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Dopamine-Glutamate in Reward and Locomotion

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ABSTRACT

Multiple neurotransmitter systems have been implicated in mechanisms underlying drug addiction and schizophrenia. Many drugs of abuse increase dopamine (DA) release in the mesocorticolimbic pathway in the brain. DA and glutamate (glu) exert important modulatory influences in the regulation of emotion, cognition, affect, movement, and reward. Systemic administration of nicotine increases extracellular levels of DA and glu in reward-related brain areas. Nicotine-induced increases of DA in the nucleus accumbens (NAS) are in part mediated by cortical glutamatergic projections to the ventral tegmental area (VTA). Enhanced locomotor responses and preference for drug associated environmental cues are considered as important behaviors in the context of animal models of drug abuse. Using nicotine and MK-801, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, the present experiments were designed to investigate glu-DA interactions in the ventral striatum with regard to both conditioned reinforcement and locomotor activity. The major findings from these experiments are: (1) systemic administration of MK-801 increased locomotor activity in both nicotine-sensitized and control rats. In conditioned reward (place preference) studies: (2) nicotine-treated rats exhibited place preference, but at a higher dose (0.8 mg kg^{-1} s.c) than the initial dose used for locomotor activity (0.4 mg kg^{-1} s.c), and (3) MK-801 blocked nicotine-induced conditioned place preference in an unbiased design but not in a biased design. These findings widen our understanding of the basis of behaviorally relevant interactions between DA and glu in regulating movement and reward and indicate strongly that further research involving other monoamines and different subtypes of glutamate receptors (GluRs) is warranted.

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5-HT	5-hydroxytryptamine, serotonin
ACh	acetylcholine
AMPA	2-amino-3-(3-hydroxy-5-methyl-4-isoxazolo)-propionic acid
AMPH	(+)-amphetamine sulphate
ANOVA	analysis of variance
CNQX	6-cyano-7-nitro-1, 2,3,4-tetrahydro-quinoxaline-2, 3-dione
CNS	central nervous system
CPA	conditioned place aversion
CPP	conditioned place preference
CSF	cerebrospinal fluid
DAT	dopamine transporter
DHßE	dihydro-beta-erythroidine
DNQX	6, 7-dinitroquinoxaline-2, 3-dione
DOPAC	3, 4- dihydroxyphenylacetic acid
GABA	γ-Aminobutyric acid
Glu	glutamate
GluR	glutamate receptor
HVA	homovanillic acid
I.P.	Intraperitoneal
mg	milligram(s)
mGluR	metabotropic glutamate receptor
MK-801	(±)-5-methyl-10, 11-dihydro-5H-dibenzo [a, b] cyclohepten-5, 10-imine

mL	millilitre(s)
mPFC	medial prefrontal cortex
nAChR	nicotinic acetylcholine receptor
NAS	nucleus accumbens septi
NBQX	2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide
NMDA	N-methyl-D-aspartic acid
PCP	phencyclidine
PET	positron emission tomography
s.c.	subcutaneous
SN	substantia nigra
VTA	ventral tegmental area

1. GENERAL INTRODUCTION

Chemical transmission is the major means by which neurons communicate with one another in the nervous system. Neuronal changes in response to psychostimulant drugs may be similar to those critical for learning and memory and may play a role in the early manifestations of drug addiction (Berke and Hyman, 2000; Hyman and Malenka, 2001; Ballas et al., 2004). Thus, neuronal pre- and postsynaptic chemical events cause neuronal changes that are essential for learning associated with drug addiction and other rewarding/motivated behaviors. Interactions between various neurotransmitters, especially DA and glu in the brain, are crucial in mediating locomotor activity, motivated behavior, and reward (Szabo et al., 2004). Such interactions are of interest in the context of drug addiction (Moghaddam and Gruen, 1991; Montogomery and Grottick, 1999; Berke and Hyman, 2000) and neuropsychiatric disorders such as schizophrenia (Hirsch et al., 1997; Tamminga, 1999; Gur and Arnold, 2004; Seeman et al., 2005). Dopaminergic projections extend from the VTA of the midbrain to the NAS, medial prefrontal cortex (mPFC), and limbic structures; these neuronal connections constitute the mesolimbic and mesocorticolimbic pathways (Phillips and LePiane, 1980; Mogenson et al., 1980; Koob, 1992; Uhl, 1999) which are considered to be the reward circuitry of the brain (Olds and Milner, 1954; Melchitzky et al., 2004). DA receptors, nicotinic acetylcholine receptors (nAChRs), and GluRs are expressed by neurons that innervate both the NAS and the VTA (Arnold et al., 2000; Kelly, 2002). It is interesting to note that rewarding compounds (such as amphetamine) capable of mimicking the positive symptoms of schizophrenia usually enhance locomotor activity (Phillips and LePiane, 1980; Wise and Bozarth, 1987). In addition, most drugs of abuse for recreational use and

many psychotropic drugs that are used to treat neuropsychiatric disorders act on neurotransmitter systems in the mesolimbic and mesocorticolimbic pathways of the brain (Wise and Bozarth, 1987; Ballas et al., 2004; Biondo et al., 2005).

1.1. Dopamine in Drug Addiction and Reward

Behavioral studies have identified neurobiological mechanisms mediating behavior that is motivated by reinforcing events associated with pleasure, and these events are termed "rewards", which are vital in governing behavior in humans and animals (Markou et al., 1993). Behavioral reinforcement which occurs after presentation of some everyday events (e.g. appetizing food, sex, etc.) increases the probability or frequency of the behavior (Schultz et al., 1997; Schultz 2000). Drug addiction and drug dependence are examples of reward and reinforcement (Wise, 1988; Kilts, 2004). As a scientific term, drug addiction is usually described as a chronically relapsing disorder of compulsive drug taking with loss of control over drug intake and emergence of a withdrawal upon cessation of drug taking (dependence). Substance dependence is a maladaptive pattern of behavior leading to clinically significant impairment or distress characterized by (1) compulsion to seek and take the drug with loss of control in limiting intake, (2) emergence of a negative emotional state (e.g., dysphoria, anxiety, irritability) when access to the drug is prevented, (3) a great deal of time spent in obtaining the substance, and (4) impairment of social and occupational functioning due to substance use (DSM IV, 1994).

The DSM-IV diagnostic criteria are exclusively operational but do not describe the complex neurobiological mechanisms involved in drug addiction. Important phenomena

associated with drugs of abuse are tolerance and sensitization (Clarke and Kumar, 1983; Benwell and Balfour, 1992). Sensitization (reverse tolerance) is defined as an increase in the effect of a certain amount of drug after repeated administration, or inversely, achievement of the same effect by a smaller dose of the substance (O'Brien, 1996; Koob et al., 1998). The physiological adaptations caused by chronic drug exposure also result in physical withdrawal symptoms, which are relieved when the drug is consumed again (Berridge and Robinson, 1998; Lyons, 2004).

Positive reinforcing effects of drugs are critical for establishment of self-administration behavior and have an important role in all aspects of drug dependence (Phillips and LePiane, 1980; Burns et al., 1994). The mechanisms underlying the transition from initial drug use to subsequent development of drug dependence are not clear. But it has been hypothesized that the transition to drug dependence involves adaptations of neurons in the brain reward pathways leading to neuronal changes and the expression of positive reinforcement. As outlined in figure 1.1 below, drug abuse is likely to be influenced by genetic and environmental factors (Nestler, 2000; Kalivas, 2002; Lyons, 2004), and learning is an integral part of induction of addictive behaviors (Berke and Hyman, 2000 for review).

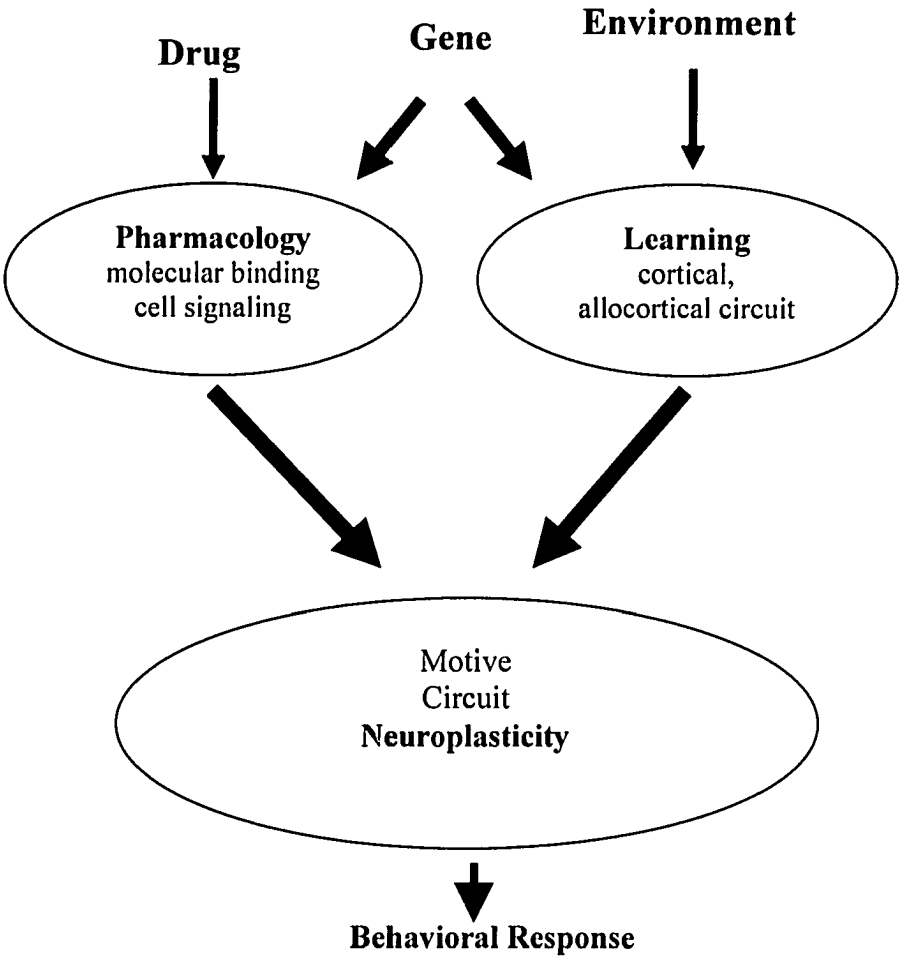


Figure 1.1. Interplay of genetics, environment, and pharmacology of abused drug in addiction. Adapted from Kalivas, 2002.

1.1.1. Dopamine-Glutamate Interactions in Reward and Addiction

A wide variety of biologically important stimuli can serve as rewards and establish adaptive behavior patterns in higher animals. DA in the central nervous system (CNS) may play a significant role in the initiation and control of movement (Beninger, 1983; Bardo et al. 1999) in addition to goal-directed behavior such as reward and reinforcement (Wise, 1978; Koob, 1992). DA is the common target for most drugs of abuse (Sidman et al., 1955; Wise, 1988; Lyons 2004).

In a pioneering discovery, it was found that direct electrical stimulation of the brain can be powerfully rewarding; rats receiving electrical stimulation in certain brain areas behaved as if they were trying to get more of it (Olds and Milner, 1954). Thus brain stimulation served as a rewarding stimulus. Subsequent mapping studies have shown that stimulation of many other seemingly disparate regions of the brain is rewarding (Phillips and LePiane, 1980; Uhl, 1999). These are considered DA enriched reward inducing areas of the brain. The most sensitive sites were along the medial forebrain bundle, particularly at its lateral hypothalamic, posterior hypothalamic, and VTA levels (Phillips and Fibiger, 1987). Electrical stimulation of the lateral hypothalamic medial forebrain bundle evoked mating responses in the opossum in the presence of another opossum or furry object (Doty, 1969; Greenshaw et al., 1983). These observations indicate that electrical stimulation of brain reward areas may mimic appetitive natural reward/reinforcement.

A rewarding stimulus that leads to behavioral reinforcement may be mediated predominantly through release of DA (Wise, 1980; Sutton and Beninger, 1999), excitatory amino acids (EAA) (Fonnum, 1984; Kilts, 2004) and other interactive

neurotransmitters from neurons mostly located in the brain reward pathways (Koob, 1992; Bardo, 1998, Kelley, 2002).

DA in the mesolimbic system increases sensitivity to stimuli of motivational significance and plays an important role in the reward mechanisms (Costal et al, 1984; Koob, 1997; Uhl, 1999). Also DA neurons in the mesolimbic pathway are activated in response to many natural events like palatable food, sex, praise, etc. (Horvitz, 2000). Many studies indicate that both DA and Glu play a major role in learning the circumstances and associated cues under which rewarding events occurred (Koob, 1997; Le Foll and Goldberg, 2005).

In recent years, previously held thoughts about the solitary role of DA in reward have been debated because DA is believed to be released in the presence of many salient and even aversive stimuli such as stress and pain (Horvitz, 2000; Kilts, 2004). So it is assumed that for induction of reward and reinforcement DA must act in collaboration with many other neurotransmitters, notably glu (Karreman et al., 1996; Kelley, 2002). Anatomically neurons within the NAS not only receive DA projecting from the VTA but also from the glutamatergic neurons from the cerebral cortex, amygdale, and hippocampus (Koob, 1992; Vanderschuren et al., 2000). Thus both DA and Glu may be involved in the establishment and maintenance of addictive behavior, reward and reinforcement (Kuhar, 1991; Nestler and Aghajanian, 1997; Wolf, 1998). Circuits implicated in generalized memory processes may play significant roles in long-term consequences of substance abuse. These circuits include those in the hippocampus, amygdala and several related cortical zones (Pierce and Kalivas, 1997).

1.1.2. Animal Models of Addiction

Much of the recent progress in understanding the mechanisms of addiction was derived from the development of animal models of addiction based on using specific drugs such as opiates, stimulants, and ethanol. Although no animal model of addiction fully emulates the human condition, animal models permit investigation of the behavioral processes of drug addiction. Data derived from animal models provide an empirical framework for a broader understanding of the scientific basis of addiction (Koob et al., 1998). In studies involving chronic exposure to psychostimulant drugs, numerous factors may profoundly influence the spectrum of behavioral and neurochemical effects of these drugs (Kilts, 2004). Such factors include dose, route, and interval between successive administrations, as well as length of exposure (Robinson and Beart, 1988; Berridge and Robinson, 1998). The addictive drugs are unique in being habit-forming and establishing learned preferences for various stimuli that are associated with their use or presentation (Smith et al., 1980; Schultz et al., 1997, Schultz, 2000).

Although the drug self-administration and the brain stimulation reward paradigms are well-investigated methods for assessing the reward-relevant properties of a substance, locomotor hyperactivity and CPP paradigm are also considered uncomplicated and reliable methods for measuring reward (Wise and Bozarth, 1987; Swerdlow et al., 1989; Lyons, 2004).

Locomotor hyperactivity occurs with most addictive drugs. Although not a specific behavioral response for addiction, it can be used in conjunction with other paradigms to determine addictive potential of a drug (Wise, 1987; Ballas et al., 2004). Laboratory animals might attempt to self-administer several classes of addictive drugs by the oral,

intraperitoneal, intravenous, or even intracranial routes and will do so to the point of physiological dependence (Olds and Williams, 1980). Ingestion of ethanol and smoking of cigarettes represent obvious human analogues of drug self- administration (Wise, 1978).

Most drugs of abuse not only possess rewarding actions of their own but they also tend to potentiate or summate with the rewarding actions of other substances or events. For example, use of cannabis is said to enhance the pleasure of music, sex, and even pain relief (Ballas et al., 2004).

The most dramatic signs of drug dependence are withdrawal syndromes. Animals withdrawn from rewarding drugs are agitated, hyperactive, and become abnormally susceptible to external stimuli (Malin et al., 1992; Schneider and Jarvik, 1985). The psychomotor agitation may result from reduction of intracellular DA levels in the brain along with a number of intracellular changes in the mesolimbic DA system, especially in the medium spiny neurons of the NAS that occur following drug withdrawal (Epping-Jordan, 1998; Kilts, 2004). Experts emphasize that these neuronal alterations and complex intracellular signal functioning changes go far beyond the widely perceived simplified notion of up- or down-regulation of receptors (Nestler and Aghajanian, 1997).

The study of animal models of addiction has contributed a number of insights into the nature of the human condition. It is probably safe to assert that all mammalian species are susceptible to the habit-forming effects of opiates, psychomotor stimulants and other drugs of abuse (Hyman and Malenka, 2001; Lyons, 2004). Also animal studies provide us with the perception that drug craving is a conditioned response because the drug solution has no distal eye-catching sensory properties that lure the animal. Thus, the significance

of the place in the environment where the drug is experienced and the maneuvers that allow the animal to earn drug injections are important components for attaining addictive behavior (Nestler and Aghajanian, 1997; Nestler, 2001). Animal studies also reveal that individual differences, deprivation states and environmental choices can influence drug self-administration (Bardo et al. 1999; Nestler and Aghajanian., 1997). More importantly, animal models of substance abuse also provide a unique opportunity to study genetic influences on drug abuse behaviors that can be ascertained by strain comparisons, selective breeding, transgenic and knockout animals (Nestler, 2000). Although animal studies do not reveal the importance of language and cultural influences (peer pressure, media advertising or attractive labeling), which play an important role in human drug abuse (Abbott, 2002), they will continue to provide indispensable vehicles to broaden our understanding of addiction. Behavioral tests used to study animal models of addiction are summarized in Table 1 below.

Table 1: Some behavioral tests commonly used to study addiction. Adapted from Nestler, 2000.	
Behavioral test	Description
Acute locomotor activation	Acute increase in locomotor activity after initial administration of a drug of abuse
Locomotor sensitization	Progressive increase in locomotor activity after repeated administration of a drug of abuse
Conditioned locomotor sensitization	Increase in locomotor activity seen in environment (for example, testing chamber) where animals received repeated administration of a drug of abuse
Conditioned place preference	Development of preference for an environment (for example, one side of testing chamber) associated with repeated administration of a drug of abuse
Oral self-administration	Development of voluntary drinking of a drug of abuse in a palatable (for example, sucrose-containing) solution
Self-administration (operant-controlled)	
Acquisition	Development of volitional (voluntary) administration (intravenous or intracerebral) of a drug of abuse by performing some task (for example, lever pressing)
Stable maintenance	Amount of drug of abuse self-administered over a range of doses, providing a measure of the acute reinforcing value of the drug
Progressive ratio	Determination of how hard an animal will work (for example, how many lever presses/unit time) to self-administer a drug of abuse
Extinction	Progressive decrease in drug-associated task (for example, lever pressing) when drug is no longer available
Relapse	Return to drug-associated task (for example, lever pressing) even when drug of abuse is not available; this can be stimulated by acute challenge with the drug itself, a drug-associated cue (for example, light or tone), or stress
Intracranial self-stimulation	Volitional (voluntary) electrical stimulation of particular brain regions by performing a task (for example, lever pressing), and potentiation of this behavior by a drug of abuse
Conditioned reinforcement	Development of volitional (voluntary) effort to receive an otherwise neutral stimulus (for example, light) associated with a reward (for example, a natural reward such as water), and potentiation of this behavior by a drug of abuse

1.1.3. Dopamine-Glutamate Interactions in Locomotor Activity

By enhancing cerebral DA release, drugs of abuse increase the locomotor activity of animals (Koob, 1992; Kilts, 2004). Interconnecting brain pathways, receptors and synapses are involved in the changes occurring during addiction as outlined in figure 1.2. It has been suggested that Glu-DA interactions in the ventral striatum mediate both locomotor activity and responding with conditioned reinforcement (Wise and Bozarth 1987; Moghaddam and Gruen, 1991; Wolf, 1998).

Repeated treatment with opioids or psychostimulants has also been shown to induce behavioral sensitization in rats, which is manifested as an enhancement of the effect of drug on horizontal locomotor activity and has been associated with increased DA release in the NAS (Kalivas and Duffy, 1997; Acquas and Di Chiara 1994; Cadoni and Di Chiara, 2000).

For instance, repeated treatment with 15 mg kg^{-1} of cocaine for 4 or 5 days resulted in behavioral sensitization associated with increased DA release in the NAS when the rats were challenged with the same dose of cocaine on the following day (Kalivas and Duffy, 1998; O'Brien, 1996). Behavioral sensitization has been observed even after a single exposure to amphetamine (Vanderschuren et al., 1999).

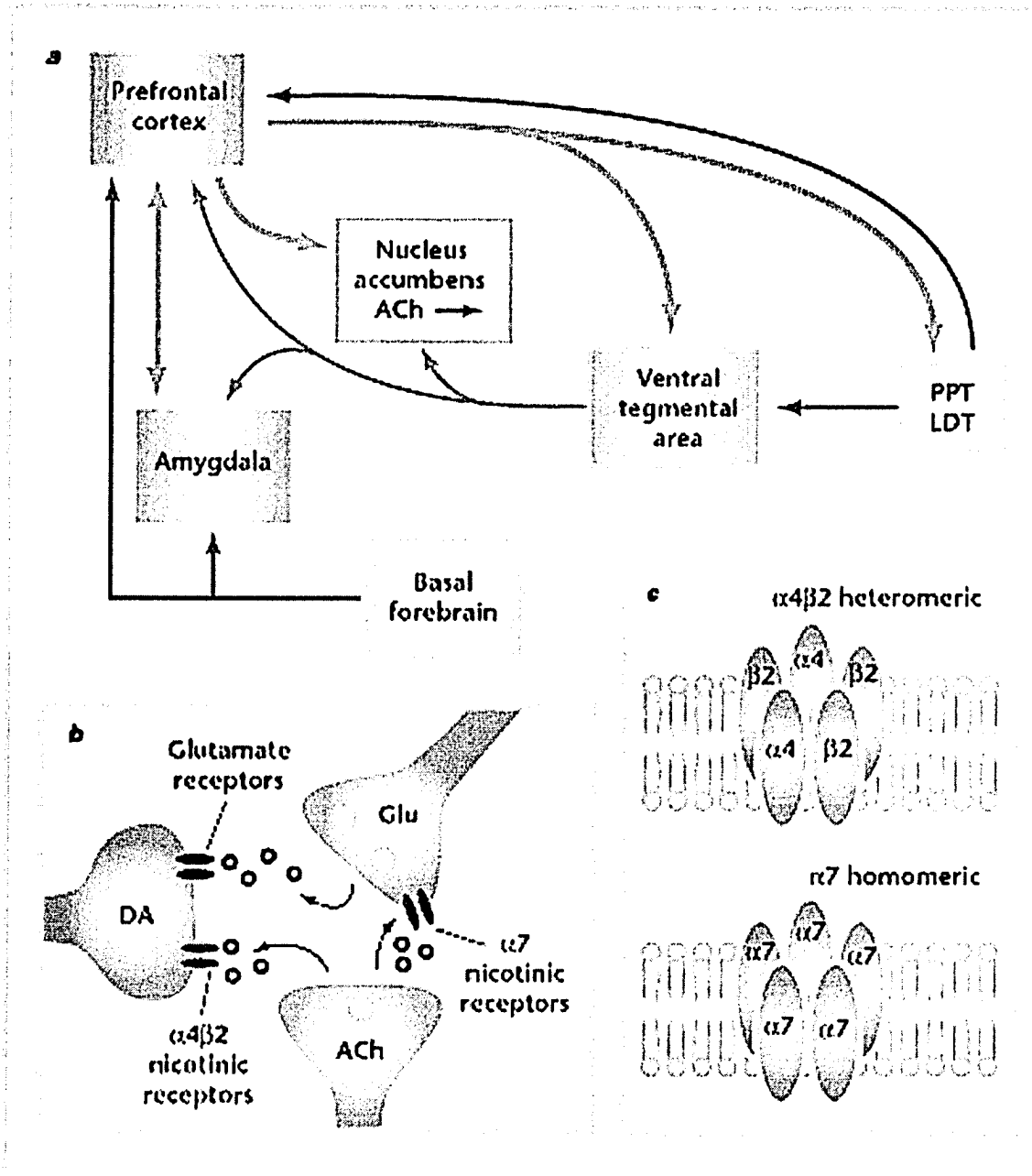


Figure 1.2. Interconnecting brain pathways, receptors and synapses mediating neuronal changes in addiction. From Kelley, 2002, reproduced with permission.

1.2. The Neurochemical Basis of Schizophrenia

Schizophrenia is a severe mental illness, affecting 0.75 to 1.5% of the adult population (Black and Anderson, 1996). The psychopathology of schizophrenia is usually described in terms of three somewhat independent syndromes, or symptom clusters, which include positive, disorganized and negative symptoms (Gur and Arnold, 2004). Positive symptoms consist of florid psychotic symptoms, mainly delusions and hallucinations. Hallucinations are usually auditory in nature and may be experienced as coming from internal or external sources. Disorganization as a syndrome of schizophrenia includes incoherence, illogicality, loose associations, inappropriate affect and poverty of thought content (DSM IV, 1994). Negative symptoms include withdrawal, impoverished emotional state, motivational difficulties, lack of energy, affective flattening, loss of spontaneity and lack of initiative. Not all of these symptoms are present at any one time, and they vary in severity over time. Cognitive impairment commonly present in schizophrenia greatly affects functional or occupational impairments (Black and Anderson, 1996).

Although the precise nature of the fundamental pathological process of schizophrenia is unknown, deregulation of neurotransmitter homeostasis in the brain (Carlsson et al., 1999) and/or a specific genetic defect (Asherson et al., 1994) may be contributing factors. Derangement of integrated functioning of the neurotransmitter systems in the CNS has been strongly implicated in the etiology of schizophrenia (Carlsson et al., 1999; Davis et al., 1991; Olney and Farber, 1995). Relative imbalances, deficiencies or excesses of neurotransmitters in the CNS may result in hyper- or hypoactivity of neurons or neuronal receptors. It has a complex mode of inheritance and variable expression. Also the

etiology of schizophrenia may involve pathological processes during brain development; disordered connectivity or neuronal localization due to faulty migration in the prefrontal cortex may be responsible for neurotransmitter derangement (Weinberger, 1987). But despite extensive efforts to discover a neuropathological basis for schizophrenia, no consistent characteristic lesions, either at the micro- or the macroscopic level, have yet been identified (Joyce, 1993). Based on the efficacy of both typical and newer antipsychotic drugs in the treatment of schizophrenia, it is assumed that in addition to DA other neurotransmitters are involved in the pathogenesis of schizophrenia.

1.2.1. Dopamine Hypothesis as the Neurochemical Basis of Schizophrenia

The DA hypothesis is the most widely investigated theory underlying schizophrenia and is the basis for explaining the mechanisms of action of most antipsychotic drugs. This DA theory proposes that schizophrenia results from an excess of DA or dopaminergic stimulation in the mesolimbic sytem in the CNS (Snyder et al. 1974).

But the DA theory of schizophrenia is almost entirely based on pharmacological evidence and is supported by two main observations. There is a high correlation between the effective dose of traditional neuroleptics (e.g. antipsychotics) and D_2 receptor blockade. In addition, symptoms similar to those of paranoid schizophrenia are seen in amphetamine and cocaine addicts and appear to be due to enhanced DA activity through activation of D_2 receptors (Seeman et al., 1976, 2005; Manschreck et al., 1988).

However, the DA hypothesis of schizophrenia has some important caveats that need to be clarified. The DA hypothesis has been tested by examining differences in concentrations of DA or its metabolites in various brain sites, induction of a paranoid form of

schizophrenia by repeated administration of high doses of amphetamine, and the ability of antipsychotic drugs to block D_2 receptors. Unfortunately, the pattern of changes in the various studies has not been consistent enough to conclude that DA hyperactivity is the sole cause of schizophrenia. It is important to note that a fair proportion of schizophrenic patients respond poorly, or not at all, to treatment with drugs that block DA activity (Davies et al., 1991). Poor response is seen especially in cases with predominantly 'negative' symptomatology characterized by poverty of speech, flattening of affect, poor social interactions etc. (Crow, 1980). Furthermore, postmortem brain studies comparing drug-free schizophrenia patients with control groups showed no difference in the density of D_2 receptors in the striatum (Davis et al., 1991). Increased DA receptor sensitivity remains another possible DA abnormality in the pathology of schizophrenia (Owen and Crow, 1987).

The lack of support to fully explain the DA hypothesis by no means suggests that DA plays no role in schizophrenia. Instead, it is assumed that the role of DA is much more complex than originally predicted. DA may exert its influence via a variety of receptor subtypes and it might interact with a variety of other neurotransmitter systems (Kebabian and Calne, 1979; Szabo et al., 2004). Some of these interactions are likely integrated into complex feedback circuits that mutually influence the activity of one another (Olney and Farber, 1995).

1.2.2. Glutamate Hypothesis as the Neurochemical Basis of Schizophrenia

Involvement of brain dopaminergic and seratonergic system in schizophrenia is well established but several lines of evidence implicate N-methyl-d-aspartatic acid (NMDA) receptor dysfunction in the pathogenesis of schizophrenia. In recent years, a growing number of studies have hypothesized that decreased tone of excitatory amino acids (EAA) may play a role in the pathophysiology of schizophrenia (Carlsson et al., 1999; Moghaddam, 2004).

The possible role of glutamate in the pathogenesis of schizophrenia has been proposed based on the finding that administration of certain types of GluR antagonists produce schizophrenia-like psychotic symptoms in normal as well as schizophrenic subjects. In humans, phencyclidine (PCP), a non-competitive antagonist acting at the ion channel site of the NMDA subtype of the GluR, produces a syndrome resembling schizophrenia, including negative and positive symptoms (Javitt and Zukin, 1991). Ketamine, a short acting anesthetic agent, also binds to the same site as PCP in the ion channel of the NMDA receptor and causes a schizophrenia-like psychosis in normal volunteers (Krystal et al., 1994) and exacerbates psychotic symptoms in patients with schizophrenia (Lahti, 1995). This pharmacological model of psychosis indicates that decreased glu activity (hypoglutamatergia) may play an important role in the pathophysiology of schizophrenia, and a DA-glu hypothesis of schizophrenia has been proposed (Carlsson et al, 1999). It is assumed that a primary deficit in corticostriatal and corticolimbic glutamatergic transmission may result in a reduction in glutamatergic inhibition of DA release, with a consequent increase in dopaminergic tone in schizophrenic patients (Carlsson et al., 1999). The finding of reduced glutamate levels in cerebrospinal fluid (CSF) of

schizophrenic subjects also supports the proposed glutamate hypoactivity theory (Kim et al, 1980). Glycine, a co-agonist of NMDA-receptor mediated neurotransmission, significantly improved negative symptoms of schizophrenia (Javitt et al., 1994). Thus, the Glu mechanisms are likely to continue to receive considerable attention in ongoing investigations related to the etiology of schizophrenia, suggesting that pharmacological manipulation of the NMDA receptor may be a feasible therapeutic strategy for treatment of schizophrenia (Moghaddam, 2004; Carlsson et al., 1999).

1. 2. 3. Animal Models of Schizophrenia

The spectrum of symptoms in schizophrenia includes cognitive and linguistic components, which make it difficult to reasonably create an animal model of schizophrenia through animal studies (Lyons, 2004). Thus behavioral measures have been used extensively to validate animal models of schizophrenia (Hartmann, 1976). Furthermore, the etiology of schizophrenia is considered a heterogeneous entity based on the existence of conceptual challenges; it does not appear possible to fit a single pharmacological model to schizophrenia (Costall et al., 1984; Gur and Arnold, 2004). However, some aspects of animal behavior such as locomotor hyperactivity, heightened response to sensory stimuli (such as sound, light, touch), stereotyped behavior, social withdrawal, and disruption of prepulse inhibition (Braff and Geyer, 1991) can be considered as an alternate comparable models of schizophrenia. Acute or continuous administration of amphetamine causes reliable behavioral expression resembling schizophrenia such as locomotor hyperactivity to bizarre behavior, abortive grooming, excessive twitching and fragmentary movement (Carlezon and Wise, 1993; Ouagazzal et

al., 1993). In animals, apomorphine or amphetamine has been shown to attenuate prepulse inhibition and overactivity of the limbic DA system is considered responsible for the loss of sensorimotor gating with consequent cognitive dysfunction (Braff and Geyer, 1991; Melchitzky, 2004). DA antagonists can inhibit the enhanced locomotor activity induced by systemic or intracranial administration of amphetamine. This outcome indicates an excellent correlation between the efficacy of traditional antipsychotic and remission of amphetamine-induced abnormal behaviors (Costall and Naylor, 1984).

In rats, acute and repeated administration of PCP and ketamine alter social behavior, impair cognitive function, and produce hyperactivity (Sherman et al., 1991). In the primate, PCP and ketamine impair learning in several paradigms. Thus behavioral measures have been used extensively to validate animal models of schizophrenia. In many species, the most potent psychosis-provoking drugs increase locomotor activity, and stereotypy, and impair sensory gating while antipsychotic drugs decrease such locomotor hyperactivity induced by PCP (Javitt and Zukin, 1991; Meador-Woodruff and Healy, 2000, 2002). Normal internal screening or sensorimotor gating is impaired in schizophrenia due to the inability of the brain to filter sensory information (Braff and Geyer, 1990). Thus findings from PCP and ketamine models of schizophrenia suggest that endogenous dysfunction of NMDA receptor mediated neurotransmission may contribute to increased DA activity in the pathogenesis of schizophrenia (Javitt and Zukin, 1991, Szabo et al., 2004).

1.3. Relevance of Locomotor Hyperactivity in Reward and Schizophrenia

An enhanced locomotor response is the most studied behavioral response following repeated exposures to drugs of abuse such as amphetamine, nicotine in animal studies (Wise, 1987; Beninger, 1983; Birrel and Balfour, 1998; Ballas et al., 2004). Manifestations of behavioral effects following systemic administration of such drugs represent the output of interactive mechanisms through the central and peripheral nervous systems (Clarke and Kumar, 1983; Wu et al., 1993). Systemic administration of these psychostimulant drugs increases the release of DA and Glu in the CNS to mediate locomotor activity and reward; coordinated action of DA and Glu is considered essential for smooth execution of these behavioral effects (Imperato et al., 1986; Karreman et al., 1996; Baik et al., 1995).

Augmented dopaminergic activity in the mesolimbic system occurs in schizophrenia and this is also the site of action of most abused rewarding drugs (Wise, 1978; Uhl, 1999). Most potential antipsychotic drugs used for the treatment of schizophrenia decrease locomotor activity in animals and the mesocorticolimbic pathway is considered as the important site of action of many antipsychotic drugs (Crow el al., 1980; Gur and Arnold, 2004). Considering involvement of both DA and Glu in drug addiction and schizophrenia (Hirsh et al., 1997; Carlsson et al 1999), the study of Glu-DA interactions in locomotor activity may broaden our knowledge of reward and motivated behavior. This insight may guide us in future investigations.

1.4. Dopamine: Neurochemistry and its Receptors

1.4.1. Dopamine (DA)

Rewarding drugs such as nicotine, amphetamine, and cocaine causes efflux of DA from the NAS, VTA, cerebral cortex, hippocampus, amygdala and other limbic structures (Costal et al, 1984; Karreman et al., 1996; Uhl, 1999). Psychostimulant drug- induced behavioral and microdialysis studies in rodent brain suggest that reward and reinforcement are associated with higher extracellular levels of DA (Dreher and Jackson, 1989; Ikemoto and Panksepp, 1999; Szabo et al., 2004).

