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THE ROLE OF NEUROKININ RECEPTORS IN DOPAMINE/ACETYLCHOLINE SIGNALING IN THE NEOSTRIATUM DURING COCAINE EXPOSURE

By
Pierre-Antoine H. Noailles

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

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ABSTRACT

THE ROLE OF NEUROKININ RECEPTORS IN DOPAMINE/ACETYLCHOLINE SIGNALING IN THE NEOSTRIATUM DURING COCAINE EXPOSURE

By

Pierre-Antoine H.Noailles

Adviser: Professor Jesus Angulo

Neurokinin receptors (NKR) are ideally located in areas of the basal ganglia on subpopulations of interneurons to modulate cocaine evoked neurotransmitter release in the rat neostriatum. Basal ganglia nuclei modulate output behaviors such as feeding, maternal and sexual behavior. The striatum is the major input nuclei of the basal ganglia and the main neurotransmitter is dopamine (DA). Altered DA activity may be responsible for plastic changes in the basal ganglia.

Rats treated acutely (10mg/kg IP) and chronically (7 days) with cocaine exhibited a dose dependant decrease in DA release in the striatum resulting from intracranial co-administration of NK-1R antagonists (WIN 51,708 , D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11} and L733,060). Administration of NK-3R antagonists (SR142801 and D-Pro^{2}, D-Trp^{6,8}, Nle^{10}) to animals that had been treated acutely with cocaine had no significant effect on cocaine evoked dopamine release. In animals treated chronically with cocaine, NK-3R antagonists reduced cocaine evoked dopamine efflux. Direct administration of WIN 51,708 (10^{-4}M) into the shell of the nucleus accumbens (NAc_{SHELL}) significantly reduced cocaine evoked DA release.

Acetylcholine (ACh) is a major modulator of DA neurochemistry in the striatum and NAc of the rat and cholinergic interneurons have NK-1R on their soma and dendrites. Direct intrastriatal administration of WIN 51,708 (10^{-4}M) caused a complete repression of cocaine evoked ACh release in that region in acutely and chronically treated rats. I used immunocytochemisty (ICC) to visualize NK-1R on cholinergic interneurons using antibodies for choline acetyltransferase (ChAt), an enzyme for the synthesis of acetylcholine, and NK-1R antibodies as markers. I found that administration of substance P (SP) caused a marked alteration in the pattern of immunoreactivity (IR) consistent with receptor internalization. Systemic cocaine administration caused a pattern of IR identical to that of the SP perfused animals. Pre-perfusion with the NK-1R antagonist WIN 51,708 blocked the cocaine evoked receptor internalization. An understanding of the role of substance P will help to elucidate mechanisms of cocaine-induced neuroplasticity and perhaps other neurochemical substrates of plasticity via peptidergic activity in other areas of the CNS.

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For my father Harry Noailles

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GLOSSARY

ACSF	artificial cerebrospinal fluid
ACh	Acetylcholine
CPu	Caudate Putamen
Ctx	Cortex
DA	Dopamine
HPLC	High performance liquid chromatograghy
IR	immunoreactivity
Meth	Methamphetamine
NAc	Nucleus Accumbens
NK-1R	Neurokinin 1 receptor
NK-2R	Neurokinin 2 receptor
NK-3R	Neurokinin 3 receptor
NKA	Neurokinin A
SNc	Substantia Nigra compacta
SNr	Substantia Nigra reticulata
SP	Substance P
VTA	Ventral Tegmental Area
WIN	WIN 57,108 antagonist

Chapter 1

INTRODUCTION

Psychostimulants and the Brain

Psychostimulants reshape brain reward mechanisms in such a way as to increase the likelihood of self-administration, and are known to have tremendous addictive potential in humans (White, 1996). The majority of the cocaine abusers in the United States are young male adults ranging from the age of 18-25 years old. Ten to 15 percent of people who try cocaine become addicted (Letters, 1999). In 1997, 1.5 million Americans were classified as chronic cocaine users according to the National Household Survey on Drug Abuse (NHSDA). Chronic abuse of cocaine can lead to irritability, gastrointestinal complications, increase in blood pressure, heart failure, stroke, psychosis, seizure, and death. In addition to these side effects, the lifestyle associated with chronic cocaine use increases the likelihood of the contraction of serious disease including, but not limited to, hepatitis B and C, and HIV, the virus that causes AIDS (Word and Bowser, 1997; Molitor et al., 1998). More so, chronic cocaine use is invariably connected with the use of alcohol and other illicit drugs.

Cocaine is a potent psychostimulant, which, when taken systemically, causes profound neurochemical changes in specific brain regions which lead to increase in the likelihood of cocaine self-administration of. Cocaine (FIG.4) is part of a class of drugs referred to as psychostimulants that include caffeine, nicotine and methamphetamine. Cocaine is referred to as an indirect dopamine (FIG.5) agonist because its pharmacological action involves the reuptake of dopamine into the synaptic terminal and thus causes an increase in the concentration of synaptic dopamine, which in turn causes an increase in post synaptic cellular activity. Cocaine also has a high affinity for serotonin and nor-epinephrine (De Felice and Adams, 2001). Cocaine effects on the cardiovascular system are mediated via its effects on Na^+ channels (Lason, 2001). Blockade of central Na channels may also have effects on centrally mediated plastic effects of cocaine (Cantrell and Catterall, 2001).

It is known that psychostimulant use can cross-sensitize with other drugs of abuse, in particular other psychostimulants (Vanderschuren et al., 1999). Cross-sensitization is the phenomenon where chronic use of a drug results in augmented reaction to the novel drug beyond what would be considered a normal acute response for an individual naïve to the use of that particular agent. It has been shown that in rats, a single administration of a psychostimulant such as amphetamine can cause long term sensitization not only to amphetamine but also to a challenge dose of cocaine several weeks later (Vanderschuren et al., 1999). Multiple drug use is correlated with an increased risk of overdose (Tardiff et al., 1996). In addition, because of the culture surrounding illicit drug use, criminality and other antisocial behaviors increase significantly with the use of psychostimulants such as methamphetamine and cocaine (Bailey and Shaw, 1989; Inciardi and Surratt, 2001). Cocaine abuse and addiction have devastating social and financial costs. Addiction to psychostimulants poses a massive public health problem. A clearer understanding of the neurochemical mechanisms underlying the addictive process and the response of the basal ganglia and all relevant brain regions to psychostimulants will help us to better understand the phenomenon of addiction and lead to more effective therapies.

Reward and Behavioral Sensitization

Chronic psychostimulant use leads to an increase in likelihood of self-administration (Robinson and Berridge, 1993). In has been proposed that the increase in the rate of self-administration resulting from exposure to the drug may be due to an alteration in reward mechanisms (Koob, 1996). Using an instrumental conditioning paradigm, where a positive reinforcer increases the likelihood of repeating a behavior that is rewarded, the association of a pleasurable effect with the self-administration of cocaine increases the likelihood of self-administration. However, positive reinforcement is not sufficient to explain the intractable problem of drug craving in the face of the terrible consequences mentioned above. One hypothesis is that in addition to being initially pleasurable, psychostimulants such as cocaine affect brain regions responsible for ascribing salience to environmental stimuli (Robinson and Berridge, 1993). When cocaine is administered chronically at the same dose the behavioral response to the same dose increases with time. This phenomenon is called behavioral sensitization. Behavioral sensitization is known to engage the circuitry of the basal ganglia (Kalivas and Duffy, 1993a). Basal ganglia circuitry is also known to subserve several complex behaviors including but not limited to feeding behavior, sexual behavior, and the association of motor programs with salient environmental stimuli (Graybiel, 1995). It has been proposed that increased craving for cocaine is related to the phenomenon of behavioral sensitization. Moreover, it is possible that chronic exposure to cocaine can sensitize motivational aspects of self-administration (Robinson and Berridge, 1993). One role of the basal ganglia is to receive inputs from the cortex and process them into a coherent signal in order to initiate complex motivational and motor programs. In this sense, one of the roles of the basal ganglia is to ascribe salience or to make certain environmental stimuli "standout" with reference to other environmental stimuli in order to better utilize sensory input and activate the appropriate motor and motivational program. Motivation is a process by which organisms adapt their behavior to control and manipulate aspects of the environment related to their needs. A basic aspect of this process involves learning the relationship between biologically significant stimuli and otherwise neutral stimuli that predict their occurrence. By this process, organisms gravitate towards useful goal stimuli, avoid harmful ones and disregard those of no use. So it can be said that the basal ganglia serves to integrate input from the environment, assign such stimuli importance or salience by association, and through that association allows them to initiate complex behavior patterns to increase the likelihood of the reoccurrence of the desirable circumstance which leads to that particular stimulus (Di Chiara, 1995). Because of the profound behavioral changes in drug seeking behavior exhibited by subjects addicted to cocaine, and the fact that cocaine acts at a site in the brain that is responsible for the integration of such complex signal processing involved in motivation and reward, this hypothesis is a powerful tool to help us characterize the complexities of the addictive process.

Cocaine and Neurochemistry

Pharmacological and behavioral studies have demonstrated that cocaine administration affects both nigrostriatal and mesocorticolimbic systems (Kalivas and Duffy, 1993a, b). Our current understanding of the biological basis of drug induced plasticity that leads to addiction implicates the activity of the mesolimbic system in part (Koob et al., 1987). The mesolimbic pathway emanates from dopamine cell bodies of the ventral tegmental area (VTA) and projects to the nucleus accumbens and prefrontal cortex (FIG.7). The mesolimbic pathway has been implicated in drug reinforcement and reward, and it is thought that all psychostimulants with addictive potential effect this circuitry (Self and Nestler, 1995). In addition, the nigrostriatal dopaminergic neurons and their targets in the striatum (neurons projecting from the striatum to the substantia nigra), are part of the direct loop as mentioned above (FIG.7). The striatum is also involved in reward but has a larger role in the phenomenon of behavioral sensitization. Sensitization to psychostimulants develops with intermittent or repeated treatment but not with continuous exposure (Post, 1980). Intermittent treatment causes progressive increases in the behavioral response to psychostimulants that can be long lasting. Some studies suggest that this change in behavior can be permanent (Paulson and Robinson, 1991). Research in this laboratory suggests that these behavioral changes are dopamine dependent (Kraft et al., 2001).

This dissertation focuses on data that elucidate the role of the neurokinin receptors with an emphasis on the NK-1 receptor in the striatum and the $\mathrm{NAc}_{\mathrm{SHELL}}$. In part, our behavioral data suggest that these receptors play a critical role in the observed dopamine dependant increase in locomotion resulting from non-contingent administration of the psychostimulant cocaine (Kraft et al., 2001). There are three main questions I sought to answer with respect to the central question of cocaine-evoked changes in basal ganglia neurochemistry.

1) What is the pattern of neurotransmitter release in the rat striatum and $\mathrm{NAc}_{\mathrm{SHELL}}$ resulting from acute and chronic cocaine administration?
2) What effect does intracranial administration of neurokinin receptor antagonists have on the pattern of cocaine-evoked neurotransmitter release in the rat striatum and $\mathrm{NAc}_{\mathrm{SHELL}}$?
3) Using receptor internalization as a marker for neurokinin binding, what activity of NK-1 receptors is elicited by the acute administration of cocaine and on what subpopulation of neurons does this occur?

My goal was to determine if the augmented dopamine (DA) release in the striatum and $\mathrm{NAc}_{\mathrm{SHELL}}$ that accompanies acute and chronic administration of cocaine is dependent on altered neurokinin receptor activity, and if alteration of neurokinin receptor activity through pharmacological targeting is sufficient to alter patterns of cocaine evoked dopamine release in rats treated acutely and chronically. We examined the effects of systemic administration of cocaine concomitant with intracranial administration of NK-1 and NK-3 receptor antagonists (WIN 51,708, [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}], L-733,060, SR142801, and D-

Pro2, D-Trp6,8, Nle10) on cocaine induced alterations in dopamine/acetylcholine overflow in the striatum and DA overflow in the NAc_{SHELL}. **Data collected for this thesis suggest a relationship between neurokinin receptors in the striatum cocaine-evoked dopamine release**. The experiments conducted provide evidence implicating the central neurokinin system in the modulation of the dopamine/acetylcholine system under conditions of cocaine exposure. Characterizing the role of neurokinin receptors in the basal ganglia will aid in defining a therapeutic target in the treatment of substance abuse as well as help us better understand the mechanisms involved in neurochemical plasticity in the central nervous system (CNS).

Basal Ganglia Circuitry

The basal ganglia consist of several functionally interconnected nuclei that span the telencephalon, diencephalon and midbrain (FIG.6). The basal ganglia include deep lying structures of the brain, incorporating the striatum, the pallidum, and the amygdala. The primary afferent structure of the basal ganglia is the striatum. In addition to sensorimotor aspects of movement programming, basal ganglia function involves conditional aspects of planning movements, program selection, and motor memory and retrieval (Graybiel, 1995). Parkinson's disease, Schizophrenia, obsessive-compulsive disorder, Huntington's disease, and Turret's syndrome are all disorders related to basal ganglia function (Calabresi et al., 2000b).

The current model of the basal ganglia suggests a cytoarchitecturally and neurochemically segregated series of inputs and outputs. The striatum is the major input area of the basal ganglia and receives significant glutamatergic excitatory input from the cerebral cortex (Centonze et al., 1999a). In contrast, the internal segment of the globus pallidum and the substantia nigra reticulata represent major output nuclei of the basal ganglia. These structures exert tonic GABAergic inhibitory influence on the excitatory premotor neurons in the thalamic nuclei (Deniau and Chevalier, 1985). Between input and output structures of the basal ganglia there are two major subdivisions, the direct and indirect pathways. The direct pathway originates from striatal neurons that contain the classical neurotransmitter GABA and the peptides SP and/or dynorphin, and project monosynaptically to the globus pallidum interna and substantia nigra reticulata. The indirect pathway consists of striatal neurons that contain GABA and enkephalin which, through a series of connections involving the external segment of the globus pallidum and the sub thalamic nucleus, connect to the globus pallidum interna and substantia nigra reticulata. In this pathway GABAergic inhibitory projections from the striatum synapse onto the globus pallidum externa; inhibitory GABAergic projections from the globus pallidum external synapse onto sub thalamic nucleus; and excitatory glutamatergic projections from the sub thalamic nucleus synapse onto the globus pallidum interna/substantia nigra reticulata (Chevalier and Deniau, 1990).

With reference to the modulatory effects of dopamine and acetylcholine in the striatum, the focus will be on the direct pathway (FIG.7a).

High concentrations of neurokinins and NK receptors have been detected in the nuclei of the basal ganglia on cells ideally located to modulate dopaminergic neurochemistry and cholinergic interneuron activity (Elliott et al., 1991; Boix et al., 1994; Steinberg et al., 1998). There are three main types of interneurons. They are cholinergic, somatostatinergic and GABAergic interneurons. All of these interneuron subtypes are innervated by cortical glutamatergic neurons. NK-1 receptors are located on cholinergic interneurons of the neostriatum (Gerfen, 1991). Because of this discrete anatomical distribution, studies have been performed in an attempt to relate mechanisms involving neurokinins and neurokinin receptors to the motor diseases mentioned above (Saria, 1999).

The basal ganglia contains 90 percent of the dopaminergic projection neurons in the central nervous system. Dopamine is synthesized in the projection neurons of the compacta and VTA (FIG.8). The majority of these emanate from the substantia nigra compacta and project into the striatum. The other large portion emanates from the ventral tegmental area and projects to the nucleus accumbens. The striatum can be divided into two broad compartments, the striosomes and matrix. In the rat, these compartments are defined by the difference in mu opioid receptor binding (Zhou et al., 2001). The striosomes receive cortical afferents from prefrontal and limbic areas while the matrix receives cortical afferents from primary motor and somatosensory cortex as well as frontal, parietal, and occipital cortex. Striatal efferents from the substantia nigra compacta also appear to be differently distributed to the striatum with different groups of midbrain dopaminergic neurons projecting to striosomes and matrix. These two striatal compartments may be linked functionally by interneurons that contain somatostatin (Testa et al., 1995). Some striatal neurons also possess dendrites that closely associate on the borders between striosomes and matrix suggesting communication between these two compartments. Matrix neurons have two subpopulations of neurons; one type contains substance P, which projects to the substantia nigra reticulata, while those containing enkephalin project mainly to the lateral globus pallidum. The substance P containing projection neurons to the substantia nigra also contain GABA (Gerfen, 1991).

Although dopamine is the primary site of action of psychostimulants, (referred to as indirect dopamine agonists), the development and expression of behavioral sensitization is likely to involve other systems that are intimately connected with the actions of the neurotransmitter dopamine. For example, the neuropeptides substance P, neurokinin A and dynorphin are positioned in a pivotal location of the basal ganglia, that is, in projection neurons of the striatum and NAc_{SHELL}. The fundamental nature of the central nervous system is that it divides its parallel incoming signals in order to combine them in novel ways (i.e. convergent and divergent processing similar to that found in the visual system). This is called a distributed system. Using a model of feedback mechanisms borrowed from engineering, neuropeptides may serve as gain control devices, "adjusting activity levels in the basic circuit and its side loops" (Graybiel, 1995). Mesencephalic dopaminergic neurons are responsive to the application of neuropeptides, which have been shown to augment dopamine release and turnover in terminal field areas of the forebrain including the accumbens (Kalivas, 1985). Substance P has been implicated as a modulator of dopamine (DA) neurotransmission in the mesolimbic DA pathway, a primary pathway implicated in the effects of cocaine related to its abuse.

