Schizophr Bull* **21**:451-461.

See RE, Toga AW and Ellison G (1990) Autoradiographic analysis of regional alterations in brain receptors following chronic administration and withdrawal of typical and atypical neuroleptics in rats. *J Neural Transm Gen Sect* **82**:93-109.

Seeburg PH (1993) The TINS/TiPS Lecture. The molecular biology of mammalian glutamate receptor channels. *Trends Neurosci* **16**:359-365.

Seeman P (1980) Brain dopamine receptors. *Pharmacol Rev* **32**:229-313.

Seeman P (1992) Dopamine receptor sequences. Therapeutic levels of neuroleptics occupy D2 receptors, clozapine occupies D4. *Neuropsychopharmacology* **7**:261-284.

Seeman P (2004) Comment on "Diverse psychotomimetics act through a common signaling pathway". *Science* **305**:180; author reply 180.

Seeman P, Chau-Wong M, Tedesco J and Wong K (1975) Brain receptors for antipsychotic drugs and dopamine: direct binding assays. *Proc Natl Acad Sci U S A* **72**:4376-4380.

Seeman P, Ko F and Tallerico T (2005) Dopamine receptor contribution to the action of PCP, LSD and ketamine psychotomimetics. *Mol Psychiatry* **10**:877-883.

Seeman P and Lasaga M (2005) Dopamine agonist action of phencyclidine. *Synapse* **58**:275-277.

Seeman P, Lee T, Chau-Wong M and Wong K (1976) Antipsychotic drug doses and neuroleptic/dopamine receptors. *Nature* **261**:717-719.

Sommer B, Kohler M, Sprengel R and Seeburg PH (1991) RNA editing in brain controls a determinant of ion flow in glutamate-gated channels. *Cell* **67**:11-19.

Stahl S (2002) *Essential Psychopharmacology*. Cambridge University Press, Cambridge.

Stanley M, Lautin A, Rotrosen J, Gershon S and Kleinberg D (1980) Metoclopramide: antipsychotic efficacy of a drug lacking potency in receptor models. *Psychopharmacology (Berl)* **71**:219-225.

Starr MS (1998) Antagonists of glutamate in the treatment of Parkinson's disease: from laboratory to the clinic. *Amino Acids* **14**:41-42.

Steinpreis RE, Parret F, Summ RM and Panos JJ (1997) Effects of clozapine and haloperidol on baseline levels of vacuous jaw movements in aged rats. *Behav Brain Res* **86**:165-169.

Svenningsson P, Nishi A, Fisone G, Girault JA, Nairn AC and Greengard P (2004) DARPP-32: an integrator of neurotransmission. *Annu Rev Pharmacol Toxicol* **44**:269-296.

Svenningsson P, Tzavara ET, Carruthers R, Rachleff I, Wattler S, Nehls M, McKinzie DL, Fienberg AA, Nomikos GG and Greengard P (2003) Diverse psychotomimetics act through a common signaling pathway. *Science* **302**:1412-1415.

Svensson A, Carlsson ML and Carlsson A (1992) Interaction between glutamatergic and dopaminergic tone in the nucleus accumbens of mice: evidence for a dual glutamatergic function with respect to psychomotor control. *J Neural Transm Gen Sect* **88**:235-240.

Svensson TH (2000) Dysfunctional brain dopamine systems induced by psychotomimetic NMDA-receptor antagonists and the effects of antipsychotic drugs. *Brain Res Brain Res Rev* **31**:320-329.

Tamminga C, Hashimoto T, Volk DW and Lewis DA (2004) GABA neurons in the human prefrontal cortex. *Am J Psychiatry* **161**:1764.

Tsai G, Yang P, Chung LC, Lange N and Coyle JT (1998) D-serine added to antipsychotics for the treatment of schizophrenia. *Biol Psychiatry* **44**:1081-1089.

Undie AS and Friedman E (1988) Differences in the cataleptogenic actions of SCH23390 and selected classical neuroleptics. *Psychopharmacology (Berl)* **96**:311-316.

Ushijima I, Kawano M, Kaneyuki H, Suetsugi M, Usami K, Hirano H, Mizuki Y and Yamada M (1997) Dopaminergic and cholinergic interaction in cataleptic responses in mice. *Pharmacol Biochem Behav* **58**:103-108.

Ushijima I, Mizuki Y and Yamada M (1995) Alteration of cataleptic responses induced by dopamine receptor antagonists after chronic cocaine administration in mice. *Eur J Pharmacol* **285**:55-59.

Vallada HP, Gill M, Sham P, Lim LC, Nanko S, Asherson P, Murray RM, McGuffin P, Owen M and Collier D (1995) Linkage studies on chromosome 22 in familial schizophrenia. *Am J Med Genet* **60**:139-146.

Van Tol HH, Bunzow JR, Guan HC, Sunahara RK, Seeman P, Niznik HB and Civelli O (1991) Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. *Nature* **350**:610-614.

Van Tol HH and Seeman P (1995) The dopamine D4 receptor: a novel site for antipsychotic action. *Clin. Neuropharmacol.* **18**:S143.

Volk DW and Lewis DA (2002) Impaired prefrontal inhibition in schizophrenia: relevance for cognitive dysfunction. *Physiol Behav* **77**:501-505.

Wang L and Pitts DK (1994) Perinatal cocaine exposure decreases the number of spontaneously active midbrain dopamine neurons in neonatal rats. *Synapse* **17**:275-277.

Wanibuchi F and Usuda S (1990) Synergistic effects between D_1 and D_2 dopamine antagonists on catalepsy in rats. *Psychopharmacology* **102**:239-342.

Westerink BH and de Vries JB (1989) On the mechanism of neuroleptic induced increase in striatal dopamine release: brain dialysis provides direct evidence for mediation by autoreceptors localized on nerve terminals. *Neurosci Lett* **99**:197-202.

Yamada K and Furukawa T (1980) Direct evidence for involvement of dopaminergic inhibition and cholinergic activation in yawning. *Psychopharmacology (Berl)* **67**:39-43.

Yamamoto BK and Cooperman MA (1994) Differential effects of chronic antipsychotic drug treatment on extracellular glutamate and dopamine concentrations. *J Neurosci* **14**:4159-4166.

Yamamoto BK and Davy S (1992) Dopaminergic modulation of glutamate release in striatum as measured by microdialysis. *J Neurochem* **58**:1736-1742.

Young AB and Penney JB (1984) Neurochemical anatomy of movement disorders. *Neurol Clin* **2**:417-433.