DA is made from the amino acid L-tyrosine, which is transported into catecholamine-secreting neurons and adrenal medullary cells by an active-transport process found on the nerve membrane. It is converted to dopa by tyrosine hydroxylase and then to DA in the cytoplasm of the cells by dopa decarboxylase (Ganong, 2001). The DA then enters the granulated vesicles, within which it may be converted to norepinephrine. The rate-limiting step in synthesis is the conversion of tyrosine to dopa. Active reuptake into the nerve terminal by DA transporter molecules is critical in terminating DA's action. The main metabolites of DA are 3, 4- dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and a small amount of 3-methoxytyramine (3-MT). DOPAC is considered to be an index of intraneuronal synthesis and metabolism of DA (Ganong, 2001; Grace, 2004). DA plays an important role in circuits implicated in generalized memory processes, may also play significant roles in long-term consequences of substance abuse involving the hippocampus, amygdala and several related cortical zones (Melchitzky et al., 2004).

1.4.2. The Dopamine Receptors

The synaptic effects of DA appear to be mediated by several pharmacologically and physiologically distinct receptors listed on table 2. Based on impressive ongoing molecular studies and findings, it is likely that not all DA receptor types have been discovered (Seeman et al., 2005; Kebabian and Calnel, 1979). Based on structure (deducted from molecular cloning) and their pharmacological properties, DA receptors can be grouped into two families: D_1 and D_2 (Seeman and van Tol, 1994). Furthermore D_1-like receptors include D_1 and D_5 whereas D_2-like receptors include D_2, D_3, and D_4 (Kebabian and Calne, 1979; Kuhar et al., 1999). The D_1 and D_5 receptors activate adenylate cyclase via G_s. The D_2 receptor inhibits adenylate cyclase and activates a voltage-sensitive K^+ channel via G_i. The precise second messenger effects of D_3 and D_4 receptors are not yet clear, but D_3 and D_4 show pharmacological similarities to the D_2 receptor subtype (Grace, 2004). Clozapine, a novel antipsychotic drug, has a high affinity for the D_4 over the D_2 receptor, suggesting that blockade of the D_4 receptor may be related to the efficacy of at least some neuroleptics (Gur and Arnold, 2004) whereas blockade of D_2 receptors may be related to their extrapyramidal side effects (Seeman, 2005; Pulvirenti et al. 1994). D_1-like receptors contribute to the negative symptoms and cognitive dysfunction seen in schizophrenia (Okubo et al., 1997). Furthermore, with the clinical success of clozapine in treating refractory schizophrenics, there has been a resurgence of interest in new strata of DA receptor subtypes and neuroleptic drug action. Over the past several years, new evidence from the application of molecular biological and gene cloning techniques suggest that the number of DA receptor subtypes appears much larger than originally envisioned (Seeman et al., 1975; 2005).

Table 2. Categorization of DA receptors (Adapted from Kuhar et al., 1999; Seeman, 1995)		
Dopamine Receptor	Location	Ligand binding effect
D_1, D_5	Brain, peripheral tissue	↑ adenyl cyclase → ↑ cAMP
D_2	Brain, pre- and postsynaptic nerve terminal Smooth musle	↓ adenyl cyclase → ↓ cAMP
D_3	Brain	↓ adenyl cyclase → ↓ cAMP
D_4	Brain and cardiovascular system	↓ adenyl cyclase → ↓ cAMP

1.4.3. Dopamine Transporter (DAT)

The reuptake process of the released DA is mediated by a specific carrier or transporter protein located on the presynaptic membrane known as the dopamine transporter (DAT) as shown in figure 1.3. The DAT takes the released neurotransmitter back up into presynaptic terminals, and is a major determinant of the intensity and duration of the dopaminergic signal. Knockout mice lacking the DAT display marked changes in DA homeostasis that result in elevated dopaminergic tone and pronounced locomotor hyperactivity (Giros et al., 1996; Fang and Ronnekleiv, 1999). DA transmission involves release of DA in the synaptic cleft to interact with specific pre- and postsynaptic receptors. The DAT is believed to control activity of released DA by rapid reuptake of the neurotransmitter from the synaptic cleft into presynaptic terminals (Ganong, 2001). The DAT is also a target for psychoactive drugs such as antidepressants and drugs of abuse, including cocaine and amphetamine, and is highly expressed in dopaminergic cells in the substantia nigra pars compacta and VTA (Kuhar et al., 1999). The DAT is considered vital target of cocaine and amphetamine, as these psychostimulants have no effect on locomotor activity or DA release and uptake in knocked out mice lacking the

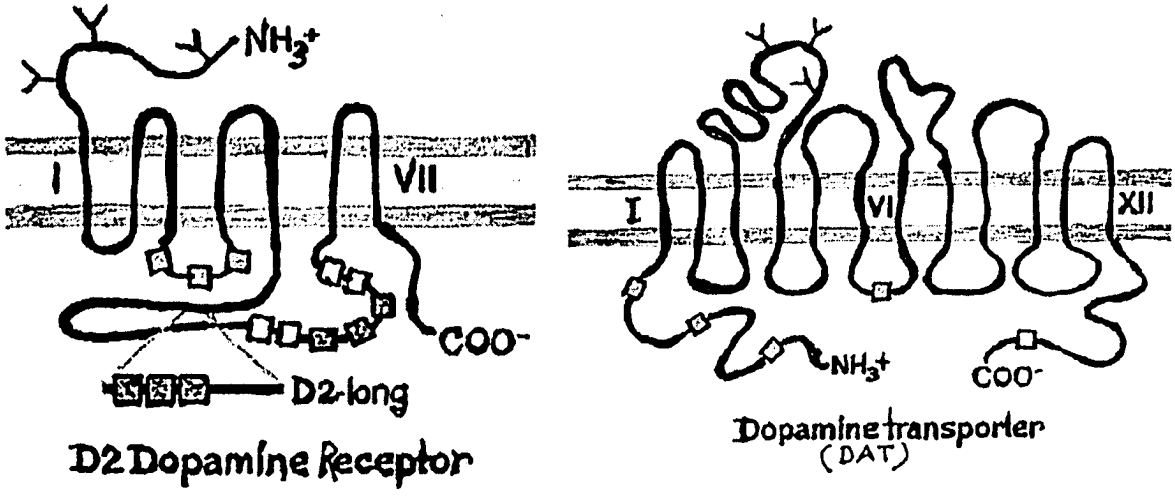


Figure 1.3. Schematic drawing of the D_2 receptor and the DA transporter. Adapted from Kuhar et al., 1999.

DAT gene because of the overabundanmce of DA in the synaptic cleft (Giros et al., 1996).

1.5. Neuroanatomical Organization of Mesocorticolimbic Dopamine System

To obtain a thorough understanding of the potential interactions between DA and glu, it is essential to have a comprehensive knowledge of the individual neurotransmitter systems, their pathways and receptors. Figure 1.4 below compares the mesolimbic DA system in rat and human brains. Mapping studies of central monoaminergic cell groups classified groups A1-A7 as noradrenergic and A8-A15 as dopaminergic (Mogenson et al., 1980; Parent, 1990).

1.5.1. Ventral Tegmental Area (VTA)

The ventral tegmental area (VTA) in the midbrain contains a large population of dopaminergic neurons. The neuronal cell bodies (A 10) form a major component of the mesocorticolimbic DA system (Oades and Hatliday, 1987). The axonal projection from these cell bodies widely innervates the NAS and lies in continuum with the pars compacta of the substantia nigra (Parent, 1990). The VTA also projects to the striatum, and many structures of the forebrain, which include the cerebral cortex, amygdala and other associated limbic structures (Uhl, 1999). Both the VTA and NAS receive EAA inputs from the mPFC and limbic structures such as the hippocampal formation and amygdala (Kalivas and Duffy, 1998).

The VTA is postulated to be involved in reward associated with newly learned behaviors in contrast to the maintenance of previously learned behaviors (Koob, 1992; Pidoplichko et al., 1997). Systemic injection of nicotine, cocaine, or amphetamine in rats produced an

increase in extracellular DA, Glu in the VTA and may underlie behavioral sensitization (Phillips and LePiane, 1980; Karler et al., 1994; Kalivas and Duffy, 1998).

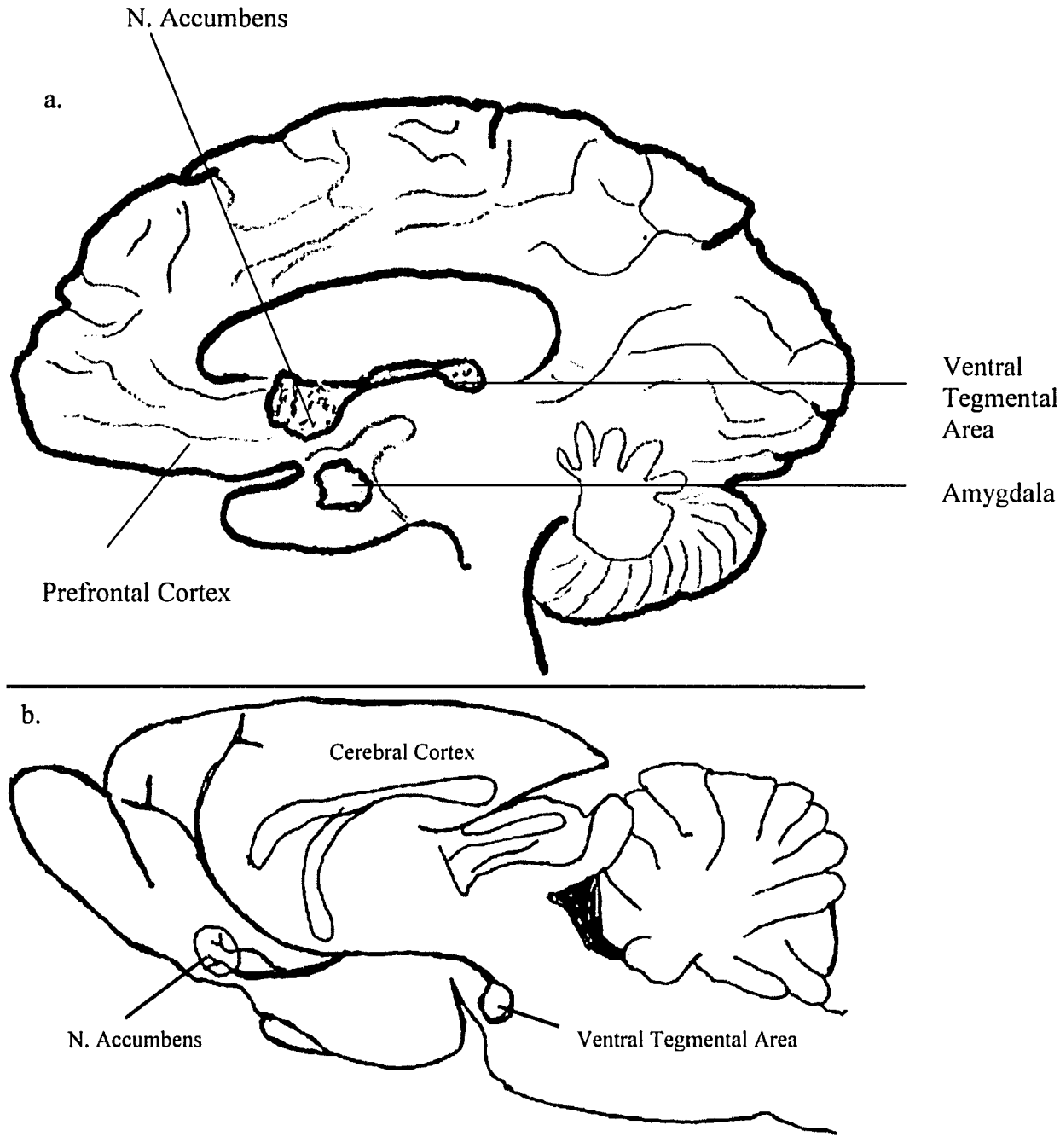


Figure 1.4. Mesolimbic DA system in (a) human brain (adapted from Abbott, 2002) and (b) rat brain (adapted from Uhl, 1999).

1.5.2. The Nucleus Accumbens (NAS)

The NAS of the basal forebrain is a major component of the ventral striatum putamen and rich in DA. The NAS is populated by a large number of medium sized spiny neurons (Parent. 1990; Hyman and Malenka, 2001). The NAS can be divided into two major sub regions: the shell and the core. The shell of the NAS surrounds the core and blends with the amygdala, providing a unique motor-limbic interface, and is involved in the processes of initiation and control of psychomotor behavior (Mogenson et al., 1980). The NAS receives excitatory glutamatergic afferents from cortical and limbic brain regions which make it an important neuronal site for mediating rewarding effects of natural reinforcers such as food, sex, and locomotor activity (Robinson and Beart, 1988; Kelley, 2002). This is also the principal site involved in the actions of many neuropsychiatric disorders (Uhl et al., 1999; Koch et al., 2000).

1.5.3. Medial Prefrontal Cortex (mPFC) and Associated Limbic Structures

The mPFC is the cortical area at the rostral end of the frontal lobe. Most connections are reciprocal, for example, while the VTA sends dopaminergic projections to the mPFC and NAS, the mPFC sends glutamatergic projections to the VTA and also to the NAS (Melchitzky et al., 2004). mPFC projections to the VTA synapse on dopaminergic as well as non-dopaminergic cells. Dopaminergic projections to the mPFC arise predominantly in the VTA, but the main transmitter of the efferent mPFC projection is thought to be glu (Fonnum, 1984; Wolf, 1998). The mPFC is the cortical region primarily rich in NMDA and AMPA receptors, and most pyramidal cells appear to express both types of receptor. Impaired functioning of the prefrontal cortex correlates with cognitive dysfunction seen

in schizophrenia (Moghaddam and Gruen, 1991). The theory of dopaminergic 'hypofrontality' in schizophrenia is supported by neuroanatomical and blood flow imaging studies and the finding of behavioral correlates (Weinberger, 1987). A reduced number of D_1-like receptors in the prefrontal cortex may underlie cognitive deficits and negative symptoms of schizophrenia (Crow, 1980). The mPFC and limbic structures are very important in the context of learning and memory and are implicated in drug abuse (Berke and Hyman, 2000 for review).

1.5.4. Nigrostriatial System

The substantia nigra (SN) consists of neuronal cell bodies (A8/9) in the midbrain with large pigmented dopaminergic neurons and provides dense dopaminergic innervations to the caudate and putamen (Parent, 1990). The SN consists of the DA-rich pars compacta and the pars reticulata with sparsely distributed cells (Alexander and Crutcher, 1990). The pars compacta fibers project to the caudate nucleus and the putamen, constituting the nigrostriatal tract (Mogenson et al. 1980; Parent, 1990). The nigrostriatial dopaminergic projection is intimately involved in the initiation and smooth execution of motor activities and forms part of the extrapyramidal system of the basal ganglia (Parent, 1990). Blockade of DA receptors by neuroleptic drugs results in extrapyramidal side effects such as tardive dyskinesia and akathisia (Burt et al., 1977).

1.6. Excitatory Amino Acids: Neuchemistry and Receptors

1.6.1. Glutamate as a Neurotransmitter

Glu is the most widely distributed excitatory transmitter in the brain and is probably involved in most aspects of normal brain function (Uhl, 1999). In contrast to monoamine neurotransmitters such as DA, EAA neurotransmitters are present in virtually all areas of the brain. Well-studied neurons that utilize glu include pyramidal cells in the cerebral cortex and in the hippocampal formation, cerebeller granule cells, and primary sensory afferents (Szabo et al., 2004) as shown in figure 1.5. Fast excitatory neurotransmission in the brain is usually subserved by GluRs that is characteristic of ligand-gated channels (Dingledine and McBain, 1999). In addition to its crucial role in fast excitatory signaling, glu is also important in synaptogenesis, synaptic plasticity, and long-term potentiation (LTP) related to learning (Conti and Weinberg, 1999).

Activation of NMDA receptors is essential for the induction of LTP that occurs in the hippocampus (Cheramy et al., 1998). Glutamatergic agonists administered locally in the NAS induce behavioral responses; glu receptors have been named for their pharmacological agonists: (NMDA, kainate, and α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) (Conti and Weinberg, 1999; see table 3).

Glu has been implicated in an increasing number of neurological and psychiatric disorders (Tamminga, 1999). Given the complex molecular structure and multiple binding sites of GluRs, it is known that behavioral effects of NMDA receptor antagonists are very diverse and depend on their action on specific sites on the receptor (Tzschenke and Schmidt, 2000).

Although acting as an excitatory transmitter in different neurons, glu is the immediate precursor of the inhibitory transmitter gamma-aminobutyric acid (GABA). GABA is derived from glucose, with α-ketoglutarate formed by the Krebs (tricarboxylic acid) cycle being transaminated to glu by GABA oxoglutarate transaminase (GABA-T) (Nedergaard et al., 2002). The key step for the generation of the transmitter pool of GABA is the enzymatic action of glutamic acid decarboxylase (GAD), which converts glu to GABA (Ganong, 2001). Glu can also be formed directly from glutamine in glial cells (Conti and Weinborg, 1999). Release of glu from terminals is regulated by autoreceptors, and this function is subserved by G-protein-coupled metabotropic GluRs (Dingledine and McBain, 1999).

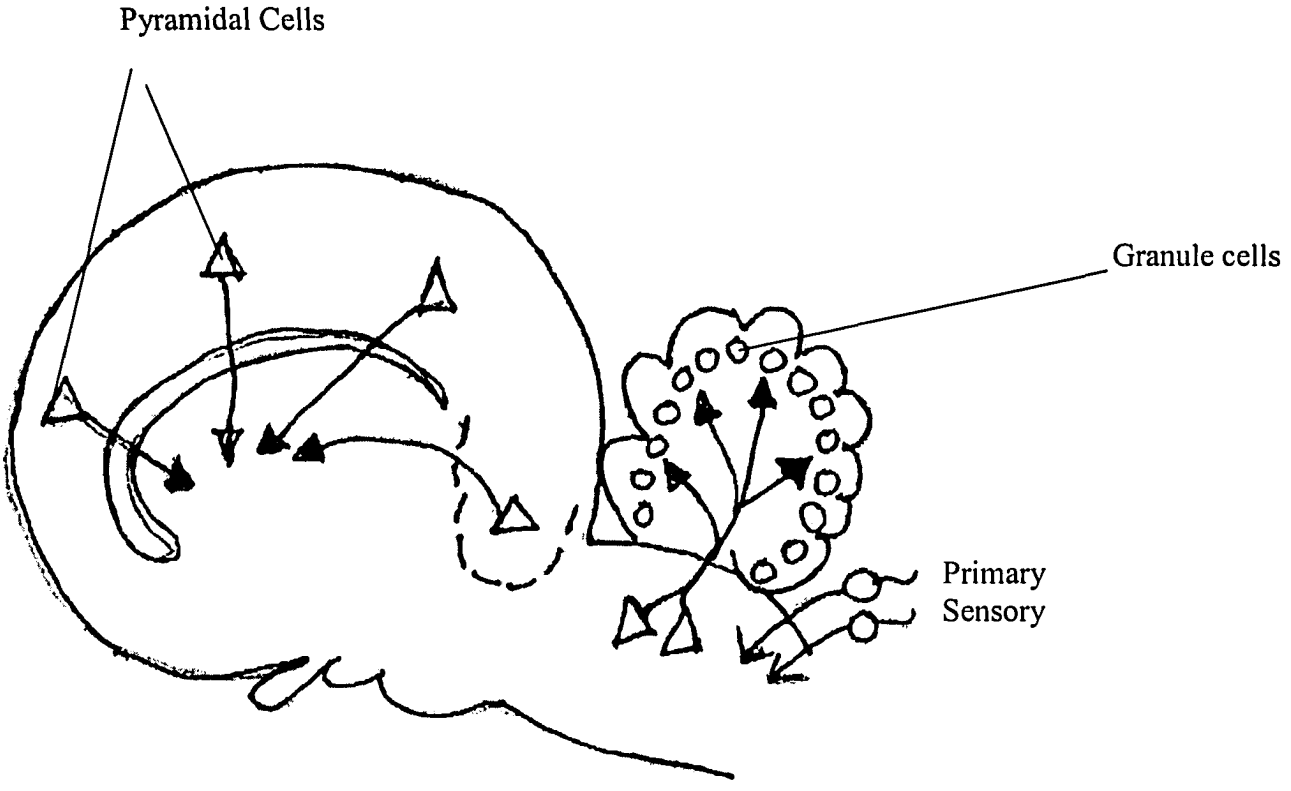


Figure 1.5. Schematic drawing of the major glutamatergic pathway in the brain. Adapted from Hyman and Coyle, 1996.

1.6.2. Glutamate Receptors (GluRs)

The term GluR refers to all EAA receptors because of its prominent role among all other EAAs. Cloning studies have identified multiple subtypes of GluRs and these are broadly classified into ionotropic and metabotropic receptor families on the basis of their mechanism of action (Malenka and Nicoll, 1993). One family of GluR is activated by the analog NMDA and these (NR1, NR2A, NR2B, NR2C and NR2D) are collectively known as NMDA receptors (Ozawa et al., 1998). Other GluRs are activated by α-amino-3-hydroxy-5-methylisoxazole propionic acid (AMPA) or by kainate, and are known as AMPA and kainate receptors respectively. The NMDA GluR has much higher affinities for glu and is more slowly inactivated than the AMPA/kainite receptors (Young and Bradford, 1986). Besides their abundance in the cortex and hippocampus, both classes of receptors are said to be present in the NAS and VTA where they modulate release of DA (Morari et al., 1998). Normally under resting membrane potentials, NMDA receptors are blocked by Mg^{2+}. Activation of NMDA receptors not only requires binding of synaptically released glu but simultaneous depolarization of the postsynaptic membrane removes Mg^{2+} blockade (Malenka and Nicoll, 1993).

The NMDA receptor also may be involved in the development of susceptibility to epileptic seizures and neurotoxicity in the presence of excess amounts of glu (Hicks and Conti, 1996). The NMDA receptor is unique as it has binding sites for glu and glycine. For activation of the receptor both glu and glycine must act in concert to open the ion channel (Malenka and Nicoll, 1993). Aspartate appears to recognize only the NMDA receptor and is inactive at AMPA and probably at kainate receptors (Dingledine and McBain, 1999).

GluRs that activate G-proteins are currently referred to as metabotropic GluRs (mGluRs). NMDA receptors are unique in that their channel can permit both Na^+ and Ca^{2+} entry and is blocked by Mg^{2+} at resting membrane potential (Ozawa et al., 1998; Moghaddam et al., 1997).

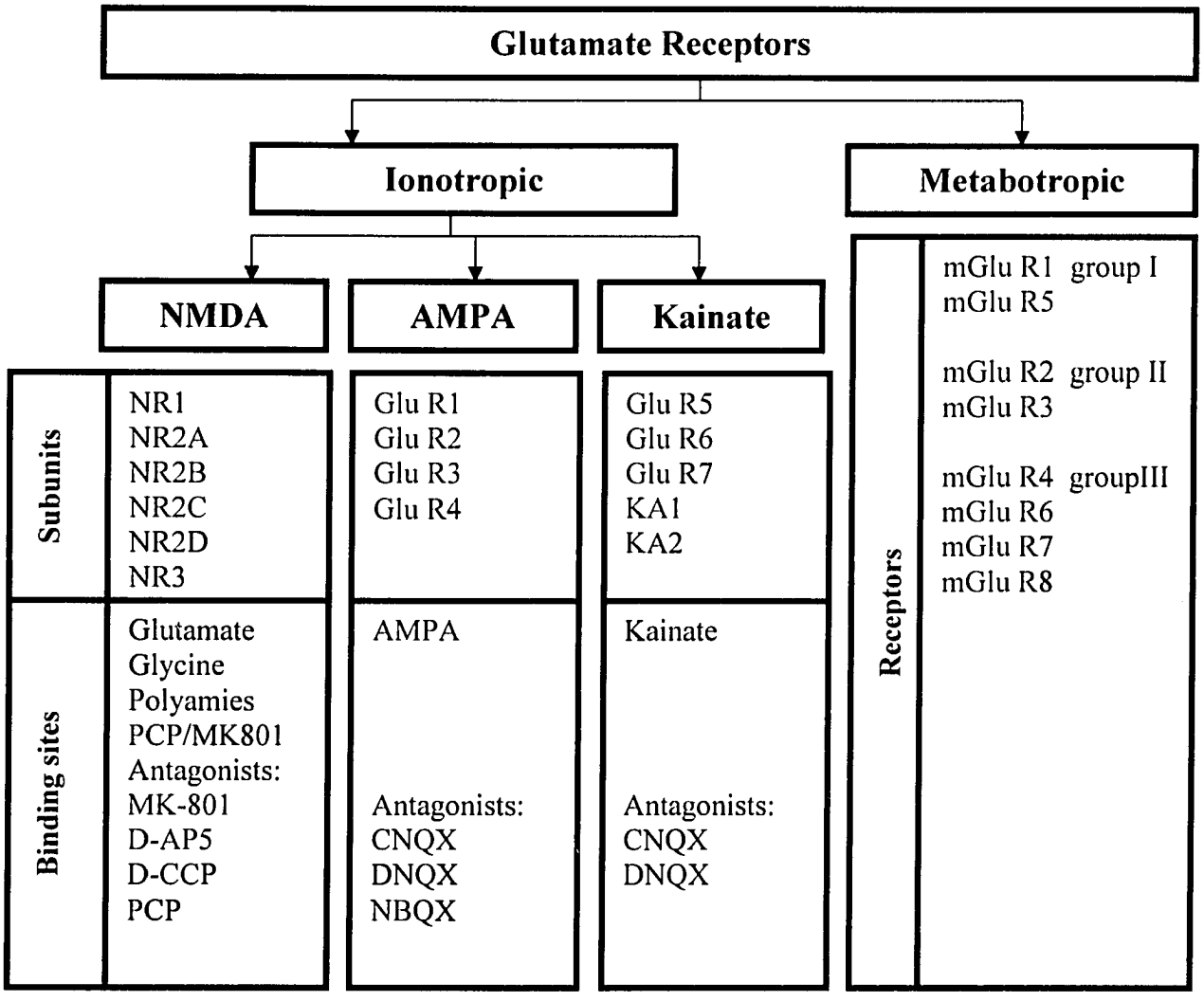


Table 3. Categorization of Glu receptors and their antagonists. Adapted from Meador-Woodruff and Kleinman, 2002.

1.6.3. Glutamate Transporters

Although glu is a major mediator of excitatory signals in the mammalian CNS, excess activation of GluRs is harmful, and glu is thus toxic in high concentration (Ozawa et al. 1998). Glu reuptake is the mechanism responsible for long term maintenance of physiologic concentrations of glu in the synaptic cleft and this uptake process is maintained via specific high affinity plasma membrane bound glu transporters (Danbolt, 2001). Five such transporters have been characterized (figure 1.7.) in the mammalian CNS, and include: the glu-aspartate transporter (GLAST), glu transporter-1 (GLT-1), and EAA transporter (EAAT1, EAAT4 and EAAT5 (Conti and Weinberg, 1999; Danbolt, 2001). Glu transporters are present both in neurons and astrocyte glial cells (Malenka and Nicoll, 1993). Astrocytes remove glu from the extracellular space and convert it to glutamine via the glu synthetase enzyme. Glu is then transferred to neurons and reused as substrate for the synthesis of glu (Melchitzky et al., 2004).

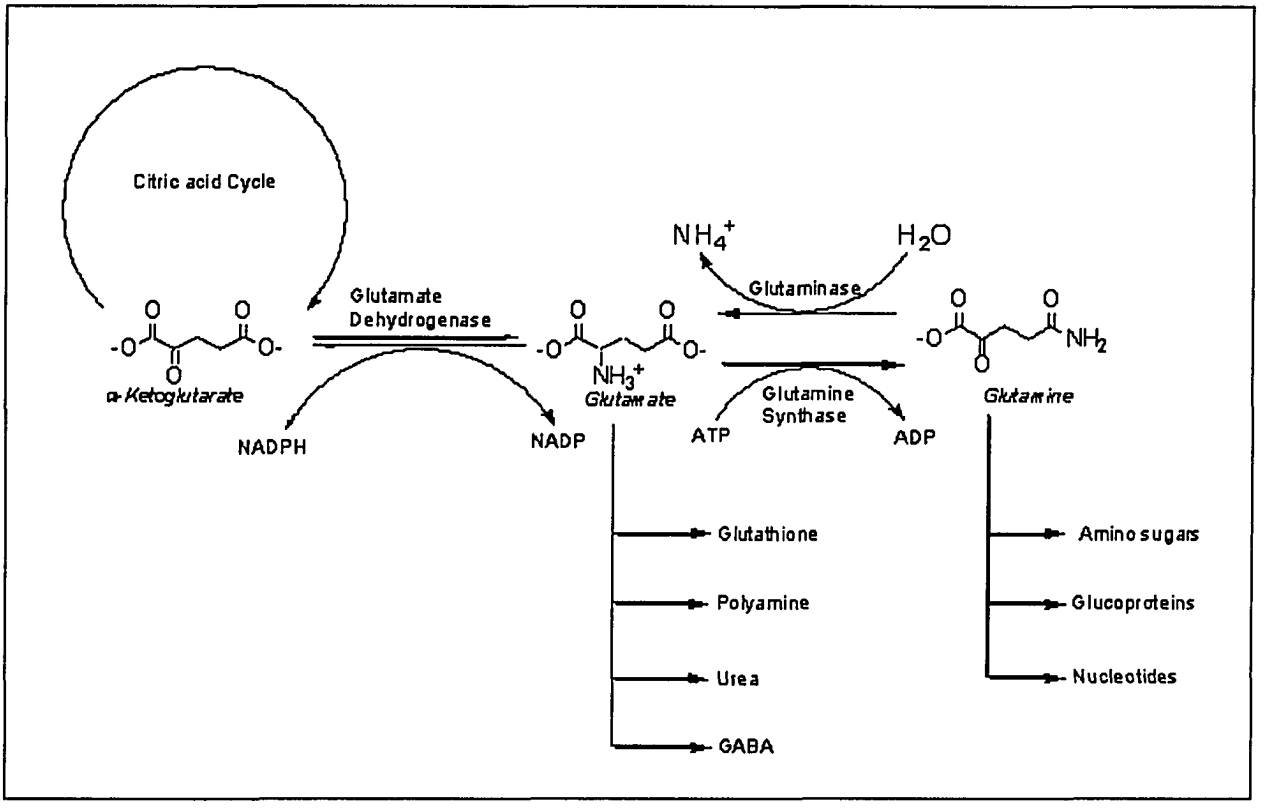


Figure 1.6. Chemical pathway of Glu synthesis. Adapted from Nedergaard et al., 2002.

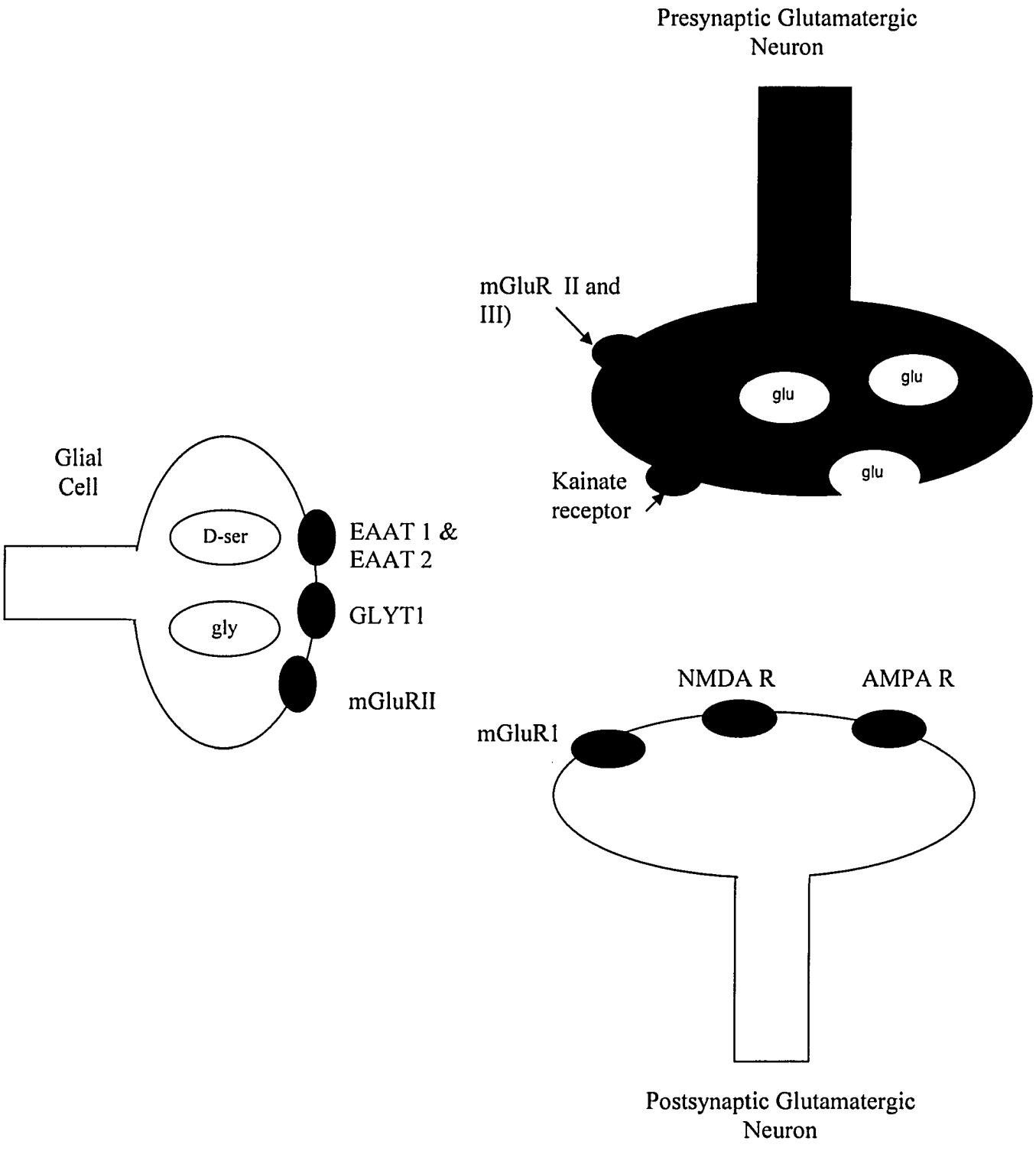


Figure 1.7. Schematic drawing of a typical glutamatergic synapse. Adapted from Meador-Woodruff and Kleinman, 2002.

1.7. Dopamine-Glutamate Integrative Neural Processes in Locomotor Activity and Reward

The DA system in the VTA and its axonal projections to the NAS plays a critical role in the mediation of the incentive and locomotor stimulating properties of psychomotor-stimulant drugs such as amphetamine and cocaine (Koob, 1992; Yamamoto and Davy, 1992). Extensive glutamarergic projections from the prefrontal cortex to NAS are implicated in the regulation of locomotor activity by interacting with dopaminergic neurotransmission (Wu et al 1993; Vanderschuren and Kalivas, 2000) as shown in figure 1.9. For example, agonists of glutamatergic ionotropic receptors, such as AMPA, kainic acid, and NMDA, stimulate locomotor activity when injected into the NAS, and drugs that interfere with dopaminergic neurotransmission inhibit these glu-mediated effects (Burns et al., 1994: Vasiliadis et al. 1999). This anatomical arrangement provides the basis for a possible interaction between DA and glu at the level of nerve terminals in the NAS.