This high level of complexity and signal processing make the basal ganglia ideal targets for modulation resulting from chronic psychostimulant administration and explains the complex array of behaviors associated with addiction.

Dopamine

Dopamine is the major catecholamine in the central nervous system (de Belleroche and Bradford, 1980). The dopaminergic system has been the primary focus of research in the basal ganglia for the past 30 years. Alterations in dopamine neurotransmission are involved, directly or indirectly, in several brain dysfunctions including Parkinson's disease, obsessive-compulsive disorder (OCP), and addiction to psychostimulants (Robinson and Berridge, 1993; Hyman, 1996). Dopamine, and specifically dopamine receptors, have been seen as therapeutic targets for the treatment of several of these pathologies. Unfortunately, alterations of dopamine neurotransmission or activity of dopamine receptors can have severe side effects. Administration of L-dopa to patients suffering from Parkinson's disease can lead to psychotic episodes and involuntary movements (Zigmond et al., 1990).

Evidence for dopamine receptors was first found in 1972 (Kebabian et al., 1972). Since then, the existence of several different subtypes of dopamine receptors has been confirmed (Raevskii et al., 1996). Dopamine receptors are part of a large family of seven transmembrane domain g-protein linked receptors. These receptors are linked through guanine-nucleotide binding proteins to effectors that translate activation of a plasma membrane receptor into an intracellular response. Of the five different subtypes of dopamine receptors found to date, there are two categories: D1-like receptors and D2-like receptors. D1 and D5 receptors are classified as D1-like because they share high sequence homology and similar pharmacology. D2, D3 and D4 receptors are considered to be D2-like, for the same reasons. The former causes an increase in adenylate cyclase (AC) activity and phospholipase C (PLC) and the latter decreases the activity of AC, as well as closing K^+ and Ca^{2+} channels. So, D1-like R are considered stimulatory in their effects while D2-like R are inhibitory. These two different receptor subtypes act as regulatory controls on the release of dopamine.

Dopaminergic neurons are localized mainly in the substantia nigra parse compacta, the ventral tegmental area and hypothalamus. There are three defined main pathways, the nigrostriatal, the mesolimbic and the tuberoinfundibular. The D1 receptor is the most common subtype and is expressed at higher levels than any other dopamine receptor. D1 mRNA is found in areas known to be under dopaminergic control such as the striatum and the nucleus accumbens. Receptor autoradiography has shown the presence of D1 receptors in the globus pallidum and in substantia nigra reticulata, where D1 receptors originate from striatal GABAergic neurons that express substance P. It has been demonstrated that mRNAs for the D1 and D2 receptors are segregated in substance P and enkephalin neurons, respectively, in the striatum and NAc_{SHELL} (Le Moine and Bloch, 1995). The D5 receptor is similar in activity to the D1 receptor but is present at much lower concentrations than the D1 receptor, and has a slightly different distribution. The presence of D1 and D5 receptors seem to be differently distributed (Tarazi et al., 1998; Filip et al., 2000). Localization of D5 is distributed to the soma and proximal dendrites of neurons in cerebral cortex, basal ganglia, basal forebrain, hippocampus, and diencephalon. The distribution of D5 in the CNS is different from that of other dopamine receptor subtypes including the D1R. This distribution supports the idea that the D5 receptor subtype has a distinct role in dopamine neurotransmission (Ciliax et al., 2000).

The D2 receptor has been found primarily in the striatum, olfactory tubercle and nucleus accumbens where they are present on the GABAergic neurons which receive dopaminergic input from the midbrain (Gifford and Johnson, 1992) . D2 receptors are also found in the substantia nigra compacta and in the ventral tegmental area, where they are present on dopaminergic projection neurons (Pierce et al., 1995). D2 receptors are found both on pre- and postsynaptic regions of projection neurons. D3 receptors are present in the NAc_{SHELL}, and the olfactory tubercle. The expression of D3 is very low in the striatum. D3 receptors also have been found in the substantia nigra compacta, the ventral tegmental area and the cerebellum (Schwartz et al., 1998). The distribution of D3 receptors in the cerebellum is of some interest and will be discussed below. D4 receptors are present in very low levels in the basal ganglia. Of all receptors, D4 has the lowest relative abundance. The D4 receptor gene displays variations in humans not demonstrated by the other receptor subtypes. In these allelic variations, insertions of repeats of 16 amino acids occur in the third cytoplasmic loop of this seven transmembrane protein receptor. These indifferent isoforms have slightly different pharmacological characteristics. D4 receptors display different affinity for the neuroleptic clozapine. However, these isoforms do not seem to be related to pathologies of the dopaminergic system such as schizophrenia (Jaber et al., 1996).

D1 and D2 receptors were cloned in 1990 (Jaber et al., 1996). The sequence homology between D1 and D2 receptors is 29 percent throughout the entire protein and 44 percent in the transmembrane domains. The D1 and D5 receptors share high sequence homology. D1 and D5 receptors have similar pharmacological profiles. The D5 receptors have a tenfold higher affinity for dopamine than D1 (Filip et al., 2000). All dopamine receptors share a high degree of sequence homology within their transmembrane domains. Members of the dopamine receptor sub-families such as D1 and D5 receptors have up to 78 percent serial homology (46 percent for D2 and D3; 53 percent for D2 and D4).

As mentioned previously, dopaminergic projection neurons are found primarily in the substantia nigra compacta, the ventral tegmental area. D1 receptors are the most common receptor subtype and are expressed at very high levels in the striatum and nucleus accumbens (Dearry et al., 1990). D1 receptors are also present in areas where no D1 mRNA is detected. This suggests that D1 receptors in these areas are only present on projections where there are no cell bodies. This is the case in the substantia nigra reticulata where D1 receptors originate from striatal GABAergic neurons that co-express substance P (Aubert et al., 2000). The D5 receptor is present at much lower levels than D1 receptors and a has a different distribution (Filip et al., 2000). D1 and D2 receptor mRNAs are present on separate neuronal populations in the rat neostriatum. D1 receptors are present on striatonigral substance P projection neurons, and striatopallidal neurons expressing enkephalin contain D2 receptors. D2 receptors are also located on cholinergic interneurons (Harsing and Zigmond, 1998). This is important because it shows a direct DA inhibitory input on these cholinergic interneurons.

Psychostimulants such as cocaine and methamphetamine increase dopamine by increasing the release of dopamine or by blocking the activity of the dopamine transporter. In the case of cocaine, this molecule binds to the dopamine reuptake carrier causing an increase in synaptic dopamine. This increase in synaptic dopamine increases postsynaptic activity. Methamphetamine both blocks the dopamine reuptake carrier and causes an increase in the release of dopamine, also increasing postsynaptic activity in addition to increasing acetylcholine (ACh) release (Ricaurte et al., 1982; Taguchi et al., 1998). Chronic administration of psychostimulants has profound effects on dopamine neurochemistry in the striatum. This alteration in dopamine neurochemistry is dependent on the activity of neurokinin receptors in the striatum (Gygi et al., 1993).

The dopamine hypothesis has dominated the theoretical outlook of the causes of addiction for years (Melichar et al., 2001). Understanding the basic dopaminergic circuitry in the basal ganglia will help us to elucidate the mechanisms of many different pathological states including addiction.

Acetylcholine

Acetylcholine neurotransmission in the striatum has a major influence on the control of movement, learning and motivation (Boix et al., 1994). Striatal cholinergic activity plays a major role in the acquisition and early maintenance stages of learned behaviors where the the mere presence of a stimulus is sufficient to elicit a learned response (Calabresi et al., 2000b; Pisani et al., 2001). Striatal dopaminergic activity influences memory consolidation in the striatum. Tonically active neurons (TAN) correspond to the giant aspiny cholinergic interneurons, which take up approximately 5 percent of the rat striatum (Pisani et al., 2001).

The striatum has one of the highest levels of acetylcholine, muscarinic receptors, and other acetylcholine related markers in the central nervous system. Acetylcholine has been implicated in the pathology of Parkinson's disease (Calabresi et al., 2000a). Early on acetylcholine receptor antagonists were used as therapies for Parkinson's disease (Carlsson and Carlsson, 1990). Parkinson's disease is marked by significant loss of dopaminergic input into the striatum. The theory states that the underlying defect is an imbalance between dopamine and acetylcholine release in the striatum (Zigmond et al., 1990). Dopamine exerts an inhibitory effect on the release of acetylcholine in the striatum (Blanchet et al., 2000). Therefore, it is suggested that this decrease in dopamine causes an unchecked release of acetylcholine in the striatal compartment, which leads to the behavioral effects of Parkinson's disease. Since the striatum is the main afferent structure of the basal ganglia and since the small percentage of cholinergic interneurons cover large areas of the striatum through extensive dendritic arborizations, cholinergic activity in the striatum may modulate inputs from various areas of the cortex, prefrontal cortex and extrapyramidal regions of the basal ganglia. The striatum receives a significant amount of glutamatergic input from the cortex (Hanania and Johnson, 1999).

Alterations in dopaminergic neurotransmission can also lead to significant changes in glutamate release in the striatum (de Belleroche and Bradford, 1980). Glutamatergic inputs synapse onto striatal cholinergic interneurons (Smith and Bolam, 1990). So it seems that ACh may have its effects in the striatum by modulating inputs from both glutamatergic cortical inputs and nigral dopaminergic inputs (Calabresi et al., 2000a). Stimulation of D1 receptors increases the release of striatal acetylcholine (Anderson et al., 1994). Stimulation of D2 receptors inhibits release of striatal acetylcholine (Acquas and Di Chiara, 1999). The D2 receptors have been localized on cholinergic interneurons in the striatum (Gifford and Johnson, 1992). However, D1 receptors seem to act indirectly on ACh interneurons (Steinberg et al., 1998). Activity of substance P efferents from the striatum to the substantia nigra may be responsible for the indirect effect of D1 agonists.

Although there are two different classes of ACh receptors, nicotinic and muscarinic, the effects of acetylcholine on striatal dopamine are mediated mainly through the activity of muscarinic receptors because atropine affects dopamine release but pimepidine does not (Harsing and Zigmond, 1998). There are five different subtypes of muscarinic receptors. Each receptor shares common features including specificity of binding for the agonists acetylcholine and carbamylcholine and the classical antagonists atropine and quinuclidinyl benzilate. Each receptor subtype couples to a second messenger system through an intervening G-protein. Activation of M_1, M_3 and M_5 receptors by acetylcholine stimulates phosphoinositide metabolism. When the receptor is activated the enzyme increases splitting of phosphatidylinositol polyphosphates of the cell membrane into (mainly) inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). IP3 is water-soluble and contains three charged phosphate groups. It is released into the interior of the cell and acts on IP3 receptors on the surface of the endoplasmic reticulum (ER), which causes an increase in the release of Ca^{2+} from the ER. Increased cytosolic Ca^{2+} is part of the intracellular message from ACh at the surface membrane. Another part of the transduced message from ACh at the cell surface is DAG. Because DAG is lipid soluble it remains in the cell membrane. Its presence in the membrane, along with increased intracellular Ca^{2+}, activates a protein kinase, protein kinase C (PKC). PKC in turn is involved in regulating a number of other enzyme activities while M_2 and M_4 receptors inhibit adenylate cyclase. The tissue distribution differs for each subtype. M_1 receptors are found in the forebrain, especially in the hippocampus and cerebral cortex. M_2 receptors are found in the heart and brainstem while M_3 receptors are found in smooth muscle, exocrine glands and the cerebral cortex. M_4 receptors are found in the neostriatum and M_5 receptor mRNA is found in the substantia nigra, suggesting that M_5 receptors may regulate dopamine release at terminals within the striatum.

Substance P terminals make contacts with cholinergic interneurons in the striatum (Bolam 1986). These projection neurons have excitatory M1 receptors on their soma (Calabresi et al., 2000a). Moreover, it has been established that NK-1 receptors, the preferential receptor for substance P, are present on cholinergic interneurons (Anderson et al., 1994). Taken together, these data suggest the presence of a "loop", which includes striatal substance P/GABA projection neurons to the substantia nigra and dopaminergic projection neurons from the substantia nigra to the striatum. This loop is involved in modulating the activity of the cholinergic interneurons (Anderson 1994).

Neurokinin Receptors and Peptides

Like many peptides, the tachykinins were not originally discovered in the central nervous system. Substance P was discovered in 1931. Mammalian tachykinin peptides consist of the neuropeptides substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) and share the amino acid carboxyl terminal sequence –Phe-X-Gly-Leu-Met-NH_2 (FIG.3). These peptides are derived from two genes: the preprotachykinin A and preprotachykinin B genes. The neurokinin peptides substance P (SP) and neurokinin A (NKA) are produced from the preprotachykinin A gene, whereas the preprotachykinin B gene encodes the precursor of the peptide neurokinin B (Angulo and McEwen, 1994).

The presence of multiple neurokinins and the fact that they have different actions suggest the existence of multiple neurokinin receptors. Three types of neurokinin receptors, neurokinin-1 (NK-1), neurokinin-2 (NK-2), and neurokinin-3 (NK-3), have been identified (Lee et al., 1986; Mantyh et al., 1989; Helke et al., 1990). These are part of a G-protein family of metabotropic receptors. The binding of neurokinins to their receptors involve the activation of phospholipase C, with the subsequent breakdown of phosphatidylinositol 4,5-bisphosphate and consequent production of inositol 1,4,5-triphosphate (IP3) and diacylglycerol. IP3 mobilizes intracellular calcium from the endoplasmic reticulum which then plays a role in the regulation of Ca-calmodulin kinases. Diacylglycerol activates specific isoforms of protein kinase C. The receptor is activated with the binding of the endogenous ligand, inducing a conformational change that results in GTP binding to the GDP or unbound heterotrimeric G-protein complex, with the subsequent dissociation of this complex from the receptor and its dissociation of the α-subunit from its $\beta\gamma$counterpart. Due to intrinsic GTPase activity, possibly along with GTPase activation proteins, the activity of G_α is reduced and it is able to re-associate with free $G_{\beta\gamma}$ subunits. Upon binding to its receptor, the ligand forms a complex with the receptor which in turn causes an event in which the receptor/ligand complex is transported to the cytoplasm for processing, There the ligand is separated from the receptor and the receptor is recycled back to the membrane (FIG.10a,b). This receptor internalization can be used to visualize the activation of the receptor. It is also an indirect marker for substance P release (Michael-Titus et al., 1999).

Based on the binding affinity, substance P, neurokinin A and neurokinin B selectively bind to NK-1, NK-2 and NK-3, respectively. Affinity for substance P is 100 times higher for the NK-1 receptor than for NKA. At the NK-3 receptor, affinity for NKA is twice that of SP although the highest affinity is for NKB. It is important to note that all tachykinins can interact with each receptor type. Therefore, the presence of any of the receptors in areas of tachykinin
implies functional relevance (Dam et al., 1990).

Areas rich in NK-1 receptors include the striatum, the nucleus accumbens, the hippocampus, the lateral nucleus of the hypothalamus, the habenula, the intrapeduncular nucleus, the nucleus of the tractus solitarius, the raphe nucleus, and the medulla oblongata.

The cloned human and rat NK-1 receptor show about 95 percent serial homology. Twenty-one out of 407 amino acid residues differ between these two species. The majority of these residues are localized at the C-terminal and N-terminal ends of the receptor protein. Only six amino acids in the transmembrane segments differ between these two species. The guinea pig tachykinins NK-1 receptor is 97 percent homologous to the human NK-1 receptor (Saria, 1999). The transmembrane segments are 100 percent identical in guinea pigs and humans. Variation of specific amino acid residues is not crucial for agonist binding. However, the effect of antagonists is highly variable depending on residue differences.

Receptor autoradiography and lesion studies indicate that both neurokinin-1 and neurokinin-3 receptors are located in intrinsic striatal neurons whereas in the substantia nigra neurokinin-receptors are located in dopaminergic projection neurons (Stoessl, 1994). NK-2 receptors are widely distributed in the peripheral nervous system. In the striatum substance P (NK-1) receptors are expressed by a large number of cholinergic interneurons (Gerfen, 1991), and activation of NK-1 receptors in the striatum elicits a dose-dependent increase in ACh release (Anderson et al., 1994). Activation of neurokinin receptors in the VTA causes an increase in striatal DA as well as behavior ambulation (Elliott et al., 1991).

The neurokinin peptides SP and NKA are located in the direct striatonigral pathway (FIG.7a). Both in vivo and in vitro studies indicate that exogenous SP and NKA can elicit dopamine release in the substantia nigra and striatum (Baruch et al., 1988), suggesting that both neuropeptides may act as neuromodulators in the direct striatonigral pathway. Although there is a prominent SP and NKA containing projection from the striatum to the substantia nigra (Smith and Bolam, 1990), the substantia nigra contains almost undetectable levels of NK-1 and NK-2 receptors (Mantyh et al., 1989). When considering this apparent mismatch one must take into account that there is significant crosstalk between the three different receptor subtypes. This suggests there is functional activity with the presence of any given receptor colocalized with any given ligand of the same tachykinin class. In addition, techniques have become increasingly sensitive and with increased sensitivity, the characterization of distribution has changed accordingly, suggesting that some putative mismatches may be due more to technological limitations than to a paucity of any given receptor or ligand. In fact, it has recently been postulated that there may be two different isoforms of the NK-1 receptor in the rat striatum (Mantyh et al., 1996). Given the location of neurokinin efferents, SP and NKA may act on the NK-3 receptors expressed on the midbrain dopamine neurons to regulate dopamine release. One can speculate that there may be a causal relationship between striatal-nigral neurokinin peptide effusion and the neural adaptation underlying augmented dopamine release in response to cocaine administration. This dissertation shows compelling evidence linking the activity of substance P receptors with the persistent changes in the pattern of dopamine and acetylcholine release resulting from cocaine administration.