Glu involvement in the regulation of locomotor activity is also inferred from observations that infusions of GluR antagonists into the NAS attenuate both the locomotor and DA-activating effects of psychomotor-stimulant drugs (Pulvirenti and Koob, 1994; Moghaddam and Bolinao, 1994).

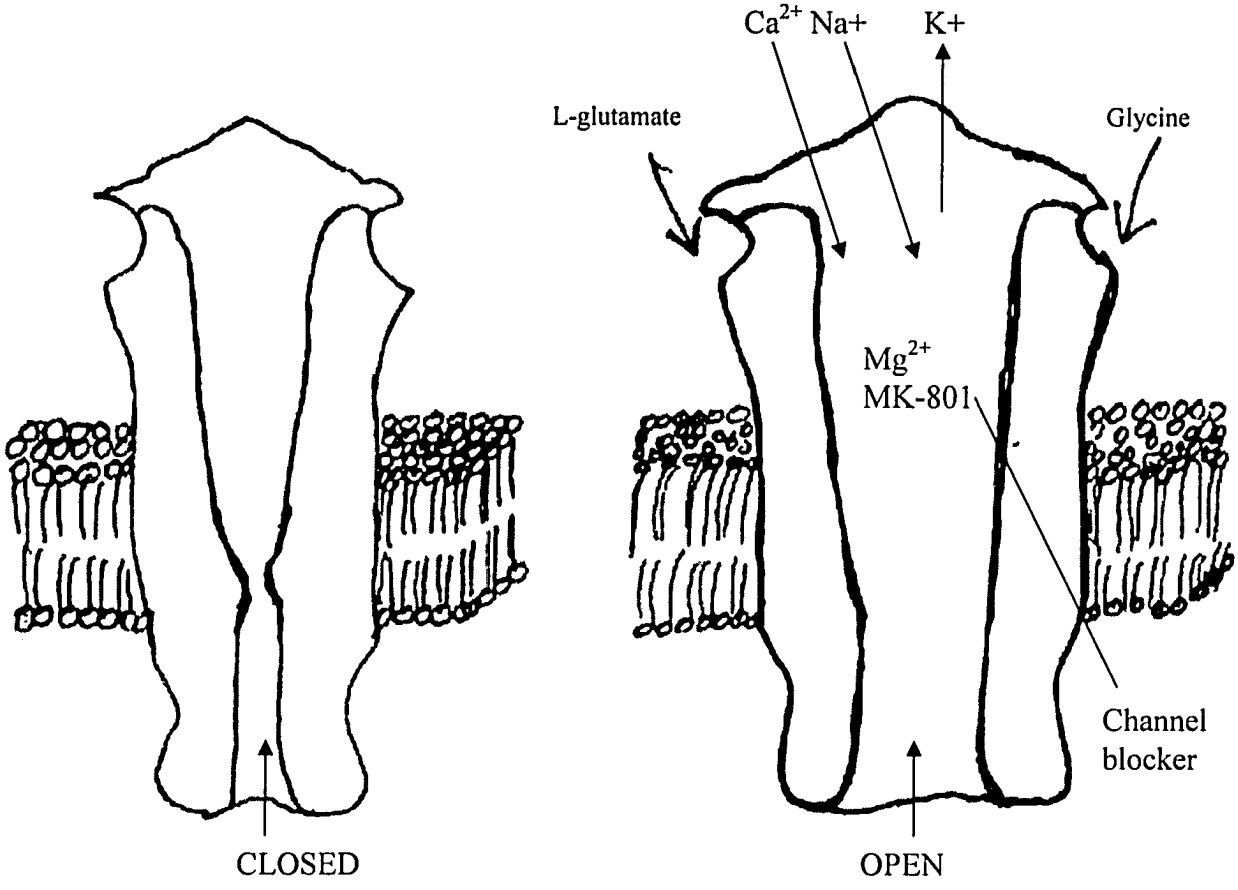


Figure 1.8. Diagrammatic representation of the NMDA receptor in open and closed state. Adapted from Ganong, (2001).

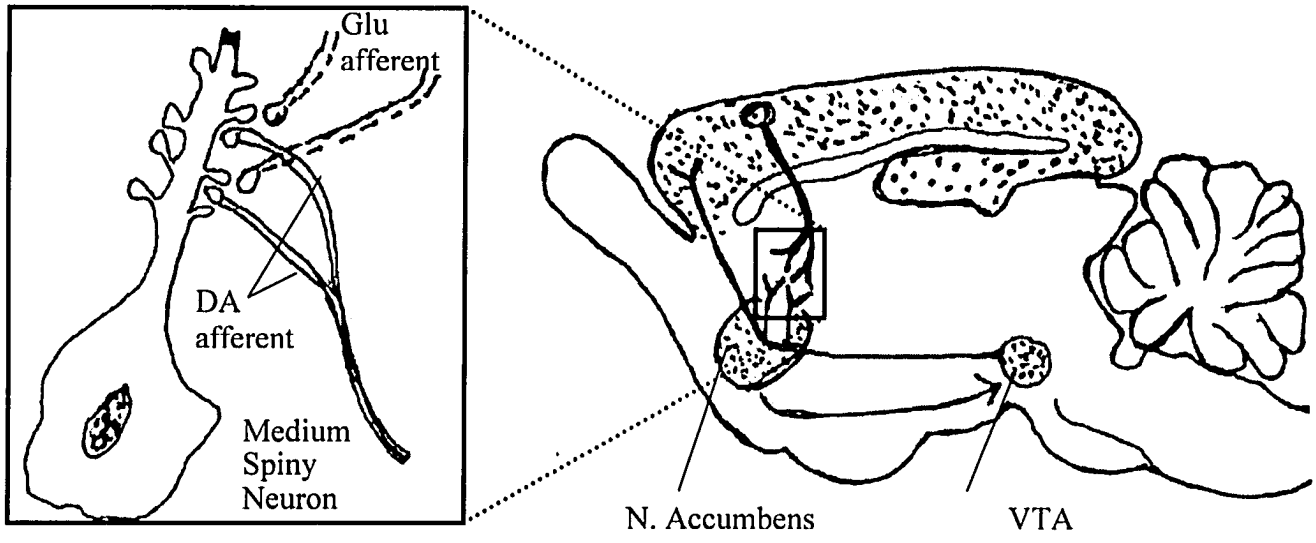


Figure 1.9. GABA-DA-Glu interaction in the striatum through GABAergic medium spiny neurons. Adapted from Hyman and Malenka, 2001.

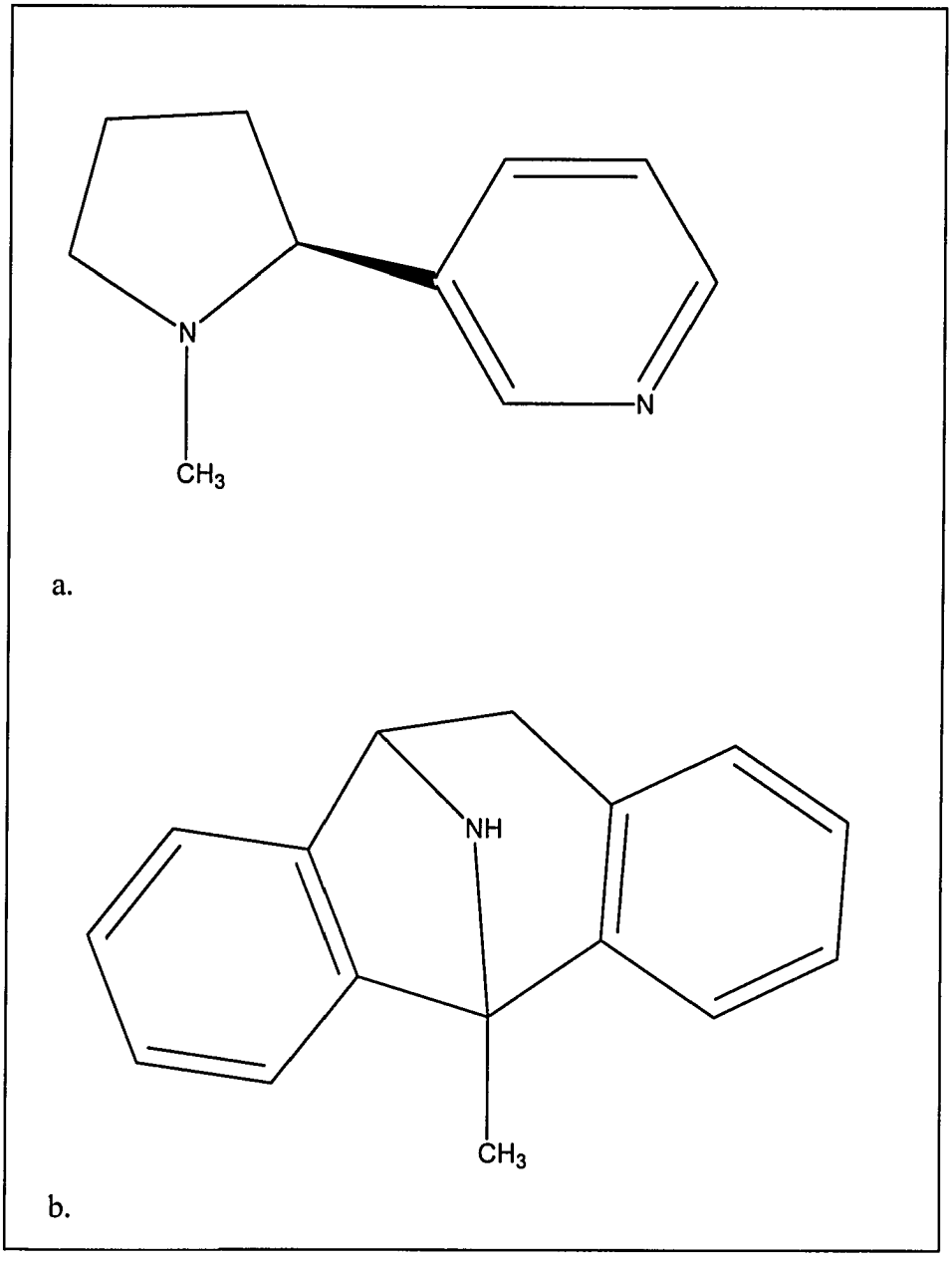


Figure 1.10. Chemical structures of (a) nicotine and (b) MK-801.

1.8. Nicotine: An Indirect Dopamine Agonist

1.8.1. Introduction

It is now widely recognized that tobacco-smoking behavior is maintained due to the presence of the psychostimulant compound, nicotine in the inhalant smoke (Stolerman and Shoaib, 1991; Yeomans and Bapista, 1997). Although nicotine (figure 1.10) is considered the principal ingredient for smoking behavior, other constituents of cigarettes and products of their combustion may also be injurious to health. Therefore, in a broader sense cigarette smoking and nicotine should not be considered synonymously (personal communication, Greenshaw, 2001). Nicotine has a wide variety of effects in humans and laboratory animals. Some of these effects are presumably important for maintaining nicotine self-administration behavior through tobacco smoking (Corrigall and Coen, 1989; Stolerman and Jarvik, 1995); other effects may have therapeutic utility (Mirza and Stolerman, 1998).

The neural mechanisms that mediate the effects of nicotine are complex and not fully understood. Nicotine seems to share properties with many of the drugs of abuse such as cocaine and amphetamine (Clarke and Kumar, 1983; Balfour et al. 1998). In particular, nicotine has been shown to stimulate the mesolimbic DA system, which is critically involved in the reinforcing effects of addictive drugs (Benwell and Balfour, 1992, Balfour et al., 1998). In the brain, effects of nicotine are mediated by the nAChRs, a subset of acetylcholine receptor. Recent findings on the structural and functional diversity of nAChRs in the brain have led to numerous attempts to clarify their specific role. Patients with mental illness have a higher incidence of smoking than the general population; the incidence of smoking is highest in schizophrenics. The incidence of

smoking is also higher in patients with depression or anxiety disorders than in the general population ((Mirza and Stolerman, 1998). These correlations are likely due to common mechanisms underlying both substance abuse and many psychiatric disorders, and smoking may be an attempt to self-medicate a neurochemical abnormality (Koelega, 1993; Levin and Simon, 1998). Also, in recent years, there has been a great deal of interest in developing nicotine analogues or agonists for therapeutic use (Mirza and Stolerman, 1998). Nicotine has cognition-enhancing effects in both humans and animals (Levin, 1992). Possible therapeutic applications of nicotine may include enhancing attention, memory, and cognition in illnesses of dementia such as Alzheimer's disease, depression, preventing immobility associated with Parkinson's disease, alleviating pain, promoting of weight loss (Paterson and Nordberg, 2000).

The reinforcing properties of nicotine resemble closely those of d-amphetamine and cocaine in a number of important aspects, particularly the locomotor response and conditioned reward effects (Birrel and Balfour1998; DiChiara, 2000 for review). Experimental impairment of DA function by 6-hydroxyDA lesions or by DA receptor antagonists show that DA is involved in nicotine-induced behavioral effects of nicotine self-administration, and of CPP (Bardo et al., 1999; Di Chiara, 2000, for review). Therefore, behavioral effects of nicotine that are most relevant for its reinforcing properties are largely dependent on mesoaccumbens DA (Imperato et al. 1986). Nicotine has been shown to co-stimulate NMDA glutamatergic receptors by increasing extracellular levels of glu (Toth et al., 1993; Reid et al., 2000). Although the molecular mechanisms underlying the pharmacological responses to cocaine and d-amphetamine differ from nicotine, repeated exposure to these three drugs involves enhancement of

locomotor activity and DA overflow in the NAS (Museo and Wise, 1990; Burns et al. 1994; Dluzen and Anderson, 1998). This evidence suggests that the mechanisms for the development of sensitization of nicotine and amphetamine are identical and involve similar neural substrates in the CNS (O'Neill et al, 1991; Stolerman and Shoaib, 1991; Pidoplichko et al., 1997). Neuroplasticity subserving learning and memory mechanisms is considered to be involved in psychostimulant-induced sensitization, and addictive behavior and nicotinic receptors in the brain play an important role in reinforcement of learning and memory (Berke and Hyman, 2000; Hyman and Malenka, 2001). nAChRs seem to be involved in the neuroadaptations induced by other stimulants such as amphetamine and cocaine (Schoffelmeer et al., 2002; Kelly, 2002.).

Neuronal nAChRs are typical ligand-gated ion channel receptors which are widely distributed in the brain (Taylor and Brown, 1999) and nAChR activation is considered to play a central role in elevated levels of DA and glu, and in the process of addiction for many other drugs of abuse in addition to nicotine (Kelly, 2002; Reid et al. 2000).

1.8.2. Nicotinic Acetylcholine Receptors: Classification and Distribution

AChRs have been divided into two main types on the basis of pharmacological properties: muscarinic and nicotinic receptors. Autoradiographic studies indicate that nAChRs are widely distributed in the mammalian CNS (Clarke and Pert, 1985); many subtypes of these nAChRs exist with distinct pharmacological and functional profiles (Paterson and Nordberg, 2000). The nAChRs are made up of multiple subunits coded by different genes; each nAChR is made up of five subunits made from a menu of 16 known subunits (α_1- α_9, β_2-β_5, γ, δ, and ε) coded by 16 different genes. The neuronal nAChRs in

the brain are made up of a variety of different subunit combinations, i.e. α_2 to α_9 and β_2 to β_4; making a multitude of different subtypes of neuronal nAChRs (Karlin and Akabas, 1995; McGehee and Role, 1995; Gotti and Clementi, 2004) as shown in figure 1.11 below; α_1 and β_1 subunits are expressed in muscle. Diverse neuronal nAChR receptors subtypes have different agonist-binding affinities and electrophysiological responses (McGehee and Role, 1995). Diverse nAChR subtypes are formed from different combinations of genetically distinct subunits. Individual neurons also express multiple types of nAChRs in different combinations (Dani et al. 2001; Paterson and Nordberg 2000). When nicotine ligand binds to its receptor, there are conformational changes in the receptor subunits, resulting in an activated state with an opening of the ion channel followed by a desensitized state with the channel closed (Sandor et al. 1991; Picciotto et al. 1998). Many of the nAChRs in the brain are located presynaptically on glutamatergic axon terminals and they facilitate the release of glu (Reid et al., 2000).

nAChRs, especially receptors containing the β2 subunit, play important roles in mediating the reinforcing properties of nicotine; genetically knocked out mice lacking the β2 subunit failed to exhibit nicotine reinforcement following systemic administration of nicotine (Picciotto et al., 1998). Muscarinic cholinergic receotors are very different from nicotinic cholinergic receptors; five types of muscarinic receptors (M_1-M_5) are encoded by five separate genes; the M_1 receptor is abundant in the brain (Ganong, 2001). Muscarinic receptors are G-protein-linked with a slower onset and offset of action compared with nAChRs. By contrast, nAChR action is predominantly excitatory, and excitation occurs very quickly within a few milliseconds (Picciotto et al., 1998; Gotti and Clementi, 2004).

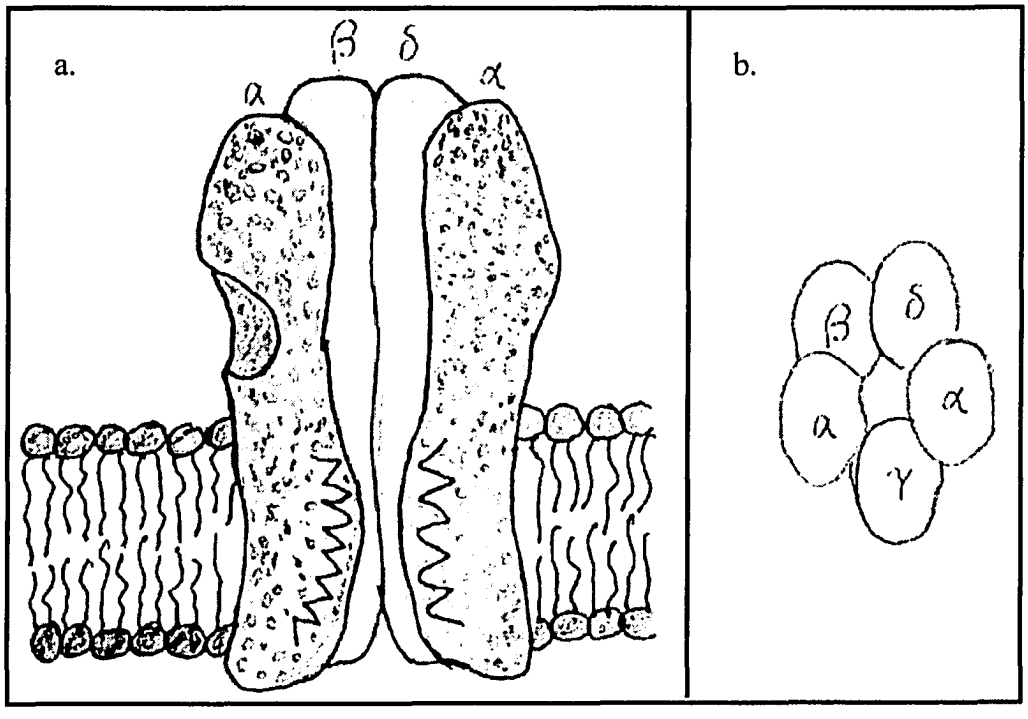


Figure 1.11. (a) Diagrammatic representation of a AChR; (b) orientation of 5 subunits of AChRs around the pore. Adapted from Uhl et al., 1999.

1.8.3. Effects of Nicotine on Midbrain Dopamine Systems and Prefrontal Cortex

Nicotinic receptors abound on both the cell soma and the terminal membranes of the mesolimbic and nigrostriatal DA neurons (Clarke and Pert 1985). Nicotine increases the firing of midbrain DA neurons both in vitro and in vivo (Pidoplichko et al. 1997). Intracranial or systemic administration of nicotine elevates extracellular DA levels in the striatum and NAS of rats (Imperato et al., 1986; Di Chiara, 2000). DA neurons, which project to the NAS, have been found to be more sensitive to the stimulatory effects of nicotine than those which innervate the dorsal striatum (Benwell and Balfour 1992). Nicotine-induced enhancement of DA release has also been observed in the prefrontal cortex (Nisell et al. 1994). Shorter treatments with once daily nicotine injections for only 5 days were shown to produce enhancement (sensitization) of nicotine-induced DA release in the NAS (Benwell and Balfour, 1992; Miller et al., 2001). Also, the development of sensitization seems to be dependent on co-stimulation of NMDA glutamatergic and nAChR receptors as the receptors are co-located (Moghaddam et al., 1997; Kelley, 2002).

1.8.4. Effects of Nicotine on Reward and Reinforcement: Relevance of Locomotor Activity

Nicotine elicits drug-seeking behavior in animal studies, as demonstrated by self-administration and place-preference experiments (Di Chiara, 2000). The conditions under which nicotine is self-administered by animals appear to be more restricted than for many other drugs of abuse. Intravenous self-administration of nicotine is best demonstrated

under conditions of limited availability (Henningfield and Goldberg, 1983). Stimulation of DA systems appears to be of critical importance for the acute positive reinforcing properties of nicotine. DA receptor antagonists attenuate nicotine self administration (Corrigall and Coen, 1989), and lesioning of the DA neurons projecting from the VTA to the NAS with the neurotoxin 6- hydroxydopa markedly attenuate nicotine self-administration as compared with control animals (Corrigall et al. 1992). Also, microinfusions of the nAChR antagonist dihydro-beta-erythroidine (DHßE) into the VTA produced a significant dose-dependent decrease in nicotine self-administration (Corrigall et al., 1994). These data strongly indicate that the mesolimbic DA system is a substrate in nicotine reinforcement, and that nicotine activates this system through the VTA. This is supported by the fact that nicotine mediated hyperactivity is considered as a DA mediated phenomenon, through release of DA.

Nicotine has variable effects on locomotor activity, depending upon factors such as dose, baseline activity, habituation to the environment, and duration of treatment. There is a good correlation between locomotor sensitization and enhancement of DA release in the NAS in response to repeated nicotine injections (Benwell and Balfour, 1992).

1.8.5. Nicotine Receptor Abnormality and Schizophrenia

Smoking rates among schizophrenic patients are estimated to be between 40 and 100%, and schizophrenic patients appear to self-administer more nicotine than the general population (Cornish et al., 1999). Some investigators have speculated that nicotine may modulate some symptoms of schizophrenia, including cognitive dysfunction. There have been numerous demonstrations of the beneficial effects of nicotine on learning and

memory from animal studies (Levin, 1992). Memory disorders are the most common cognitive deficits experienced by schizophrenic patients. Nicotine may also enhance some cognitive processes in schizophrenia. This high level of smoking in people with schizophrenia may be linked to abnormalities in the nicotinic-cholinergic system and associated receptors.

Given beneficial effects of nicotine in learning and memory in both animal and human studies and role of α7 nicotinic receptors in auditory gating, nAChR agonists would seem to be reasonable candidates for the treatment of cognitive and perceptual disturbances in people with schizophrenia. Some of the nAChR agonists are being tested in clinical trials (Levin and Simon, 1998; Jones and Benowitz, 2004).

1.8.6. Nicotine Withdrawal

Nicotine is considered a strongly reinforcing substance, which is further evidenced by its robust withdrawal effects. The nicotine withdrawal syndrome in both humans and rodents has somatic and affective aspects (Kenny and Markou 2001). In rat, withdrawal from continuous nicotine infusion involves abstinence symptoms such as shakes, ptosis, teeth chatter, and changes in locomotor activity (Malin et al., 2001; Hildebrand et al., 1997; Epping-Jordan et al., 1998). This syndrome was observed during the first 2 days of withdrawal, and alleviated by a single dose of nicotine. A decrease in DA output in the NAS and amygdala was observed in chronically nicotine-treated rats when treated with nAChR antagonists (Hildebrand et al., 1997; Nomikos and Spyraki, 1988). Humans generally consume nicotine by smoking or other means over a period measured in years. Therefore, it is important to also treat experimental animals for a 5 weeks or more period

to observe the withdrawal effects of nicotine (Brown and Kolb, 2001). A significant drop in locomotor activity on the first day of withdrawal and an increase in weight gain were also observed (Malin et al. 1992). In addition, a nicotine abstinence syndrome was also precipitated by administration of various nAChR antagonists such as mecamylamine and DHßE to rats after chronic nicotine treatment (Hildebrand et al., 1997; Malin, 2001). Spontaneous and precipitated nicotine withdrawal results in elevations in brain reward thresholds, which reflect diminished sensitivity to rewarding stimuli, similar to that seen with other drugs of abuse (Epping-Jordan et al., 1998). Similarly, nicotine-induced increases in VTA electrical self-stimulation thresholds can be blocked by haloperidol and mecamylamine (Ivanova and Greenshaw, 1977). In addition, precipitated nicotine withdrawal induces decreased tissue levels of DA in the striatum and NAS; such changes have been found during spontaneous nicotine withdrawal in rats (Fung, 1996).

1.9. Methodological Aspects of Measuring Reward in Drugs of Abuse in Animal Models

The connotation of reward or reinforcement (often used synonymously) has its origin in the early works of Pavlov and Skinner. Conditioned place preference, is a variant of Pavlov's conditioning paradigm and represents a measure of incentive stimuli (Martin-Iverson et al., 1985). Stimulant drugs increase locomotor activity, which is used as a measure of stimulant or reinforcing effects of a rewarding drug (Wise and Bozarth, 1987).

1.9.1. Locomotor Activity

Results of a number of experiments have shown that rats previously exposed to amphetamine or cocaine subsequently showed enhanced locomotor responses.

The most widely used test to measure rewarding effects of a drug is determination of locomotor activity (Wise and Bozarth, 1987; Berridge and Robinson, 1998). Although the relationship between locomotor responses and reward and addiction remains a matter of debate, locomotor responses are thought to be mediated by the mesolimbic DA system (via nigrostriatal projection), which is also implicated in reward and addiction (Benwell and Balfour, 1992; Di Chiara, 2000). These findings are consistent with the view that repeated administration of a stimulant drug enhances sensitization of midbrain DA neurons and promotes their responsiveness when challenged subsequently (Stolerman and Jarvik, 1995).

The relevance of locomotor responses and reward is also evidenced by the ability of neuroleptic agents to consistently decrease stimulant induced locomotor activity and reduce reward-seeking behavior (Costall et al., 1984).

Although both locomotor activation and rewarding effects have been reported with nondopaminergic drugs like PCP, the stimulation produced by these compounds seems to be accomplished through indirect activation of the mesolimbic DA system (French and Vantini, 1984; Koob, 1992; Kato and Niwa, 2000).

1.9.2. Conditioned Place Preference:

Conditioned place preference (CPP) is a direct measure of drug reward where an animal learns to prefer an environment that was paired with drug exposure (Spyraki et al., 1982; van Der Kooy, 1983). Conditioned place preference is also mediated partly by the mesolimbic DA system and is thought to model some of the powerful conditioning effects of drugs that are seen in humans (van Der Kooy, 1982). This experimental procedure provides an animal model that expresses the subjective effects of a drug. In CPP a drug is injected and the subject is placed in a test chamber with distinctive environmental cues; the procedure is repeated for several days (Bozarth, 1987). During these conditioning trials the animal develops an association between the subjective state produced by the drug (e.g., reward comparable to mood elevation and euphoria in humans) and the environmental cues where the animal enjoyed the euphoria in the drug state (Carr and White, 1983). When the subject is tested in an apparatus that contains the drug-related environmental cues in one compartment and dissimilar cues in another compartment, the animal tends to move toward the compartment with drug-related cues

as experienced previously (Spyraki et al., 1982; Bardo and Brevins, 2000). This learned integration of environmental stimuli and the effect of a rewarding drug in a behavioral repertoire provide the basis for CPP experiments.

Although the CPP method does not directly measure drug reinforcement, the concordance between CPP and intravenous self-administration and locomotor hyperactivity studies provides a fairly comparable measure of reward (Tzschenke, 1998; Bardo et al., 1999).

1.9.3. Novelty Seeking and Conditioned Place Preference: Biased and Unbiased Design

Environmental novelty is an important consideration in behavioral studies, including CPP. Besides the need to balance pairing between the two dissimilar environmental cues in a CPP apparatus, another important behavioral issue is the novelty effect that might compromise the validity of CPP as a measure of reward and reinforcement. Both humans and animals have a natural tendency to search for novel stimuli (Bevins and Bardo, 1999). The experience of novelty is rewarding via the activation of the mesolimbic dopaminergic system (Rebec et al. 1997). It is reported that if a rat is given the opportunity to choose a novel versus a familiar environment, they will spend more time in the novel environment. Thus relative novelty alone can induce place preference for novel environmental cue (Bardo et al., 1984).

The preference for novel stimuli in a choice situation has been described as 'novelty seeking' whereas the term "neophobia" refers to an animal's avoidance of new food (Marcontell et al., 2003). In the counterbalanced design of CPP, animals will spend half of the conditioning time in the novel environment that might affect place preference after

conditioning (Phillips and Le Paine, 1980; Bozarth, 1987). CPP is done in both unbiased and biased designs. The biased design was developed to alleviate the novelty effect in while determining rewarding effects of stimulant drugs (Bozarth, 1987). Biased design provides a control over novelty, thus tends to produce robust CPP with rewarding stimuli (Marcontell et al., 2003; Bevins and Bardo, 1999).

1.10. Thesis Objectives

The experiments described in this thesis were designed to investigate DA-glu interactions in the ventral striatum in both conditioned reinforcement and locomotor activity. Previous research has shown that the coadministration of the glu NMDA receptor blocker MK-801 (dizocilpine) with drugs of addiction can block the expression of behavioral adaptations, such as locomotor sensitization, produced by repeated administration of nicotine (Schoffelmeer et al., 2002)..

These findings have been interpreted to represent a blockade of the development of these adaptations by inhibitory actions of MK-80 l on glu systems of the central nervous system. Furthermore, we sought to determine if there was any rewarding effect of MK-801 on its own (Vanderscuren et al., 1998) without the combination of nicotine that has been reported in various studies (Ranaldi et al., 2000; Spripada et al., 2001).

Glu receptor antagonists were administered to nicotine-sensitized rats, and the effects of these interactions on responding with conditioned reinforcement and locomotor activity were measured (Shoaib et al.1994). Nicotine was used as a DA indirect agonist that enhances DA as well as glu release in the brain and MK-801, a non-competitive NMDA receptor antagonist, was intended to block nicotine's locomotor activity (Svensson et al., 1992; Museo and Wise, 1990). No direct glu/NMDA receptor agonists were administered because nicotine is thought to release both DA and glu through interconnected mesocorticolimbic pathways (Mogensen et al., 1980; Kelly, 2002).

The objectives of this thesis were:

1. To replicate the induction of locomotor sensitization by repeated administration of nicotine.

2. To replicate the effectiveness of mecamylamine and haloperidol in blocking nicotine induced hyperactivity.
3. To investigate the effect of MK-801 on locomotor activity and CPP (a) alone, and (b) in nicotine-sensitized (e.g. nicotine and MK-801 combination) rats.
4. To investigate the effects of MK-801 and nicotine on CPP in both biased and unbiased designs to rule out any role of novelty effects.

2. MATERIALS AND METHODS

2.1. Drugs

(-)-Nicotine ([–]-1-methyl-2-[3-pyridyl] pyrrolidine) as the hydrogen tartrate, mecamylamine (2-[methylamino (isocamphane; N, 2, 3, 3-tetramethyl-bicyclo[2.2.1] heptan-2-amine]) as the hydrochloride, and haloperidol (4-[4-(p-chlorophenyl)-4-hydroxy-piperidinol]-4'-flurobutyrophenone) were obtained from Sigma Chemical Co., St.Louis, MO, USA. (+)-Amphetamine [S (+)-1-methyl-2-phenylethylamine sulfate] was obtained from SmithKline Beecham Pharmaceuticals, Mississauga, ON, Canada. Dizocilpine maleate: (5S, 10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzol [a,d]cyclohapten-5,10-imine maleate) was purchased from Tocris, Ellisville, MO., USA.

2.2. Animal Subjects

Male Sprague-Dawley rats (200-250 g) were obtained from Health Sciences Laboratory Animal Services, University of Alberta. The animals were individually housed in Plexiglas cages on wood chip bedding in a temperature-(21±l °C) and humidity-controlled environment with a 12-h light/dark cycle (7:00-19:00 h). The rats had free access to food and water in their home cages. They were fed with standard rodent chow (Lab-Diet 5001 Rodent Diet, PMI Nutrition International Inc. Brentwood, MO, USA) composed of 4% crude fat, 4.5% crude fibers and 24% crude protein. Procedures involving the use and care of rats in conducting all the experiments related to this thesis were carried out in accordance with institutional guidelines set forth by the University of

Alberta Health Sciences Animal Welfare Committee and the Canadian Council of Animal Care.

2.3. Locomotor Activity Measurements

2.3.1. Locomotor Activity Monitoring System: Photobeam Apparatus

The locomotor activity of the rats was measured by using photobeam activity boxes (I. Halvorsen System Design, Phoenix, AZ, USA). Six photobeam activity boxes interfaced with a microcomputer system were used to measure spontaneous locomotor activity. Each box consisted of a Plexiglas test cage (43 cm L x 43 cm W x 30 cm H) placed in two parallel infra-red grids (12 x 12 diode beams, Infra-red beam Grid Model 17-12) 2.5 cm above the floor as well as 12 vertical sensors 12 cm above the floor (Arnold et al., 1995).

Following systemic injection of intended drug or drug combinations, each animal was placed in the photobeam activity box and spontaneous locomotor activity was measured. Three locomotor activity measures were obtained based on the number of infra-red beam interruptions and comprised of: 1) ambulatory activity corresponding to the total number of beam breaks indicating all locomotor behavior, 2) vertical activity corresponding to the number of upper beam breaks indicating rearing behavior, and 3) consecutive activity corresponding to repetitive breaking of the same beam, representing stereotyped behaviors.

Throughout the experiment each rat was assigned to a particular locomotor activity box to maintain identical environmental cues and the boxes were cleaned with soap and water

between test intervals. Test sessions were conducted under red light illumination to induce higher basal activity in accordance with prior studies from this laboratory (Arnold et al. 1995).

2.3.2. Microcomputer Used

The behavioral measures were digitally recorded by a computer system (PC486) for temporal analysis of activity.

2.4. Conditioned Place Preference (CPP)

2.4.1. CPP Apparatus

Six CPP apparati (I. Halvorsen System Design, Phoenix, AZ, USA) were used. These place-preference apparatus units each consisted of a rectangular clear Plexiglas box with dimensions of 30L X 30W X 25 H (cm) partitioned into two compartments of equal size (figure 2.1). Sidewalls and the removable roof (ceiling) of each unit were transparent and identical; but the compartments were distinctive, with floors of dissimilar textures. The distinct floor cues served as conditioned stimuli (CS) and allowed rats to be in direct contact with a CS to experience its conditioned effect during preference testing (Martin-Iverson et al. (1985). One compartment had a grate floor consisting of 1-cm squares while the floor of other compartment contained 14 horizontal bars arranged in parallel 1.25 cm apart. The compartments were separated by an opaque plastic partition with a 7.5-cm long tunnel in the base. The tunnel had two removable doors, one on each end. Opening the doors allowed free movement of animals between the compartments through

the tunnel. The doors were closed for confinement of the animals in a single compartment for drug conditioning trials.