Mammalian substance P derives from the pre-protachykinin-A gene that originates from a common ancestral gene by duplication (Angulo and McEwen, 1994). The PPT-A gene encodes neurokinin A, neuropeptide K and Neuropeptide γ. Alternative RNA splicing of the PPT-A gene transcript results in three distinct mRNAs (αPPT-A, βPPT-A and γPPT-A) All three encode for the substance P precursor sequence. The NKA precursor sequence is present only on αPPT-A and βPPT-A mRNAs. αPPT-A is far more abundant in the CNS. Synthesis of substance P occurs in the ribosomes. Substance P is then packed into vesicles and axonally transported to terminal endings for final enzymatic processing (Harrison and Geppetti, 2001). Extracellular hydrolysis of neuropeptides is accomplished by a limited number of peptidases, which, by acting in concert can terminate the biological activity of these peptides. All of these enzymes are metalopeptidases. Endopeptidases are anchored to the membrane and are non-specific with regard to particular peptides. There are several enzymes involved in substance P metabolism. These include neutral endopeptidase (NEP) substance P degrading enzyme (SP-DE EC.3.4.24), angiotensin-converting enzyme (ACE: EC.3.4.15.1) dipeptidyl aminopeptide IV (DPIV: EC.3.4.21.26) cathespin-D (EC.3.4.3.23) and cathespin-E (EC.3.4.23.34). Although all of these enzymes have an established enzymatic activity in vitro, NEP is the most probable candidate for metabolism of substance P in the CNS, although ACE has been found in the substantia nigra (Harrison and Geppetti, 2001). NEP can hydrolyze cholecystokinin, enkephalin, as well as tachykinin (Hooper and Turner, 1985). NEP is present in high concentrations in the striatum and the substantia nigra and has been colocalized with sites of neurokinin release (Zhou et al., 2001). This is a promising avenue of inquiry for elucidating the complex mechanisms that may be involved in extra-synaptic neuropeptide regulation.

Substance P

Substance P and its C-terminal sequence have reinforcing properties when injected systemically or centrally and is know to increase dopamine release in the striatum (Boix 1994). Approximately 90 percent of the cells present in the striatum are GABAergic medium-sized spiny neurons. These GABAergic neurons consist of two major efferent pathways that project to the substantia nigra reticulata and the globus pallidum externa. These GABAergic neurons receive major dopaminergic input from the substantia nigra compacta (Anderson and Reiner, 1991). GABAergic spiny projection neurons are targets for cholinergic interneurons. Acetylcholine release acts primarily on muscarinic receptors located on projection neurons, which stimulate dopamine release (Calabresi et al., 2000a). GABA inhibits striatal cholinergic interneurons whereas acetylcholine exerts both excitatory and inhibitory control on GABAergic activity through the activity of the different classes of muscarinic receptors (Harsing and Zigmond, 1998).

Substance P release leads to release in dopamine outflow in striatal slices. M1 and M2 have opposing influences on striatal dopamine release (Khan et al., 2000). The modulatory effect of substance P on striatal dopamine release depends upon the activity of M1 and M2 receptors. 6-OHDA lesions show that a significant proportion of striatal muscarinic receptors may be located on nigrostriatal afferents (Calabresi et al., 2000a). These presynaptic receptors may be activated by acetylcholine release in the terminals. The presence of M1 receptors on dopamine terminals has been established (Raiteri 1982). Muscarinic receptors are also found on substance P containing striatal neurons (Calabresi et al., 2000a). The modulation of dopamine release by substance P appears to be determined ultimately by the balance between the activity of M1 and NK-1 receptors. This suggests that the modulatory effects of neurokinins on dopamine outflow in the striatum depend upon muscarinic receptors and are limited by M1/M2 receptor balance. Substance P increases dopamine release via activation of cholinergic cells via M1 receptors, however, the effects appear to be self-limiting, as high concentrations of acetylcholine may trigger activity of M2 receptors and the subsequent inhibition of dopamine.

Tachykinins are involved in the central control of stress (Vassout et al., 2000). Antagonists for the NK-1 receptor also facilitate defensive rage behavior in cats induced by stimulation of the amygdala. The substance P antagonist MK-869 is effective in alleviating depression and anxiety in patients with major depressive disorder. The fact that MK-869 is an NK-1 receptor antagonist with an IC 50 in the 0.1 nanoM range and has no significant affinity for monoamine receptors and reuptake sites suggests a whole new mechanistic approach to alleviate depression and associated anxiety disorders (Lieb et al., 2000; DeVane, 2001). The site of action of substance P antagonists with respect to the mediation of emotional disturbances in humans is unknown. However, NK-1 receptors are highly expressed in the striatum, amygdala, septum, hippocampus, hypothalamus, reticulopontine nucleus and periaqueductal gray (Saria, 1999). These are areas associated with the processing of emotions as well as stress. Direct central injection of substance P C-terminal sequence produce place aversion in rats (De Araujo et al., 2001). Remarkably, substance P and its C-terminal sequence have reinforcing properties when injected directly into the striatum (Boix 1994). Substance P antagonists resemble antidepressant and anxiolytics drugs and are able to inhibit neonatal distress vocalizations in guinea pig pups separated from their mother and littermates. Systemic administration of substance P antagonists abrogate cocaine induced behavioral sensitization. (Kraft et al., 2001). Local application of NK-3 neurokinin receptor antagonist in the substantia nigra and VTA regions of the basal ganglia enhance release of dopamine and acetylcholine in the striatum and nucleus accumbens, suggesting a positive link between the NK-3 receptors and striatal neurotransmitter activity (Marco et al., 1998).

Dopamine/Acetylcholine Coupling

Cholinergic inter-neurons represent less than 5% of the neuronal population of the rat striatum (Gerfen and Young, 1988; Gerfen, 1991). These neurons receive input from both dopaminergic neurons of the substantia nigra and collaterals of striato-nigral neurons that release substance P as well as receive significant glutamatergic input from the cortex and prefrontal cortex (Kaneko et al., 2000). Histological analyses demonstrate that these cholinergic interneurons display extensive dendritic arborizations, which span large areas of the striatum and the NAc_{SHELL}, placing them in an ideal position to modulate neurotransmitter response to cocaine (Gerfen, 1991). A significant percentage of these interneurons express the mRNA for both NK-1 and D2 receptors (Jaber et al., 1996). Direct administration of the D2 agonists cause a dose dependant decrease in the methamphetamine-induced release of acetylcholine (DeBoer et al., 1996). Direct administration of D1 agonists increases the release of acetylcholine (Steinberg et al., 1998). This increase may result from indirect excitation via the D1 receptors on the GABAergic substance P releasing neurons whose collaterals synapse onto cholinergic interneurons and which project to the SN (FIG.7a,b)(Steinberg et al., 1995). Data shown in this thesis suggest that the neurokinin class of receptors play a pivotal role in cocaine-induced alterations in dopamine release as well as behavioral response. Since NK-1 receptors are found primarily on cholinergic interneurons, it suggests that the pattern of acetylcholine release may also be altered in the basal ganglia as a result of cocaine administration.

A significant portion of the striatal medium spiny neurons are GABAergic projection neurons. There is the possibility that non-synaptic mediated intracellular communication might occur in the striatum through gap junctions. Dopamine may exert its physiological role by modulating the electrical coupling among sets of striatal neurons (Onn and Grace, 1994). Dopamine exerts a prominent inhibitory effect on the release of acetylcholine within the striatum. This is consistent with the dopamine/acetylcholine balance hypothesis and is mediated via D2R. The loss of dopaminergic nerve terminals causes hyperactivity in cholinergic neurons that leads to an abnormal striatal output to the globus pallidum and the substantia nigra (Pisani et al., 2001). This is common in pathological states of the striatum such as Parkinson's disease.

Acetylcholine mediated innervation is thought to have a primary role in determining the final activity of striatal neurons that project to the output structures of the basal ganglia such as the substantia nigra and the ventral tegmental area. However, recent findings have challenged this hypothesis. The activity of different dopamine receptor subtypes can elicit opposite effects of acetylcholine release in the striatum, suggesting that the loss of dopaminergic nerve terminals interferes with the activity of cholinergic neurons in a less pronounced manner than proposed in the past. Traditionally, neuroanatomical and functional studies demonstrate that de-innervation of dopaminergic neurons leads to major changes in striatal neurotransmitter activity (Nikolaus et al., 1997). The specific mechanisms underlying these adaptations to the cholinergic system remain undefined.

Corticostriatal glutamatergic inputs are considered a major influence on striatal neuron activity in the intact animal. In addition, increases in the release of glutamate from cortical striatal terminals has been reported in the striatum following dopaminergic neuron de-innervation (Burrows and Meshul, 1999). This is seen in Parkinson's disease patients. In these individuals, there is a significant increase in length of postsynaptic densities of the corticostriatal synapses suggesting that the synapses are hyperactive (Carlsson and Carlsson, 1990). Electrophysiological recordings taken after nigral lesions show a prominent enhancement of spontaneous depolarizing glutamate mediated postsynaptic potentials (Centonze et al., 1999b). This suggests that excessive glutamate mediated neurotransmission may be part of the pathogenesis of Parkinson's disease. Blockade of glutamate receptors stimulate locomotor behavior and potentiate the anti-Parkinson's effect of levodopa in both animals and individuals. Anti-cholinergic drugs were initially used as treatment for Parkinson's disease (Pisani et al., 2001). The efficacy of these drugs might be explained by the interaction with glutamatergic transmission.

The intrinsic neural network that comprises interneurons represents the main source of acetylcholine in the striatum. Cholinergic interneurons are large (20 -50 microns) aspiny neurons that account for 1-5% of neurons in the striatum. In spite of their small numbers, axonal fields of the cells are extensive and possess much more widespread trees than that of the medium spiny projection neurons. Cholinergic efferents do not leave the striatal compartment and their exclusive role seems to control the modulation of striatal input and striatal neurotransmission. This cytoarchitectural organization suggests that striatal cholinergic neurons can integrate synaptic inputs over relatively large regions, and influence wide areas of the striatum. Nevertheless, the majority of dopaminergic projections to the striatum synapse directly onto projection cells of the striatum (Blanchet et al., 1998). Cholinergic cells receive their main input in the form of glutamatergic thalamic and cortical projections (Christie et al., 1987). The main synaptic targets of striatal cholinergic neurons are projection neurons (Centonze et al., 1999a). The cells are GABAergic medium-sized spiny neurons that constitute the vast majority of the striatal population. Medium spiny neurons receive connections from dense excitatory glutamatergic fibers originating from the cortex and thalamus. In addition, there is a close association between glutamatergic and cholinergic inputs converging on striatal spiny neurons (Blanchet et al., 2000). Glutamatergic nerve terminals make asymmetrical synaptic contacts on the heads of dendritic spines of the cells, whereas cholinergic nerve terminals synapse symmetrically on their soma, dendrites and spines. Both cholinergic interneurons and projection cells receive glutamatergic input from the cortex and, in turn, cholinergic interneurons innervate projection neurons to the substantia nigra. Thus, striatal cholinergic interneurons may be involved in mediation of processing input from the cortex to projection cells. Studies have also shown that cholinergic neurons may modulate release of other neurotransmitters within the striatum via a presynaptic mechanism, through a non-junctional or volumetric mode of transmission (Contant et al., 1996).

Release of GABA and glutamate in the striatum is inhibited by the activation of presynaptic muscarinic type 2 receptors. The effects of acetylcholine are mediated primarily by activation of different classes of muscarinic receptors. M1 receptors are coupled negatively to adenylate cyclase activity. In addition, M1 receptors exert both presynaptic and postsynaptic effects on striatal cells and mediate opposite effects on striatal synaptic plasticity.

Activation of different receptor subtypes effect the release of acetylcholine variously. D1 receptor blockade reduces acetylcholine release while D2 receptor blockade stimulates acetylcholine release (Acquas and Di Chiara, 1999). It has been suggested that endogenous dopamine controls acetylcholine release in a reciprocally symmetric manner through stimulatory D1 and inhibitory D2 receptors (Di Chiara 1994). However, both D1 and D2 receptor agonists and antagonists cause major changes in endogenous dopamine release which might indirectly alter changes in acetylcholine release making it difficult to isolate the foundation of this effect. Feedback induced release of dopamine onto D1 receptors on projection neurons to the SN might account for the stimulation of acetylcholine release elicited by D2 receptor blockade (Damsma et al., 1991). Similarly, a decrease in dopamine release leading to less activity of D2 receptors has been suggested to account for the increase of acetylcholine release following D1 receptor stimulation (Acquas et al., 1997). Application of agonists for D2 and D3 receptors cause an increase in the release of striatal acetylcholine (DeBoer et al., 1996). The application of D1 receptor antagonist causes a reduction in the striatal concentration of acetylcholine which coincides with an increase in striatal dopamine (Acquas and Di Chiara, 1999). This could be the result of the feedback stimulation of dopamine release by the D1 receptor antagonist. It seems that endogenous dopamine release in response to D1 receptor stimulation counteracts indirect effects of D1 receptor blockade on acetylcholine release, suggesting that endogenous dopamine acting on D1 receptors control acetylcholine release in a fashion independent from D2 receptors.

Stimulation of D1 receptors increases the release of striatal acetylcholine in vivo but has no effect on acetylcholine release in vitro in striatal slices. Stimulation of D2 receptors inhibits the release of striatal acetylcholine both in vivo and in vitro (Anderson et al., 1994). This effect seems to be direct because D2 receptors have been localized to cholinergic interneurons in the striatum. D1 induced release of acetylcholine in the striatum seems to be indirect because the effect is observed only in vivo and not in striatal slices. In addition, D1 receptors have not been found on striatal cholinergic interneurons. The NK system is one possible pathway that mediates the effects of D1 agonists on acetylcholine release. Axon collaterals of striatonigral neurons arise extensively within the striatum and substance P containing terminals have been shown to make synaptic contacts with cholinergic cell bodies in the striatum. Stimulation of NK receptors by substance P has been shown to increase the release of striatal acetylcholine in vivo (Anderson et al., 1993) and in vitro (Arenas et al., 1991). Blockade of striatal NK-1 receptors reduces the increase of acetylcholine release evoked by striatal D1 stimulation in both intact rats and rats with unilateral 6-OHDA lesions of nigrostriatal pathway (Alberch et al., 1993). NK-1 receptor antagonists block the D1 receptor related to increase in acetylcholine release (Anderson et al., 1994). This is consistent with the hypothesis that D1 receptors mediate increases in acetylcholine due to the indirect pathway, which includes the NK-1 cholinergic receptors. Substance P mediation of striatal acetylcholine release via D1 receptor stimulation provides evidence of one mechanism to explain interactions between D1 and D2 receptors in the basal ganglia at the level of the striatum. It appears that D1 and D2 receptors are differentially expressed on striatal output neurons. D1 receptors are primarily located on striatonigral neurons and D2 receptors are localized to striatopallidal neurons (Gerfen et al., 1990). This receptor balance in part modulates the interaction of substance P, dopamine and acetylcholine release in the striatum.

Stimulation of D1 receptors on striatonigral neurons stimulates the release of substance P in the striatum, which binds to NK-1 receptors on cholinergic neurons and enhances the release of acetylcholine. Acetylcholine in turn may stimulate muscarinic receptors on striatopallidal neurons and alter the activity of the striatopallidal pathway. Release of acetylcholine induced by substance P from striatonigral collaterals may serve as a functional link between striatonigral and striatopallidal pathways (Gerfen, 1992).

6-OHDA lesions and D2 antagonist induced increases in striatal PPE mRNA were reduced by muscarinic receptor blockade, and since D2R are present on PPE postive/pallidal projection neurons, this suggests that D2 receptors' regulation of striatopallidal activity is mediated in part through cholinergic interneurons (Pollack and Wooten, 1992). D1 receptor stimulation may influence activity of the striatopallidal neurons indirectly through the substance P cholinergic circuit whereas D2 receptors may influence striatopallidal neurons directly by stimulation of these receptors located on cholinergic interneurons.

Blockade of NK-1 receptors reduces D1 receptor stimulated release of striatal acetylcholine and suggests that increases in acetylcholine concentration induced by D1 agonists may be mediated indirectly through the local release of substance P acting at the NK-1 receptors on striatal cholinergic interneurons. These findings provide evidence for the role of substance P in neurotransmission within a local circuit (striatonigral axon collateral cholinergic interneurons) within the striatum.