In the unbiased paradigm care is taken to choose conditioning stimuli that produce approximately equal preferences for the two sides of the test compartment with naive, untreated rats. This permits counterbalancing of drug pairing with one side of the conditioning box within a group and also obviates the need for pretesting subjects before training begins. In the unbalanced paradigm, subjects are pretested and usually show a substantial preference for one side of the test box. Drug pairings are then always on the least preferred side. Because counterbalancing is impossible, a separate control group is often run with vehicle injections to demonstrate that increases in time on the least preferred side are not as large with saline as with morphine (Bozarth and Wise, 1981; Katz and Gormezano, 1980; Phillips & Le Paine, 1980, 1982).

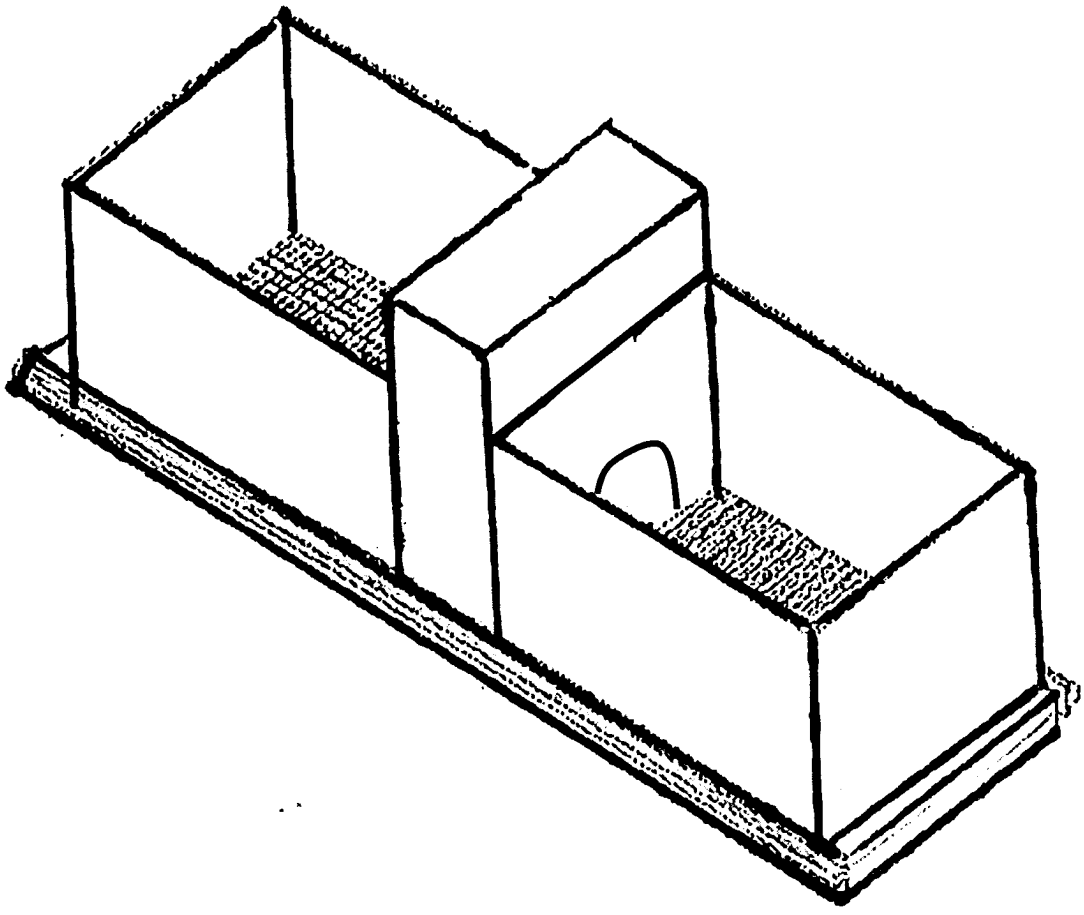


Figure 2.1. Schematic layout of the CPP apparatus. Modified and adapted from Kling-Peterson, 2000.

2.4.2. Behavioral tests procedures in CPP

The behavioral procedure consisted of three phases: Pre-exposure (phase 1), conditioning with drug-compartment pairings (phase 2), and post conditioning expression test (phase 3) as represented in figure 2.2.

Phase 1:

Prior to beginning the experiments, newly purchased rats were handled adequately to alleviate fear of human contact and to make them familiar to the examiner. The rats were taken out of their home cages, held on the palm wrapped in a towel and softly touched and stroked over the head and body in a playful manner for about 15 minutes for three days. Human contact was maintained throughout the experiment as the rats were handled before placing in CPP apparatus.

Afterward starting from 5^{th} day, the rats were laid on the floor of one compartment of the CPP apparatus in a counterbalanced order and were allowed to roam freely in both compartments through the tunnel. Time spent in each compartment and in the tunnel was recorded over a 15-minute period for three consecutive days. Thus, in this phase initial place preference was determined as the rats became acquainted with the CPP apparatus and could exhibit preference for a particular compartment of the CPP box.

Phase 2:

Place conditioning has been studied most commonly by adopting two experimental designs: unbiased and biased. In phase I it is probable that the naive rats when exposed to the CPP test box may exhibit approximately equal (Martin-Iverson, 1997) or unequal preferences (in our lab) for two sides of the CPP test compartment. In order to create an unbiased or a biased CPP design, rats were then randomly assigned to drug groups

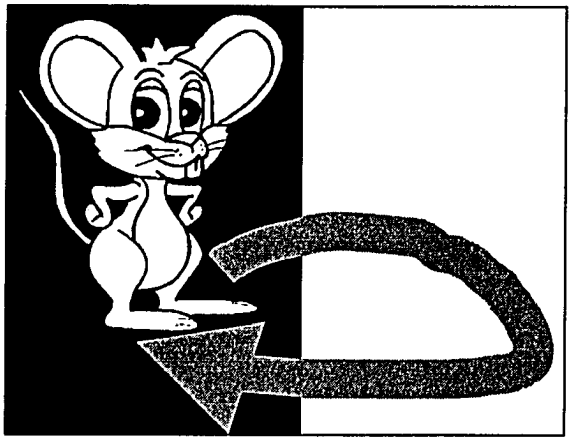
matched for baseline compartment preferences as determined in phase 1. In the unbiased design each group of rats may be counterbalanced in order to ensure that equal numbers of animals in each group received drug pairings and conditioning in both preferred and non preferred compartments.

In the unbiased paradigm of CPP, regardless of initial side preference in the CPP box, the conditioning of rats with drug or non-drug pairing is done in a counterbalanced manner. Each group was counterbalanced so that an equal number of animals received drug pairings in either compartment. Counterbalancing provided an opportunity to condition the animals to both compartments of the CPP apparatus. Thus the animals are conditioned to both compartments in both the drug and control states for an equal number of days. During this phase the animals were conditioned by injecting a drug or a non-drug substance (normal saline) followed by confining them in a CPP box for 30 minutes. Administration of drug or non-drug substances serves as a conditioning stimulus (based on Pavlovian conditioning). On alternate days, all rats were injected with vehicle prior to confinement in the second compartment for 30 minutes. This totaled 4 days of vehicle injections (days 1, 3, 5 and 7) and 4 days of drug injections (days 2, 4, 6, and 8). In the biased design the rats were given a drug or saline injection before confinement for conditioning, but unlike the unbiased design they were confined to their less preferred compartment (i.e. the compartment in which they spent less time) as determined in the initial place preference test. Thus in the biased design no counterbalancing procedure was followed.

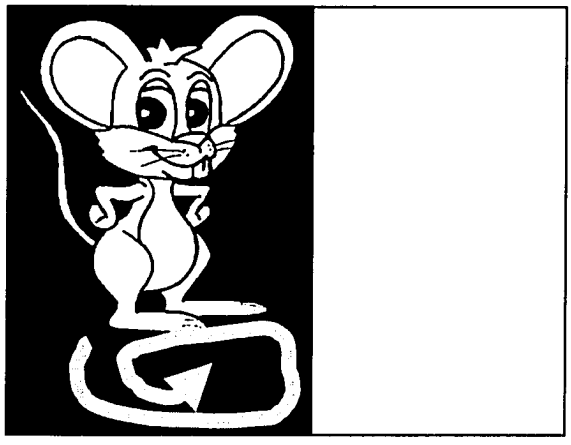
The duration of confinement for conditioning was 30 minutes each day for pairing with both drug and vehicle injections.

Phase 3:

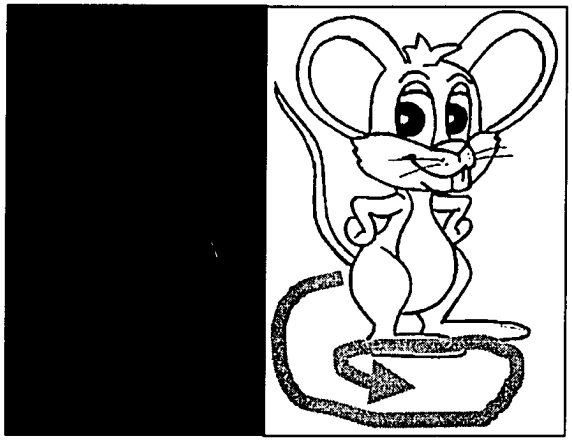
Following conditioning, animals were tested in a drug-free state but still having the effect and memory of their conditioning experience. This test was done by allowing free access of the rats to both compartments by removing the barriers on both entrances of the tunnel in CPP box. The amount of time spent in each side of the box and in the tunnel was recorded manually by a digital timer. Place preference was said to occur if the rats spent more time in the drug-paired compartment than in the vehicle-paired compartment. Throughout the experiment each animal was assigned to a particular CPP box. The boxes were kept free of urine and droppings by cleaning the cages between runs with ammonia based cleaning fluid (No Name, Club Pack, Glass Cleaner obtained from Superstore, Edmonton) that was diluted with four parts water to one part cleaner. Once started, all experiments were conducted on a daily basis under red light illumination between 9:00 A.M. and 7:00 P.M.



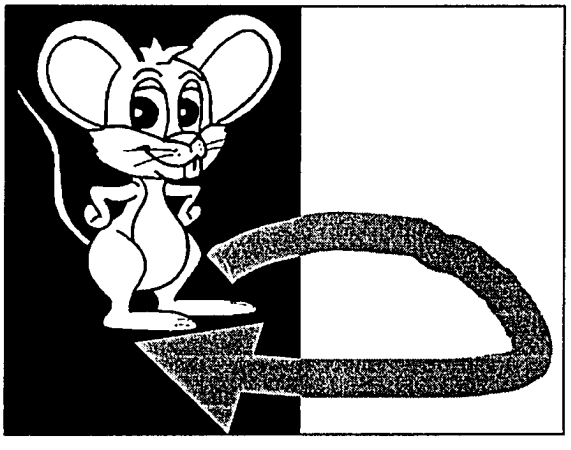
Days 1, 2,3
Baseline Preference



Days 4, 6, 8, 10
Drug Injection



Days 5, 7, 9 &11
Saline Injection



Days 12, 13 & 14
Postconditioning days

Figure 2.2. Simple version of the CPP paradigm. Adapted from Swerdlow et al., 1989.

2.5. Preparation of Drug Solutions

Vehicle and drug solutions were prepared on the day of injection. All the drugs used for systemic injection were dissolved in 0.9% saline (Fisher Scientific, Nepean, Ontario) and adjusted to pH 7.4 with sodium hydroxide. The drug was given as free base weight per kg body weight and the injection volume was 1 ml kg^{-1} body weight. Saline (0.9%) was used as the control solution.

2.6. Systemic administration of drugs

Drugs were administered either subcutaneously in the gluteal region or intraperitoneally in the mid-abdomen with a 1ml syringe (Becton Dickinson & Co, Rutherford, NJ, USA.) at appropriate intervals.

2.7. Statistics

Conditioned place preference data for nicotine, MK-801, amphetamine and the nicotine-MK-801 combination were analyzed using paired-sample t-tests with statistical significance based on a probability value of $P<0.05$. For locomotor activity, dose-response data were analyzed using two-way repeated measures ANOVA with Greenhouse-Geisser correction (drug x time), with a probability value of $P<0.05$ representing statistical significance. The finding of a significant F-ratio was followed by Turkey's HSD. Local time course data were analyzed using one-way ANOVA across all treatments at each 5-minute interval. The finding of a significant F-ratio ($P<0.05$) on any 5-minute interval was followed by a *post hoc* comparison of each drug treatment with vehicle using Turkey's HSD test with a significance criterion of $P<0.05$. All statistical analyses (except Tukey's HSD) were completed using SPSS 11.0 statistical software (SPSS Inc. Chicago, IL, USA) and Graph Pad Prism 3.0 (San Diego, CA, USA).

3. EFFECTS OF DIZOCILPINE MALEATE (MK-801) ON NICOTINE INDUCED LOCOMOTOR HYPERACTIVITY

3.1. Introduction

Repeated administration of psychostimulants such as nicotine, cocaine and amphetamine induces progressive increases in certain behaviors, including locomotor hyperactivity and reward-related behavior (Clarke and Kumar, 1983; Corrigall et al. 1992; Kalivas et al., 1993; Miller, 2001). Behavioral sensitization is the consequence of drug-induced neuroadaptive changes in the brain circuits involving DA and glutamatergic interconnections between the VTA, NAS, mPFC and amygdala (Ballas et al., 2004; Kelly, 2002). Nicotine has a pharmacological profile that is characteristic of a psychostimulant drug of abuse and can serve as a reinforcer in self-administration experiments (Corrigall and Coen 1989; Di Chiara, 2000). Abstinence after chronic nicotine exposure causes a dramatic decrease in brain reward function as measured by elevations in intracranial self-stimulation (ICCS) brain reward thresholds (Ivanova and Greenshaw, 1997; Epping-Jordan et al., 1998) whereas slow and continuous administration of nicotine decreases craving for smoking cigarettes as evidenced by use on nicotine patch. Even once weekly administration of nicotine produces long lasting locomotor sensitization in rats (Miller et al., 2001). Acute nicotine administration to rats produces a dose sensitive increase in locomotor activity (Clarke and Kumar, 1983; Ksir, 1994). Nicotine-induced locomotor hyperactivity is a DA-mediated phenomenon, which is blocked by D1 or D2 DA receptor antagonists (O'Neill et al., 1991). Nicotine can act directly on DA cells in the VTA to augment their rate of firing and subsequently increase DA release in the NAS (Impereto et al., 1986; Pidolplichko et al., 1997). Mecamylamine,

a classical antagonist of central nicotinic receptors inhibits the effects of acute and chronic nicotine treatment on locomotor activity in rodents (Kempsill and Pratt, 2000; Jones and Benowitz, 2004).

Thus, the effects of nicotine on locomotor activity following acute and chronic administration appear to result from nAChR-mediated DA and EAA release and their interaction in the VTA, NAS and the interconnected areas of the brain (Birrell and Balfour, 1998; Lanca et al., 2000). Chronic behavioral effects due to repeated nicotine administration are associated with significant increases of nAChRs in the brain as measured by quantitative autoradiography (Clarke and Pert, 1985).

Glu is widely accepted as the major excitatory neurotransmitter in the CNS, possibly global and is central to neuronal plasticity. Glu is intimately implicated in behavioral reinforcement, memory and related cognitive functions. A growing body of evidence suggests that glu neurotransmission and subsequent glu-DA interactions involving VTA, mPFC and limbic structures are crucial for the development and expression of nicotine-induced locomomotor hyperactivity (Toth and Lajtha, 1993; Moghaddam and Gruen, 1991; Morari et al., 1998). It is generally believed that certain neural substrates are collectively involved in psychomotor-induced locomotor activity, and NMDA receptors appear to be common substrates critical for behavioral sensitization (Imperato et al., 1990; Wolf, 1998; Kelly, 2002). Results of studies indicate that nicotine might mediate some of its excitatory effect on DA neurons indirectly through enhancement of glu release (Karreman et al., 1996; Karreman and Moghaddam, 1996; Kalivas and Duffy, 1997). It is reported that electrical self stimulation of lateral hypothalamus in rats causes persistent activation of the mesolimbic DA system with significant increases in DA

(332%) and glu release (150%) in the VTA as determined by in vivo microdialysis measurements (Xue et al., 1996; You et al., 1998). The stimulant-induced increase in glu level was blocked by mecamylamine (Reid et al., 2000).

Also, co-administration of NMDA glu receptor antagonists may block induction of stimulant-induced locomotor activity. Amphetamine-induced sensitized locomotor activity was blocked by intraVTA administration of the competitive NMDA receptor antagonist AP-5 (Vezina and Queen, 2000). But the behavioral profiles of antagonists of all subtypes of GluRs are not uniform, and studies involving various NMDA receptor antagonists have provided different behavioral effects (Tzschenke and Schmidt, 2000 for review).

The most common approach to study NMDA receptors in locomotor activity has been to use MK-801, a non-competitive antagonist of the NMDA receptor. Based on earlier reports, it was hypothesized that the locomotor effects of nicotine would be blocked by coadministration of MK-801 as blockade of the NMDA receptor might block the sequential neural plastic events that result in sensitization to stimulants (Shoaib et al., 1994).

The present study investigated the effects of repeated exposure of nicotine on locomotor activity and effects of MK-801 in blocking nicotine-induced locomotor activity. We also examined the effect of mecamylamine and haloperidol on nicotine-induced locomotor activity to compare with the blocking effect of MK-801.

3.2. The Experimental Methods

3.2.1. Repeated Systemic Administration of Nicotine

Experimentally naïve Sprague-Dawley rats (250-300 gm) were handled and placed in photobeam activity boxes for one hour daily for three days (habituation) to decrease unconditioned effects of the novel environment on behavior. Following habituation the rats were randomly assigned to two groups: nicotine and saline (NIC, $n = 9$, SAL, $n = 9$). Rats in the NIC group were injected (1 ml kg^{-1}, s.c) with nicotine (0.4 mg kg^{-1}) and the SAL group was injected with 0.9% saline each day for 7 days. The sites of injection were alternated daily to the left and right gluteal region. Ten minutes after injection each rat was transferred to its assigned photobeam activity box and spontaneous locomotor activity was monitored for 60 minutes. A microcomputer interfaced to the activity chamber recorded movement at 5 minutes intervals over 60 minutes. For all experiments, the photobeam activity boxes were cleaned with soap and water between testing of individual animals.

3.2.2. Administration of Mecamylamine in Nicotine-Sensitized Rats

Following treatment with nicotine or saline for 7 days each rat received an IP injection of mecamylamine (1 mg kg^{-1}) or 0.9% saline vehicle on each of two days (days 8 and 9) in a counterbalanced design. The design is illustrated in the table below.

Table 4. Counterbalanced design

TREATMENT GROUP	DRUG GIVEN ON DAY 1	DRUG GIVEN ON DAY 2
NIC	MEC	VEH
SAL	VEH	MEC

Mecamylamine was injected 40 minutes prior to and nicotine 10 minutes prior to behavioral testing. Animals remained in the home cage after injection until the time of testing. Locomotor activity was then recorded for 60 minutes in the photobeam activity box.

3.2.3. Administration of Haloperidol to Nicotine-Sensitized Rats

Following treatment with nicotine or saline for 7 days, each rat received an IP injection (1 mg/ml) of haloperidol (0.1 mg kg^{-1}) or 0.9% saline vehicle on each of two days in a counterbalanced design as illustrated in Table 4. Haloperidol was injected 30 minutes prior to, and nicotine 10 minutes prior to, behavioral testing. Animals remained in the home cage after injection until the time of testing. Locomotor activity was then recorded for 60 minutes in the photobeam activity box.

3.2.4. Administration of Four Doses of MK-801 in Nicotine-Sensitized Rats

Following treatment with nicotine or saline for 7 days each rat received an IP injection of MK-801 (0.01, 0.03, 0.1 and 0.3 mg kg^{-1}) or 0.9% saline vehicle on each of two days. In this experiment the dose order on each of these two-day probe tests was randomly assigned; each animal received a total of 4 probe tests in this dose-response analysis. Five days of baseline nicotine testing occurred between successive doses of MK-801. MK-801

was injected 30 minutes prior to, and nicotine 10 minutes prior to, behavioral testing. Animals remained in the home cage after injection until the time of testing. Locomotor activity was then recorded for 60 minutes in the photobeam activity box.

3.2.5. Administration of MK-801 (0.2 mg kg^{-1}) and Nicotine (0.8 mg kg^{-1}) at Doses Similar to Those Used in CCP Experiments 4 and 5

The apparatus and procedure for this experiment were identical to that of experiment 3 using doses of MK-801 (0.2 mg kg^{-1}) and nicotine (0.4 mg kg^{-1}). Sensitization to nicotine was done by administering nicotine (0.8 mg kg^{-1}, s.c) for seven days, and a single dose of MK-801 (0.2 mg kg^{-1}, IP) was injected to nicotine-sensitized rats. These are exactly the same doses we used in CPP study. This experiment was done to generate locomotor activity data by matching doses of MK-801 and nicotine used in the CPP study.

3.3. RESULTS

3.3.1 Effects of Nicotine on Locomotor Activity

Ambulatory (Horizontal) Activity

The effect on locomotor activity of systemic administration of nicotine (0.4 mg kg^{-1}, s.c.) and saline was compared. As shown in figure 3.1 acute treatment with nicotine (0.4 mg kg^{-1}, s.c.) significantly increased total ambulatory locomotor activity as compared to saline over 60 minutes of recording [F (1,16)=73.718, $p<0.05$]. Post-hoc analysis ($P<0.05$) conducted on group mean values indicated a significant increase in total horizontal activity compared to saline. In addition, there was no significant main effect of day [F (6, 96) =2.638, $p <0.05$]; but significant interaction between nicotine and day [F (6, 96) =5.895, $p<0.05$], and time [F (11,176.)=147.355, $p <0.000$]; nicotine x time [F (11,176)=3.998, $p <0.000$]; and day x time (66,1056)=2.127, $p<0.000$]; day x time x nicotine [F (66,1056)=2.448, $p<0.000$].

Local time course data for ambulatory activity is shown in figure 3.2. Analysis of local time course data by independent t tests at each 5-minute interval showed a significant difference ($p< .05$).

Ambulatory Locomotor Activity of Saline/Nicotine (0.4 mg kg $^{-1}$) Treated Rats

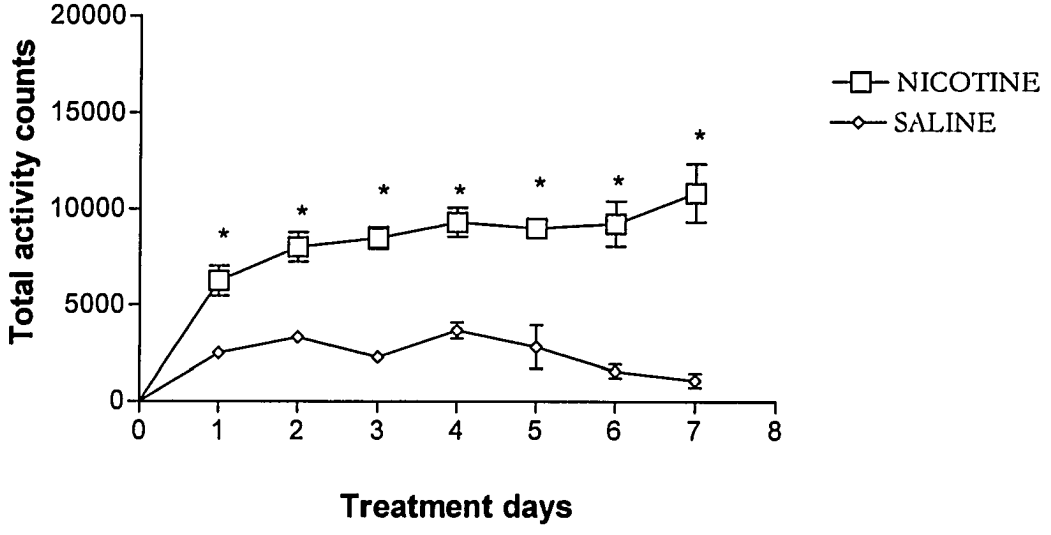


Figure 3.1. Repeated injections of 0.4 mg kg^{-1} of nicotine (n=9) or saline (1 ml kg^{-1}) for seven days progressively increased ambulatory activity. Data are means ± S. E. M. * Significant at $p<0.05$, relative to saline (n=9).

Time Course in Ambulatory Activity on Day 7 of Saline/Nicotine (0.4 mg kg $^{-1}$) Treated Rats

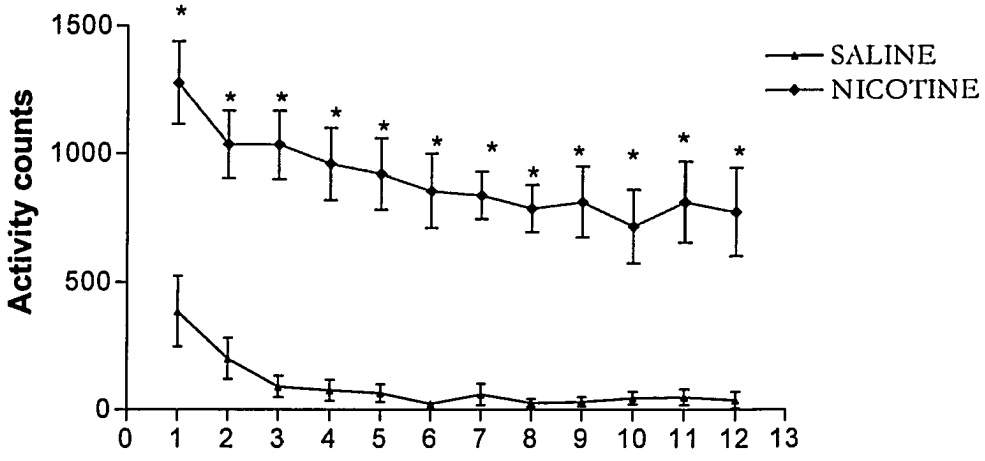


Fig 3.2. Time course effects of nicotine (0.4 mg kg^{-1}) or saline on total ambulatory activity in a 60-minute test session (each bin 5 min.). Data are means±S.E.M. *Significant at $P<0.05$, relative to saline (n=9).

Consecutive Activity

The effect on locomotor activity of systemic administration of nicotine (0.4 mg kg^{-1}, s.c) and saline (1 ml kg^{-1}, s.c) was compared in consecutive activity. As can been seen in figure 3.3, acute nicotine treatment (0.4 mg kg^{-1}; s.c) significantly increased consecutive locomotor activity as compared to saline over 60 minutes/day of recording [F (1,16)=21.656, p<0.000]. Post-hoc analysis (P<0.05) conducted on group mean values indicated a significant increase in total consecutive activity compared to saline. There was a no main effect of day [F (6, 96) =1.723, p >0.05] but the following significant interactions were observed: nicotine and day [F (6, 96) =4.776, p<0.0001], nicotine and time [F (11,176.)=48.573, p <0.0001]; nicotine x time [F (11,176) =9.729, p <0.000]; day x time (66, 1056) =1.657, p<0.001]; and day x time x nicotine [F (66, 1056) =2.136, p<0.0001].

Consecutive Locomotor Activity in Saline/Nicotine (0.4 mg kg $^{-1}$) Treated Rats

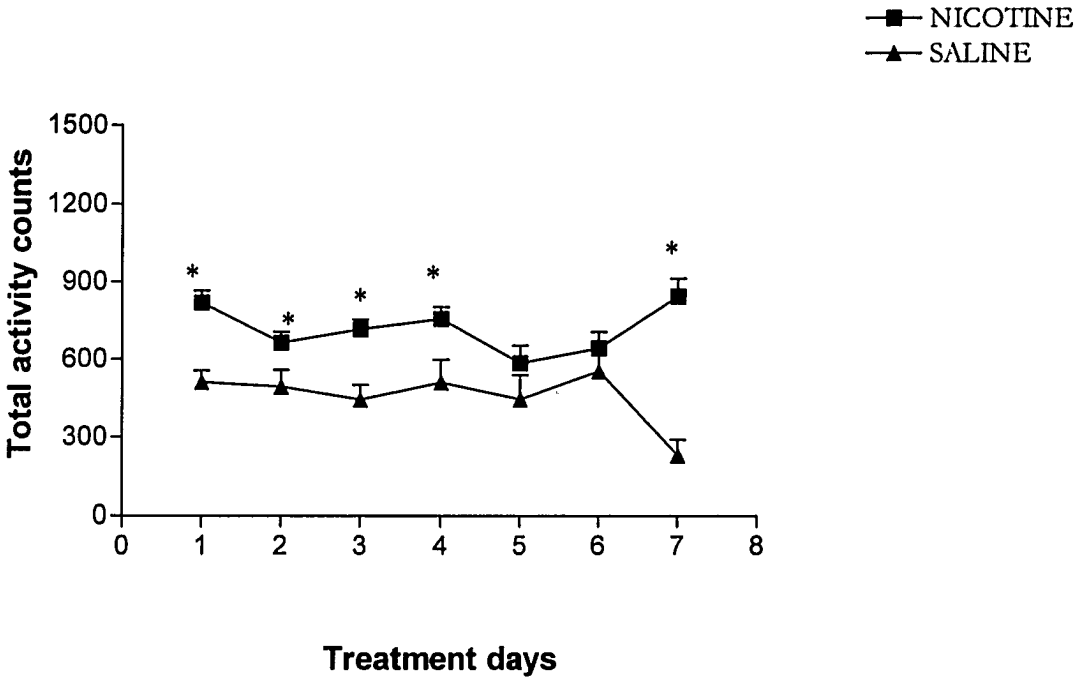


Figure 3.3. Effect of repeated injections nicotine 0.4 mg kg^{-1} or saline on consecutive locomotor activity for seven days. Data are means ± S. E. M. * Significant at $p<0.05$, relative to saline (n=9).

Time Course in Consecutive Activity on Day 7 of Saline/Nicotine (0.4 mg kg $^{-1}$) Treated Rats

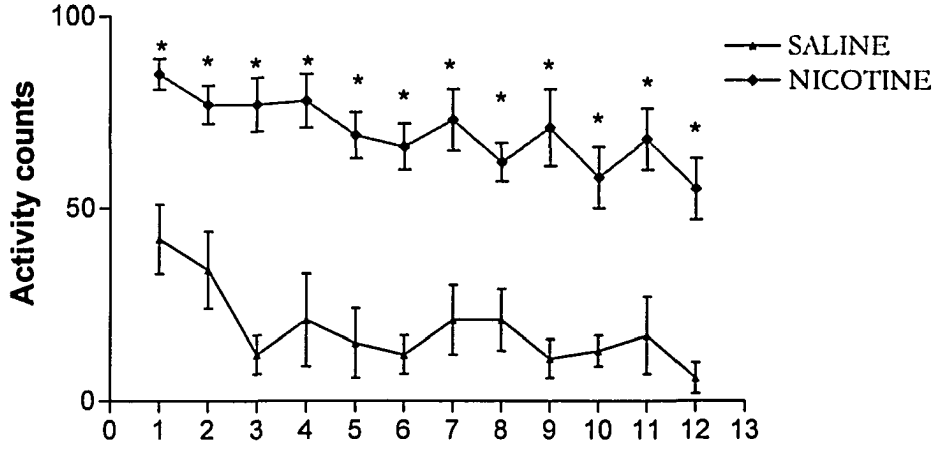


Fig 3.4. Time-course effects of nicotine 0.4 mg kg^{-1} or saline on consecutive activity in a 60 minute test session. Data are means±S.E.M. *Significant at $P<0.05$, relative to saline (n=9).

Local time course data of consecutive locomotor activity are shown in figure 3.4; analysis of local time course data by independent t tests at each 5-minute interval showed a significant difference ($p<0.005$).

Vertical activity

The effect on locomotor activity of systemic administration of nicotine (0.4 mg kg^{-1}, s.c) and saline (1 ml kg^{-1}, s.c) was compared. As can been seen in figure 3.5, acute nicotine treatment (0.4 mg kg^{-1}; s.c) increased total vertical locomotor activity as compared to saline over 60 minutes/day of recording [$F(1,16)=2.681$, $p<0.05$]. As there was no significant effect of nicotine in vertical activity, tests of simple effects were not suitable. In addition, there was a no significant main effect of day [$F(6,96)=1.077$, $p>0.05$] but the following significant interaction were observed between nicotine and day [$F(6,96)=8.761$, $p<0.000$] with time [$F(11,176.)=66.342$, $p<0.000$]; nicotine x time [$F(11,176)=3.183$, $p<0.001$] and day x time ($66,1056)=3,468$, $p<0.000$]. There was no significant interaction of day x time x nicotine [$F(66, 1056)=1.054$, $p>0.05$]. Local time course data are shown in figure 3.6; analysis of local time course data by independent t tests ($p<0.005$) at each 5-minute interval showed a significant difference. Enhanced locomotor activity on day 7 in nicotine treated rats represents development of sensitization/reverse tolerance whereas decrease in locomotor activity of saline treated rats indicate return to initial baselie activity due habituation to test apparatus.

Vertical Locomotor Activity in Saline/Nicotine (0.4 mg kg $^{-1}$) Treated Rats

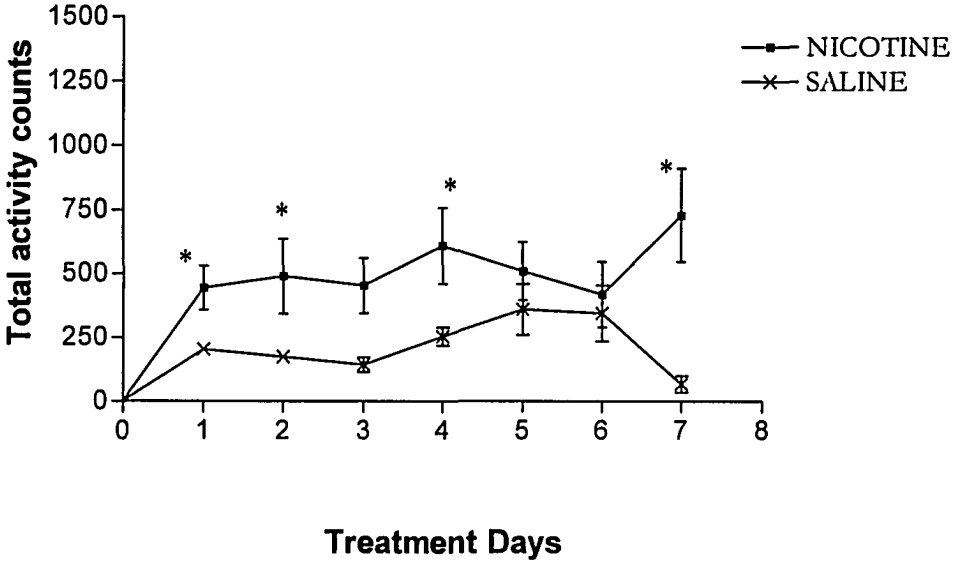


Figure 3.5. Effect of repeated injections nicotine 0.4 mg kg^{-1} or saline on vertical locomotor activity for seven days. Data are means ± S. E. M. * Significant at $p<0.05$, relative to saline (n=9).