Pharmacological and lesion studies demonstrate that dopaminergic activity augments production of striatal substance P and NKA (Gerfen et al., 1991). There are also high levels of expression of protachykinin A mRNA (Gerfen et al., 1991). Increase in cholinergic activity results in higher levels of protachykinin A mRNA. Blockade of postsynaptic dopamine receptors by neuroleptic drugs reduces concentrations of substance P, NKA and protachykinin A mRNA in the striatum of the rat (Angulo and McEwen, 1994). 6-OHDA lesions of nigral dopaminergic neurons results in significant reductions of substance P NKA and protachykinin A mRNA (Gerfen et al., 1991), suggesting that dopaminergic input has a major influence on SP striatal release. The direct dopamine agonist methamphetamine, when injected systemically, elevated striatal protachykinin A mRNA levels significantly, further strengthening the supposition that DA is intimately involved with SP modulation in the direct loop of the basal ganglia (FIG.7a,b). Concurrent administration of either D2 agonists or D1 antagonists blocks this effect (Lindefors, 1992). This gives strong evidence that the neurokinin system in the striatum is regulated by the interaction of dopamine D1 and D2 receptors. D1 agonists cannot effect changes in striatal PTA mRNA levels but can alter activity initiated by D2 receptor activation. This suggests that D1 receptor action provides an enabling tone. These results strongly suggest that dopaminergic D1 and D2 receptors subserve the regulation of striatal and accumbens substance P activity (Angulo and McEwen, 1994). Two separate populations of medium-sized neurons containing substance P immunoreactivity have been described. Substance P containing terminals have been reported to synapse onto somatodendritic trees of cholinergic neurons (Bolam et al., 1986) and on the medium-sized neurons (Izzo and Bolam, 1988). In addition, acetylcholine release was elicited from rats' striatal slices by potent NK-1, NK-2 and NK-3 agonists (Arenas et al., 1991).

The cytoarchitectrural organization of the striatum can be distinguished by its afferent and efferent pathways. There are two main populations of efferent GABAergic neurons in the matrix, those that innervate the substantia nigra reticulata and/or the internal globus pallidum and contain dynorphin, substance P and NKA, and those that project to the external globus pallidum and are positive for enkephalin and NKB. The cell bodies of cholinergic interneurons are also localized to the matrix. These cells are close to the striosomes and may innervate both striatal territories, which suggests cholinergic interneurons also play a role in the communication between the two striatal compartments (Blanchet et al., 2000).

Striatal interneurons and projection neurons all possess NMDA receptors that are innervated by glutamatergic neurons originating from the cortex and thalamic nuclei (Standaert et al., 1996). It is thought that dopamine is involved in inhibitory regulation of the evoked release of acetylcholine under potent stimulation of NMDA receptors. This effect is mediated primarily by D2 receptors located on cholinergic interneurons and occurs in both matrix and striosomal striatal compartments (Hanania and Johnson, 1999). Neurokinins contribute to dopamine dependent inhibition of acetylcholine release evoked by the potent stimulation of NK receptors (Boix et al., 1994). Due to its increased inhibitory synaptic effects on evoked release of dopamine release, GABA indirectly facilitates the evoked release of acetylcholine in both striatal compartments. Tachykinins (substance P and NKA), which are present in GABAergic projection neurons, can be released from collaterals of these neurons. However, it cannot be concluded that GABA is released as well from GABAergic interneurons. Through a dopamine dependent process, endogenously released GABA tachykinins exert opposite effects on the regulation of acetylcholine release from cholinergic interneurons. While the facilitating effects of GABA on acetylcholine release occur under the activity of NMDA receptor activation, inhibition by tachykinins seems to occur only under conditions of intense NMDA receptor activation (Blanchet et al., 2000).

Matrix neurons are thought to regulate NMDA evoked release of acetylcholine in the striatum. In the matrix, substance P and NKA exert an inhibitory role on NMDA evoked release of acetylcholine due to an indirect effect mediated by dopamine release from nerve terminals in the nigrostriatal dopaminergic neurons. Inhibitory processes contribute to the regulation of NMDA evoked release of acetylcholine when NMDA receptors are stimulated. Dopamine, primarily through D2 receptors but to a lesser extent through D1 receptors, is primarily responsible for this inhibitory regulation. GABA pre-synaptically reduces evoked release of dopamine and subsequently the inhibitory dopaminergic regulation of acetylcholine release. The blockade of NK-1 and NK-2 receptors is associated with a marked enhancement of NMDA evoked release of acetylcholine (Blanchet 1998).

The data available suggests that dopamine/acetylcholine signaling are mediated through the activity of NK-1 receptors on cholinergic interneurons. These data further support the hypothesis that a balance of the activity of dopamine and acetylcholine receptor subtypes has a major effect on the activity of the striatum and that the cholinergic interneurons act as a linchpin in modulating the various inputs into the striatum.

Background Data

Previously in our lab, we found that the systemic application of NK antagonists significantly altered behavioral response resulting from acutely and chronically administered cocaine. Systemic administration of NK-1 antagonists significantly reduced acute cocaine-induced hyperlocomotion. In addition, neurokinin-3 antagonists had no effect on acute cocaine induced locomotor response but robustly facilitated the development and expression of cocaine induced locomotor sensitization. Chronic systemic cocaine administration increased neurokinin mRNA expression but after three days of withdrawal, mRNA levels returned to baseline (data not shown here). After chronic treatment with cocaine, co-administration of the NK-1 antagonist seemed to facilitate the sensitization with cocaine. Interestingly, chronic administration of the NK-3 antagonist alone had no effect on behavior. When a challenge dose of cocaine was given however, the animals behaved as if sensitized. These behavioral data led me to speculate what the role of NK receptors in the neostriatum might be on cocaine evoked DA and ACh release. To that end, using in vivo microdialysis and histology, I examined the role of NK-1 and NK-3 receptors on altered cocaine evoked neurochemistry in the rat neostriatum.

Chapter 2

SPECIFIC AIMS

Specific Aim I **Is the pattern of dopamine and acetylcholine release resulting from acute cocaine administration related to the activity of neurokinin receptors?**

a) Establish pattern of dopamine outflow in the striatum and shell of the NAc following acute exposure to cocaine.

b) Establish pattern of acetylcholine outflow in the striatum following acute exposure to cocaine

c) Measure effects of local perfusion of NK-1 and NK-3 antagonists into the striatum on dopamine overflow in rats treated acutely with cocaine using three different concentrations of the NKR antagonists.

Substance P is present in areas of the central nervous system including but not limited to the neostriatum of the basal ganglia. To determine if substance P may be acting to modulate the dopamine response to cocaine we applied the neurokinin receptor antagonists directly into the striatum and shell of the nucleus accumbens (NAc) using in vivo microdialysis while simultaneously measuring dopamine release in these areas. In vivo microdialysis was utilized to assess the effect of the NK-1 and NK-3 receptor antagonists on neurotransmitter release in the dopamine terminal field regions, which contains neurokinin receptors.

Male Sprague Dawley rats (~350gms) had a cannula surgically implanted according to coordinates referred to in the Methods section. The animals were given 7 days to recover after which treatment was initiated. Approximately 18 hrs before the experiment the animals were brought to the procedure room where they were placed in the testing cage to habituate. The cannula guidepost was removed and the dialysis probe inserted. Artificial CSF (145mM NaCl, 2.7mM KCl, 1.2nM CaCl, 1mM MgCl, pH5.2) was then perfused at a rate of 0.5 ul/min through the probe overnight. The next day the flow rate was increased to 1 ul/minute and dialysate was collected every half-hour for 2 hours. Each 1/2-hour sample will be assessed for concentrations of dopamine and acetylcholine using HPLC. Animals were then injected intraperitoneally with 10mg/kg body weight cocaine dissolved in 0.5 mls PBS. Samples were collected every half-hour for 2 hours. At the conclusion of the experiment the animals were sacrificed in a manner described in the Methods section below, the brains removed and examined to verify for probe placement.

Sprague Dawley rats will be treated the same as in **Specific Aims Ia and Ib**. However, after the baseline is established, three different groups of rats were perfused with three different concentrations of the NK-1 antagonist dissolved in CSF for 1/2 hour to allow thorough receptor saturation. The rats were then injected intraperitoneally with 10mg/kg body weight cocaine dissolved in 0.5 mls of PBS. Samples were collected every half hour for two hours. In this manner the optimal dose for the receptor antagonist was determined.(Table 1).

Table1 Treatment Paradigm for acutely treated animals.

Region	Antagonist		Dose				
		1mM Ant/Coke	10mM Ant/Coke	100mM Ant/Coke	Cocaine Alone	Optimal Concentration Alone	CSF
Striatum	NK-1	8 rats	8 rats	8 rats	8 rats	8 rats	8 rats
NAc	NK-1	8 rats	8 rats	8 rats	8 rats	8 rats	8 rats
Striatum	NK-3	8 rats	8 rats	8 rats	8 rats	8 rats	8 rats
NAc	NK-3	8 rats	8 rats	8 rats	8 rats	8 rats	8 rats

Specific Aim II Is the pattern of dopamine and acetylcholine release resulting from chronic cocaine administration related to the activity of neurokinin receptors in the basal ganglia?

a) Establish the pattern of dopamine and acetylcholine release in the striatum and shell of the NAc resulting from chronic treatment with cocaine

b) Measure the effects of local perfusion of NK-1 antagonists on dopamine and acetylcholine overflow in the striatum and shell of the NAc in chronically treated rats using the optimal dose established in **Specific Aim Ib**

Male Sprague Dawley rats (~350gms) had a cannula surgically implanted according to coordinates referred to in the Methods section. The animals were given 7 days to recover after which treatment began. These animals were then injected once daily (10mg/kg of body weight @ 10:00am) with cocaine dissolved in 0.5 mls PBS for seven days. On the seventh day, dialysate was collected in a manner identical with those treated acutely with cocaine (Table 2). After the baseline was established the optimal concentrations of the NK-1 or NK-3 antagonist as determined in Specific Aim Ic were perfused through the dialysis membrane for 1 hour to allow thorough receptor saturation in the area of administration. The rats were injected intraperitoneally with 10mg/kg body weight cocaine dissolved in 0.5

mls of PBS. Samples were collected every half hour for two hours. After the experiment the animal were sacrificed in the prescribed manner. (Table 3).

Table 2 Treatment schedule for chronically treated animals

DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Injection	Injection	Injection	Injection	Injection	Injection and Habituation	Injection and Microdialysis

Table 3 Paradigm for chronically treated animals

Region	Antagonist	Cocaine Alone	Optimal concentration/ Cocaine	CSF
Striatum	NK-3	8 rats	8 rats	8 rats
Striatum	NK-1	8 rats	8 rats	8 rats
NAc	NK-1	8 rats	8 rats	8 rats

Specific Aim III Is Substance P release in the striatum altered as a result of systemic cocaine administration?

a) Measure the effects of acute cocaine administration on NK-1 receptor internalization in the striatum on cholinergic interneurons.

b) Determine if the effect is reversible with the administration of the NK-1 receptor antagonist WIN51,708.

The surgical procedure will be the same as in Specific Aims I and II. The animals will be given 7 days to recover after which treatment began. These animals were brought to the procedure room approximately 18 hrs before the experiment and placed in individual testing cages to habituate. The cannula guidepost was removed and the dialysis probe inserted. Artificial CSF (145mM NaCl, 2.7mM KCl, 1.2nM CaCl, 1mM MgCl, pH5.2) was then be perfused at a rate of 0.5 ul/min through the probe overnight. The next day the rate was increased to 1 ul/minute and dialysate was collected every half-hour for 1.5 hours. Each 1/2-hour sample was assessed for concentrations of dopamine and acetylcholine using HPLC. Group I animals were injected intraperitoneally with 10mg/kg body weight cocaine dissolved in 0.5 mls PBS and animals will be perfused with CSF at 1ul/minute for 15 minutes, removed for the dialysis apparatus and cage, then deeply anaesthetized with isoflourane, put on ice and fixed with 4% formaldehyde/12.5% picric acid. The brain tissue was then be subjected to ICC as described in Methods section. Animals in Group II were subjected to same treatment as Group I however, they received a saline injection. Group III will receive a saline injection, however in this group, substance P was administered via the dialysis probe at a concentration of 10^{-6}M. Group IV had the substance P antagonist WIN 51,708 administered via the microdialysis probe for 1 hour and then received an intraperitoneal injection of cocaine. Fifteen minutes later animals in Group IV will be deeply anaesthetized and prepared for ICC in the manner described above.

Table 4 Paradigm for immunocytochemistry

		Group I	Group II	Group III	Group IV
		CSF perfusion/ Cocaine Inj	CSF perfusion/ Saline Inj	Sub. P perfusion/ Saline Inj	NK-1 Ant./ Cocaine Inj.
NK-1	Striatum	8	8	8	8

In all cases a dose of 10 mg/kg was administered IP because our preliminary behavioral data demonstrated this dose was ideal for inducing behavioral sensitization. Higher doses were assayed but it was found that anything higher than 10mg/kg administered once daily induced stereotypy. In order to study the neurochemical underpinnings of behavioral sensitization I found it most effective to use a dose of cocaine previously administered in a behavioral paradigm in which the use of neurokinin antagonists administered peripherally had a significant effect in reducing the sensitization response.

My hypothesis is that **Neurokinin receptors present on cholinergic interneurons of the rat neostriatum play a significant role in the cocaine evoked changes in dopamine and acetylcholine concentration in animals treated acutely and chronically.**

Chapter 3

MATERIALS AND METHODS

Stereotaxic Surgery and In Vivo Microdialysis

Dopamine Assay

In vitro recovery experiment

In order to evaluate the efficiency of DA passive diffusion through the microdialysis membrane, recovery experiments were performed at room temperature, both in the absence and in the presence of the drugs used in the experiments. The recovery of DA from the dialysis membrane in the absence of drugs was 10-45% (N =6). All the drugs tested did not modify DA release recovery.

Dopamine measurement

Microdialysis in awake and freely moving rats was used to determine dopamine content in the striatal and NAc_{SHELL}(FIG.11,12). Rats were deeply anesthetized with isoflourane (5% at 0.5 liters O_2/minute) and placed in a Kopf stereotaxic apparatus. An incision was made in the scalp to expose the saggital, lambdoidal, coronal sutures and bregma. The stainless steel outer-housing of the cannula was affixed to the skull with two screws and dental cement at the appropriate stereotaxic coordinates (caudate-putamen, A=1.6mm, L=2.5mm, D=4.5mm; nucleus accumbens, A=1.6mm, L=0.9mm, D=6mm) (Paxinos and Watson, 1986). The incision was sutured and treated with topical antibiotic. A steel guard post was placed in the guide cannula to prevent dust and pathogens from entering and the animal was allowed to recover from surgery for 7 days before psychostimulant treatment. Subsequent to implantation of the guide post, animals were single housed to assure that the wound would be allowed to heal properly. Test subjects were then housed in a modified operant conditioning chamber, given food and water ad libidum, and connected to an infusion pump (CMA 102) via a double-channel fluid swivel (Instech 375/D/22QE) that permitted relatively unrestricted movement (FIG.13). Artificial CSF (145 mM NaCl/2.7 mM KCl/1.2 mM $CaCl_2$ /1 mM $MgCl_2$, pH 5.2) was perfused through the probe at a rate of 1ul/min. The guide post was removed from the animals skull and the microdialysis probe (20kDa cut off semi-permeable membrane CMA 14/12 3mm) implanted into the brain and the test subject was perfused with artificial CSF overnight at a low flow rate (0.6 ml/minute) for approximately 18 hours. The next day, at approximately 10a.m. after the flow rate had been increased to 1 microliter per minute, fractions of the dialysate were collected every 30 minutes in plastic 250 □L vials containing 3.0 □l of 0.95 M perchloric acid solution (containing 500 mg/L EDTA and 500 mg/L sodium metabisulfite) to inhibit the degradation of dopamine dissolved in a total of 1ML solution. Dopamine was measured by high-performance liquid chromatography (HPLC) with electrochemical detection using a Choulochem II Detctor with a ESA 5020 guard cell set at 300 mV, and a graphite coulometric analytic cell (5014b analytic cell with platinum target (electrode 1:-150 millivolts, electrode 2:200 millivolts)) with a reverse phase sep-stick microbore column. 10microL samples were taken out of the 30 microL aliquots with a Waters 717plus autosampler. The mobile phase consisted of 9g/L NaH_2PO_4, 520 mg/L octane sulfonic acid, 8% (vol/vol) acetonitrile, 250 mg/L EDTA, and 175 ul/L triethylamine (modeled after Jedema and Moghaddam, 1994). Quantification of the peaks were made using Millennium integrating software (Waters Corp.). Concentrations of dopamine were expressed as percent of baseline. Probe placement was verified in coronal sections of brain tissue (20 um) stained with Cresyl violet. Brain dialysate fluid was obtained from the following regions and coordinates: caudate-putamen, A=1.6mm, L=2.5mm, D=4.5mm; nucleus accumbens, A=1.6mm, L=0.9mm, D=6mm (Paxinos and Watson, 1986).

Changes in concentration of dopamine released between experimental groups were expressed as percent change relative to the baseline.

Acetylcholine Assay

In vitro recovery experiment

In order to evaluate the passage of ACh through the microdialysis membrane, recovery experiments were performed at room temperature, both in the absence and in the presence of the drugs used in the experiments. The average recovery of ACh from the dialysis membrane in the absence of drugs was 20 and 25%, respectively (N =4). None of the drugs tested modified ACh recovery. The ACh values were not corrected for recovery. Changes in concentration of acetylcholine were expressed as percent change relative to baseline.