Time Course in Vertical Activity on Day 7 in Saline/Nicotine (0.4 mg kg $^{-1}$) Treated Rats

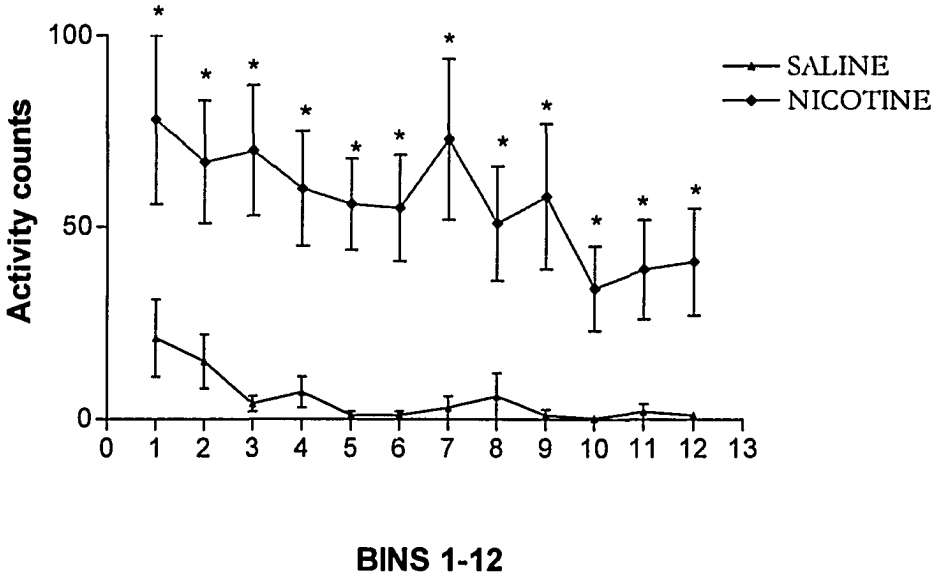
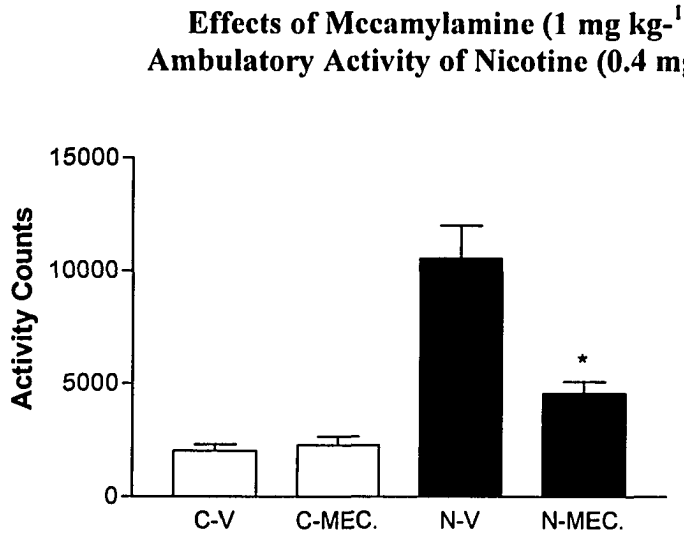


Fig 3.6. Time course effects of nicotine 0.4 mg kg^{-1} or saline on vertical activity in a 60 minute test session. Data are means±S.E.M. *Significant at $P<0.05$, relative to saline, (n=9).

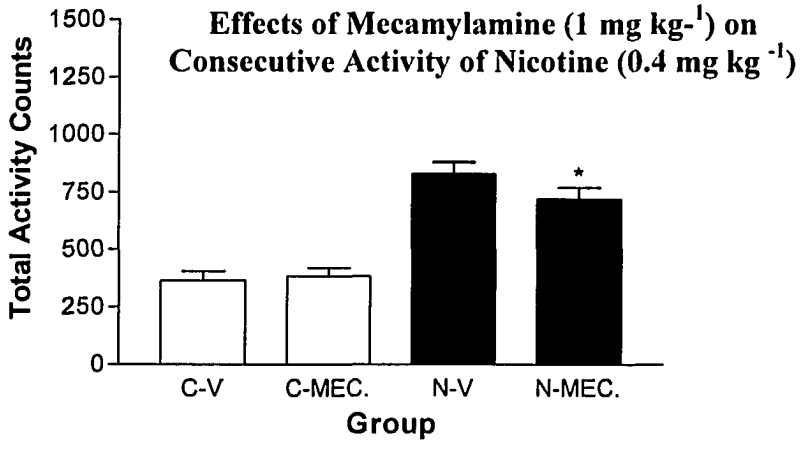
3.3.2. Effects of Mecamylamine on Locomotor Activity of Nicotine

Three-way repeated measures ANOVA with Greenhouse-Geisser correction showed significant main effects of mecamylamine [F (1, 14)=14.006, p<.002, nicotine [F (1,14) =40.918, p<0.000], and of time [F(11,154)=52.583, p<0.000]. In addition, there was significant interaction between nicotine and mecamylamine [F (1, 14) =16.457, p<.001) seen in ambulatory locamotor activity. There were no significant differences with mecamylamine x time and mecamylamine x nicotine x time. An interaction between nicotine and mecamylamine was revealed in vertical activity but not in consecutive activity. Post-hoc analysis (P<0.05) conducted on group mean values revealed a significant interaction between nicotine and mecamylamine. These effects are illustrated by the data displayed in figure 3.7.

a)



b)



c)

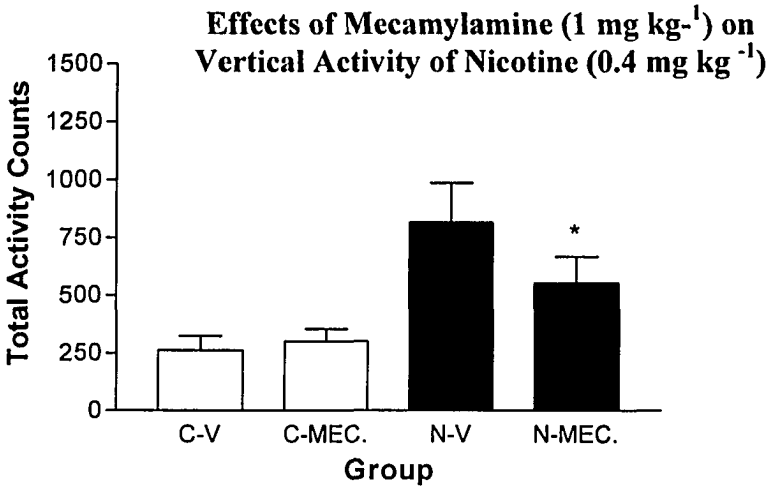
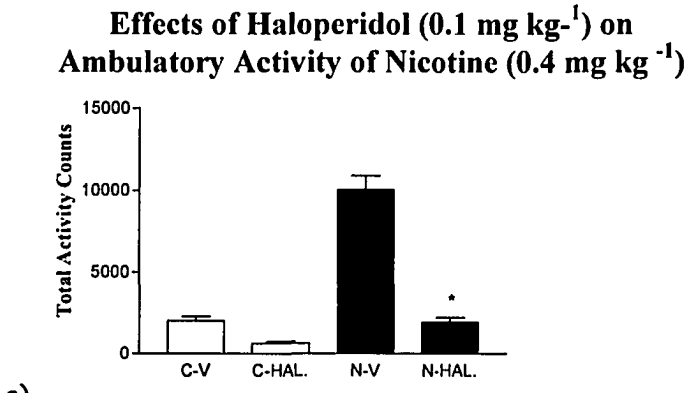


Figure. 3.7. Effect of mecamylamine (1.0 mg kg^{-1}) in blocking a) ambulatory, b) consecutive, and c) vertical locomotor activity produced by nicotine (0.4 mg kg^{-1}) or saline. Data are means±S.E.M. *Significant at $P<0.05$, (n=9).

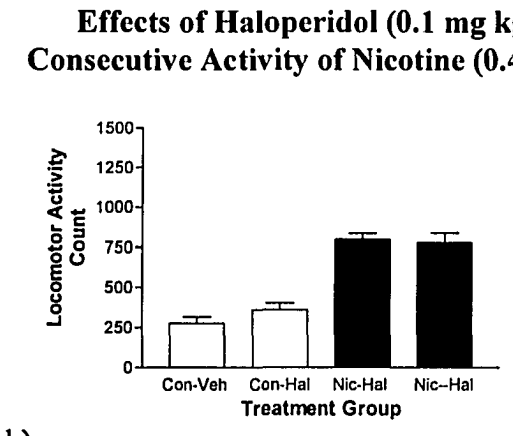
3.3.3. Effects of Haloperidol on Locomotor Activity of Nicotine

Three-way repeated measures ANOVA with Greenhouse-Geisser correction showed significant main effects of haloperidol [$F(2,28)=52.541$, $p<.0001$, nicotine [$F(1,14)=218.889$, $p<0.0001$], time [$F(11,154)=84.432$, $p<0.0001$], and haloperidol x time[$F(22,79.953)=8.303$, $p<0.0001$].

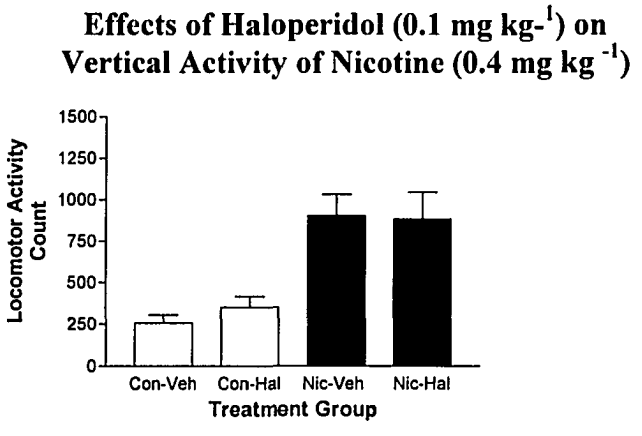
In addition, there was a significant interaction between nicotine and haloperidol [$F(2,28)=25.272$, $p<.0005$] seen in ambulatory locomotor activity. Significant interaction between nicotine and haloperidol was not revealed in consecutive and vertical activity. Post-hoc analysis ($P<0.05$) conducted on group mean values revealed significant interaction between nicotine and haloperidol. These effects are illustrated by the data displayed in figure 3.8.



a)



b)



c)

Figure 3.8. Effect of haloperidol (0.1 mg kg^{-1}) in blocking a) ambulatory, b) consecutive, and c) vertical locomotor activity produced by nicotine (0.4 mg kg^{-1}) or saline. Data are means±S.E.M. *Significant at $P<0.05$, (n=9).

3.3.4. Effects of MK-801 on Nicotine Induced Locomotor Activity

Ambulatory activity

Three-way repeated measures ANOVA with Greenhouse-Geisser correction showed significant main effects of MK-801 [$F(4, 56) = 14.810$, $p<0.0001$], and time [$F(11,154)=63.521$, $p<0.0001$]. The MK-801 and nicotine combination showed increases in locomotor activity but the increase in locomotor activity due to the said interaction between MK-801 x nicotine did not attain the level of significance [$F(4, 56) = 2.200$ $p=0.125$] shown in figure 3.9. In addition, there was no significant interaction between MK-801 x time [$F(44,154=1.193$, $p>0.05$] nor amongst MK-801 x nicotine x time [$F(44,616)=1.124$, $p=0.355$]. As there was no significant interaction between MK-801 and nicotine, tests of simple effects were not suitable. Analysis of local time course data by one-way repeated measures ANOVA across all drugs at each 5-minute interval (figure 3.10) with significant F ratios are indicated by significance mark (*).

Ambulatory Activity of Nicotine (0.4 mg kg $^{-1}$) and Four Doses of MK-801

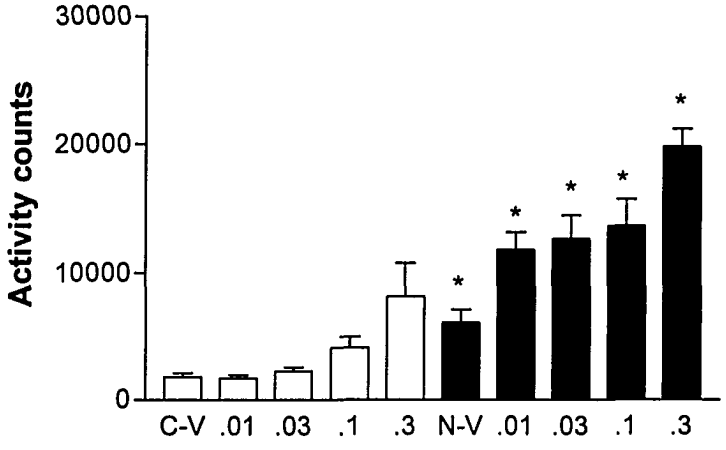


Fig. 3.9. Effects of MK-801 (0.01, 0.03, 0.1, 0.3 mg kg^{-1}) on ambulatory locomotor activity induced by repeated administration of nicotine (0. mg kg^{-1}). (n=9). Data are means ± S.E.M. (*) Significant at $P<0.05$, relative to saline/MK

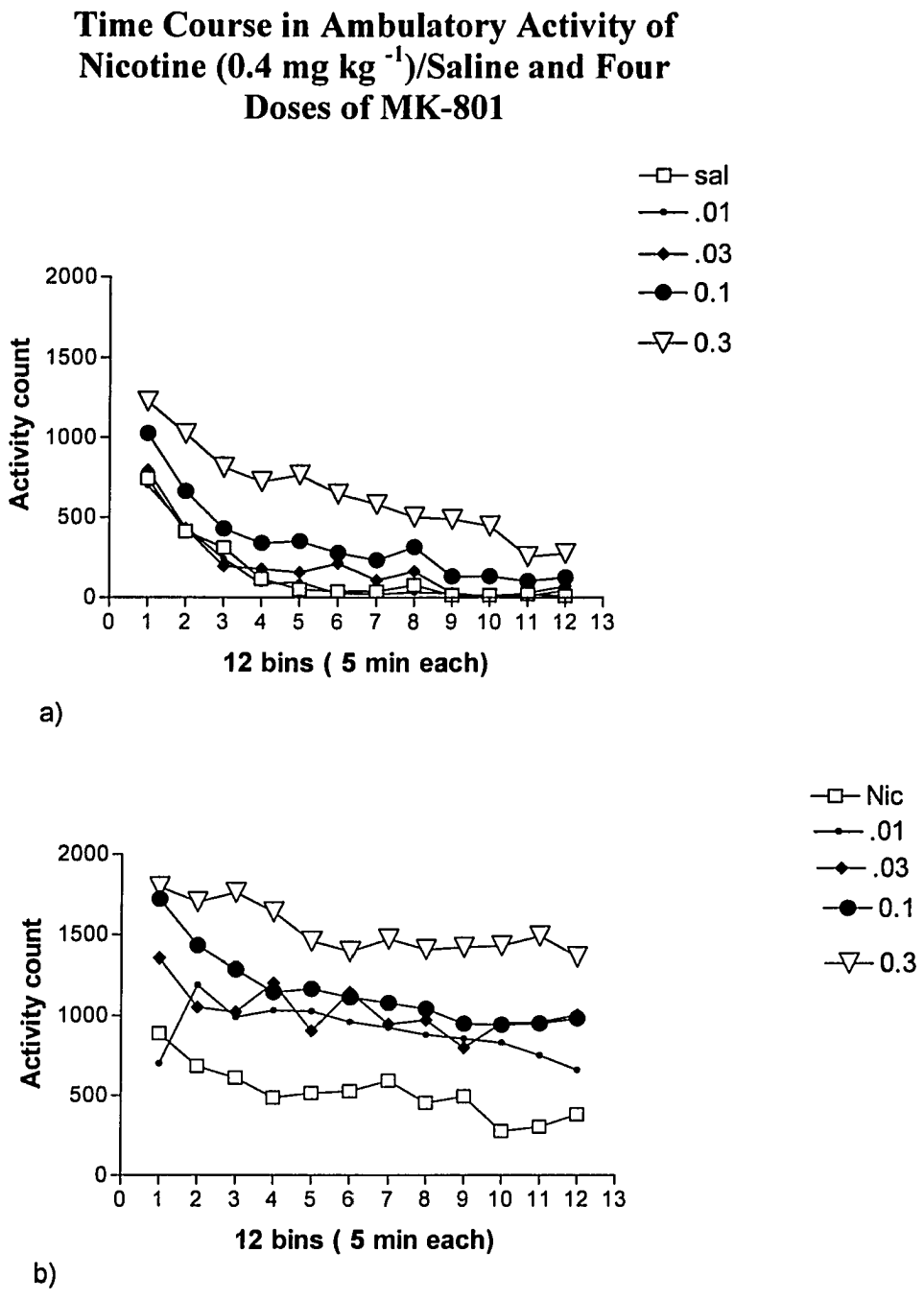


Figure 3.10. Time course effects of MK-801 (0.01, 0.03, 0.1, 0.3 mg kg^{-1}) alone (a); and in combination with nicotine (b) on ambulatory locomotor activity in a 60 minute test session (n=8). Data are means.

Consecutive Locomotor Activity

Three-way repeated measures ANOVA with Greenhouse-Geisser correction showed significant main effects of MK-801 [$F(4,56)=8.130$, $p<0.001$], and time [$F(11,154)=16.101$ $p<0.000$] as shown in figure 3.11.

In addition, there was a significant interaction between MK-801 and nicotine [$F(4,56)=9.100$, $p=0.000$]. However, there was no significant interaction between MK-801 x time [$F(44,154=1.824$, $p>0.05$] nor amongst MK-801 x nicotine x time [$F(44,616)=1.160$, $p=0.328$]. Post hoc tests ($P<0.05$) conducted on group mean values indicate significant interactions. Analysis of local time course data was done by one-way repeated measures ANOVA across all drugs at each 5-minute interval (figure 3.12)

Consecutive Activity of Nicotine (0.4 mg kg $^{-1}$) and Four Doses of MK-801

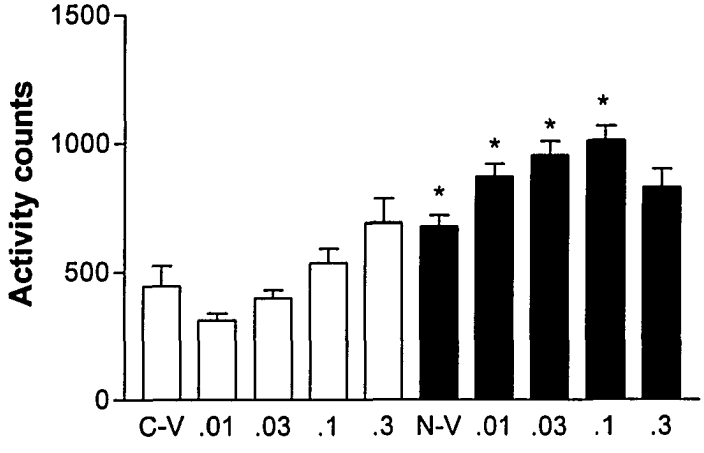


Figure 3.11. Effects of MK-801 (0.01, 0.03, 0.1, 0.3 mg kg^{-1}) on consecutive locomotor activity induced by repeated administration of nicotine (0.4 mg kg^{-1}) (n=8). Data are means ± S.E.M. (*) Significant at $P<0.05$, relative to saline/MK-801

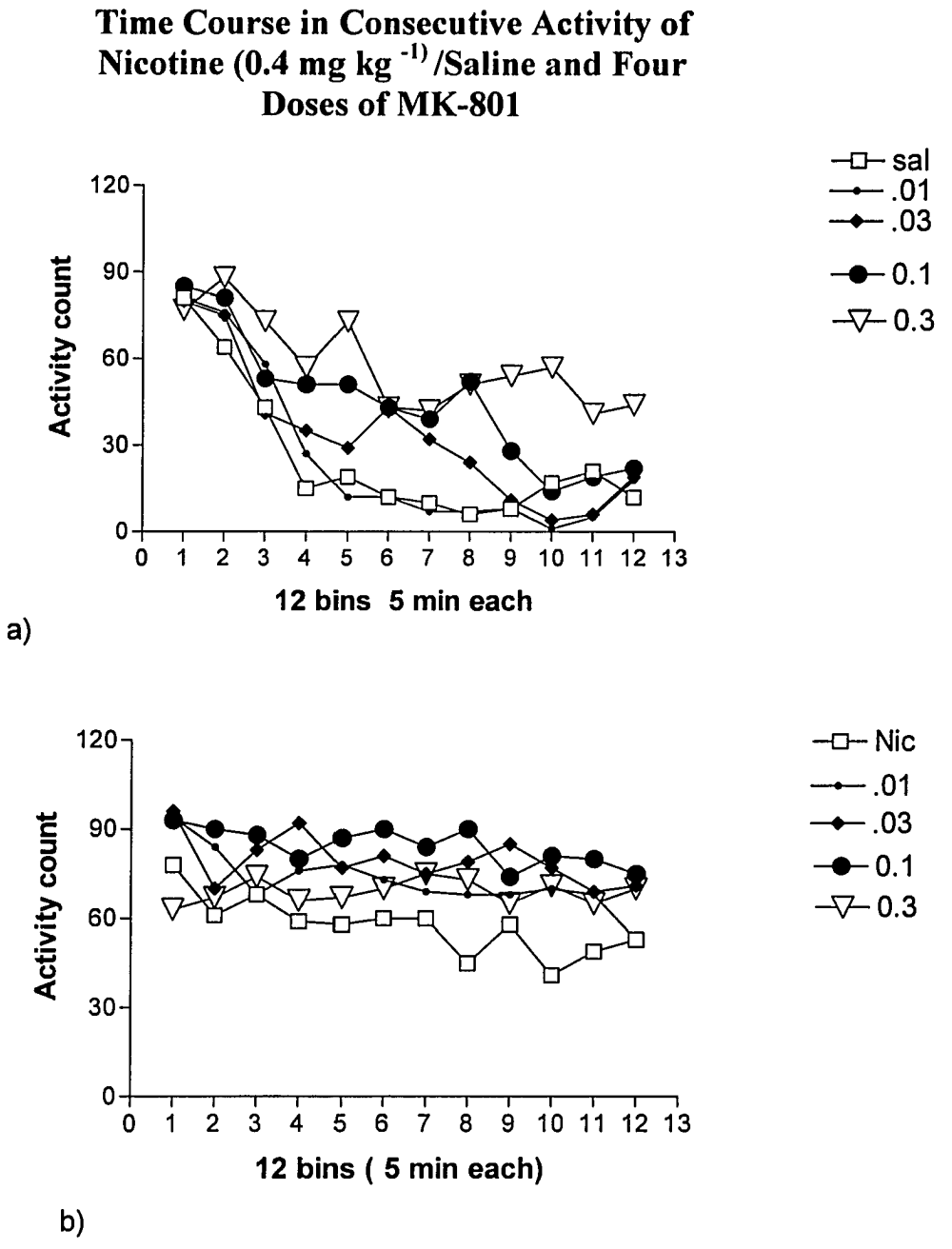


Figure 3.12. Time course effects of MK-801 (0.01, 0.03, 0.1, 0.3 mg kg^{-1}) alone (a) and in combination with nicotine (b) on consecutive locomotor activity in a 60 minute test session (n=8). Data are means.

Vertical Locomotor Activity

Three-way repeated measures ANOVA with Greenhouse-Geisser correction showed no significant main effects of MK-801 [$F(4,56)=1.872$, $p=0.167$]. There was no significant interaction between MK-801 and nicotine [$F(4,56)=.889$, $p=0.431$]. Significance was revealed in time [$F(11,154)=22.587$ $p<0.0001$] and MK-801 x time [$F(44,616=2.870$, $p<0.05$], but not amongst MK-801 x nicotine x time [$F(44,616)=1.004$, $p=0.467$] as shown in figure 3.13. As there was no significant interaction between MK-801 and nicotine, tests of simple effects were not suitable. Analysis of local time course data was done by one-way repeated measures ANOVA across all drugs at each 5-minute interval (figure 3.14).

Vertical Activity of Nicotine (0.4 mg kg $^{-1}$) and Four Doses of MK-801

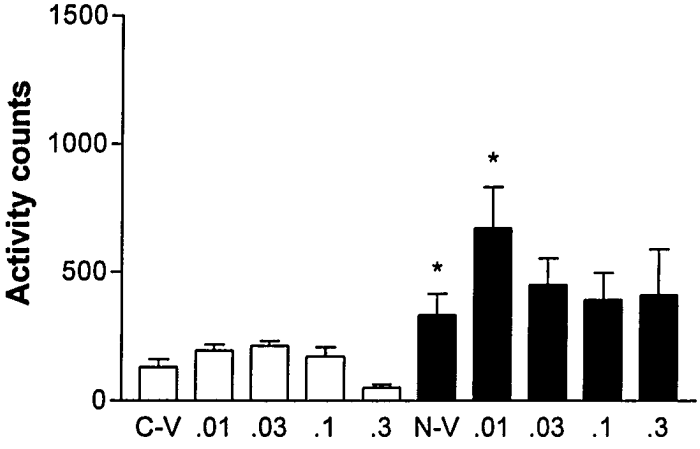


Figure 3.13. Effects of MK-801 (0.01, 0.03, 0.1, 0.3 mg kg^{-1}) on vertical locomotor activity induced by repeated administration of nicotine (0.4 mg kg^{-1}). (n=8). Data are means ± S.E.M. (*) Significant at $P<0.05$, relative to saline/MK

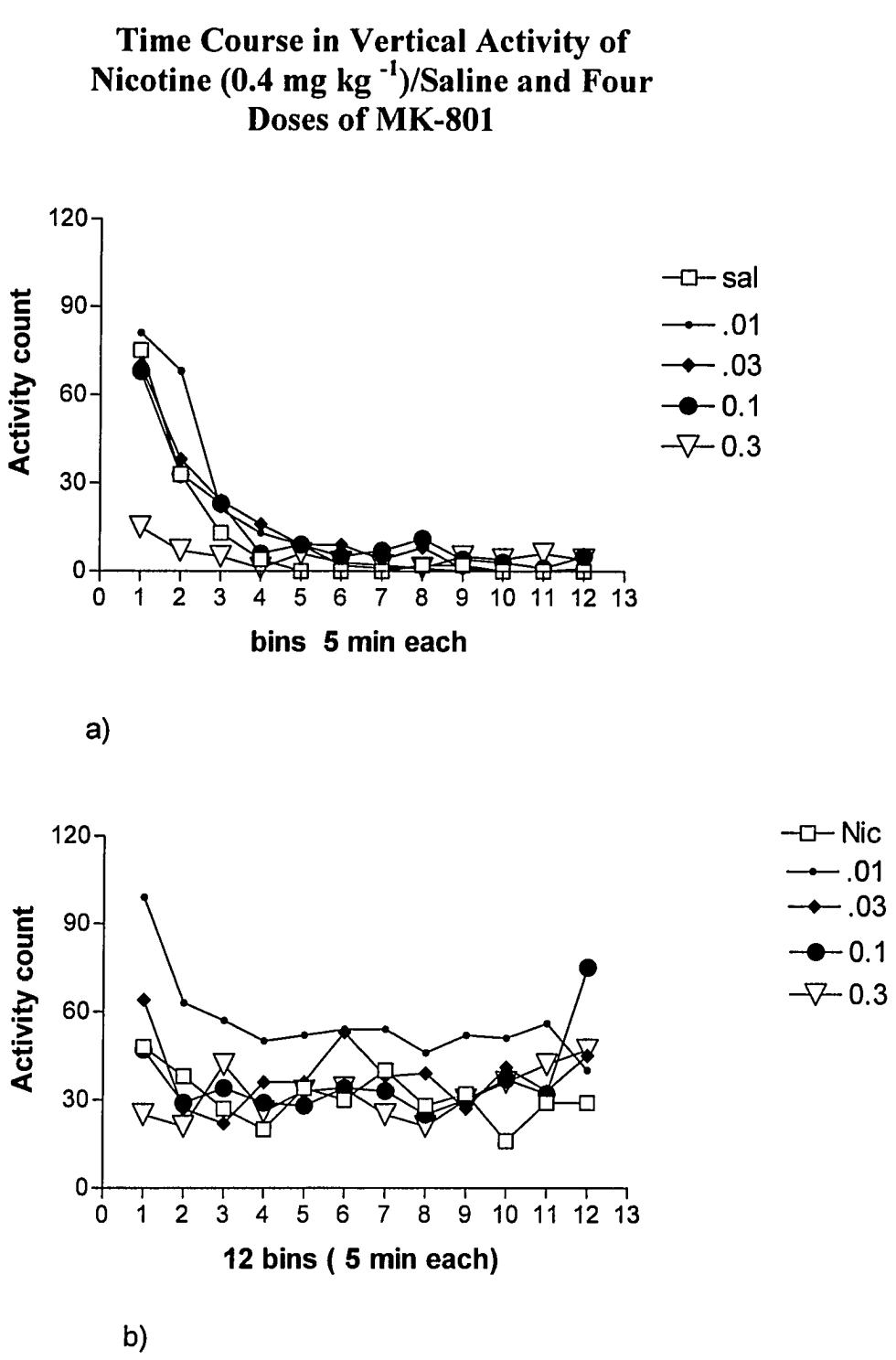


Figure 3.14. Time course effects of MK-801 (0.01, 0.03, 0.1, 0.3 mg kg^{-1}) alone (a); and in combination with nicotine (0.4 mg kg^{-1}) (b) on vertical locomotor activity in a 60 minute test session (n=8). Data are means.

3.3.5. Effects of a Higher Dose of Nicotine (0.8 mg kg^{-1}) on Locomotor Activity

Ambulatory (horizontal) Locomotor Activity

The effect on locomotor activity of systemic administration of nicotine (0.8 mg kg^{-1}, s.c.) and saline was compared. As can been seen in figure 3.15, repeated injection of nicotine over seven days (0.8 mg kg^{-1}; s.c) significantly increased total horizontal locomotor activity [F (1,16)=73.427, p<0.0001]. Post-hoc analysis (P<0.05) conducted on group mean values indicated a significant increase in total horizontal activity compared to saline. In addition, there was a significant main effect of day [F (6,96)=3.030, p <0.02]; a significant interaction between nicotine and day [F (6,96)=16.715, p<0.0001], and time [F (11,176.)=91.408, p <0.0001]; nicotine X time [F (11,176)=13.031, p <0.0001]. There was no significant interaction between day X time (66, 1056) =1.269, p>0.05]; or day X time X nicotine [F (66,1056)=1.529, p>0.05]. Analysis of local time course data (shown in figure 3.16) by independent t tests (p<. 05) of nicotine and saline groups at each 5-minute interval showed significant difference.

Ambulatory Locomotor Activity in Saline/Nicotine (0.8 mg kg $^{-1}$) Treated Rats

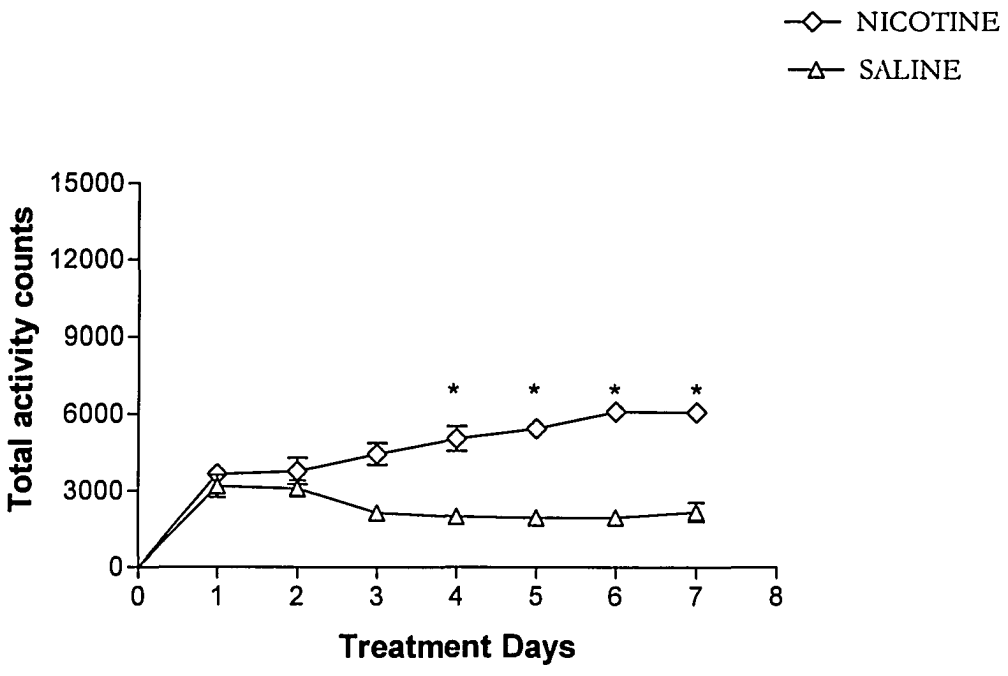


Figure 3.15. Effects of repeated administration of nicotine (0.8 mg kg^{-1}) for seven days on ambulatory locomotor activity in a 60 minute test session (n=8). Data are means ± S.E.M. *Significance at ≤ 0.05, relative to saline

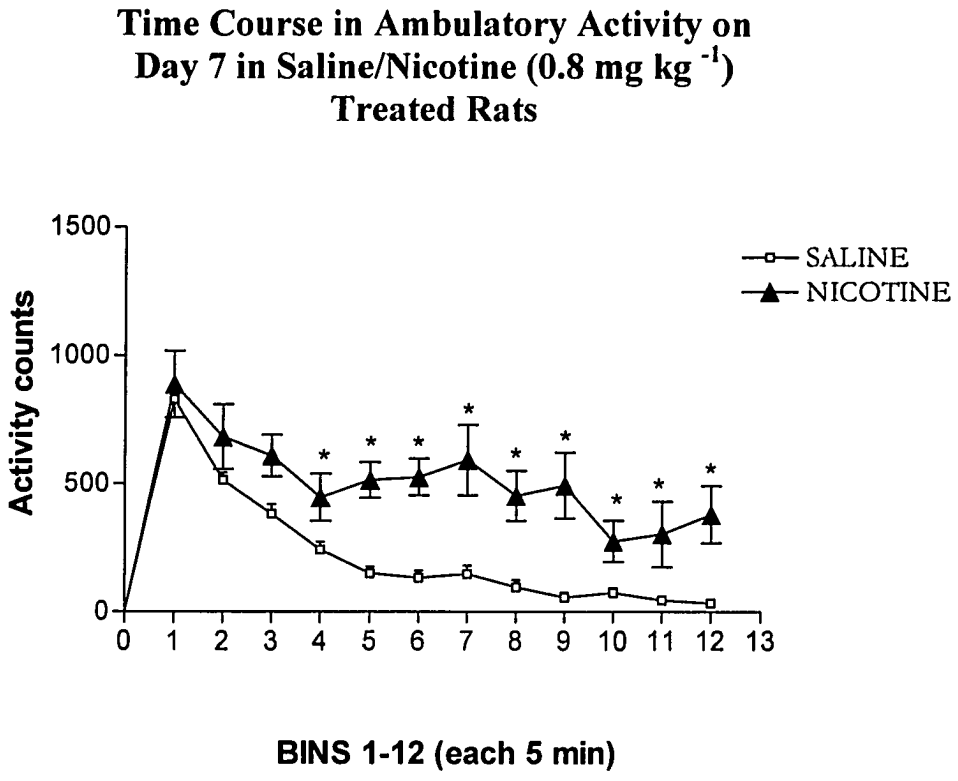


Figure 3.16. Time course effects.of nicotine (0.8 mg kg^{-1}) on ambulatory locomotor activity in a 60-minute test session on day7; n=8.
Data are means ± S.E.M. (*) Significance at ≤ 0.05, relative to saline

Consecutive Locomotor Activity

The effect on locomotor activity of systemic administration of nicotine (0.8 mg kg^{-1}, s.c) and saline (1 ml kg^{-1}, s.c) was compared. As can been seen in figure 3.17, repeated injection of nicotine over seven days (0.8 mg kg^{-1}; s.c) significantly increased total consecutive locomotor activity [F (1,16)=127.013, p<0.000]. Post-hoc analysis (P<0.05) conducted on group mean values indicated a significant increase in total consecutive activity compared to saline. In addition, there was no significant main effect of day [F (6,96)=. 759, p >0.05], but significant interactions between nicotine and day [F (6,96)=3.297, p<0.010], time [F (11,176.)=31.257, p <0.000]; nicotine X time [F (11,176)=6.015, p <0.000] and day X time (66,1056)=1.861, p<0.05]. There was no significant main effect of day X time X nicotine [F (66, 1056) =1.105, p>0.05]. Analysis of local time course data (shown in figure 3.18) by independent t tests (p<. 05) of nicotine and saline groups at each 5-minute interval showed significant differences.