Assay of ACh

ACh was directly assayed in the dialysate using the HPLC method with post-column enzyme reactor and ESA Coulochem II electrochemical detector with a platinum target. ACh and choline (Ch) were separated on an ESA ACH-SPR, 70-0640 3cm ACh solid phase reactor column kept at 35° C by the Keystone HotPocket column heater. The mobile phase consisted of 100 mM di-Sodium hydrogen phosphate anhydrous Fluka, Microselect 71639, 250 g 2.0 mM 1-Octanesulfonic Acid Acros Organics, 25 g 0.005% Reagent MB ESA 70-1025, 9 ml Adjust to pH=8.00 Phosphoric Acid Fisher Scientific.

The flow rate was 0.35 µL/min (injection volume 10µL). ACh was hydrolyzed by acetylcholinesterase (AChE) to acetate and Ch in a post-column enzyme reactor; Ch was oxidized by Ch oxidase to produce betaine and hydrogen peroxide. Hydrogen peroxide was electrochemically detected by an electrochemical detector (Model 5200A Electrochemical Detector) equipped with a cell (M5040 Analytical cell) with a platinum target at +300 mV.

Determination of AUC

Area under the curve (AUC) of dopamine and ACh concentrations in the dialysate of brain region was calculated by adding total DA release across the 2 hour post- injection period in each subject using 4 consecutive 30-min samples. These were then averaged and differences in effects of concentration of antagonists were examined by two-way ANOVA, followed by Newman–Keuls post hoc tests.

Systemic Drug Treatment

Drugs were dissolved in phosphate-buffered saline (pH 7.4) and injected intraperitoneally with plastic 1 ml syringes using a 25-gauge 0.5 in. needle. Control animals received saline vehicle. Animals received drug or vehicle in a total volume of 0.5 ml. Cocaine was obtained from Sigma Chemical Co. and was administered at a dose of 10 mg/kg of body weight. Animals were weighed daily to ensure proper growth, monitor health and determine appropriate dose of drug.

Intracranial Drug Treatment

After being perfused overnight the flow rate was adjusted to one microliter per minute and the microdialysis probe was allowed to equilibrate for approximately 90 minutes. Two 30 minute baseline samples were collected, then the syringe with artificial CSF was changed and replaced with a syringe in which the proper antagonist had been dissolved in artificial CSF. Two more 30 minutes samples were collected, then cocaine was administered intraperitoneally and four 30 minutes samples were collected.

L-733,060 was obtained from RBI pharmaceuticals and was dissolved in DMSO or in the case of WIN51,708 (FIG.14), dissolved in the non-toxic solvent 2-Hydrocypropyl-B-Cyclodextrin (Sigma Chemicals), as a stock of 10mg/ml. Then, it was diluted to the proper molar concentration in aCSF. Substance P was obtained from Sigma Chemical and dissolved in H_2O as a stock solution. SR142801 was obtained with the permission of Xavier Emond-Alt from Sanofi Recherche.

Immunocytochemistry

Fifteen minutes after systemic cocaine administration the animals were deeply anesthetized with isoflourane (5% at 0.5 liters O_2/minute) and perfused intracardially with 200 ml of 0.1 M PBS followed by 500 ml of a solution of 4% formaldehyde and 12.5% picric acid in 0.1 M PBS. The brain was then removed and post-fixed for 16 hr in the same fixative and cryoprotected for 24 hr in 30% sucrose in 0.1 M phosphate buffer. Serial sections, 40-µm-thick, were cut with a Vibrotome and collected in TBS to be processed immunohistochemically as free-floating sections.

The tissue sections were incubated for 5 minutes in H_2O_2 to quench any endogenous peroxidase activity, incubated for 30 min at room temperature in a protein blocking solution of 1% normal goat serum in PBS, and then incubated for 4 hr at room temperature. Tissue samples were then incubated in the primary antiserum (SPR antibody) with 0.3% Triton X-100 overnight @ 4°C. SPR was detected with a polyclonal rabbit anti-SPR antibody (1:250, Chemicon). ChAt was detected with a monoclonal mouse anti-SP antibody (1:1000, Biocare Medical). The incubated sections were washed three times for 10 min in TBS, incubated in the goat anti-mouse biotinylated secondary antibody solution for 2 hr at room temperature, and then incubated with a streptavidin-DAB solution for 25 minutes. The sections were then incubated for 5 minutes in the DAB reactant initiating a chromogenic response. The sections were washed three times for 10 min in TBS. Then the sections were incubated again in a protein blocking solution of 1% normal goat serum in PBS, incubated for 4 hr (ChAt antibody) at room temperature, and then in the primary antiserum with 0.3% Triton X-100 overnight. They were washed three times for 10 minutes and incubated in the goat anti-rabbit biotinylated secondary antibody solution for 2 hours at room temperature. After this, the sections were incubated with streptavidin-ALP solution (Biocare Medical) for 25 minutes and then for 5 minutes in the ALP reactant (Biocare Medical) in low light conditions for 30 minutes. Sections to be used for fluorescence were incubated with both primary antibodies simultaneously (both at a concentration of 1:100). Secondary antibodies conjugated to fluorescent markers TRITC (used with SPR) and FITC (used with ChAt) were used at 1:600. The slides were washed three times in TBS, then once in distilled deionized H_2O and finally, mounted on gelatin-coated slides, dehydrated via an alcohol gradient (70, 90, and 100%), cleared in Histoclear, and coverslipped. To confirm the specificity of the primary antibody, controls were generated; omission of primary antibody in the protocol abolished the staining. Because staining intensity might vary between experiments, control sections were included in each run of staining.

Statistical Analysis

Analysis of variance (ANOVA) was used to analyze drug treatment effects. Newman-Keuls test was used for post hoc analysis. Neurotransmitter release was expressed as percent change relative to baseline. This was necessitated by the observation that although values fall within a certain broad range baseline values are highly variable between animals. However, percent differences in neurotransmitter release relative to baseline were highly comparable. Thus, I averaged percent values from 10 animals, performed a paired Student's t-test, and obtained P values smaller than 0.05. In order to perform comparisons between neurotransmitter levels and behavioral scores, I utilized percent changes from individual subjects and performed a bifactorial analysis.

Chapter 4

RESULTS

Dopamine

Effects of Acute Cocaine administration on Striatal Dopamine release

WIN 51,708

In three groups, after the first two initial baseline measurements, artificial CSF with three different doses of the non-peptide antagonist WIN 51,708 (FIG.14) (10^{-4}, 10^{-5}, and 10^{-6} M) was administered in three separate groups of rats. In animals treated with WIN 51,708 alone, there was no effect on basal release of dopamine in the striatum in individual animals for the first hour post-antagonist intracranial administration regardless of concentration in all groups. However, when compared to control measures WIN 51,708 had a significant effect on the reduction of cocaine evoked dopamine release at the highest concentration (118.5% of baseline measure; a non-significant increase compared to basal measures of DA. (N=8, F=1.649; P=.1649;Power.545). In animals treated with WIN 51,708 the animals showed a dose-dependent response to abrogation of cocaine evoked dopamine release in the striatum when compared to groups treated with cocaine alone (FIG.15) (N= 8, F=4.998; P=.0045;Power =.926)

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D-Arg^1, D-Pro^2, D-Trp^{7,9,} Leu^{11}

After 4 baseline samples were colleced, cocaine was administered intraperitoneally (10 mg/kg body weight). Samples were collected every half hour for two more hours. Cocaine administration alone caused a significant increase in the release of striatal dopamine in the rat neostriatum. The increase in dopamine release in the first half hour time period post-cocaine injection was more than two-fold (213% of baseline measure; (N=8 $P < 0.0001$). In the other three treatment groups, after the first two half hour baseline measurements were collected, artificial CSF with the NK-1 receptor antagonist D-Arg^1, D-Pro^2, D-Trp^{7,9,} Leu^{11} (FIG.16) was administered in three separate groups of rats (10^{-3}, 10^{-5}, and 10^{-6} M). As in the first group, cocaine was administered systemically at 10 mg/kg bodyweight after 2 hours of samples had been collected. In animals treated with D-Arg^1, D-Pro^2, D-Trp^{7,9,} Leu^{11} , the antagonist alone had no effect on basal release of dopamine in the striatum in individual animals for the first hour post-antagonist administration regardless of concentration in all groups treated with D-Arg^1, D-Pro^2, D-Trp^{7,9,} Leu^{11}. However, when compared to the control group, D-Arg^1, D-Pro^2, D-Trp^{7,9,} Leu^{11} had a significant effect on the reduction of cocaine evoked dopamine release at the 10^{-3}M concentration (118% of baseline measure; a non-significant increase compared to basal measures of DA. (N=8, F=1.672; P=.1773;Power.501). The intracranial administration of D-Arg^1, D-Pro^2, D-Trp^{7,9,} Leu^{11} elicited a dose-dependent abrogation of cocaine evoked dopamine release (FIG.17).

SR142801

Using three different doses of the NK-3 nonpeptide receptor antagonist SR142801, results showed no significant dose-dependent response to the abrogation of cocaine evoked dopamine release in the striatum of the rat (N=8, F=1.693; P=.1659;Power=.522). This suggests nonspecific action of this experimental non-peptide receptor antagonist (FIG.18). However, there was a trend towards an increase in cocaine evoked dopamine release in the 10^{-5}M concentration.

L733,060

Administration of the NK-1 receptor antagonist L733,060 (FIG.19), intracranial at a concentration of 10^{-4}M, caused a significant decrease in cocaine evoked dopamine release in the rat neostriatum (FIG.20). Use of this antagonist was used to establish the specificity of the effect with other neurokinin-1 receptor antagonists. The fact that all neurokinin 1 receptor antagonists had a significant effect on the reduction of cocaine evoked dopamine release suggests the mechanism of cocaine evoked dopamine release is specific to the neurokinin receptors (N=8, F=2.366; P=.0345;Power=.824).

D-Pro^{2}, D-Trp^{6,8}, Nle^{10}

The non-peptide NK-3 receptor antagonist was administered intracranially in the same manner described in the methods section above at three different concentrations. In animals treated with NK-3 receptor antagonist alone there was no effect on basal release of dopamine in the striatum and individual animals the first hour post-antagonist administration. When compared to control groups where cocaine alone was administered systemically, the NK-3 peptide receptor antagonist had no significant effect on the reduction of cocaine evoked dopamine release, unlike the NK-1 receptor antagonists. (FIG.21) (N=9, F=1.677; P=.1610;Power=.541).

Effects of chronic cocaine administration on striatal dopamine release

WIN 51,708

Rats were treated daily with the same dose (10 mg per kg) of cocaine at the same time each day for five days. On the sixth day 10 mg /kg of cocaine was administered systemically and 4 hours were allowed to pass. Animals were then transported to the test cages. After overnight habituation (approximately 18 hours), animals were treated identically to those in acute groups. After chronic treatment with cocaine, rats were subjected to in vivo microdialysis and systemic administration of cocaine (10 mg/kg IP) in one group (N=10, F=5.606; P=.0013;Power=.967) and administration of non-peptide NK-1 receptor antagonists WIN 51,708 intracranially in another (N=10, $P < 0.1677$). The non-peptide receptor antagonist caused a significant reduction in cocaine evoked dopamine release in chronically treated rats with respect to controls (FIG.22).

D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9} Leu^{11}

Cocaine administration on the seventh day of treatment caused a significant increase in dopamine release. Administration of the peptide antagonist for the NK-1 receptor D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9} Leu^{11} , had limited effect in animals treated chronically with cocaine. Administration intracranially of the NK-1 receptor antagonist did not significantly reduce cocaine evoked dopamine release in the striatum in rats treated chronically (FIG.23). Although there was a trend toward reduction, this was not enough for a significant effect.

SR142801

Intracranial administration of the experimental NK-3 non-peptide receptor antagonist SR142801 reduced cocaine evoked dopamine release in the striatum of the rat significantly. This is in contrast to the acute results which showed that the NK-3 receptor antagonist had no significant effect on cocaine evoked dopamine release and, in fact, showed a trend towards an increase in cocaine evoked dopamine release (FIG.24) (N=10, F=4.102; P=.0312;Power=.526).

D-Pro^2, D-Trp^{6,8}, Nle^{10}

After chronic systemic administration with cocaine, rats were subjected to in vivo microdialysis administration of peptide NK-3 receptor antagonist intracranially in the manner consistent with other groups. Data show that the NK-3 peptide receptor antagonist had a profound effect on the reduction of cocaine evoked dopamine release. Dopamine release was brought to basal levels. However, NK-3 receptor antagonist administered alone had no effect on basal dopamine release (FIG.25) (N=10, F=1.606; P=.0281;Power=.383).

Effects of acute and chronic cocaine administration on nucleus accumbens dopamine release

Effects of WIN 51,708 on NAc$_{SHELL}$ DA release in rats treated acutely with cocaine

In animals in which the cannula was placed in coordinates consistent with the nucleus accumbens shell, the 10^{-4}M concentration of WIN 51,708 was used. When NK-1 receptor antagonist WIN 51,708 was administered intracranially into the NAc$_{SHELL}$, systemic administration of cocaine alone caused a significant increase in basal levels of dopamine. Systemic administration of cocaine caused a significant increase in concentrations of dopamine in the first and second half hour time points measured (N=8,F=4.456; P=.0018;Power=.970). Co-administration with the antagonist WIN 51,708 at a 10^{-4}M concentration caused a reversal of cocaine evoked DA release in the striatum. In fact DA was reduced below basal levels (N=8, F=4.162; P=.0023;Power=.926) (FIG.26).

Effects of WIN 51,708 on nucleus accumbens DA release in rats treated chronically with cocaine

In rats treated chronically with cocaine, administration on day seven of treatment caused a significant increase in the concentration of DA (F=4.456; P=.0018;Power=.970).

Intracranial administration of the NK-1 receptor non-peptide antagonist caused a significant decrease in cocaine evoked dopamine release in all half hour time points post-cocaine injection. Basal levels of dopamine efflux were suppressed significantly, suggesting tonic release of dopamine in the striatum controlled by the NK-1 receptors (FIG.27) (N=7, F=4.335; P=.0022;Power=.965).

Acetylcholine

Effects of acute and chronic cocaine administration on striatal acetylcholine release

Effects of WIN 51,708 on rats treated acutely with cocaine

Animals were perfused for two hours prior to sample collection with the acetylcholinesterase inhibitor neostigmine (FIG.28) at a concentration of 1nanoM. Acute systemic cocaine administration caused a significant increase in acetylcholine concentration in the rat striatum at all time intervals post-cocaine administration (N=7, F=6.417; P=.0053;Power=.937). The administration of the non-peptide antagonist WIN 51,708 caused a significant treatment effect in the rat neostriatum. Administration of WIN 51,708 alone had no significant effect on basal acetylcholine concentrations in the rat striatum. In comparison to animals with systemic administration of cocaine alone, NK-1 receptor antagonist WIN 51,708 caused a decrease in cocaine evoked acetylcholine release in the rat striatum. In fact, intrastriatal administration of WIN 51,708 brought acetylcholine concentrations in the striatum down to basal levels in rats treated with systemic cocaine (FIG.28) (N=10, (F=.481; P=.7852;Power=.139).

Effects of WIN 51,708 on rats treated chronically with cocaine

In rats treated with a challenge dose of cocaine after chronic administration there was a significant and profound increase in basal concentration of acetylcholine. This increase was reduced significantly with the intracranial application of the NK-1 receptor non-peptide antagonist WIN 51,708 at 10^{-4}M (N=6, F=2.502; P=.0440;Power=.749)(FIG.30). The antagonist alone had no effect on basal acetylcholine levels.

AUC measures

Dose response to application of WIN 51,708 on striatal cocaine evoked DA release

When areas under the curve (AUC) were added together for all dopamine measures post-cocaine administration within each group, the dose response to the WIN 51,708 became very apparent. The 10^{-6}M concentration had no effect on striatal cocaine evoked DA release while the 10^{-5}M showed a trend towards reduction. The only significant reduction in total cocaine evoked DA release occurred with the 10^{-4}M concentration of WIN 51,708 (FIG.31) (N=10 $P < 0.0001$).

Dose response to application of D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9,} Leu^{11} on striatal cocaine evoked DA release

AUC measures indicate that the administration of the peptide antagonists *D-Arg*^{1}, *D-Pro*^{2}, *D-Trp*^{7,9,} *Leu*^{11} caused a dose dependant reduction in cocaine evoked striatal DA release. The two highest concentrations (10^{-5}M and 10^{-3}M) caused significant reductions in total post injection DA release. The 10^{-4}M concentration was used for chronically treated animals because it had the most profound effect (FIG.32) (N=8 ,F=1.672; P=.1773;Power.501)

Dose response to application of SR142801 on striatal cocaine evoked DA release

AUC measures show that the non-peptide antagonist SR142801 data do not vary significantly with respect to cocaine evoked striatal dopamine release at any concentration. In fact, the middle dose caused a trend towards an increase in striatal dopamine release at the 10^{-5}M concentration (F=5.181; P=.0015;Power =.978)(FIG.33).

AUC of acute vs. chronic striatal dopamine release

AUC measures demonstrate a significant increase in cocaine evoked dopamine release in the striatum resulting from chronic cocaine administration. Although both acute and chronically treated animals showed a significant increase in cocaine evoked dopamine release in the striatum, chronic administration caused a significantly larger increase in evoked dopamine release, indicating neurochemical sensitization (FIG.34)(N=10 $P < 0.009$).

AUC of acute vs. chronic striatal ACh release

AUC measures were taken of acetylcholine release resulting from systemic cocaine administration. Both acute and chronic systemic cocaine administration caused significant increases in striatal acetylcholine concentrations. However, chronic administration caused a significantly higher concentration of striatal acetylcholine (FIG.35). This shows clearly that cholinergic neurotransmission was altered with chronic systemic cocaine administration (N=10 P < 0.012).

Immunocytochemistry

NK-1R IR in the striatum

Please note that all results and immunohistological micrographs attained here were from rats treated acutely (refer to methods section).

Rats were injected with 10 mg/kg body weight of cocaine. Ten minutes subsequent to injection, rats were perfused and sacrificed. Brains were removed and sections (40 microns) were made and subjected to ICC analysis.

Effects of aCSF perfusion on the pattern of NK-1R IR in the rat striatum

In the first group, rats were treated by being perfused intracranially with artificial CSF. In these groups, tissue was treated using NK-1 receptor monoclonal antibody using DAB as a chromogenic marker. Note that in both magnifications the pattern of immunoreactivity (IR) is consistent with the presence of NK-1 receptors on the surfaces of the cells and dendrites (FIG.36b). These sections show cytoarchitecture consistent with the presence of large aspiny cholinergic interneurons. Note the presence of large areas of the striatum devoid of any patterns of immunoreactivity roughly oval in shape (FIG.36a). Higher magnification in the same section shows cells with a distinct morphology characteristic of aspiny cholinergic interneurons (FIG.38). Both cytoarchitectural and morphological analysis provide strong evidence in identifying these neurons as cholinergic interneurons (FIG.37a,b).

Effects of substance P perfusion on the pattern of NK-1R IR in the rat striatum

Rats were perfused intracranially with the peptide substance P (10^{-6}M). In these rats the pattern of immunoreactivity in the same brain region showed a significant alteration in dendritic and somal distribution of NK-1 receptor patterns of immunoreactivity. Substance P perfusion caused an increase in the phenomenon of receptor internalization that can be readily identified and is characterized by a "beads on a string" morphology of dendrites, and radical change in distribution of NK-1 immunoreactivity on the soma (FIG.39). The internalization response to substance P administration is complete throughout all quadrants of the striatum. With administration of substance P, I was unable to detect soma or dendrites without the pattern of immunoreactivity consistent with receptor internalization (FIG.40).

Administration of cocaine caused a radical change in the pattern of immunoreactivity compared with rats treated with artificial CSF alone. Similar to subjects treated with substance P intracranially, cocaine caused a significant increase in the pattern of immunoreactivity consistent with receptor internalization in the rat striatum (FIG.41). This effect was complete throughout areas of the striatum examined, and was indistinguishable from rats perfused intracranially with substance P (FIG. 42).

Immunofluorescence

Effects of aCSF perfusion on the pattern of NK-1R IR in the rat striatum

In rats treated with aCSF perfused intracranially, a pattern of NK-1 receptor IR was consistent with morphological and histological landmarks suggesting cholinergic interneurons. Note the pattern of IR suggests an even distribution across the dendrites and soma of large (20-50 microns) neurons (FIG.43a,b). These neurons also had one to three dendrites that exhibited extensive arborizations throughout areas of the striatum (FIG.44). 37 1c). Note the tubular, almost three-dimensional effect of the pattern of IR.

Effects of substance P perfusion on the pattern of NK-1R IR in the rat striatum

Rats were perfused intracranially with substance P at a concentration of 10^{-6}M for fifteen minutes. This time point was used because the half-life of the internalization event is such that animal tissue must be fixed shortly after administration otherwise the effect cannot be observed. Animals were treated as mentioned in the methods section above. Note the radical change in the pattern of IR and the apparent shift in morphology. This suggests the phenomenon of receptor internalization that is consistent with the binding of substance P with its endogenous receptor, the NK-1R. These micrographs show that this effect is specific to cholinergic interneurons (FIG.44a,b,c).

Effect of systemic cocaine administration on the pattern of NK-1R IR in the rat striatum

This group of rats was perfused with artificial CSF alone and injected with 10 mg/kg of cocaine. Within fifteen minutes, there was a radical shift in the pattern of IR consistent with receptor internalization. When compared with rats perfused with substance P, the extent and pattern of IR throughout the striatum is similar. Cocaine administration caused a pattern of IR consistent with receptor internalization. This phenomenon was found throughout the entire striatum (> 95% of neurons which showed positive IR for the NK-1R showed a pattern consistent with receptor internalization) (FIG.45a,b,c).

Effects of systemic cocaine administration and WIN 51,708 on the pattern of NK-1R IR in the rat striatum

Subjects were perfused with the NK-1R antagonist WIN51,708 30 minutes prior to systemic cocaine administration. Cocaine was then administered systemically. Note that the distribution of NK-1 IR is consistent and similar to those of control animals. This suggests a total blockade of NK-1 receptor binding induced internalization. Ninety-five percent of cells in the area surveyed show the blockade of NK 1 receptor internalization patterns of IR (FIG.46a,b,c). These cells and the morphology of the area were indistinguishable from controls.

Chapter 5

CONCLUSIONS

Addiction is a behavioral phenomenon that is characterized by uncontrolled self-administration of known drugs of abuse. There are three facets of addiction that define it: tolerance, dependence, and sensitization. Tolerance is defined as the diminished effect of a given substance with chronic intermittent administration. Since the primary hedonic effect of the substance is reduced in the phenomenon of tolerance, this may lead to increased self-administration simply to maintain the initial effect (Berridge and Robinson, 1998). When a substance is administered chronically and then removed, it can lead to behavioral and hedonic depression. This is the hallmark of dependence (Di Chiara, 1995). Sensitization, also known as reverse-tolerance, is defined as an increase in behavioral or neurochemical effect of a drug with chronic administration. It has been postulated that this sensitization extends to motivational aspects of drug taking and drug seeking and is a crucial mechanism for the maintenance of addiction when the hedonic aspects of drug taking have a minimal impact (Berridge and Robinson, 1998). Intermittent systemic administration of cocaine causes a progressive increase in behavior resulting from the neurochemical effects of cocaine with each administration (Post, 1980). Since the half life of cocaine in the rat is approximately one half hour, these alterations in cocaine evoked behavior must be due to constitutive changes in the neurochemistry of the animal. Administration of NK-1 non-peptide receptor antagonist causes an abrogation of this cocaine evoked behavioral response (Kraft et al., 2001). I sought to elucidate the underlying neurochemical changes that subserve this behavioral phenomenon. Neurokinins and their receptors are present on centrally located, functionally relevant cells (cholinergic interneurons) which effect dopaminergic tone in the striatum. I conducted this investigation to elucidate the underlying mechanisms leading to alterations in the rat neostriatum in order to characterize the relationship of the activity of NK receptors on cholinergic interneurons and their role in cocaine evoked neurotransmitter release.

Behavioral Sensitization

Dopamine is considered the driving force behind behavioral sensitization (Kalivas and Duffy, 1993a). It is also assumed to be the essential neurotransmitter responsible for changes in neurochemistry and the driving force of neurochemical changes in this part of the brain leading to addiction. However, it seems that dopamine alone cannot be responsible for the behavioral output that leads to the addictive state. Although dopamine release is directly related to increases in behavior resulting from cocaine administration in the early stages of the development of behavioral sensitization, we have found that in the period of abstinence following chronic exposure, an increase in dopamine is not necessary for the sensitization phenomenon to occur (Zhang et al., 2001).

Acetylcholine and its markers are present throughout the striatum at very high levels (Angulo and McEwen, 1994; Calabresi et al., 2000a). Most importantly, cholinergic interneurons seem to be ideally placed to modulate inputs from several distinct brain regions including but not limited to the cortex, thalamus, hippocampus, and the striatum (Calabresi et al., 2000b). The interplay between acetylcholine and dopamine may elucidate discrete circuitry in the basal ganglia and will help us to better understand the neurochemical changes subserving addiction and other pathological states.

Basic Structure of Basal Ganglia

The striatum is a functionally and morphologically heterogeneous nucleus of the basal ganglia. It can be separated into two gross morphological regions: the matrix and the striosome. Both contain the projection neurons (Blanchet et al., 1998). Our histological data show clearly the distinct distribution of NK-1 positive cholinergic interneurons with respect to these two striatal territories (FIG. 36a). The functionally relevant circuit that we are examining is the direct loop of the basal ganglia. This consists of the set of functionally interconnected nuclei that are striato-nigral and nigro-striatal. The striatum contains approximately 90% medium spiny neurons; of these, there is a population of SP/GABA positive neurons that project to the substantia nigra reticulata. Dopaminergic neurons from the substantia nigra compacta project to the striatum onto SP/GABA positive neurons. They also make connections with the cholinergic interneurons that synapse with the SP/GABA positive neurons that in turn project to the SN. Substance P projection neurons have collaterals that also make connections with cholinergic interneurons (Gerfen, 1991). This circuit is referred to as the direct loop.

Cholinergic Interneurons

There are three different types of interneurons in the striatum. They are somatostatin interneurons, GABA interneurons, and cholinergic interneurons. Somatostatin is found in the neostriatum in high concentrations and is synthesized in medium-sized aspiny interneurons (Di Figlia and Aronin,1982; Chesselet and Graybiel, 1982; Galarraga et al., 1993). Striatal somatostatin modulates GABA release in the neostriatum (Meyer et al., 1989). If this action occurs through a direct action on spiny neurons, it may have a role in regulating motor functions (Aronin et al., 1983). However, direct actions of somatostatin on striatal neurons are not well characterized. GABA interneurons receive input from the cortex and may effect release of ACh in this brain region via the $GABA_A$ receptors present on cholinergic interneurons (Calabresi et al., 2000a). The cholinergic interneurons are of greatest interest in this investigation because of the presence of NK-1 receptors on their soma and dendrites, and because they make synaptic connections with the medium spiny projection neurons to the midbrain nuclei that contain SP, dynorphin and GABA (Arenas et al., 1991). In addition, these interneurons receive dopaminergic input from nigral projection neurons. Cholinergic interneurons are ideally placed to modulate the interactions between cortical inputs and the direct loop of the basal ganglia. The major neurotransmitter in terms of the modulation of cholinergic tone seems to be substance P since NK-1 receptors are present primarily on the surfaces of cholinergic interneurons (Arenas et al., 1991). Cholinergic interneurons also contain glutamate receptors, D1 receptors, in addition to NK-1 receptors (Steinberg et al., 1998). The presence of these neurokinin receptors on cholinergic interneurons and their relationship with release of acetylcholine and dopaminergic input from substantia nigra is of primary interest.

The presence of D2 receptors on the cholinergic interneurons and muscarinic receptors on the SP positive projection neurons suggests a functional link between these three neuronal subpopulations. At low concentrations, release of DA inhibits the release of ACh via the D2 R present on cholinergic interneuron soma (Calabresi et al., 2000a). ACh release can also mediate the effects of DA by indirect action on the striatal projection neurons (de Belleroche and Bradford, 1980). My hypothesis is that neurokinin receptor activity modulates the activity of the direct loop because of the presence of neurokinin receptors on cholinergic interneurons, the presence of muscarinic receptors on substance P projection neurons to the substantia nigra, and the presence of D1 and D2 receptors on substance P projection and cholinergic neurons respectively. This functional interaction provides ideal location for neurokinin receptor modulation of dopaminergic and cholinergic transmission.

Discussion

Previous data collected in this laboratory suggest that striatal neurokinin receptors play a major role in behavioral alterations resulting from cocaine administration in rats (Kraft et al., 2001). Using highly specific peptide and non-peptide receptor antagonists, I sought to elucidate the neurochemical mechanisms underlying this plasticity. An examination of neurokinin receptor pharmacology in the striatum helped to characterize the role of NK-1 and NK-3 receptors in acute and chronic cocaine evoked neurotransmitter release in the rat neostriatum. Use of histology helped to establish locale of this effect on a neuroanatomical level and was beneficial in informing the current proposed model of the activity of NK-1R activity on cocaine evoked DA release. The data presented here implicate the neurokinin receptors as major intermediaries in cocaine evoked changes in striatal neurochemistry.

Acute systemic cocaine administration caused a 213% increase in extra striatal dopamine concentration in the first half hour after systemic cocaine administration. Intracranial administration of the non-peptide NK-1 receptor antagonist WIN 51,708 reduced cocaine-evoked dopamine release by 95%. The two other concentrations, 10^{-5}M and 10^{-6}M, showed a trend toward reducing dopamine efflux, but did not have as profound an effect as the highest concentration of WIN 51,708. AUC was used to determine the effects of the antagonists on total cocaine evoked DA and ACh release in the striatum post injection. This measure gives us a clearer view of overall neurotransmitter release and the effects of antagonists on striatal neurochemistry without the higher temporal resolution of the 30 minute measures and the concomitant fluctuations in measurememts. When total dopamine efflux over the two-hour period following post cocaine injection was measured, the lowest concentration had no effect on cocaine evoked dopamine release while the next highest concentration showed a trend toward reducing total post cocaine injection dopamine release. This was in contrast to the results using the NK-1 peptide receptor antagonist D-Arg^1, D-Pro^2, D-$Trp^{7,9}$, Leu^{11}

It is of note that administration of the non-peptide antagonist alone had no significant effect on DA release.

Similar to the result of the non-peptide antagonist, D-Arg^1, D-Pro^2, D-$Trp^{7,9}$, Leu^{11}

administered at the highest concentration (10^{-3}) had the most profound effect on cocaine evoked dopamine release in the striatum and reduced dopamine efflux by 95%. The lowest concentration, 10^{-6}M, had absolutely no effect on cocaine evoked dopamine release. At the middle concentration, 10^{-5}M, D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11} reduced dopamine efflux significantly. AUC measures were used to show effects of treatment on total DA and ACh release in the brain regions assayed. AUC measures showed total post injection dopamine efflux was brought to basal levels with the highest concentration of the peptide antagonist, while the 10^{-6}M of D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11} concentration had no effect. In acutely treated animals, the peptide antagonist was slightly less effective at the highest concentration in reducing dopamine efflux resulting from systemic cocaine administration. Intracranial administration of the non peptide antagonist L733,060 at a concentration of 10^{-4}M also showed a significant reduction in cocaine evoked dopamine efflux in the striatum. This confirmed our results that effects were specific to the NK-1 receptors in this region. Intracranial administration of the NK-3 non-peptide antagonist SR142801 resulted in an inverted U-shaped response to concentration of antagonist used in AUC measures (FIG.32). At the highest and lowest concentration, 10^{-4}M and 10^{-6}M respectively, they had no effect on cocaine evoked dopamine release. However, in the middle dose, intracranial perfusion caused a non-significant increase in the effect of cocaine evoked dopamine release. When the highest concentration of SR142801 was administered intracranially to rats treated chronically with systemic cocaine, SR142801 application caused an 88% reduction in cocaine evoked DA release in the first half hour post cocaine injection (FIG.18). AUC measures showed that the highest and lowest dose had similar effects on cocaine evoked dopamine release and both measures of total dopamine release were higher than in the animals treated with cocaine alone. This is an interesting contrast with the NK-3 peptide receptor antagonist D-Pro^2, D-Trp^{6,8}, Nle^{10} which had no significant effect on cocaine evoked dopamine release at a dose of 10^{-5}M in animals treated acutely with cocaine (FIG.21). However, chronically, this peptide antagonist reduced cocaine evoked dopamine release 157% in the rat striatum. These results show that NK-1 and NK-3 receptors may have differential effects and counterbalance each other in their activity in the rat striatum. The peptide antagonist data is especially interesting when contrasted to the non-peptide antagonist data. The peptide antagonist is not as effective as the non-peptide antagonist when administered chronically. I hypothesize that this difference in efficacy is due to endopeptidase activity which increases with chronic stimulation of neurons in the brain regions I examined (Zhou et al., 2001). This increase in endopeptidase activity effected the efficiency of the peptide antagonist, but had no effect on the non-peptide antagonist. This may be a fruitful avenue for further investigation as well as another target for therapeutic intervention.

Systemic acute administration of cocaine caused a significant increase in cocaine evoked dopamine efflux in the NAc$_{\text{SHELL}}$. There was a 30% increase in the first half hour time point and a 40% increase in the second half hour period. By the end of the sample period dopamine had returned to normal levels. Unlike the effects of antagonists in the striatum, administration of the nonpeptide antagonist WIN 51,708 directly into the NAc$_{\text{SHELL}}$ caused a significant reduction in basal dopamine levels (FIG.26). In addition, levels were kept below baseline even after cocaine injection. In chronically treated animals, challenge dose on day seven of administration caused a significant but small increase in cocaine evoked dopamine release (FIG.27). However, the non-peptide antagonist alone had no effect on basal levels of dopamine release in that area. In addition, after cocaine administration in animals intracranially perfused with antagonists, dopamine levels were brought below baseline in a manner similar to those animals treated acutely.