Consecutive Locomotor Activity in Saline/Nicotine (0.8 mg kg $^{-1}$) Treated Rats

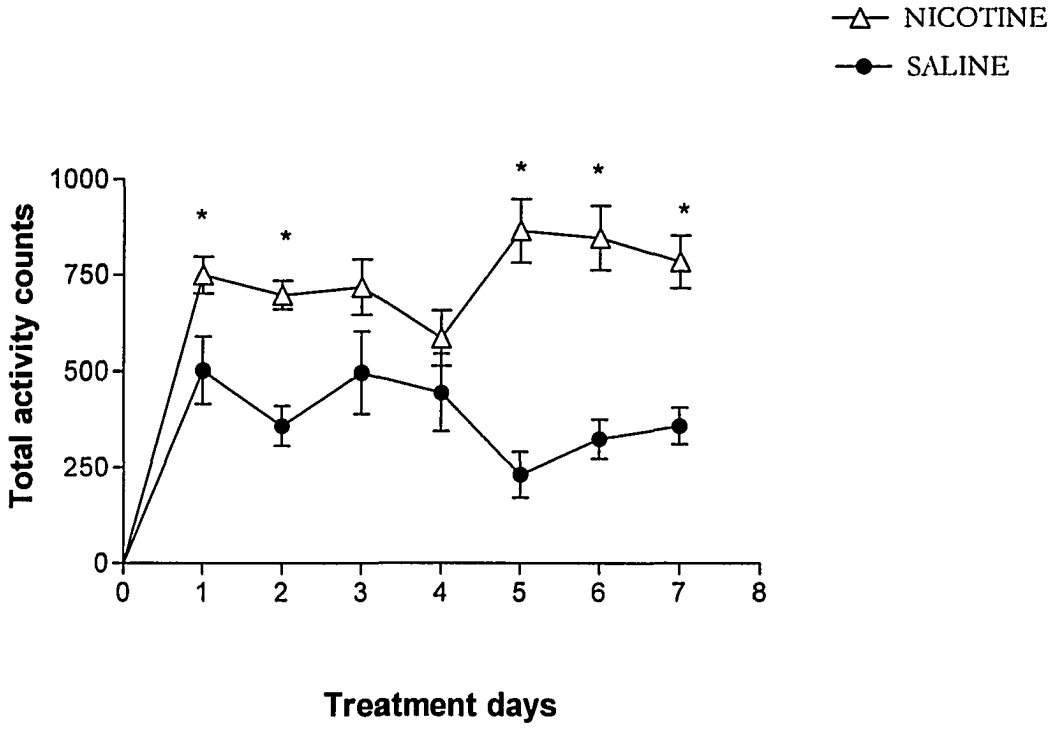


Figure 3.17. Effects of repeated administration of nicotine (0.8mg kg^{-1}) for seven days on consecutive locomotor activity in a 60-minute test session (n=8). Data are means ± S.E.M. (*) Significance at ≤ 0.05, relative to saline.

Time Course in Consecutive Activity on Day 7 in Saline/Nicotine (0.8 mg kg $^{-1}$) Treated Rats

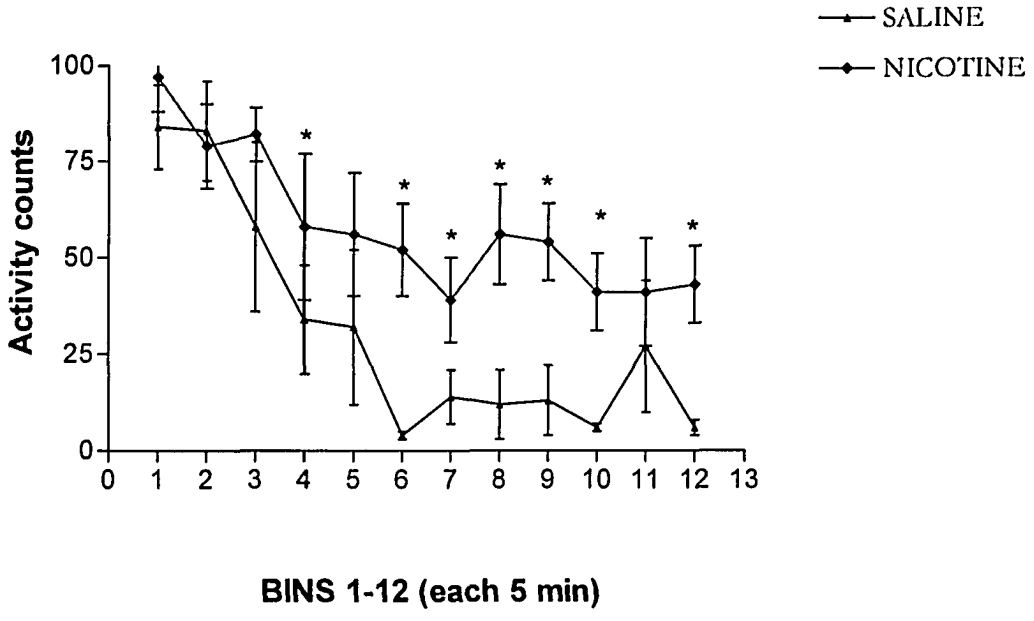


Figure 3.18. Time course effects.of nicotine (0.8 mg kg^{-1}) on consecutive locomotor activity in a 60-minute test session on day 7; n=8.
Data are means ± S.E.M. (*) Significance at ≤ 0.05, relative to saline

Vertical Locomotor Activity

The effect on locomotor activity of systemic administration of nicotine (0.8 mg kg^{-1}, s.c) and saline (1 ml kg^{-1}, s.c) was compared. As can been seen in figure 3.19, repeated injection of nicotine over seven days (0.8 mg kg^{-1}; s.c) significantly increased total vertical locomotor activity [F (1,16)=21.633, p<0.0001]. Post-hoc analysis (P<0.05) conducted on group mean values (collapsed across time) indicated a significant increase in total vertical activity compared to saline. In addition, there was no significant main effect of day [F (6,96)= 1.556, p >0.05] but significant interactions between nicotine and day [F (6,96)=2.924, p<0.038], and time [F (11,176.)=12.800, p <0.0001]. There was no significant main effect of nicotine x time [F (11,176)=. 845, p >0.05], day x time (66,1056)=1.1377, p>0.05] or day x time x nicotine [F (66,1056)=1.576, p>0.05]. Analysis of local time course data (shown in figure 3.20) by independent t tests (p< .05) of nicotine and saline groups at each 5-minute interval showed a significant difference. This time course results indicate that that throughout the 60 minutes activity period vertical activity of nicotine-treated rats was higher than in the saline-treated rats.

Vertical Locomotor Activity in Saline/Nicotine (0.8 mg kg $^{-1}$) Treated Rats

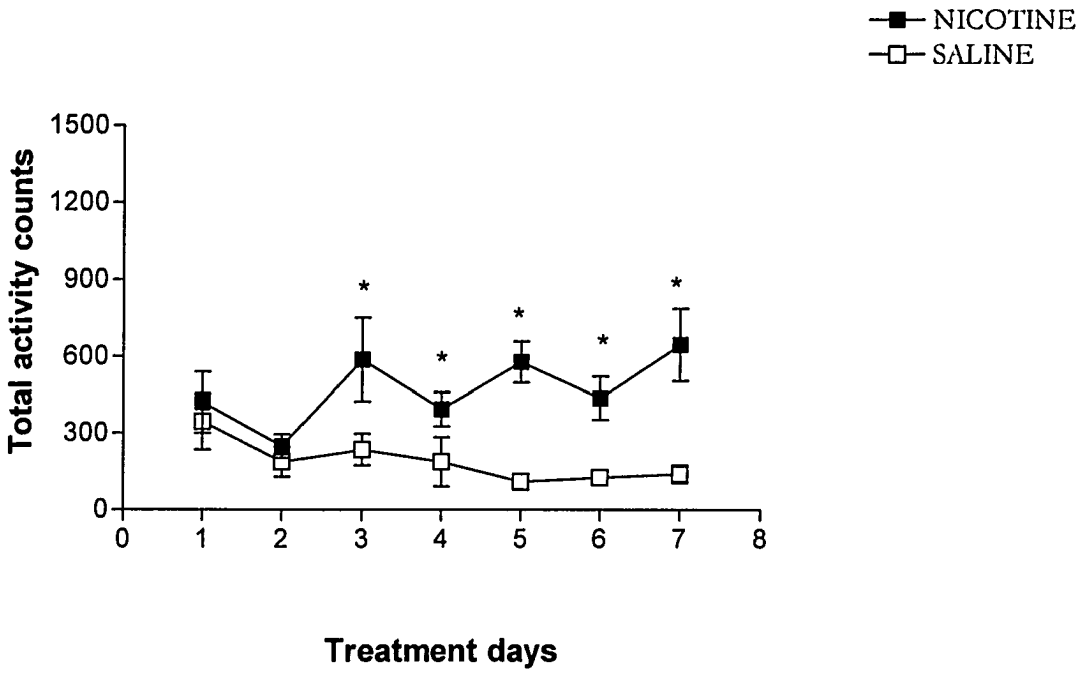


Figure 3.19. Effects of repeated administration of nicotine (0.8mg kg^{-1}) for seven days on vertical locomotor activity in a 60-minutesute test session (n=8) . Data are means ± S.E.M. (*) Significance at ≤ 0.05, relative to saline

Time Course in Vertical Activity on Day 7 of Saline/Nicotine (0.8 mg kg $^{-1}$) Treated Rats

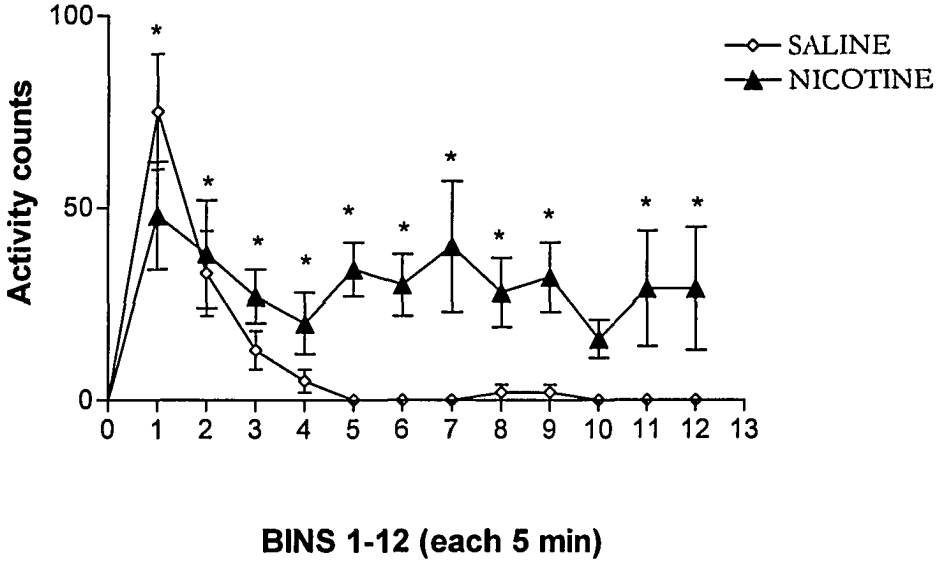


Figure 3.20 Time course effects.of nicotine (0.8 mg kg^{-1}) on vertical locomotor activity in a 60-minute test session on day 7; n=8.
Data are means ± S.E.M. (*) Significance at ≤ 0.05, relative to saline

3.3.6. Effects of Nicotine (0.8 mg kg^{-1})-MK-801 (0.2 mg kg^{-1}) Interaction in Locomotor Activity

Ambulatory Locomotor Activity

Three-way repeated measures ANOVA with Greenhouse-Geisser correction showed significant main effects of MK-801 [$F(1,16=55.527, p<0.0001$], and time [$F(11,176)=20.872, p<0.0001$] shown in figure 3.21. MK-801 and nicotine in combination showed increases in locomotor activity, but the increase in locomotor activity due to the said interaction between MK-801 x nicotine did not attain the level of significance [$F(1,16)= 2.580$ $p=0.128$]. In addition, there was no significant interaction between MK-801 X time [$F(11,176=1.006, p>0.395$] nor amongst MK-801 X nicotine X time [$F(11,176)$, $p=0.627$]. As there was no significant interaction between MK-801 and nicotine, tests of simple effects were not suitable. Efffects of MK-801 and nicotine seems to be additive. The local time course data of this interaction in ambulatory activity are shown in figure 3.22.

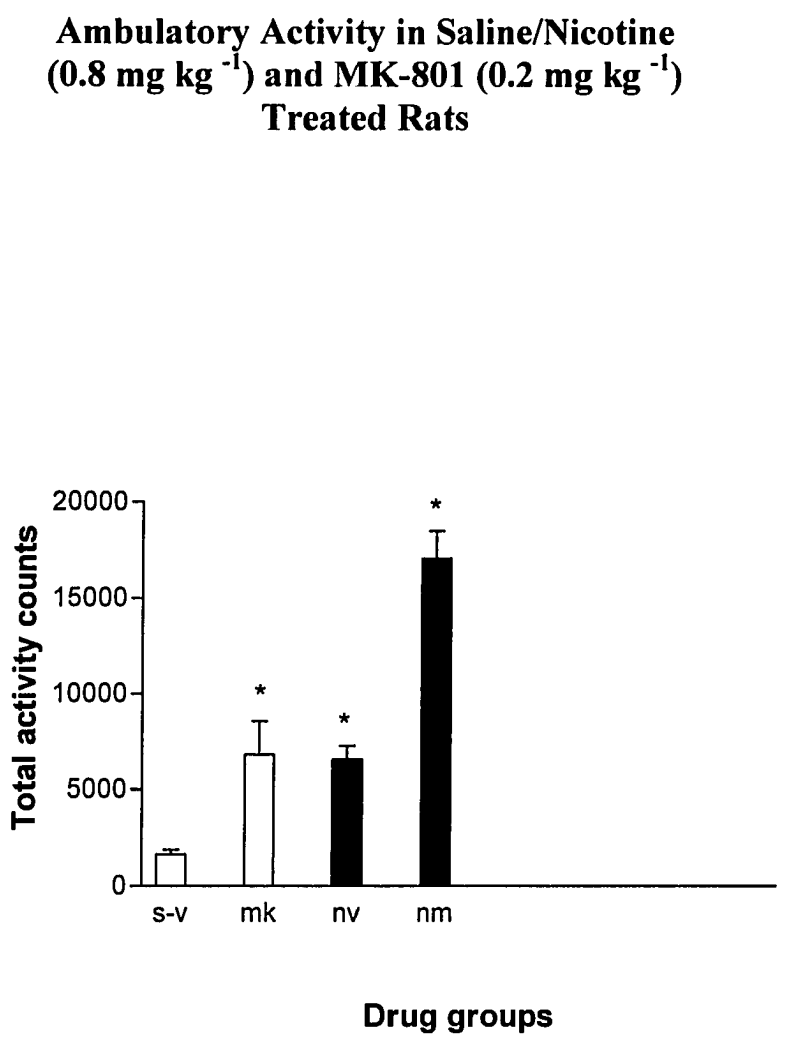


Figure 3.21. Effects of MK-801 (0.2 mg kg^{-1}) on ambulatory locomotor activity of nicotine (0.8 mg kg^{-1}) in a 60 minute test session. n=8. Data are means ± S.E.M. *Significance at ≤ 0.05, relative to saline.

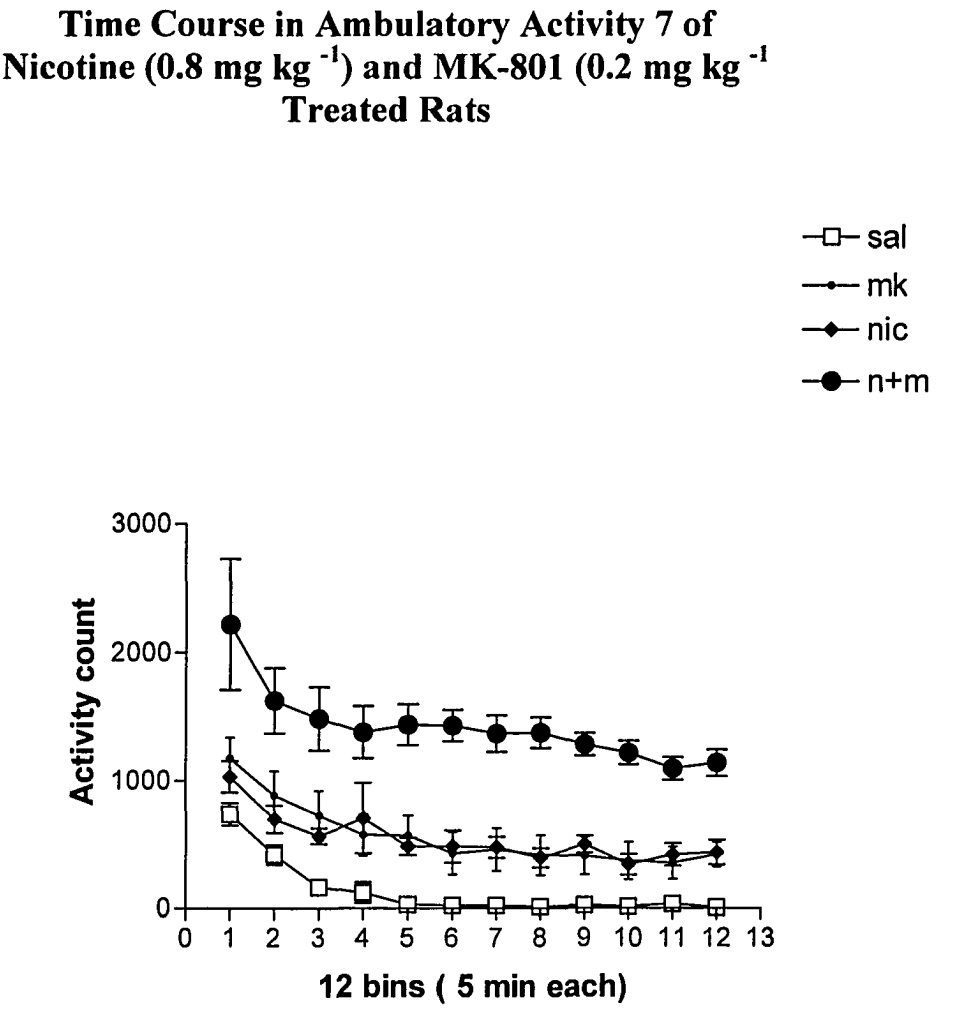


Figure 3.22. Time course effect of MK-801 (0.2 mg kg^{-1}) and nicotine (0.8 mg kg^{-1}) interactions on ambulatory activity in a 60 minute time session n=8. Data are means ± S.E.M.

Consecutive Locomotor Activity

Three-way repeated measures ANOVA with Greenhouse-Geisser correction showed significant main effects of MK-801 [F (1,16)=15.777, p<0.001], and time [F(11,176)=6.812, p<0.000]. The MK-801 and nicotine combination showed an increase in locomotor activity and there was significant interaction between MK-801 x nicotine [F(1,16)= 2.608, p=0.022] shown in figure 3.23. In addition, there was significant interaction between MK-801 x time [F (11,176=2.541, p>0.032] but not amongst MK-801 x nicotine x time [F (11,176), .510, p=0.777]. Post-hoc analysis (P<0.05) conducted on group mean values (collapsed across time) indicated a significant interaction between MK-801 x nicotine. The local time course data of this interaction in consecutive activity are shown in figure 3.24.

Consecutive Activity in Saline/Nicotine (0.8 mg kg $^{-1}$) and MK-801 (0.2 mg kg $^{-1}$) Treated Rats

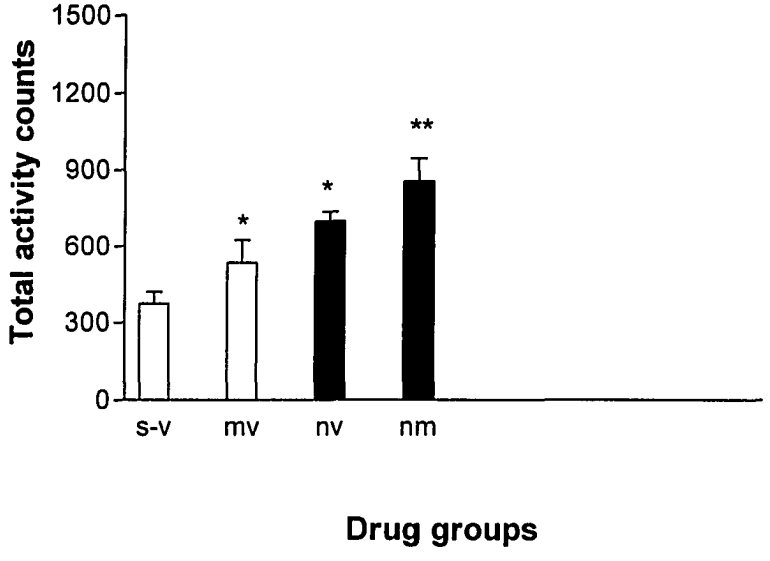


Figure 3.23. Effects of MK-801 (0.2 mg kg^{-1}) on consecutive locomotor activity of nicotine (0.8 mg kg^{-1}) in a 60 minute test session n=8. Data are means ± S.E.M.*Significance at ≤ 0.05, relative to saline; ** significance relative to nicotine/vehicle.

Time Course in Consecutive Activity on Day 7 of Nicotine (0.8 mg kg $^{-1}$) and MK-801 (0.2 mg kg $^{-1}$)

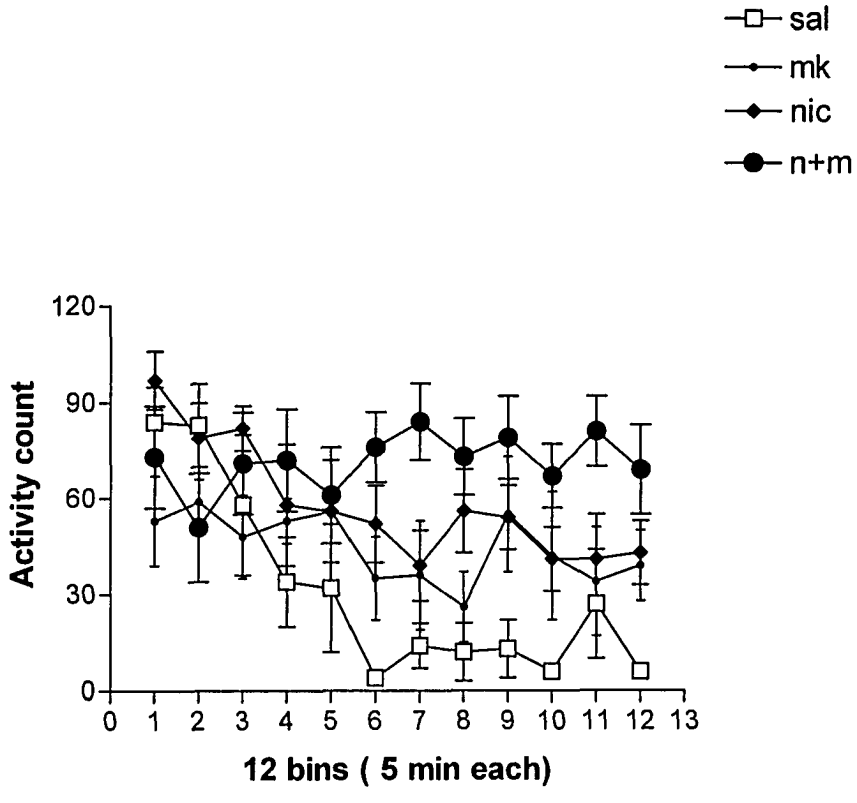


Figure 3.24. Effects of MK-801 (0.2 mg kg^{-1}) on consecutive locomotor activity of nicotine (0.8 mg kg^{-1}) in a 60 minute test session n=8. Data are means ± S.E.M.

Vertical Locomotor Activity

Three-way repeated measures ANOVA with Greenhouse-Geisser correction showed no significant main effects of MK-801 [F (1, 16) = .573, p<=.460], time [F (11,176) =63.521, p<0.061]. The MK-801 and nicotine combination showed increases in locomotor activity but the increase in locomotor activity due to the said interaction between MK-801 x nicotine did not attain the level of significance [F (1, 16) = 1.961 p=0.130] shown in figure 3.25. In addition, there was no significant interaction between MK-801 x time [F (11,176=1.165, p>0.334] nor amongst MK-801 x nicotine x time [F (11,176), =.461, p=0.736]. As there was no significant interaction between MK-801 and nicotine, tests of simple effects were not suitable. The local time course data of this interaction in vertical activity are shown in figure 3.26.

Vertical Activity in Saline/Nicotine (0.8 mg kg $^{-1}$) and MK-801 (0.2 mg kg $^{-1}$) Treated Rats

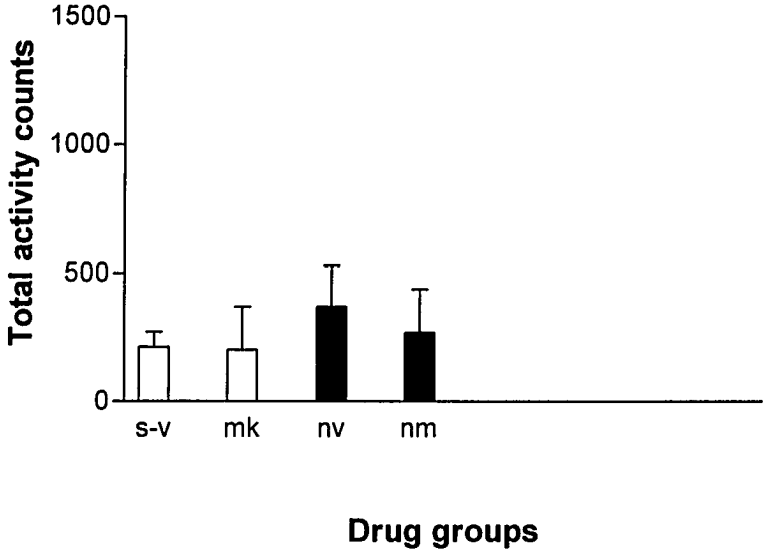


Figure 3.25. Effects of MK-801 (0.2 mg kg^{-1}) on vertical locomotor activity of nicotine (0.8 mg kg^{-1}) in a 60 minute test session. Data are means ± S.E.M.

Time Course in Vertical Activity on Day 7 in Nicotine (0.8 mg kg $^{-1}$) and MK-801 (0.2 mg kg $^{-1}$) Treated Rats

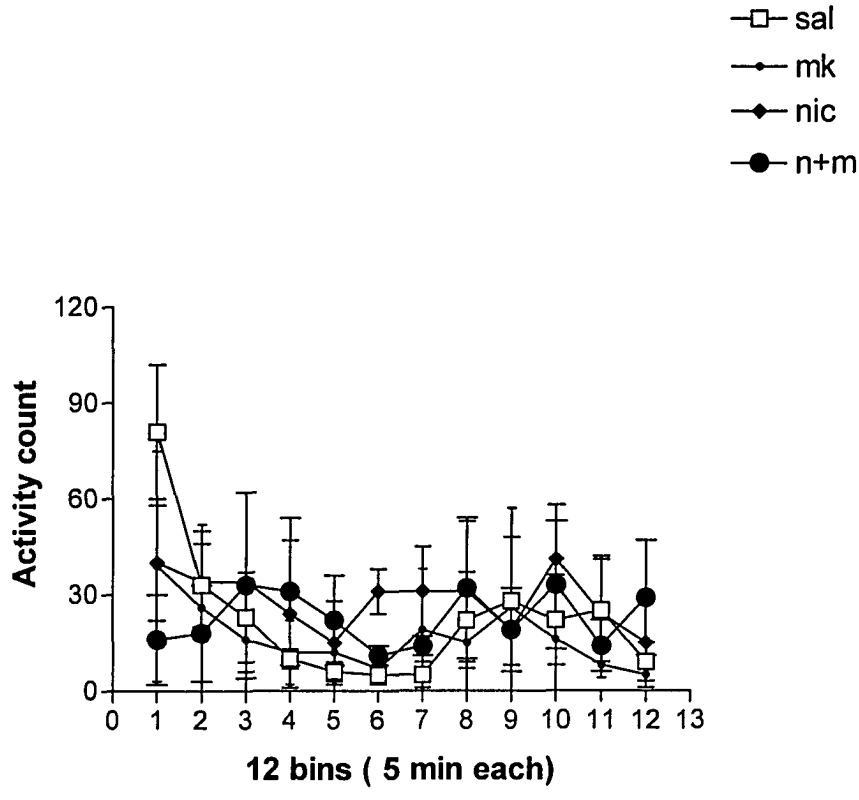


Figure 3.26. Effects of MK-801 (0.2 mg kg^{-1}) on vertical locomotor activity of nicotine (0.8 mg kg^{-1}) in a 60 minutes test session. n=8. Data are means ±S.E.M.

3.4. Discussion

In these experiments the repeated administration of nicotine in two doses (0.4 mg kg^{-1}, 0.8 mg kg^{-1}, s.c) to male Sprague-Dawley rats caused locomotor sensitization. Repeated administration of nicotine for 7 consecutive days produced a progressive increase in locomotor activity similar to the finding of previous studies (Clarke and Kumar, 1983; Belfour et al., 1998). The increase in locomotor activity in nicotine-treated rats was consistent throughout the 60 minute period each day and the total locomotor activity counts increased significantly each day although the dose of nicotine remained the same. It is generally well accepted that chronic administration of nicotine produces augmented locomotor responses (Stolerman et al. 1973; Clarke and Kumar, 1983; Kisr 1994). The phenomenon of behavioral sensitization was replicated in our study. The activity count was higher at lower dose (0.4 mg kg^{-1}) as compared with the higher dose (0.8 mg kg^{-1}) of nicotine (0.4 mg kg^{-1}, s.c) because at a higher dose excess release of DA and other neurotransmitters affect post-synaptic potential, thus decreses locomotor activity.

Nicotine, the active compound of tobacco, is the subject of considerable scientific and public discussion regarding its addictiveness (Stolerman and Jarvis, 1995). It is now widely recognized that tobacco-smoking behavior is maintained due to the presence of the psychostimulant nicotine in the inhalant smoke. Although considerable functional and neurochemical evidence provides commonalties between nicotine and other addictive drugs, the positive reinforcing property of nicotine is considered weaker as compared with other addictive drugs such as amphetamine and cocaine (Pontieri et al., 1996; Cadoni et al., 2000). Nevertheless, the reinforcing effect of nicotine has been

demonstrated in laboratory animals using the intravenous self-administration paradigm (Corrigall and Coen, 1989).

The increase in locomotor activity as seen from day 1 to day 7 suggests both development and expression of enhanced DA neurotransmission and subsequent long-term neuronal changes (Wise and Bozarth 1987; Koob 1992; Pidoplichko et al., 1997). Both D1 and D2 receptors of the mesolimbic DA system play an important role in mediating psychostimulant-induced locomotor hyperactivity, which can be blocked by D1 and D2 receptor antagonists (Dreher and Jackson, 1989; Imperato and Di Chiara, 1986; O'Neill et al., 1991). A strong argument for the relevance of locomotor sensitization to addiction comes from the observation of locomotor hyperactivity following administration of all three prototype stimulant drugs, namely nicotine, amphetamine and cocaine (Wise and Bozarth, 1987; Vezina and Queen, 2000).

In a study by Donny et al. (2000) both male and female Sprague-Dawley rats were allowed to self-administer different doses of nicotine and the results indicated that reliable rates of nicotine self-administration were observed in both male and female rats. Nicotine self-administration is decreased by the nicotine receptor antagonist mecamylamine (McCallum et al., 1999), indicating that nicotinic receptors are involved in self-administration. Intracranial microinjection of the nicotine agonist cytosine into midbrain DA terminals increases locomotor activity and these data support the notion that systemic nicotine interacts with the DA projections to the NAS to produce an increase in locomotor activity (Museo and Wise, 1990; Di Chiara, 2000) and enhances sustained attention and vigilance (Mirza and Stolerman, 1998).

Initiation and expression of sensitization have been reported to be behaviorally, neurochemically, and temporally distinct (Pierce and Kalivas, 1997). The present replication study demonstrates that expression of previously developed sensitization to nicotine was blocked by mecamylamine administered 40 minutes prior to nicotine. Unlike the case in nicotine-treated rats, the locomotor responses of saline-treated rats were not altered by mecamylamine. This finding of mecamylamine clearly indicates the involvement of nicotinic receptors consistent with the current findings; mecamylamine is also reported to inhibit physiological as well as other behavioral effects of nicotine (Henningfield and Goldberg 1983, Malin, 2001).

Nicotine-induced hyperactivity is a DA- mediated phenomenon (Fung, 1990). In order to confirm and extend this finding we studied the effect on nicotine hyperactivity of acute administration of the D_2 receptor antagonist, haloperidol at a dose of 0.1 mg kg^{-1}, s.c in both saline- and nicotine-treated rats in a counterbalanced order. In agreement with previous studies, haloperidol significantly decreased locomotor activity of nicotine-treated rats (Drehar and Jackson, 1989). No significant change occurred in the locomotor activity of saline-treated rats following treatment with haloperidol.

Considering the DA-glu interactions in nicotine-induced locomotor hyperactivity as presented in experiment 3.1, we wanted to examine the role of a NMDAR antagonist on expression of nicotine sensitization. We expected that co-administration of MK-801 would block the development of locomotor sensitization to nicotine; perhaps by blocking the neural changes (Shoaib et al. 1994). It is hypothesized that NMDA receptor antagonists can block or reduce the occurrence of the neural changes that underlie the

development of locomotor sensitization to a variety of drugs and imply that NMDA receptors are critical for the development of locomotor sensitization (Wolf, 1998). Results of our study indicate that co-administration of MK-801 did not prevent the locomotor hyperactivity of nicotine; rather MK-801 was shown to enhance locomotor hyperactivity in combination with nicotine. It seemed that acute co-administration of MK-801 after development of nicotine sensitization did not block the expression of the previously developed sensitization to nicotine; the rats injected with the MK-801/nicotine combination increased their locomotion *even more.* However, it was also noted that injections of MK-801 alone (MK-801/saline) showed increased locomotion compared to rats treated with saline only.