Systemic acute administration of cocaine caused a significant increase in evoked striatal acetylcholine content. Using a 1nM concentration of neostigmine (FIG.28) to eliminate the effects of acetylcholine transferase, systemic cocaine administration caused a 300% increase in acetylcholine efflux. Administration of the WIN compound at 10^{-4} M caused a 250% reduction in cocaine evoked acetylcholine release (FIG.29). Levels were brought down to basal concentrations. The antagonist had a profound effect on suppression of acetylcholine release in this brain region. A challenge dose administered to animals treated chronically with cocaine caused a 400% increase in cocaine evoked acetylcholine efflux (FIG.30). Administration of the WIN compound caused a complete reversal of this effect and brought levels down to basal concentrations as in the acutely treated animals. AUC measures showed that total acetylcholine efflux over the two-hour period post-cocaine injection was significantly higher in chronic than in acutely treated animals, suggesting a neurochemical sensitization of the cholinergic system in this brain region (FIG.35).

Histology

Using antibodies for makers of cholinergic interneurons NK-1 receptor antibodies, I found that there where a great deal of NK-1 positive neurons associated with striosomes of the striatum. I administered substance P into the striatum to establish if this administration could cause an internalization event. I then administered cocaine at the same dose that was used for the microdialysis and found that administration caused a profound internalization response that was indistinguishable from animals perfused with substance P. This established the effect. Since it was important to establish what population of neurons was undergoing internalization, I used a double label. Using confocal microscopy to visualize the sections, I double labeled tissue for NK-1 and ChAt. I found the same results, a population of interneurons labeling positive for both NK-1 and ChAt, the rate-limiting enzyme for ACh synthesis. These cells had extensive dendritic arborizations and were the appropriate size and shape. Application of SP caused a profound change in the pattern of immunoreactivity consistent with receptor internalization. Systemic administration of cocaine caused the same response showing that cocaine did indeed effect NK-1 receptor activity. I then pre-perfused animals in the same manner as in the microdialysis experiments with the WIN compound. The application of WIN to the striatum before cocaine administration caused a radical reduction in receptor internalization, indistinguishable from controls. With the dose used in this series of experiments the response was complete. The tissue showed complete internalization with the administration of substance P and cocaine, or the morphology was indistinguishable from control.

Implications

Several factors make the findings presented in this thesis significant with respect to the effects of cocaine on the cholinergic and dopaminergic system. One of the factors that stands out most is that with systemic administration of cocaine whether this administration is acute or chronic has no effect on basal extra-synaptic concentrations of DA or acetylcholine. This suggests that collaterals from SP/GABA positive projection neurons are not releasing SP at basal levels of activity thus blockade of NK-1 receptors has no significant effect on the circuit at basal levels of activity.

The direct loop of the striatum is mainly inhibitory (Deniau and Chevalier, 1985; Chevalier and Deniau, 1990). Tonic release of ACh and DA in the striatal compartment causes a tonic GABAergic signal to be released onto dopaminergic projection neurons in the substantia nigra (Blanchet et al., 2000). This GABAergic input may keep the dopamine projections neurons from becoming too active. Tonic release of ACh onto the terminals of SP projection neurons activates M1 and M2 receptors on the soma of the substance P projection neurons (Arenas et al., 1991; Galarraga et al., 1999). Depending on the concentration of the ACh this modulated the response of SP positive projection neurons. At low levels, the M1 receptors have the most significant effect. This causes an increase in the release of GABA onto DA neurons in the SN, with which these SP/GABA positive neurons make synaptic connections (Deniau and Chevalier, 1985). This in turn keeps DA release in the terminal field regions of the striatum within a normal basal range that we measured using in vivo microdialysis.

In order for substance P to be released from projection neurons, there must be intense sustained stimulation of these neurons. It is at this high level of stimulation that the direct loop goes from being a primarily inhibitory to an excitatory projection. This occurs at several points. Within the striatum, the main transmitter, GABA, ceases to have the primary effect on cholinergic interneurons that contain $GABA_A$ receptors. Substance P release onto cholinergic interneurons causes ACh to be released by cholinergic interneurons (Steinberg et al., 1995; Blanchet et al., 2000).With the initial increase in SP release the primary effect of SP is to inhibit the ACh release (Boix et al., 1994). This may be due to the tonic release of GABA from SP/GABA collaterals onto GABA R on ACh interneurons. Increased stimulation of the projection neurons causes SP to be release in the striatal compartment via the SPergic collateral contained within the striatal compartment (Anderson et al., 1994). The release of substance P onto cholinergic interneurons causes an increase in ACh release(Arenas et al., 1991). This acts as a small feed forward loop causing the increase of the activity of the SP projection neurons. SP is also release in the substantia nigro onto SNr and VTA neurons that have functional neurokinin receptors. The SNc extends mossy fibers into the SNr compartment that allow these two compartments to communicate (Arenas et al., 1991). This causes an increase in the activity of the projection neurons that in turn increases the release of both ACh and DA in this brain region. At basal levels this circuit is inhibitory, however with the administration of cocaine, this inhibition is overridden and excitatory SP activity takes over via the neurokinin receptors.

What are the implications of this activity? It has been established that novel stimulus causes an increase in the release of DA and ACh (Steele et al., 1996; Wu et al., 1999). The activation of this initially inhibitory feed forward loop and its conversion to an excitatory loop may in part explain the activation motivational aspects of drug addiction. The activation of this loop that engages aspects of the basal ganglia responsible for some of the complex behaviors that subserve addiction may help inform our present model of addiction and define clear therapeutic targets.

The alteration in the pattern of cocaine evoked neurotransmitter release combined with the histological evidence implicates the neurokinin system as an important modulatory mechanism of psychostimulant induced changes in neurotransmitter release. These results characterize substance P, ACh and DA as a triumvirate of neurotransmitters presiding over neurochemical alterations in the rat neostriatum resulting from systemic cocaine administration, and point to a potentially fruitful avenue for the development of treatments for addiction and other diseases of the basal ganglia.

Figures

ACETYLCHOLINE

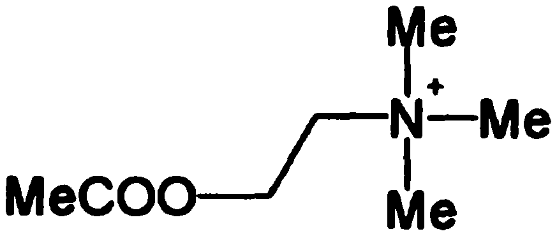

FIG.1 Molecular structure of acetylcholine

ACh
SYNTHESIS

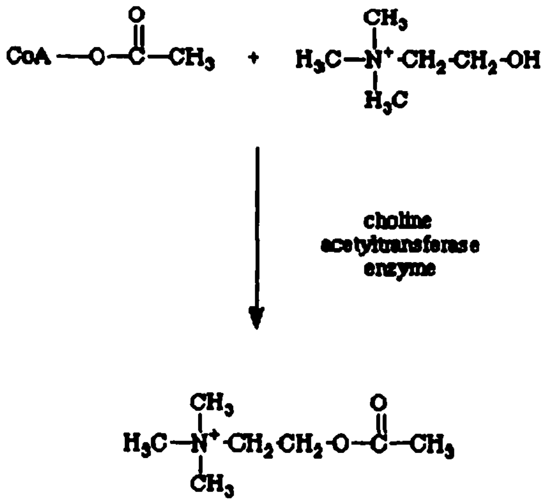

FIG.2 Metabolic pathway of acetylcholine synthesis

SUBSTANCE P

Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met(NH_2)

FIG.3 Amino acid sequence of substance P

COCAINE

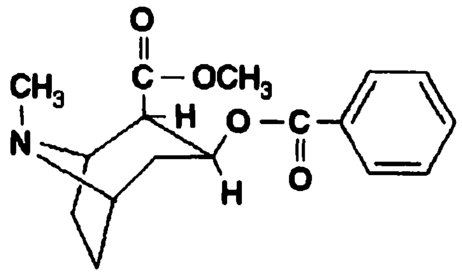

FIG.4 Molecular Structure of Cocaine

DOPAMINE

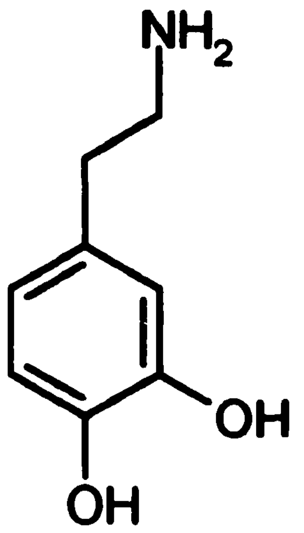

FIG.5 Molecular structure of dopamine

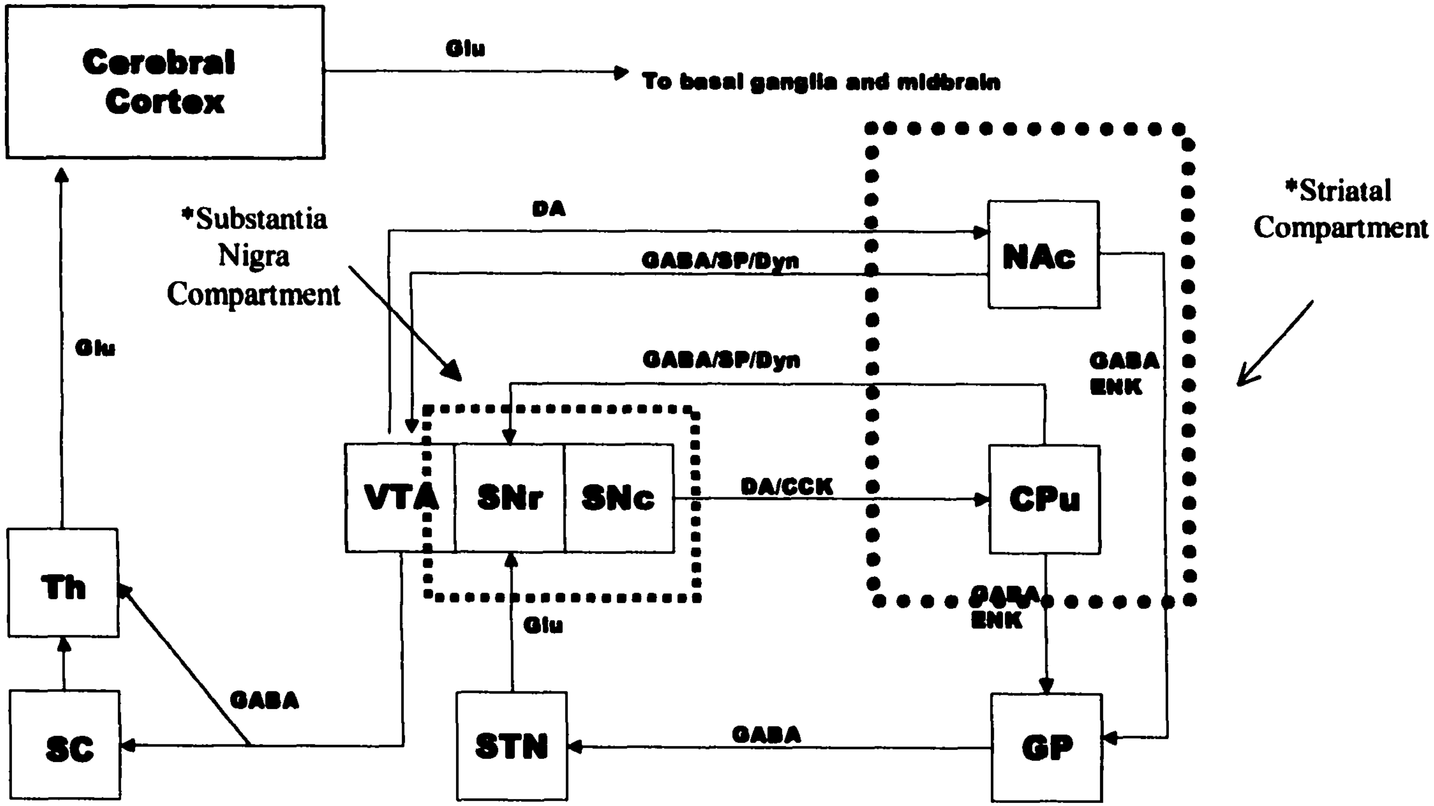

FIG.6 Schematic of the basal ganglia. Note the substance P projection neurons from the nucleus accumbens (NAc) and the caudate putamen (CPu). Areas shown include: Thalamus (Th), Ventral tegmental area (VTA), Substantia nigra reticulata (SNr), Substantia nigra compacta (SNc), Caudate putamen (CPu), Nucleus accumbens (NAc), Globus pallidum (GP), Sub-thalamic nucleus (STN) (Angulo and McEwen, 1994). The boxes with dotted lines denote nuclei that are merged and treated as one in figures 7a and 7b.

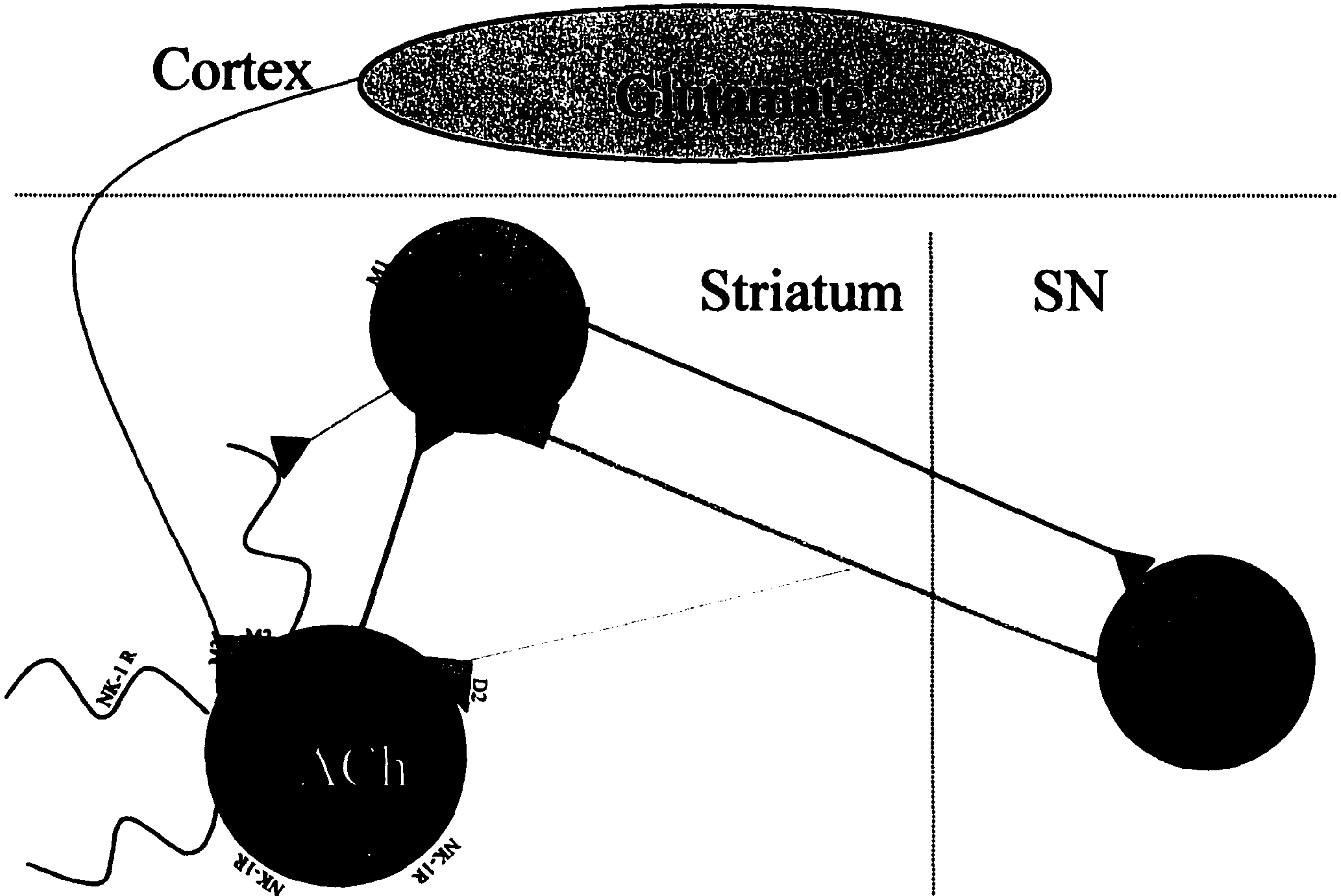

FIG.7a The Direct Loop of the Basal Ganglia. This schematic shows the relationship between the DA, ACh and SP/GABA neurons in a quiescent circuit.