The enhanced locomotor response was observed with all doses of MK-801 combined with nicotine, but significant interactions between nicotine and MK-801 were seen in consecutive locomotor activity only. We recorded locomotor activity for a period of 60 minutes, 30 minutes after the injection of MK-801. Higher locomotor activity was recorded throughout the 60 minutes duration.

The precise neurobiological mechanisms by which MK-801 enhance the hyperlocomotion is currently unknown. Despite the existence of such convincing data on EAA and motor activity, studies using MK-801 to block stimulant induced locomotor sensitization have shown inconsistent and contradictory findings (Karibura et al., 1992, 1994; Tzschenke and Schmidt, 2000). In some rats higher locomotor activity was associated with unsteadiness and hyper-responsiveness to sensory stimuli such as touch and light. This behavioral observation may suggest that this model could be an experimental model of schizophrenia due to hypoglutamatergic transmission (Carlsson et

al., 1999). As there was no interaction with nicotine and ambulatory activity, it is possible that the enhanced locomotor observed in the MK-801/nicotine combination was due to an additive effect.

Our data address the important issue of how MK-801 can produce locomotor sensitization (when it is presumed to block neural mechanisms that are critical for sensitization). Data from our study suggest that the effects of MK-801 in blocking nicotine sensitization may be confined to the development of neuronal changes for sensitization. MK-801 given after the previously developed sensitization to nicotine does not block the expression of the sensitized response to a challenge injection of nicotine administered acutely. Whatever the resolution of these more general controversies, our data indicate that acute administration of MK-801 in an already nicotine sensitized (induced by repeated injections of nicotine) rat neither blocks the development of sensitization nor prevents the expression of sensitized behavior, i.e.locomotor hyperactivity. This was a unique experimental design to compare the effects of NMDA, DA (D_2), and nAChR antagonists (MK-801, mecamylamine, and haloperidol respectively) in blocking nicotine-sensitized locomotor activity. As shown in figures 3.8 and 3.9 both mecamylamine and haloperidol evidently decreased the locomotor hyperactivity of nicotine when administered acutely in nicotine-treated rats after development of sensitization (i.e. after seven days of nicotine treatment). As expected, both mecamylamie and haloperidol had no effect on saline-treated control rats. Thus it is assumed that both mecamylamine and haloperidol can prevent the development as well as the expression of behavioral sensitization. Based on the demonstrated results of mecamylamine and haloperidol, we tried to compare behavioral effects of MK-801,

mecamylamine and haloperidol in blocking nicotine-induced locomotor hyperactivity. Despite the expectation of comparable behavioral profiles of the three drugs based on theoretical concepts, the results of the present study indicate that it is very unlikely that blocking effect/behavioral profile of MK-801 is similar to mecamylamine and haloperidol in blocking nicotine-induced locomotor hyperactivity. Thus we conclude that the behavioral effect of MK-801 on the nicotine-sensitized rat is different from that of mecamylamine and haloperidol (Cole, 1993; Al-Khatib, 1995; Carey et al., 1998).

4. DIZOCILPINE MALEATE (MK-801) AND NICOTINE INTERACTION IN CONDITIONED PLACE PREFERENCE: UNBIASED DESIGN

4.1. Introduction

Behavioral effects related to Pavlovian cues are considered to be mediated through the integrated functioning of several neurotransmitters in the brain involving the reward circuitry connectivity of the NAS, VTA, mPFC, and limbic structures (Swerdlow et al., 1989; Mogenson et al., 1980). It is currently hypothesized that concurrent DA and NMDA receptor activation in the NAS is required for CPP (Spyraki et al., 1982; Carr et al., 1989; Bardo et al., 1998). Establishment of CPP is blocked by D1 and D2 receptor blockers (Ranaldi and Beninger, 1993) and by some NMDA (Sukhotina et al., 1998) and other GluR antagonists (Kaddis et al., 1995). Animal models of cocaine-induced neuroplasticity have demonstrated that increases in both DA and glu transmission in the NAS are important for the mechanisms that underlie drug addiction (Pierce and Kalivas, 1997; Kelly, 2002).

Administration of nicotine stimulates nAChRs to facilitate DA and glutamatergic neurotransmission with reciprocal inhibition of GABA tone of the medium spiny neurons in the NAS as illustrated in figure 1.9 (Carlsson, 1999; Hyman and Malenka, 2001). Activation of brain nicotinic receptors may represent a common denominator involved in the process of sensitization induced by protypical psychostimulants (Hoffman and Beninger, 1989; Pontieri et al., 1996; Marshall et al., 1997). DA and glu play a key role in mediating acquisition and expression of Pavlovian appetitive conditioned responses (Mucha and Iverson, 1984; Mithani et al., 1986).

Glu transmission in drug addiction has long been thought to be involved in learning and plasticity within the mesocortical and limbic system (Museo and Wise, 1994; Papp et al., 1996; Wolf, 1998 for review; Hyman and Malenka, 2001). Previous studies showed that systemic administration of glu receptor antagonists blocked stimulant-induced locomotor sensitization, acquisition of CPP and self-administration (Museo and Wise, 1994; Cervo and Samanin 1995; See, 2002).

Although it has been suggested that glu transmission in the VTA is involved in the addictive properties of cocaine (Unglass et al., 2001), the role of this input in conditional reinforcement is relatively unexplored. Considering DA-glu interactions in nicotine-induced behavioral sensitization and acquisition of CPP, we sought to determine if NMDA glutamate receptor activation is crucial to the development of nicotine-induced CPP by treating nicotine-sensitized rats with the NMDA receptor antagonist MK-801. To the best of our knowledge this is the first study to examine the effects of MK-801 on acquisition and expression of CPP in nicotine-treated rats.

4.2. Method

Unbiased design of CPP: Animals were randomly assigned to drug groups. In the unbiased design each group was counterbalanced so that an equal number of animals in each group received drug pairings in either the grate or bar floored compartment, thus exposing them to two distinct environments. Baseline place preference was not taken into consideration in this unbiased design of CPP.

4.3. Experiment:

Phase 1

Rats (n=36) were randomly assigned to four groups: Nicotine (NIC) n = 9, MK-801 (MK) n = 9, Amphetamine (AMPH) n = 9, or Nicotine + MK-801 (NIC+MK) n = 9. After initial handling (15 minutes/day for 3 days) by holding on palm wrapped with towel the rats were given free access to both compartments of the CPP apparatus (by keeping the tunnel open) to assess their baseline preference for any specific compartments. Total time spent on each compartment of the shuttle boxes was recorded over 15 minutes/day for 3 days.

Phase 2

The NIC and NIC+MK groups received daily injections of nicotine (0.8 mg kg^{-1}, s.c) while the MK-801 and AMPH groups received saline injections for 7 days.

Following this the groups of rats were injected with drugs on days 1, 3, 5 and 7 in the following manner:

- MK group: MK-801 (0.2mg kg^{-1}, IP) followed by saline injection at 20 minutes.
- MK+NIC group: MK-801 (0.2 mg kg^{-1}, IP) followed by nicotine (0.8 mg kg^{-1}, s.c) at 20 minutes.
- NIC group: Saline (1 ml kg^{-1}) followed by nicotine (0.8 mg kg^{-1}, s.c) at 20 minutes. and,
- AMPH group: Saline (1 ml kg^{-1}) followed by (+)-amphetamine (1.5 mg kg^{-1}, IP) at 20 minutes.

Thirty minutes following the injection of MK-801 or vehicle the rats were confined to a compartment of the CPP box in a counterbalanced design. Thus half of the rats were confined in one compartment of the box with the grid floor and the remaining half were confined to the other compartment of the box with the bar floor for a total duration of 30 minutes in each compartment.

On days 2,4,6, and 8 all groups of rats were treated with saline injections in the same manner as with the drug days and were confined for 30 minutes to the side of the box that was not paired with drug (as illustrated in figure 2.2).

Phase 3

In this phase the rats were given free access to both compartments for 15 minutes. Total time spent in each compartment and in the tunnel was recorded manually by a digital timer. Total time spent in the tunnel (if any) was deducted from total time.

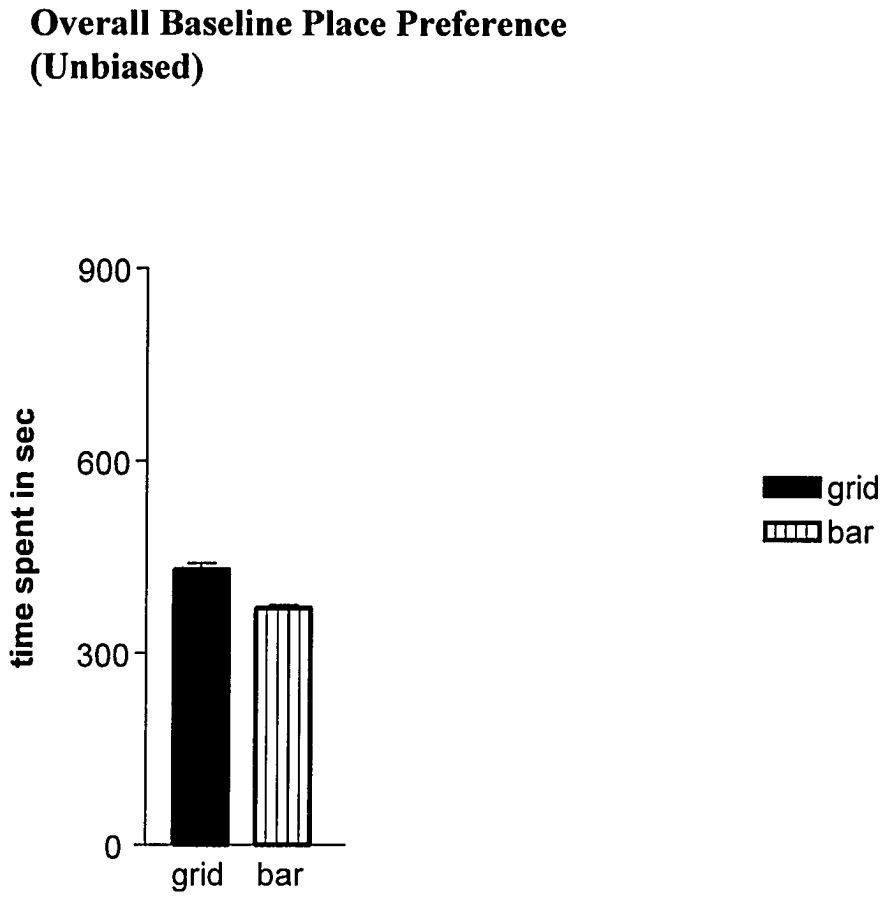


Figure 4.1. Overall baseline place preference of naïve rats: unbiased. Animals spent more time on the grid floor compartment as compared to the bar floor compartment [$t(8) = 6.438$, $P>0.05$]. Following habituation, animals were tested for initial baseline place preferences in a drug-free state for three days. Data are means±S.E.M. *Significant at $P<0.05$.

4.4. Results

4.4.1. Effects of Nicotine on Conditioned Place Preference

In their overall baseline place preference observed for three days, naïve rats preferred to spent more time on the grid floor compartment as compared to the bar floor but the difference was not statistically significant (figure 4.1).

Systemic administration of nicotine at a dose of 0.8 mg kg^{-1} did induce CPP in the unbiased design [$t(8) = 2.8701$, $P<0.05$] shown in figure 4.2. There was significant change in the amount of time spent in the conditioned compartment before or after drug treatment on day 1 only. Time spent in the conditioned compartment revealed reduction of nicotine place preference on postconditioning days 2 and 3.

Unbiased CPP Test with Nicotine (0.8 mg kg $^{-1}$)

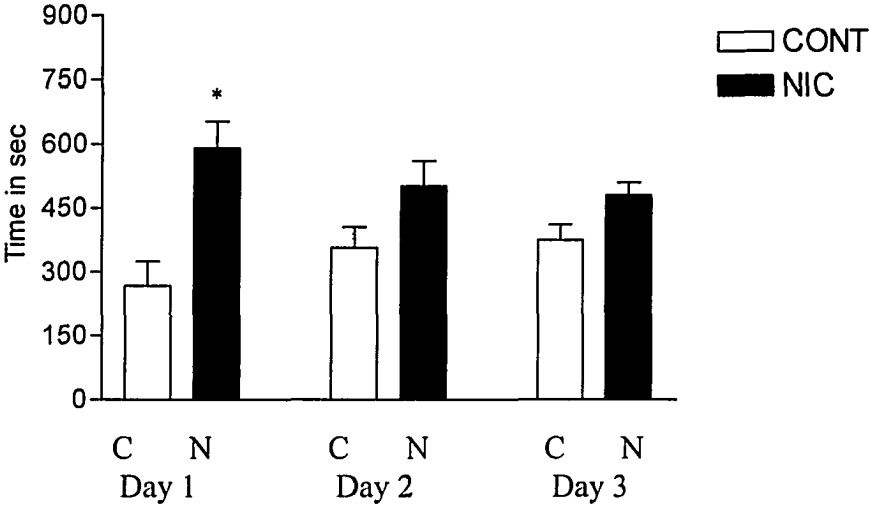


Figure 4.2. Effects of nicotine (0.8mg kg^{-1} s.c.) on unbiased CPP test. Nicotine induced a significant place preference [$t(8) = 5.047$, $P<0.05$] on day one. Each animal received four drug and four vehicle injections on alternating days (n=9). Following conditioning, animals were tested for CPP in a drug-free state. Data are means±S.E.M. *Significant at $P<0.05$.

4.4.2. Effects of Amphetamine on Conditioned Place Preference

Systemic administration of amphetamine at a dose of 1.5 mg kg^{-1} did induce CPP in the unbiased design [$t(8) = 4.406$, $P<0.05$] shown in figure 4.3. There was significant change in the amount of time spent in the conditioned compartment before or after drug treatment. Additional tests performed on time spent in the conditioned compartment revealed significant place preference on postconditioning days 1 and 2. CPP was extinct on day 3.

Unbiased CPP Test with AMPH (1.5 mg kg $^{-1}$)

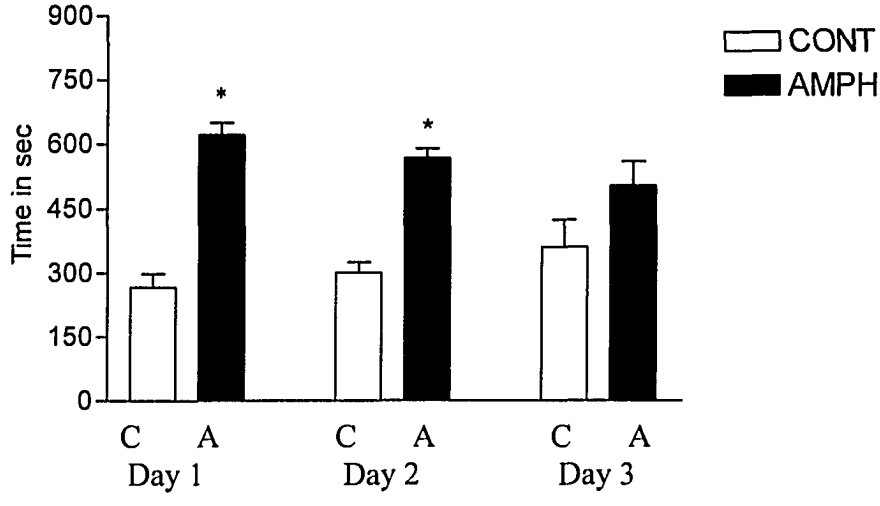


Figure 4.3. Effects of amphetamine (1.5 mg kg^{-1} s.c) on unbiased CPP test. Amphetamine-induced CPP [t (8) = 4.406 $P<0.05$]. Each animal received four drug and four vehicle injections on alternating days (n=9). Following conditioning, animals were tested for CPP in a drug-free state. Data are means±S.E.M. *Significant at $P<0.05$.

4.4.3. Effects of MK-801 on Conditioned Place Preference

Systemic administration of MK-801 alone at a dose of 0.2 mg kg^{-1} did not induce CPP in the unbiased design [t (8) = 1.748, P>0.05] shown in figure 4.4. There was no significant change in the amount of time spent in the conditioned compartment before or after drug treatment in all 3 post conditioning days.

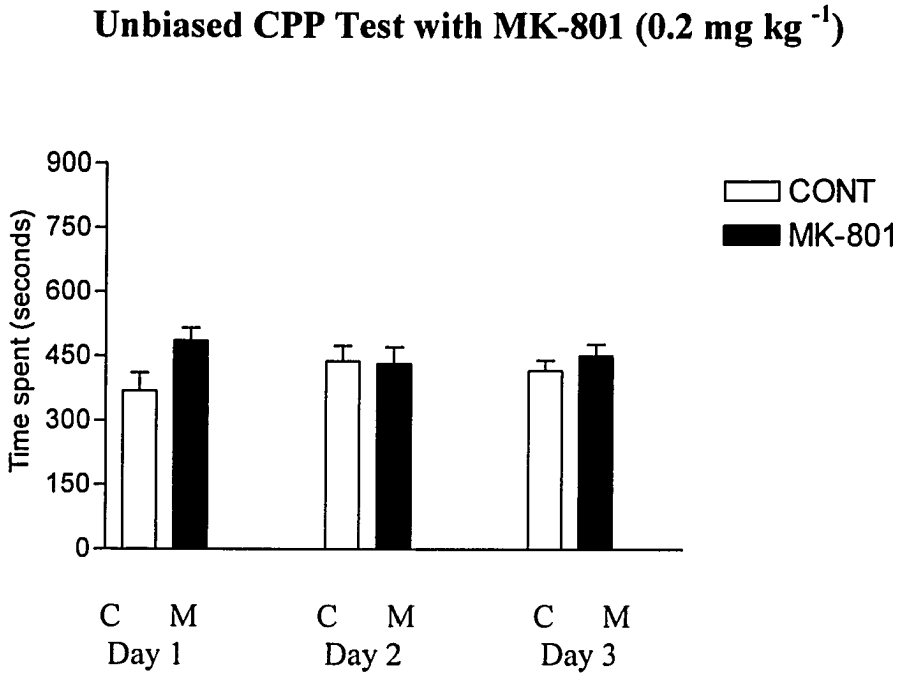


Figure 4.4.Lack of effects of MK-801 (0.2 mg kg $^{-1}$) on CPP test [t (8) = 1.748 P<0.05]. Each animal received four drug and four vehicle injections on alternating days (n=9). Following conditioning, animals were tested for CPP in a drug-free state. Data are means±S.E.M. *Significant at P<0.05.

4.4.4. Effects of MK-801 in Inducing Conditioned Place Preference in Nicotine-Sensitized rats

Systemic administration of MK-801 at a dose of 0.2 mg kg^{-1} in nicotine-sensitized rats neither produced CPP nor CPA [t (8) = 1.061, P>0.05] shown in figure 4.5. There was no significant change in the amount of time spent in the conditioned compartment before or after drug treatment. Time spent in the conditioned compartment revealed neither CPP nor CPA on all 3 postconditioning days.

Results of all unbiased CPP experiments are discussed with the results of biased CPP in section 5.4.

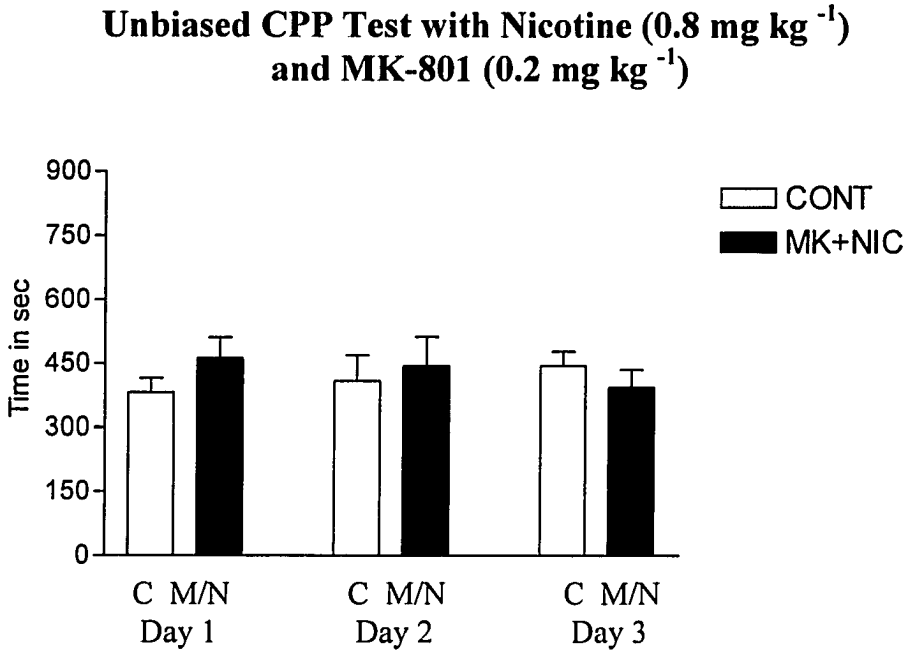


Figure 4.5. Lack of effects of a combination of MK-801 (0.2 mg kg $^{-1}$mg) and nicotine (0.4 mg kg $^{-1}$) in CPP test. [t (8) = 1.061, P>0.05]. Each animal received four drug and four vehicle injections on alternating days (n=9). Following conditioning, animals were tested for CPP in a drug-free state. Data are means±S.E.M.

5. DIZOCILPINE MALEATE (MK-801) AND NICOTINE INTERACTION IN CONDITIONED PLACE PREFERENCE: BIASED DESIGN

5.1. Novelty Seeking Effect in CPP and Biased Design

Rats given free access to a novel environment and a familiar environment will spend more time in the novel environment, and this is known as the novelty effect. Access to novel objects, similar to drugs of abuse, can enhance a place preference in rats (Bardo and Brevins, 2000). It is well established that animals have a tendency to prefer novel stimuli. It is important to note that relative novelty can enhance induction of CPP (van der Kooy, 1982). This important behavioral characteristic of the novelty effect might compromise the validity of CPP as a measure of reward and reinforcement. In order to demonstrate that a drug has reinforcing properties using the place conditioning procedure, it would seem necessary to establish a preference that differs from that which could be produced by novelty alone (Spyraki et al., 1982).

If rats are given free access to a novel and a familiar environment, they will spend more time in the novel environment (Bardo and Brevins, 2000). It is reported that if rats are given a choice between a novel and a familiar object they spend more time interacting with the novel object. The preference for novel stimuli in a choice situation has often been described by researchers as 'novelty seeking'. The term 'neophobia' should not be confused with fear of a new environment as the term refers to avoidance of novel food (Galloway et al., 2003). Novelty seeking is thought to be maintained by some appetitive or rewarding aspect of novel stimulation. Access to novel stimuli has an appetitive quality comparable to drugs of abuse. Like access to novel stimuli, drugs of abuse such as

morphine, nicotine, cocaine, and amphetamine produce an increase in preference for an environment in which they are administered (Spyraki et al., 1982).

During this free-choice test, rats spend more time in the novel environment and treatment with the non-specific dopamine antagonist haloperidol or the D1 antagonist SCH-23390 blocks the preference for the novel environment (Bardo et al., 1999). The biased design of CPP was developed to alleviate the novelty effect in determining rewarding effects of stimulant drugs. In the biased paradigm subjects are pretested to determine the animal's side preference (Bozarth, 1987).

Thus, in order to minimize the novelty effect, the drug was consistently paired with the least preferred compartment because it is considered that pairing drug to the less preferred compartment and pairing saline in the preferred compartment on alternate days might alleviate the novelty seeking effect (Bozarth & Wise, 1981; Phillips and Le Paine, 1980). Regarding the demonstration of initial place preference, some researchers reported that naïve rats show preference for one side of the CPP box but others (Carr and White, 1983) found no preference for any particular compartment of the box. Thus, the biased design is well suited to deter the novelty effect in CPP.

However, both biased and unbiased designs have met with some criticism. In the biased design the obligatory drug conditioning in the less preferred compartment may create an inequality in exposure during conditioning (Swerdlow et al., 1989). This inequality of exposure might bias the result of place preference. But despite criticism, considering the powerful effect of novelty in CPP, the biased place-conditioning paradigm should be used to compare and confirm the positive reinforcing or aversive properties of both peripherally and centrally administered drugs of abuse (Spyraki et al., 1982).

5.2. Experiment

The initial place preference (phase 1) was done for 3 three days. In this biased test design, animals are first tested for their baseline preference between two environments and are confined to the less preferred compartments following drug treatment for conditioning. Like the unbiased design, in this experiment rats were randomly assigned to four groups: Nicotine (NIC) n = 9, MK-801 (MK) n = 9, Amphetamine (AMPH) n = 9, and Nicotine + MK-801 (NIC+MK) n = 9. After initial handling for habituation the rats were given free access to both compartments of the CPP apparatus (by keeping the tunnel open) to assess their initial preference for any specific compartments. Total time spent in each compartment of the shuttle boxes was recorded over a 15 minute period for 3 days. Nicotine and nicotine + MK-801 groups received chronic injection of nicotine (0.8 mg kg^{-1}, s.c) while the MK-801 and amphetamine groups received saline injection for 7 days. In phase 2 of the experiment rats were injected with drugs on days 1,3,5 and 7 in the following order:

- MK group: MK-801 (0.2 mg kg^{-1}, IP) followed by saline injection at 20 minutes
- MK+NIC group: MK-801 (0.2 $mgkg^{-1}$, IP), followed by nicotine (0.8 $mgkg^{-1}$, s.c) at 20 minutes.
- NIC group: Saline injection (1 ml kg^{-1}) followed by nicotine (0.8 mg kg^{-1}, s.c) at 20 minutes and,
- AMPH group: Saline injection (1 ml kg^{-1}) followed by amphetamine (1.5 mg kg^{-1}, IP) at 20 minutes.

At 30 minutes time the rats were confined to the less preferred compartment of the CPP box, in a counterbalanced design.

On days 2, 4, 6, and 8 all groups of rats were treated with saline injection and were confined for 30 minutes to the preferred side of the box. In phase 3 rats were given free access to both compartments for 15 minutes. Time spent in each compartment and in the tunnel was recorded manually. Total time spent in the tunnel (if any) was deducted from total time.

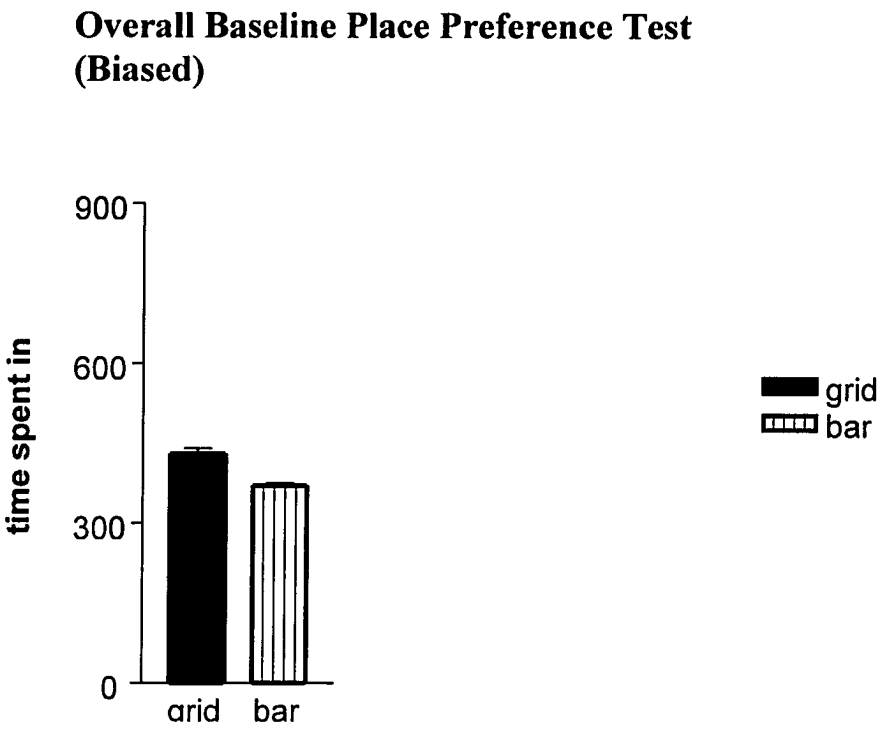


Figure 5.1. Overall baseline place preference of naïve rats: biased design. Animals spent more time on the grid floor compartment as compared to the bar floor compartment [$t(8) = 2.925$, $P>0.05$]. Following habituation, animals were tested for initial baseline place preferences in a drug-free state for three days. Data are means±S.E.M. *Significant at $P<0.05$. .

5.3. Results

5.3.1. Effects of Nicotine on CPP

In their three days overall baseline place preference naïve rats spent more time on the grid floor compartment as compared to the bar floor but it was not statistically significant (figure 5.1).

Systemic administration of nicotine at dose 0.8 mg kg^{-1} did induce CPP in the biased design paradigm [$t(8) = 2.449$ $P<0.05$] shown in figure 5.2. There was significant change in the amount of time spent in the conditioned compartment before or after drug treatment. Additional tests performed on time spent in the conditioned compartment revealed significant extinction of nicotine place preference by postconditioning day 3.

Biased CPP Test with Nicotine (0.8 mg kg $^{-1}$)

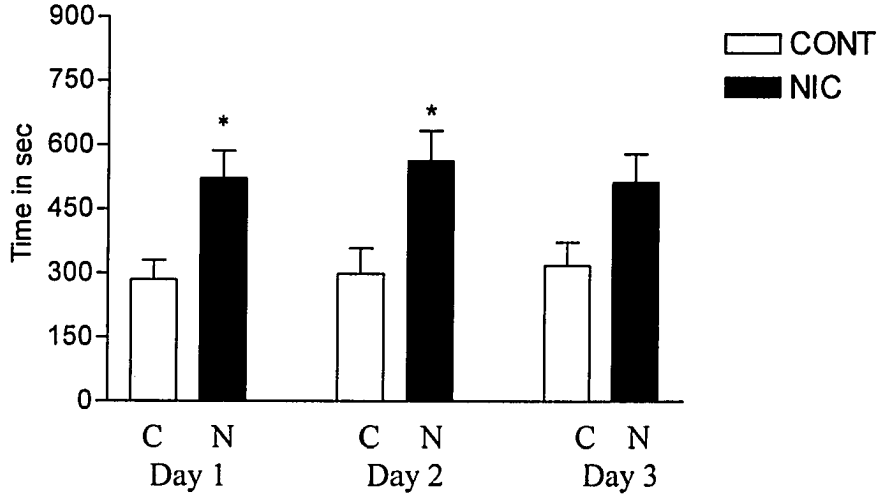


Figure 5.2. Effects of nicotine (0.8mg kg^{-1} s.c.) on biased CPP test. Nicotine induced CPP [t(8) = 2.449 P<0.05].Each animal received four drug and four vehicle injections on alternating days (n=9). Following conditioning, animals were tested for CPP in a drug-free state. Data are means±S.E.M. *Significant at P<0.05.

5.3.2. Effects of Amphetamine on Conditioned Place Preference

Systemic administration of amphetamine at 1.5 mg kg^{-1} induced CPP in the biased design CPP [$t(8) = 5.047$ $P<0.05$] shown in figure 5.3. There was significant change in the amount of time spent in the conditioned compartment before or after drug treatment. Tests performed on time spent in the conditioned compartment revealed significant place preference on all 3 postconditioning days.

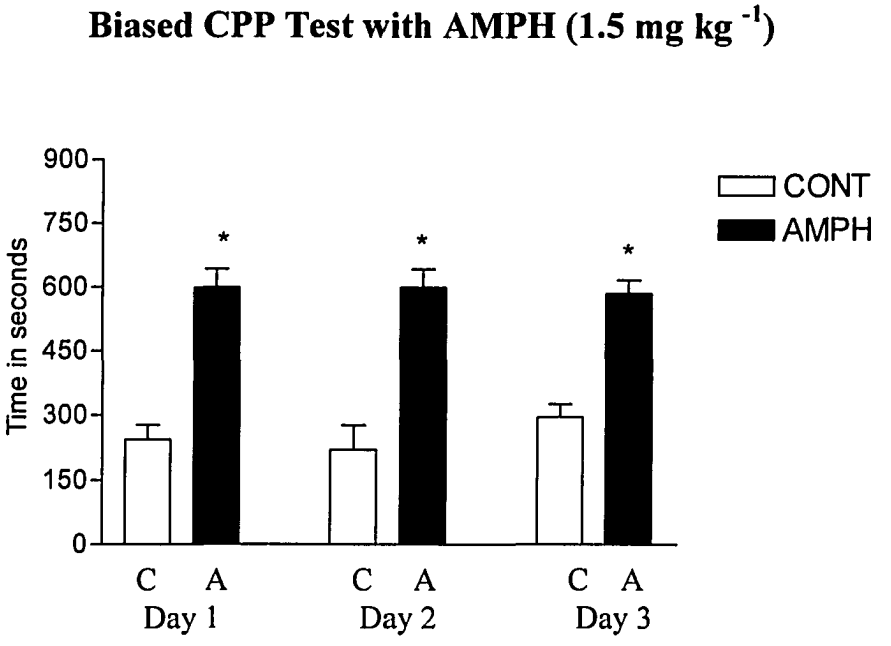


Figure 5.3. Effects of amphetamine (1.5mg kg^{-1} IP) on biased CPP test. Amphetamine induced CPP [t (8) = 5.047 $P<0.05$]. Each animal received four drug and four vehicle injections on alternating days (n=9). Following conditioning, the animals were tested for CPP in a drug-free state. Data are means±S.E.M. *Significant at $P<0.05$.

5.3.3. Effects of MK-801 on Conditioned Place Preference

Systemic administration of MK-801 alone at a dose of 0.2 mg kg^{-1} did not induced CPP in the biased design [t (8) =. 750 P>0.05], shown in figure 5.4. There was no significant change in the amount of time spent in the conditioned compartment before or after drug treatment. There was no place preference on all 3 postconditioning days.

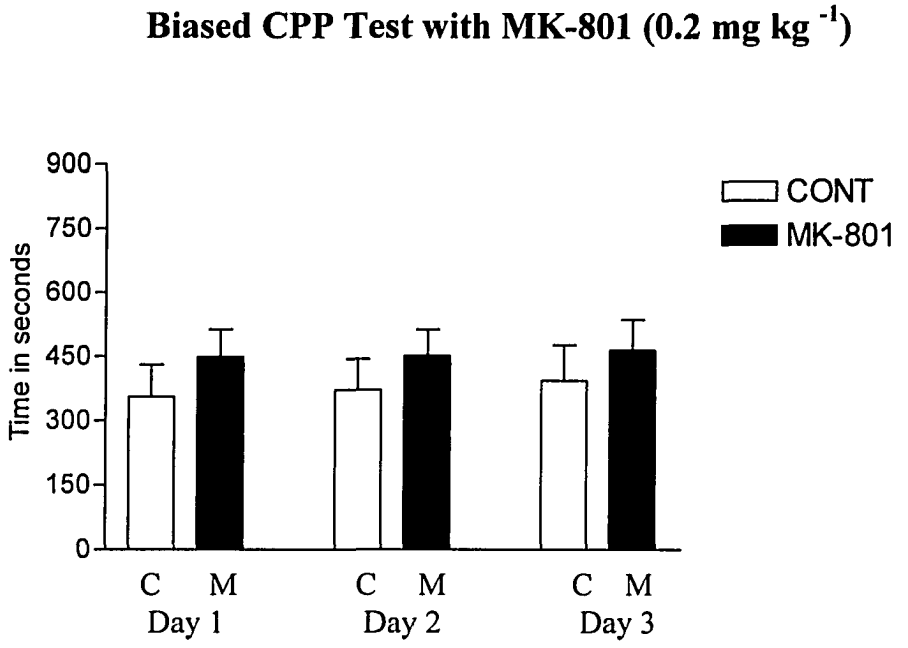


Figure 5.4. Lack of effects of MK-801 (0.2mg s.c.) on biased CPP test. MK-801 induced neither CPP nor CPA place preference [t (8) =. 750 P>0.05]. Each animal received four drug and four vehicle injections on alternating days (n=9). Following conditioning, animals were tested for CPP in a drug-free state. Data are means±S.E.M.

5.3.4. Effects of MK-801 in Inducing CPP in Nicotine-Sensitized Rats

Systemic administration of MK-801 at a dose of 0.2 mg kg^{-1} in nicotine sensitized rats produced CPP on first day only [$t(8) = 4.189$, $p<0.05$]. Tests performed on time spent in the conditioned compartment revealed neither CPP nor CPA on postconditioning days 2 and 3.

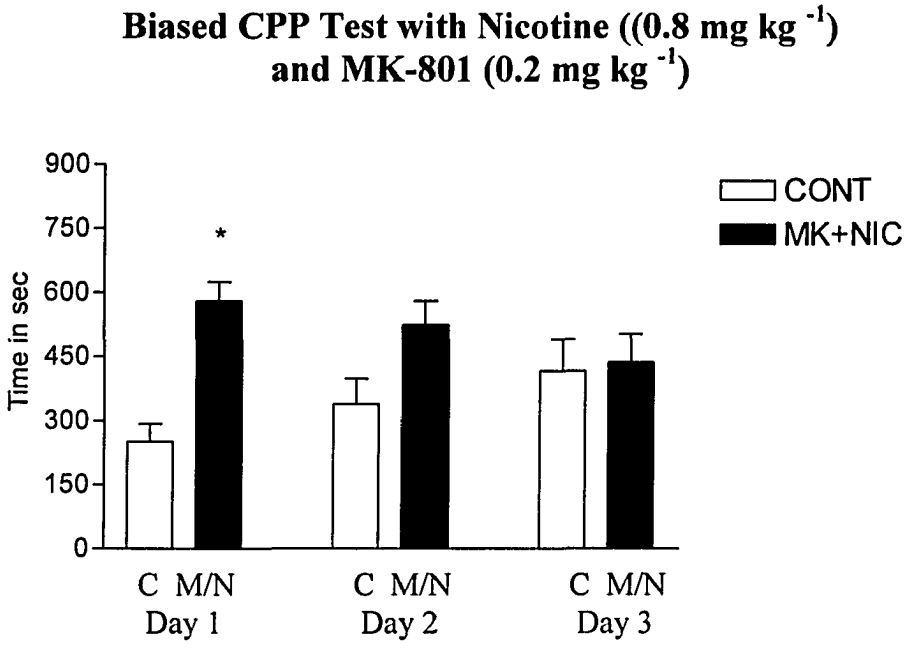


Figure 5.5. Effects of MK-801 (0.2 mg kg $^{-1}$IP) and nicotine (0.4 mg kg $^{-1}$g s.c) on biased CPP test. The drug combination induced CPP on day 1 [t (8) = 4.189, $p<0.05$]. Each animal received four drug and four vehicle injections on alternating days (n=9). Following conditioning, animals were tested for CPP in a drug-free state. Data are means±S.E.M. *Significant at $P<0.05$.

5.4. Discussion on Both Unbiased and Biased CPP

Our result indicating that prior exposure of nicotine in rats induces CPP is consistent with the findings of prior studies (Shoaib et al 1994). At 0.8 mg kg^{-1} nicotine induced CPP, but failed to induce CPP at a dose of 0.4mg kg^{-1} in other studies (Clarke and Fibiger 1987). It is generally expected that under appropriate conditions, drugs that have rewarding effects such as cocaine (Nomikos and Spyraki, 1988), amphetamine (Spyraki et al. 1982), methamphetamine (Martin-Iverson et al. 1985), morphine (Bardo et al. 1984), and nicotine (Shoaib et al. 1994) may induce CPP. With rewarding drugs, there appears to be reasonable concordance between self-administration and CPP (Bardo et al. 1999). Considering the important interrelationship between DA and glu (Kelley, 2002) in inducing CPP it is expected that MK-801 might block the rewarding effects of nicotine by preventing glutamatergic transmission (Vasiliadis et al., 1999). As expected, amphetamine induced a robust CPP in both the biased and unbiased designs. In our unbiased CPP study, nicotine induced CPP but the reinforcing effect of nicotine was less robust than amphetamine. Also, MK-801 failed to block reinforcing effect of nicotine on CPP when used in combination with nicotine. In the biased design of study MK-801 in combination with nicotine induced CPP; this induction of CPP in combination was observed at day one only.

With the CPP paradigm it has been difficult to demonstrate unequivocally nicotine's reinforcing effects; previous studies intended to induce CPP with nicotine provided equivocal results. For example Fudala et al., (1985) and Feudala and Iwamoto (1986) observed a CPP at a nicotine dose of 0.8mg / kg^{-1} and CPP was not induced when the dose was increased to 1.5 mg kg^{-1}. On the other hand Clarke and Fibiger (1987) found no

evidence of CPP at different doses nicotine such as 0.2, 0.4, and 0.8 mg kg^{-1}. Nicotine-induced CPP is very sensitive to dose of the drug and environmental cues (Risinger and Oakes, 1995). Prior exposure to nicotine for 6 days at a dose of 0.6 mg Kg-1 did not induce CPP or CPA following subsequent conditioning with 0.6 mg kg^{-1} nicotine (Jorenby et al., 1990).

Nevertheless, many other studies have confirmed that nicotine may exhibit locomotor hyperactivity, CPP, self-administration, self-stimulation and produce characteristic signs and symptoms when withdrawn after chronic use (Hildebrand et al, 1997; Koob et al. 1998; Di Chiara, 2000). Age of the animal subject (rats) may be an important factor in revealing rewarding effects of nicotine because it is argued that their brains are differently sensitive to the addictive effects of nicotine at different stages of life. For example, Belluzi et al., (2004) reported that during early early adolescence (age 28-30) a single injection of nicotine (0.5 mg kg^{-1}) induced CPP. In contrast, during late adolescence (age 38-41days) or adulthood (age 90-94 days) nicotine did not induce CPP after either one or four conditioning trials.

On the basis of this finding it is clear that nicotine has strong reinforcing properties but this depends on subject selection and experimental paradigms used (Le Foll and Goldberg, 2005).

Considering DA and glu interactions in inducing CPP by nicotine we sought to determine whether MK-801 would block nicotine-induced CPP. In our locomotor study we found enhanced locomotor activity when nicotine was combined with MK-801, which indicates that MK-801 did not block nicotine-sensitized locomotor activity. In this CPP study I tried to elucidate the possible blocking effect of MK-801 on the conditioned reward of

nicotine. At the same time I was curious to examine the reported rewarding effect (if any) of MK-801 (Tzschentke and Schmidt 2000). To the best of my knowledge, this was the first study on nicotine and MK-801 interactions in a CPP paradigm.

In the unbiased design of the CPP test, co-administration of MK-801 and nicotine did not induce CPP. In a similar study using amphetamine and MK-801 in combination, CPP induced by amphetamine was not prevented by MK-801 (Hoffman, 1994). In the biased design of my study nicotine when combined with MK-801 induced CPP. These results indicate that CPP still can occur in the presence of NMDA receptor blockade by MK-801, especially if MK-801 is administered acutely after development of nicotine sensitization.

GluRs are very complex, with different binding sites, and various NMDA receptor antagonist drugs have different behavioral profiles (Cole et al. 1993). For example, while the NMDA receptor blocker MK-801 enhances locomotor activity, AP5 decreases locomotor activity (Jerram et al., 1996). Obviously, further experiments involving manipulation of other subtypes of GluR are needed to fully understand their behavioral effects.

Some studies reported that MK-801 might have rewarding effects as demonstrated in CPP, locomotion and even self administration (Carlezon and Wise, 1996; Ouagazzal et al., 1993). Furthermore, microdialysis studies indicate that systemic administration of MK-801 releases DA in the brain (Yan et al., 1997; Wedzony et al., 1993). NMDA receptors have been shown to play a critical role in the acquisition of a number of learned behaviors. Blockade of NMDA receptors with both competitive and non-competitive antagonists may lead to deficits in the acquisition and in the extinction of conditioned

behavior and spatial learning (Carey et al., 1998). Rats treated with MK-801 alone in both biased and unbiased place conditioning experiments, I found neither place preference nor place aversion; these results extend the findings of Tzschenke (1998) and Sufka (1994). It is possible that MK-801-induced increased locomotor activity is not relevant to rewarding properties (Tzschenke, 1998).

If MK-801 possesses rewarding effects of its own then synergistic effects of the MK-801 and other rewarding drugs in combination would be expected to induce CPP. Sukhotina et al. (1998) reported induction of CPP with MK-801 but the experimental protocol, selection of animal subjects and design of the entire CPP apparatus was different from my study. Based on the available data it can be argued that reinforcing properties of MK-801 in the place preference paradigm are not always revealed but may be subject to experimental paradigm, environmental cues and dose of the drug (Tzschenke, 1998).

6. GENERAL DISCUSSION

6.1. General Discussion on Locomotor Activity

In agreement with previous studies, nicotine at two doses (0.4/kg and 0.8 mg kg^{-1}, s.c) increased locomotor activity (Benwell and Belfour, 1992). Repeated administration of both doses of nicotine for seven consecutive days induced a progressive increase in total locomotor activity. This effect was largest following the 0.4 mg kg^{-1} dose. The nicotine induced increase in locomotor activity was observed on each treatment day. Increased motor activity was also evident from the measures of consecutive and vertical motor activity although this was not significant in all days. In the nicotine treated rats increased motor activity was sustained throughout the 60-minute test period. The total activity of the saline-treated rats did not change over the seven days. It is worth noting that the term psychomotor hyperactivity should not be confused with circumstantial agility or hyperactivity associated with sensory stimuli such as pain (Wise and Bozarth, 1987; Ballas et al., 2004). We observed that in our experiments nicotine treated rats were more vigilant and responsive to the environment such as touch and sound (Mirza and Stolerman, 1998); this observation strongly supports the numerous reports suggesting nicotine or nAChR agonist as possible targets for treatment of dementia, depression and cognitive abnormalities (Koelega, 1993; Levin, 1992; Martin 2004). It is expected that nicotine research will open new frontiers for therapeutic applications of nicotine receptor ligands in a wide array of diseases. For example, smoking in people with schizophrenia may be linked to abnormalities in the nicotinic-cholinergic system (Dalak et al., 1998; Dar and Frenk, 2004). These abnormalities in nicotinic receptor functioning may have

effects on multiple neurotransmitter systems. Alpha 7 (α7) nicotinic receptor agonists appear to be a candidates for studies involving the enhancement of cognition in humans and are considered as a potential therapeutic target in the treatment of schizophrenia (Martin 2004; Ballas et al., 2004).

Based on the theoretical concept of nicotinic nAChR-mediated glu and DA release and subsequent locomotor hyperactivity, in our next experiment we studied the effect of nAChR antagonist on the locomotor activity of both saline- and nicotine-treated rats. Unlike saline-treated rats, locomotor activity of nicotine-sensitized rats was noticeably inhibited by both the nicotinic receptor antagonist mecamylamine and the D_2 receptor antagonist haloperidol. We replicated these studies to compare and contrast the expected blockade effect of the NMDAR antagonist (MK-801) on nicotine-induced locomotor activity. Since mecamylamine was given acutely to rats already sensitized by repeated administration of nicotine (i.e. after development of the neuronal adaptations related to nicotine sensitization) it appears that acute administration of mecamylamine blocked the expression of locomotor activity. Our results are consistent with the previous studies and reaffirm that both mecamylamine and haloperidol block nicotine-mediated hyperlocomotion through their action on central nAChR and DA (D_2) receptors (McCallum et al. 1999; Fung, 1990). My results concur with the finding of Ivanova and Greenshaw (1997) who demonstrated that repeated daily injection of nicotine increases rewarding effects of electrical self-stimulation as evidenced by a decrease in VTA electrical self-stimulation. This electrophysiological effect was blocked by the DA receptor antagonist haloperidol and the nAChR antagonist mecamylamine. The muscarinic AChR antagonist, scopolamine and the serotonin 5-HT_3 receptor antagonist

ondansetron did not change VTA electrical self-stimulation thresholds which suggest a major involvement of DA in nicotine sensitization (Ivanova and Greenshaw, 1997). Also, nicotine self-administration is decreased by the nicotine receptor antagonist mecamylamine (McCallum et al. 1999). Clearly, effects of nicotine are mediated by many endogenous neurotransmitters including DA, ACh, and glu functioning in an integrated fashion. Nicotine-induced hyperactivity was also blocked by systemic administration of selective D_1 antagonist SCH 23390, selective D_2 antagonist raclopride and the D_1/D_2 antagonist fluphenazine, which indicate the involvement of both D_1 and D_2 DA receptors in mediating motor activity (O'Neill et al, 1991). But nicotine's hyperactivity was not blocked by systemic administration of the $5HT_3$ receptor antagonist, ondansetron (Arnold et al., 1995), suggesting that nicotine-induced hyperactivity is mainly under dopaminergic control and 5HT is less likely to be directly involved in nicotine-induced hyperactivity.

Withdrawal from chronic use of nicotine results in an abstinence syndrome that peaks within 24 hours (Malin et al, 1992). Nicotine withdrawal is similar to withdrawal from other addictive drugs also found to decrease in brain reward function as evidenced by elevation in intracranial self-stimulation (ICSS) brain reward thresholds (Epping-Jordan et al., 1998).

Although locomotor hyperactivity in rats cannot explain many aspects of addiction-related behavior, it provides an animal model for induction of changes in the neural circuitry of motivation and reward as a result of chronic exposure to drugs of abuse (Wise and Bozarth, 1987; Taetavarapruk et al, 2000; Kilts, 2004). Locomotor hyperactivity is usually, but not always, associated with reward-related phenomena.

Both the VTA and NAS receive EAA input from numerous brain regions, notably the mPFC which is mainly responsible for locomotor sensitization induced by psychomotor stimulants (Hyman and Coyle, 1996; Park et al., 2002). Bilateral lesions of the mPFC prevent the induction of locomotor sensitization by stimulants (Wolf and Xue, 1999). Similarly, systemic administration of DNQX, a non-NMDA receptor antagonist, blocked locomotor activity and stereotypy produced by amphetamine (Karler, 1991).

Thus it is generally conceived that locomotor sensitization by psychomotor stimulant drugs requires co-activation of NMDA receptors in the VTA (Kalivas and Duffy, 1997; Karler et al., 1994). Based on the reports suggesting DA-glu interactions (Karler et al., 1989) and glu modulation of DA release in nicotine-induced locomotion, (Shimuzu et al., 1990), reward and motivated behaviors I studied the effect of MK-801 on nicotine-mediated locomotor activity. My results indicate that MK-801 enhanced locomotor activity in both saline- and nicotine-treated groups in a dose-dependent manner, which contradicts the finding of Karler (1989) and Shoib et al. (1997). It is conceivable that blockade of glu transmission by a NMDA receptor antagonist might prevent development of behavioral sensitization of locomotor hyperactivity induced by stimulant drugs (Dal'olio et al. 1992). Karler et al (1998) first reported that MK-801 blocks amphetamine-induced sensitization of locomotion; a considerable number of other studies have shown that the interference with glutamatergic transmission at NMDA receptors can prevent the induction and expression of locomotor activity of stimulant drugs (Wolf, 1998 for review). Carlson and Carlsson (1989) contradicted the result of the study by Karler (1989) and reported that MK-801 is capable of inducing motor activity even in completely monoamine-depleted mice. Similarly, both systemic and intra-VTA

administration of MK-801 increases locomotor activity (Brosnan-Watters et al, 1996; O'Neil and Shaw, 1999). On the basis of findings obtained with MK-801, behavioral pharmacologists have raised questions about the ability of NMDA receptor antagonists to block psychostimulant-induced sensitization.

Tzschenke (2000) in his review article titled "Blockade of behavioral sensitization by MK-801: fact or artefact?" addressed clearly the concern of contradictory results; he additionally summarized findings that are both consistent with and in contradiction to the view that MK-801 blocks behavioral plasticity. MK-801-induced hyperlocomotion may be partly mediated by the DA system (Ouagazzal et al., 1993) and it elevates the extracellular concentration of DA in the mPFC (Yan et al., 1997; Wedzony et al., 1993). If the mechanism of MK-801-induced locomotor activity is the same as that of DA-enhancing drugs (such as nicotine, amphetamine and cocaine), repeated administration of MK-801 would be expected to augment locomotor activity. The reported blockade of behavioral sensitization by MK-801 may not have been unequivocally revealed, but studies have consistently confirmed that MK-801 has locomotion-stimulant properties when given alone or in combination with stimulant drugs (Bristow et al., 1993; Hargreaves and Cain, 1995; O'Neill and Shaw, 1999).

It is also important to note that different receptor antagonists have different effects on behavioral profiles. The locomotor activity elicited by amphetamine and cocaine is enhanced by MK-801; on the contrary, AP5 (a competitive NMDA receptor antagonist) and DNQX (AMPA/kainite receptor antagonist) attenuate the locomotor activity of amphetamine and cocaine (Kaddis et al. 1995). Similarly, contrasting effects of the

competitive NMDA receptor antagonist CPPenne and the non-competitive NMDA receptor antagonist MK-801 on matching performance was observed (Cole et al. 1993). Mele et al. (1998) found that focal administration of MK-801 into the NAS enhances spontaneous locomotor activity in rats; the glycine/NMDA receptor antagonist HA-966 can block MK-801- and PCP-induced hyperactivity (Bristow et al. 1993). Results of these studies suggest the likelihood of different neuronal mechanisms responsible for MK-801-induced locomotor activity (Mele et al., 1998).

Hargeaves and Cain (1998) studied the duration of MK-801-induced hyperactivity at a dose of 0.5 mg kg^{-1}; behavioral activation occurred 30 minutes after administration and lasted 3 hours before measures returned to baseline. In my study, I recorded locomotor activity for 60 minutes, 30 minutes after the injection of MK-801, and uninterrupted hyperactivity was observed throughout the 60 minutes period.

The explanations offered for MK-801-induced hyperactivity are also diverse. There appears to be a reciprocal interaction between glutamatergic deficiency and enhanced dopaminergic transmission in the basal ganglia, and a so-called "brake and accelerator" model has been suggested by Carlson et al. (1999) and is rather convincing (see figure 6.1.). It is suggested that the psychotomimetic action of glu antagonists (producing a low glutamatergic tone) could mediate a countersurge of an increased catecholaminergic (DA) activity. DA neurons, like other monoaminergic brainstem neurons, seem to be controlled by corticofugal glutamatergic neurons either directly or via GABAergic intereurons. Corticofugal glutamatergic neurons acting directly on brainstem DA neuron function as 'accelerators' whereas cortical glutamatergic neurons acting indirectly via GABAergic intemeurons are considered 'brakes'. An enhanced dopaminergic activity,

mediated via low glutamatergic tone could be induced by a failure of the brake i.e. low glutamatergic tone, on GABAergic neurons. Normally there seems to exist a delicate balance between dopaminergic and glutamatergic transmission. In the case of enhancement of DA function by a DA releasing agent such as nicotine or amphetamine, a negative feedback is probably activated, leading to a strong effect. Thus, the enhanced DA release may be due to a glu deficiency, leading to a weakened-negative feedback control.

Other probable explanations suggest that blockade of NMDA receptors by channel blocking drugs like PCP or MK-801 may indirectly promote compensatory hyperfunction of the AMPA and/or kainate receptors within the VTA, leading to enhanced DA release in the NAS (Mathe et al., 1998). Systemic injection of MK-801 evoked locomotor hyperactivity and enhanced extracellular concentrations of DA in the mPFC in a dose-dependent manner; selective antagonists of D_1 and D_2 receptors prevent MK-801-induced locomotor hyperactivity and stereotyped behavior (Wednozy et al., 1993). A microdialysis study in freely moving rats following systemic administration of MK-801 (0.3 mg kg^{-1} IP) found increases in extracellular concentrations of DA, norepinephrine, and 5HT in the NAS (Yan et al., 1997). Although both PCP and MK-801 might modulate DA release, we should bear in mind that DA release in many non-salient events including aversive conditions like pain and hyperactivity, through activation of sigma 1 and sigma 2 receptor subtypes is a possibility (personal communication with Prof. Greenshaw, 2001; Horvitz, 2000;Ault et al., 1999).

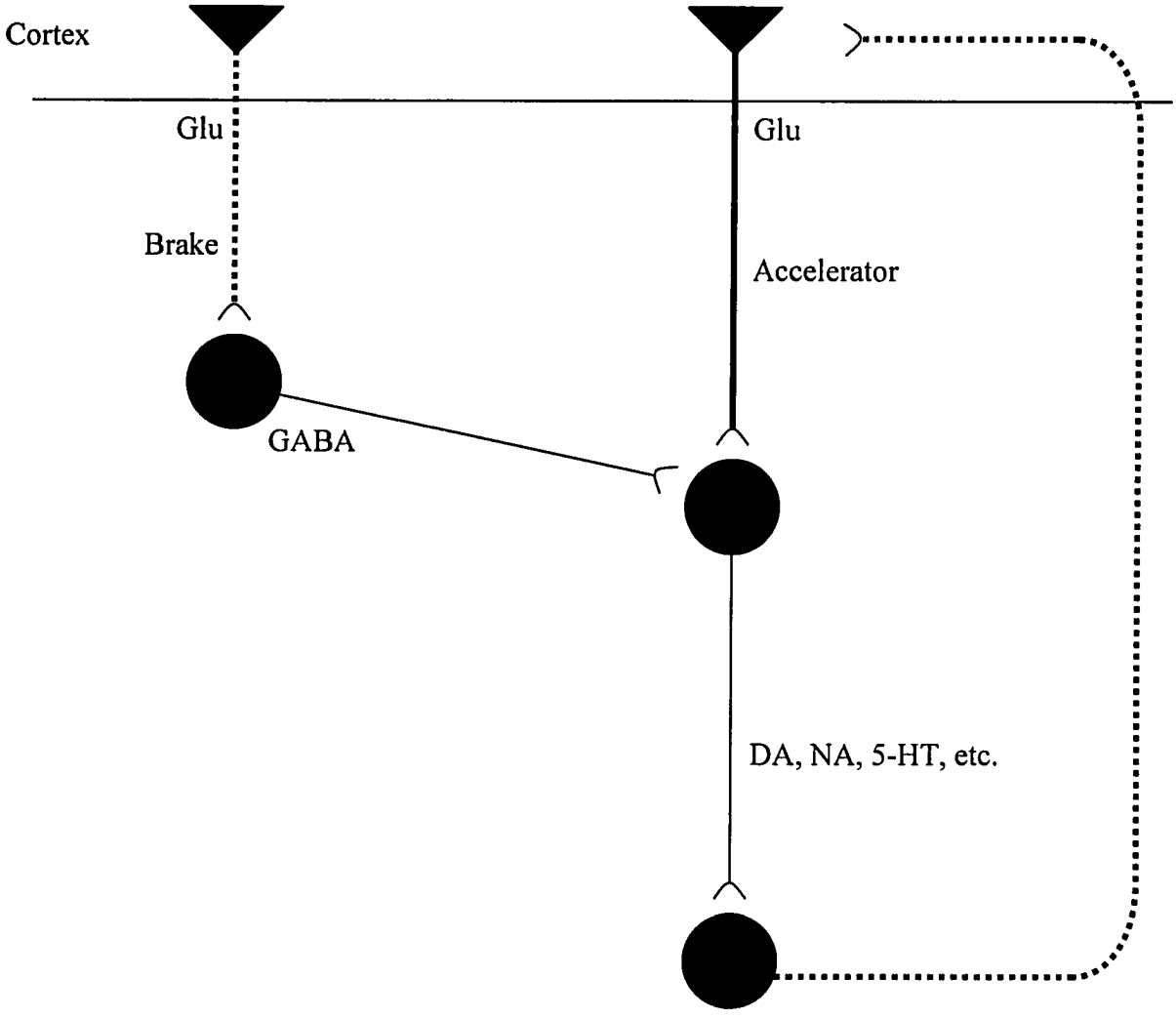


Figure 6.1. GABA, DA and Glu feedback pathway. Adapted from Carlsson et al., 1999. 156

The present results and data from other studies clearly demonstrate that behavioural expression of sensitization to the psychomotor stimulant effects of nicotine can still occur despite NMDA receptor channel blockade by MK-801.These findings imply that NMDA receptors may be critical for the development of locomotor sensitization, but are less critical (or not required at all) for the expression of previously sensitized locomotion (Wolf, 1998). Further research is thus needed to determine the role of MK-801 receptors in regulating glutamatergic and dopaminergic neurotransmission in various motor circuits of the brain in the presence or absence of psychostimulant drugs. Such studies will hopefully shed light on differential effects of MK-801 on spontaneous versus psychostimulant-induced locomotor activity. Nevertheless, the possibility still exists that the effect of MK-801 could be mediated through yet unknown mechanisms unrelated to either NMDA or AMPA receptors (Vanderschuren and Kalivas, 2000).

Effects of blockade of NMDA and other GluR seemed to be different and complex and do not fit a single conceptual framework. In conclusion, the present study of an animal model of behavioral sensitization to locomotor hyperactivity purportedly due to nicotine addiction (Hyman and Malenka, 2001) and MK-801 failed to block the expression of nicotine-induced behavioral sensitization. To the best of my knowledge, this is the first study on the effects acute systemic administration of a NMDA receptor antagonist (MK-801) in nicotine-stimulated locomotor activity. Whether the effect of MK-801 is specifically due to a blockade of NMDA receptors cannot be fully clarified on the basis of these current findings.

6.2 General Discussion on CPP

Accumulated evidence shows that nicotine facilitates glutamatergic transmission; NMDA and other glutamate receptors are involved in the reinforcing properties of nicotine. One of the major findings in the present study was that systemic administration of the non-competitive NMDA receptor antagonist MK-801 did not prevent behavioural sensitization to nicotine's effect on locomotor activity. Considering the important interrelationship between DA and glu, it is expected that MK-801 might block the rewarding effect of nicotine by preventing glutamatergic transmission (Vasiliadis et al., 1999; Pierce and Kalivas, 1997; Vanderschuren and Kalivas, 2000). On the other hand, based on results of its own rewarding effect in place preference, MK-801 may enhance CPP in the MK-801 and nicotine combination. We found induction of CPP with amphetamine and nicotine-treated rats; MK-801 alone did not induce CPP in either the biased or unbiased design. I did not replicate the CPP study on effects of mecamylamine and haloperidol in nicotine sensitized rats because of the existence of well established resuts with predictive validity.

Under appropriate conditions, drugs such as cocaine (Nomikos and Spyraki, 1988), amphetamine (Spyraki et al. 1982), methamphetamine (Martin-Iverson et al. 1985), morphine (Bardo et al. 1984), nicotine (Shoaib et al. 1994) and MK-801 (Sukhotina, 1998; Layer et al 1993) have rewarding effects, as indexed by CPP. There appears to be a reasonable concordance between drugs that produce CPP and drugs that are self-administered (Bardo et al., 1998).

The ability of a drug to establish CPP does not infer an abuse potential to the drug unless intravenous self-administration, drug discrimination and/electrical brain stimulation

experiments generate similar conclusions (Spyraki et al., 1982; Sukhotina, et al., 1998). As mentioned, nicotine produces intravenous self-administration and drug discrimination but in the CPP paradigm it has been difficult to demonstrate unequivocally nicotine's reinforcing effects; studies intended to induce CPP with nicotine have yielded conflicting results. For example Fudala et al. (1985) and Fudala and Iwamoto (1986) found CPP with nicotine as the nicotine dose of nicotine was increased to 0.8 mg kg^{-1}; further increase of dose inhibited nicotine induced CPP. On the contrary, Clarke and Fibiger (1987) found no evidence of nicotine-induced CPP even though they used same nicotine doses (0.2, 0.4, and 0.8 mg kg^{-1}. Nicotine-induced CPP is very sensitive to dose of the drug and environmental cues (Risinger and Oakes, 1995). Successful induction of CPP with nicotine was reported in a study where rats were pretreated with nicotine (dose of .8 mg kg^{-1}, s.c) for 7 days (Shoib et al. 1994). However, prior exposure to nicotine for 6 days at a dose of .6 mg kg^{-1} did not induce CPP or CPA following subsequent conditioning with 0.6 mg kg^{-1} nicotine (Jorenby et al. 1990). In my study we sensitized the rats with nicotine at a dose of 0.8 mg kg^{-1} for 7 days. Then the animals were conditioned using both biased and unbiased protocols of CPP. My results extend the finding of Shoaib et al. (1994) confirming the ability of nicotine to induce CPP at a dose of 0.8 mg kg^{-1}. Neurons in the NAS and VTA express high levels of the nAChR (Koob et al., 1998, for review); nAChRs containing β_2 subunit are involved in the reinforcing properties of nicotine (Picciotto et al. 1998). Nicotine may activate both the DA system and some opioid peptide neurons in the same neural circuitry (Koob et al. 1998, for review; Kelley, 2002). Repeated nicotine injection produced a selective enhancement of responding with conditioned reinforcement (Belluzzi et al., 2004; Fudala et al., 1985). These findings

demonstrate that acute exposure to nicotine augments the control over behaviour by a conditioned reinforcer, suggesting that nicotine may enhance motivational processes. Repeated exposure to drugs of abuse produces long-lasting, and perhaps permanent, neuroadaptations within brain circuits involved in motivation that may underlie alterations in learning and memory processes relevant to drug craving and compulsive aspects of addiction (Berke and Hyman 2000; Hyman and Malenka 2001). Exposure to environmental stimuli associated with the subjective effects of drugs can enhance motivation to use a drug, and drug-associated stimuli results in cue-induced craving (Bardo and Bevins, 2000). Moreover, in my CPP study we have found that prior repeated nicotine exposure facilitates subsequent reward-related Pavlovian associative learning (i.e. stimulus-reward learning). Nicotine may therefore enhance the incentive motivational properties of reward-related stimuli.

Since little is known about the direct effects of nicotine on conditioned reinforcement, we tested the hypothesis that chronic nicotine would enhance responding with conditioned reinforcement (Hemby et al., 1992; Pidoplichko et al., 1997; Pontieri et al., 1996). These data suggest that nicotine augments the reinforcing properties of conditioned stimuli previously associated with reward. Nicotine-induced enhancement of responding with conditioned reinforcement was also blocked by the nAChR antagonist mecamylamine, indicating that actions mediated via nAChRs are involved in this effect. These results may have important implications for studies investigating the effect of nicotine on motivational processes and have potential relevance to our understanding of nicotine dependence and smoking (Henningfield and Heishman, 1995).

It is worthy to note that nicotine produces locomotor hyperactivity, CPP, self-administration, self-stimulation and produce characteristic signs and symptoms when withdrawn after chronic use. On the basis of these behavioral profiles it is convincing that nicotine has strong reward and reinforcing properties. A withdrawal reaction of nicotine can be elicited in rat by termination of chronic nicotine administration (Hildebrand et al, 1997).

Based on the locomotor activity data of nicotine obtained from my earlier study, we studied the rewarding effect of MK-801 in inducing CPP alone and in combination with nicotine. In both biased and unbiased designs of CPP we found that MK-801 did not induce place preference or place aversion; my results extend the findings of Sufka (1994). On the contrary, although MK-801 induced locomotor hyperactivity its failure to establish CPP raises a question of about its ability to establish reward and reinforcement. Systemic administration of MK-801 reversed CPP induced by cocaine in mice (Del Pozo et al., 1996).

Sukhotina et al., (1998) reported finding CPP following MK-801 but the experimental protocol, selection of subjects and design of the total apparatus were different from our study. Based on the available data, it can be argued that reinforcing properties of MK-801 in the place preference paradigm do not seem to be dependent on NMDA receptor blockade or simply that the reward-potentiating effects of MK-801 are not always revealed in CPP but are dependent on experimental paradigm, environmental cues and dose of the drug.

Various NMDA receptor antagonist drugs have different behavioural profiles (Cole et al. 1993; Jerram et al. 1996). Sukhotina et al., (1998) found CPP with MK-801 but not with

eliprodil, another NMDA receptor antagonist. So it is arguable that glu receptor action should be considered on the basis of: 1) the action of the particular drug, 2) activity on specific sites of the NMDA receptor molecule, and 3) specific sub-type of three-glu receptor class. It is possible that by blocking one receptor subtype may not necessarily confer blockade of all other receptors subtypes. Clearly, more studies are required to clarify the role and mechanisms of VTA glutamatergic transmission involving NMDA receptor in drug reinforcement especially the development as well as expression of previously sensitized locomotor behavior.

6.3. General Conclusions and Future Research

Interactions between DA and glu systems are involved in the reinforcing properties of drugs of abuse, motivated behaviors and motor activity in laboratory animals. However, the fact that MK-801 did not attenuate the locomotor stimulant effects of nicotine suggests complex interactions between glu, DA and possibly other monoamines in the regulation of psychostimulant-induced locomotor activity. Further research is thus needed to determine the role of other GluR, most importantly mGluR5 receptors, in regulating glutamatergic and dopaminergic neurotransmission in various motor circuits of the brain in the presence or absence of psychostimulant drugs. Such studies will hopefully shed light on differential effects of glu on psychostimulant-induced locomotor activity. More recently, mGluR5 has been implicated in psychostimulant-induced hyperlocomotion, as genetic deletion of the mGluR5 in mice abolishes the hyperlocomotion produced by cocaine (Chiamulera et al., 2001). Thus a role for the mGluR5 in the reinforcing and conditioned rewarding effects of nicotine is worth investigating in further research.

Similarly, studies on involvement of the serotonegic system (5HT), especially 5-HT_{2C} receptors, in mediating mesolimbic DA functioning as assessed by changes in behaviors indicative of nicotine reward are warranted. Also, it is known that glu afferents synapse directly onto inhibitory GABAergic interneurons that might play an important role in drug reinforcement (Hyman and Malenka, 2001) and deserve further elucidation. Future research protocols should address the issue of timing of the administration of antagonists in relation to the stimulant drug because efficacy of the blocking effects of an antagonist largely depends on timing of administration (acute administration with stimulant vs. administration after the development of sensitization). Several lines of evidence implicate NMDA receptor dysfunction in the cognitive deficits of schizophrenia, suggesting that pharmacological manipulation of the NMDA receptor may be a feasible therapeutic strategy for treatment of these symptoms (Moghaddam, 2004). The level of smoking in people with schizophrenia may be linked to abnormalities in the nicotinic cholinergic system, which merits undertaking further research regarding involvment of nAChR in cognition. Locomotor and rewarding effects of the various subtypes of nAChR agonists and antagonists and their interaction with glu, DA, and 5HT, GABA remain at large an exciting area of research. Certainly, our research involving nicotine-MK-801 interactions has invoked ample critical thinking about DA and glu.

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