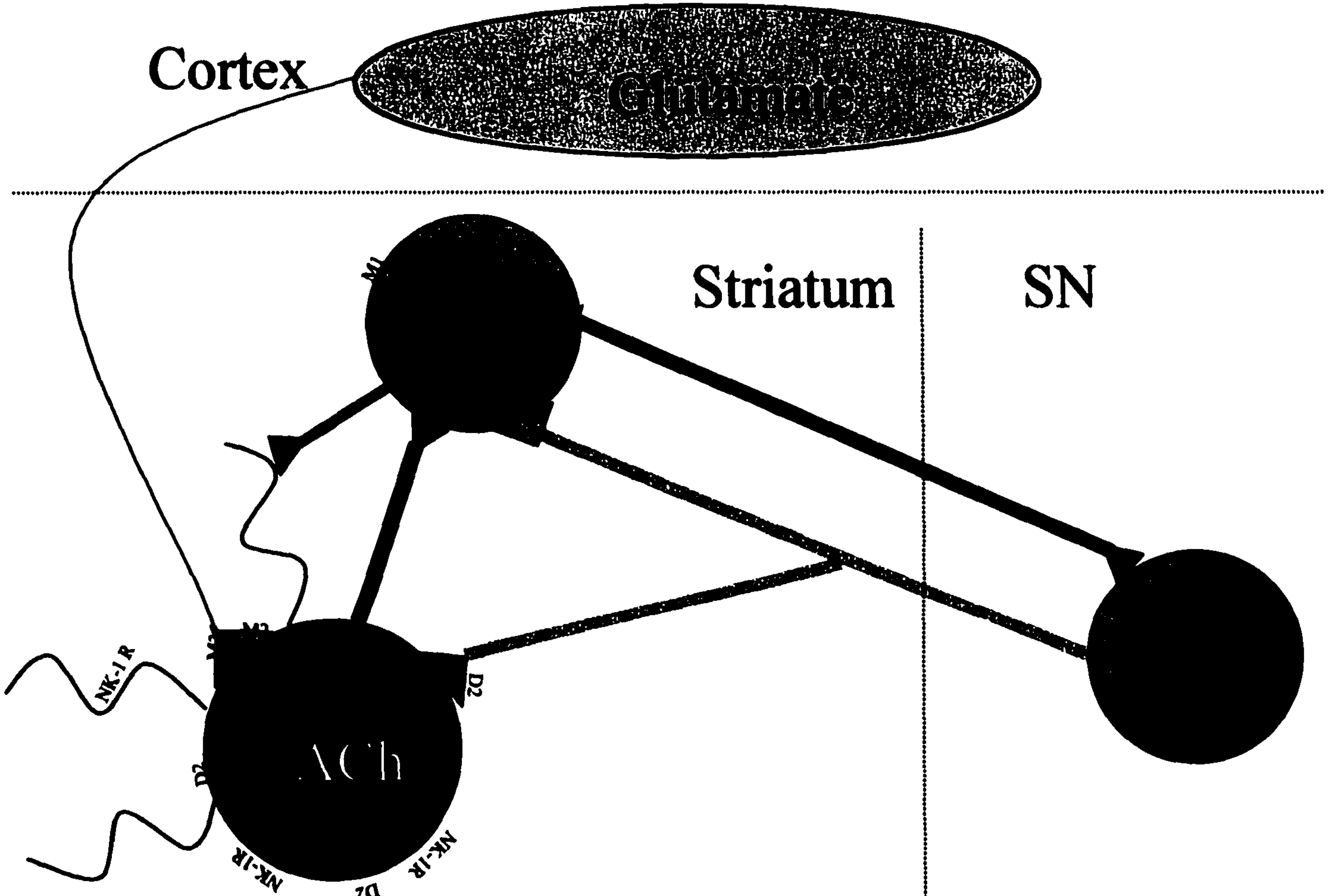

FIG.7b The Direct Loop of the Basal Ganglia. This schematic shows the relationship between the DA, ACh and SP/GABA neurons in a circuit activated by cocaine. The cholinergic neurons, activate the feed forward loop, which leads to an increase in DA release in the striatum. Thick lined represent increase in neurotransmitter release

DA SYNTHESIS

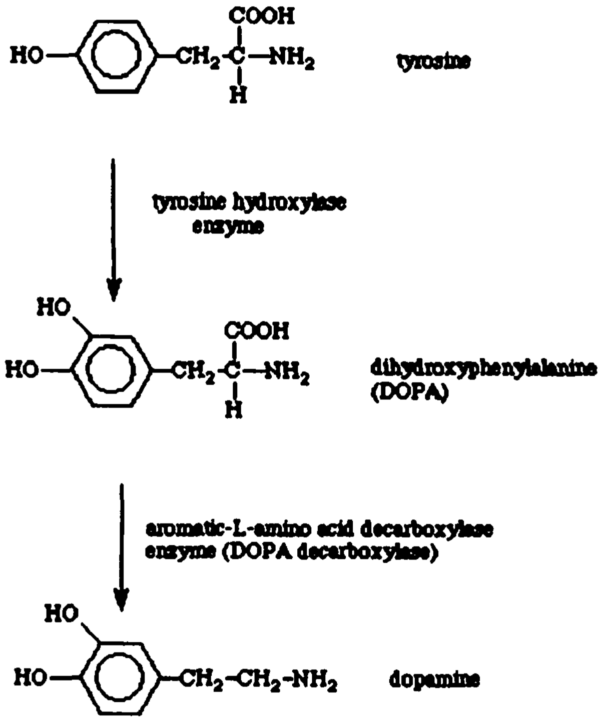

FIG.8 Metabolic pathway of dopamine synthesis

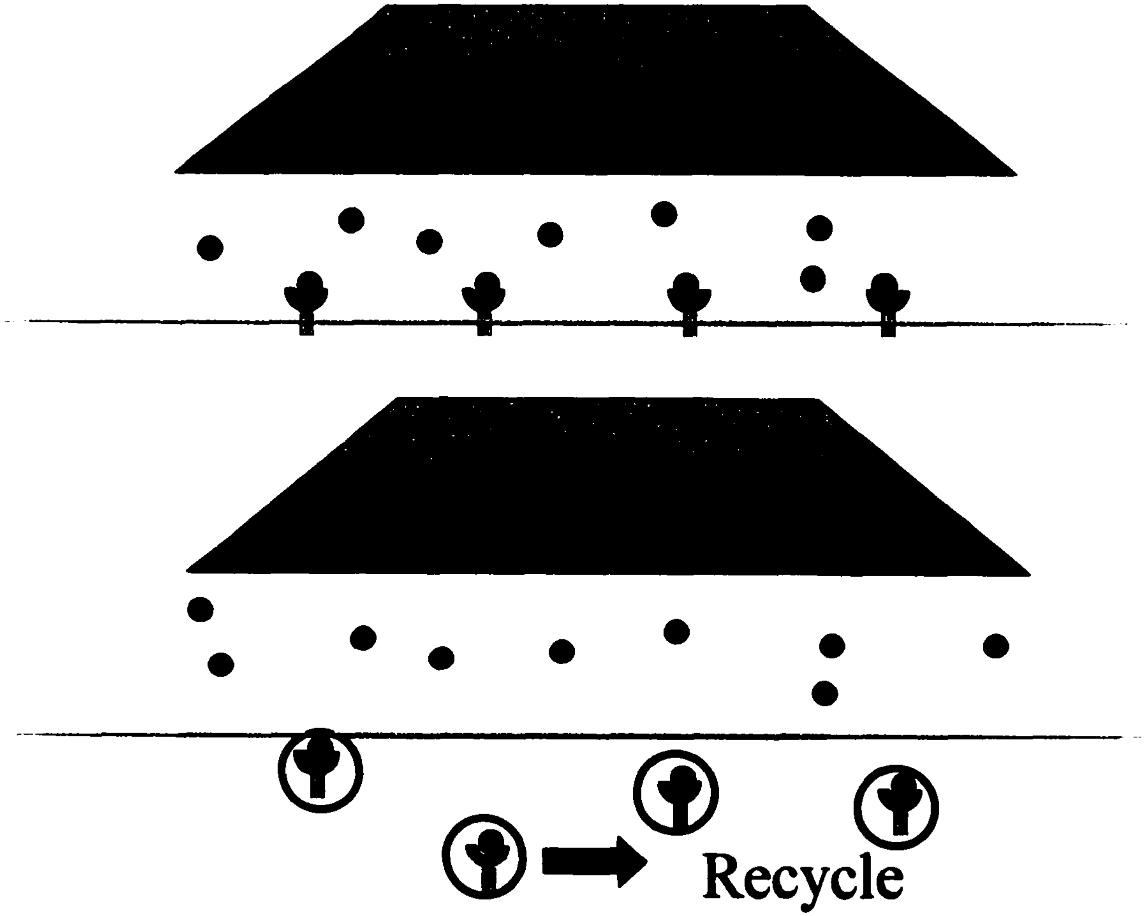

FIG.9.10 After substance P binds to the NK-1 Receptor, the receptor/ligand complex is packaged into endosomes and transported back to the soma for recycling

Striatum Microdialysis Area

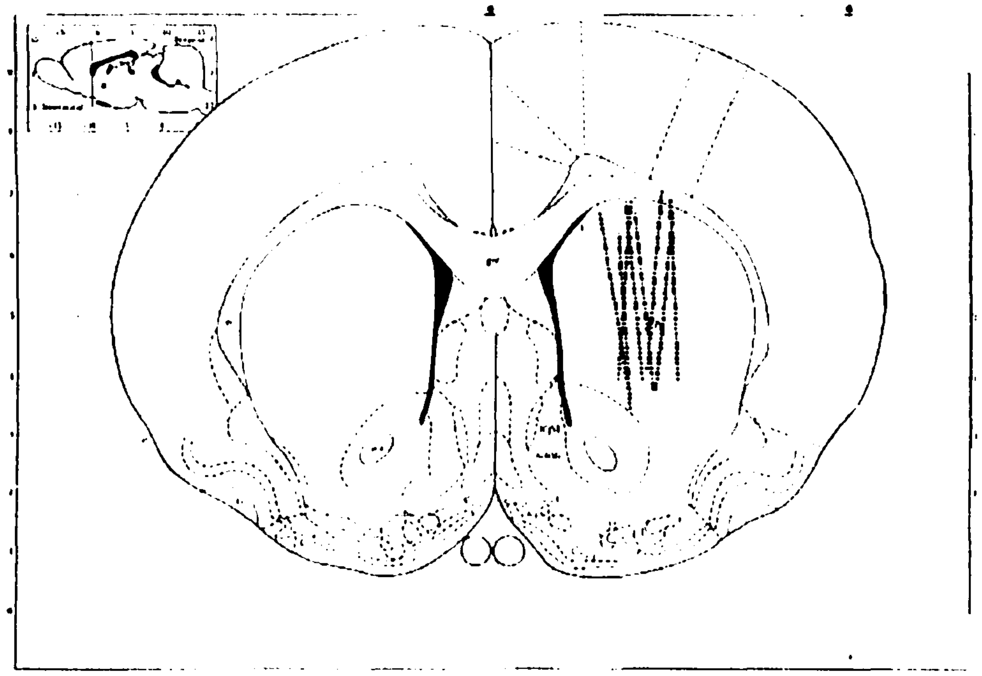

FIG.11 Insertion tracts of microdialysis probes active membrane area. (Adapted from Paxinos and Watson 1986)

NAc$_{\text{SHELL}}$ Microdialysis Area

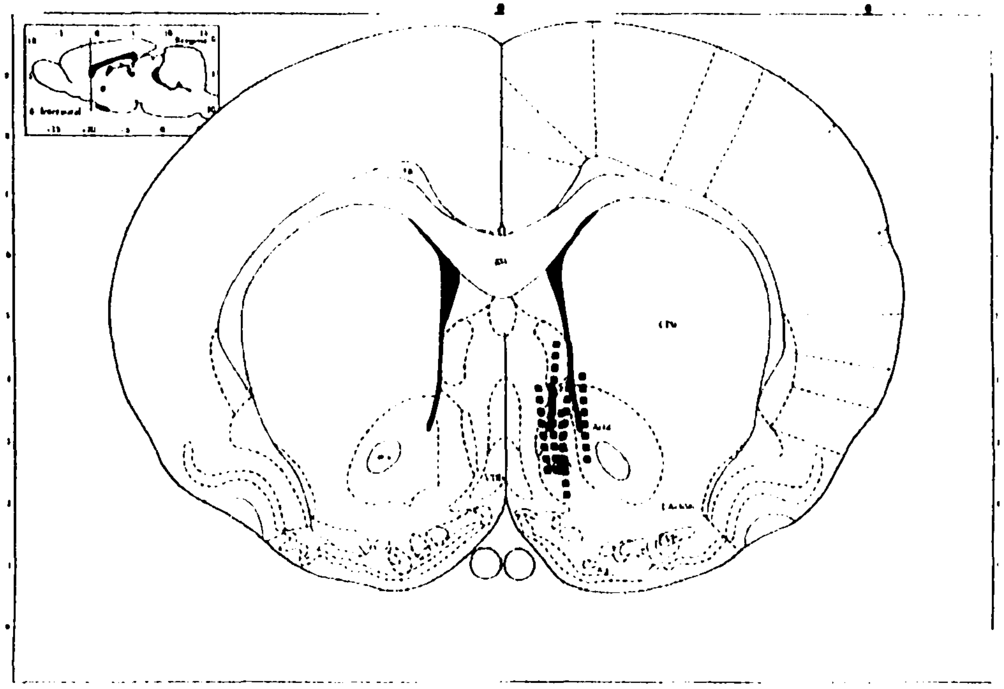

FIG.12 Insertion tracts of microdialysis probes active membrane area. (Adapted from Paxinos)

Microdialysis Setup

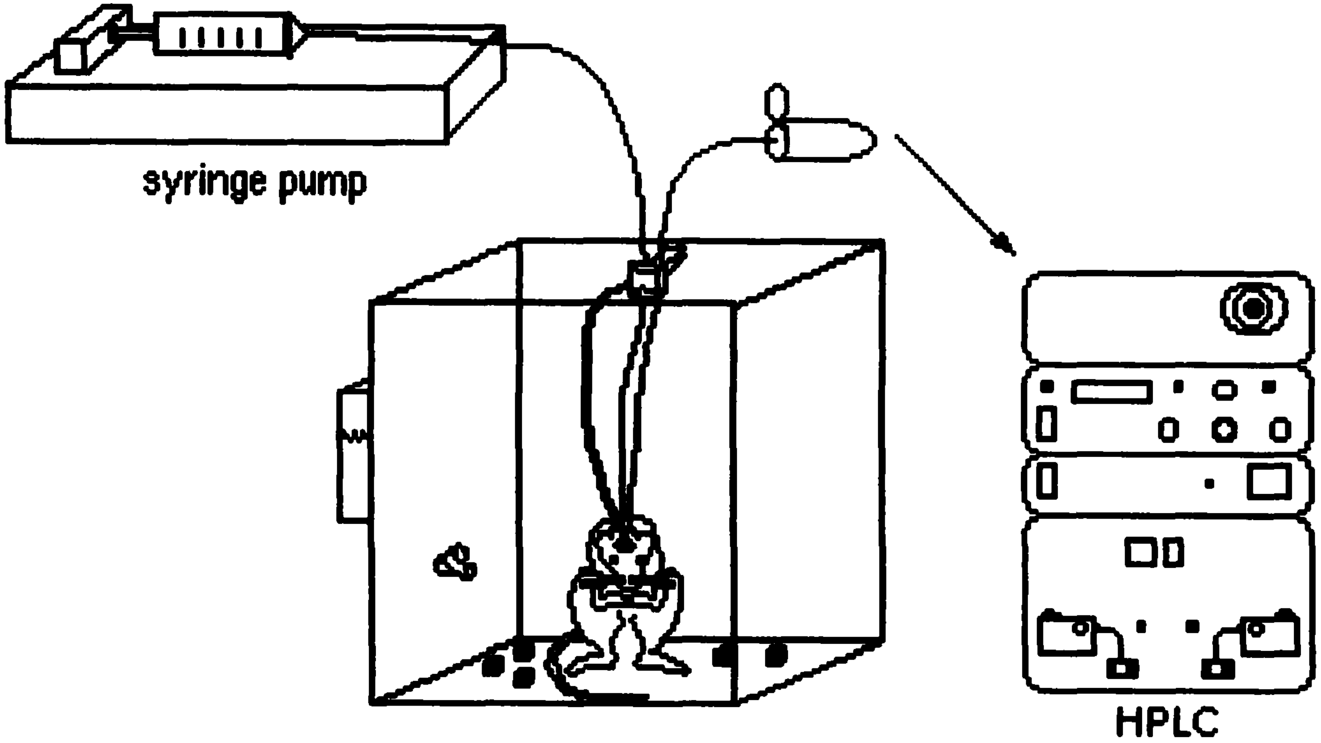

FIG.13 Microdialysis configuration. Animals are awake and freely moving, with open access to food and water as they are perfused in the modified operant conditioning chamber. Samples are collected and subjected to HPLC analysis.

WIN 51,708

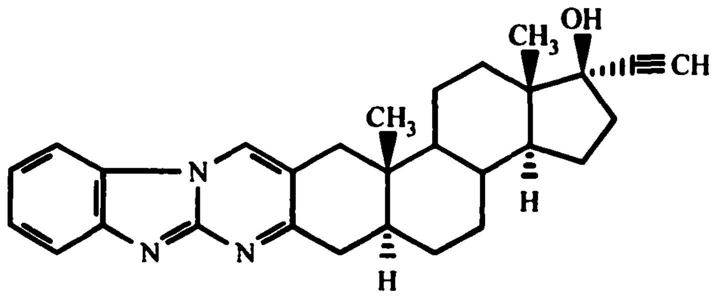

FIG.14 Molecular structure of non peptide antagonist WIN 51,708

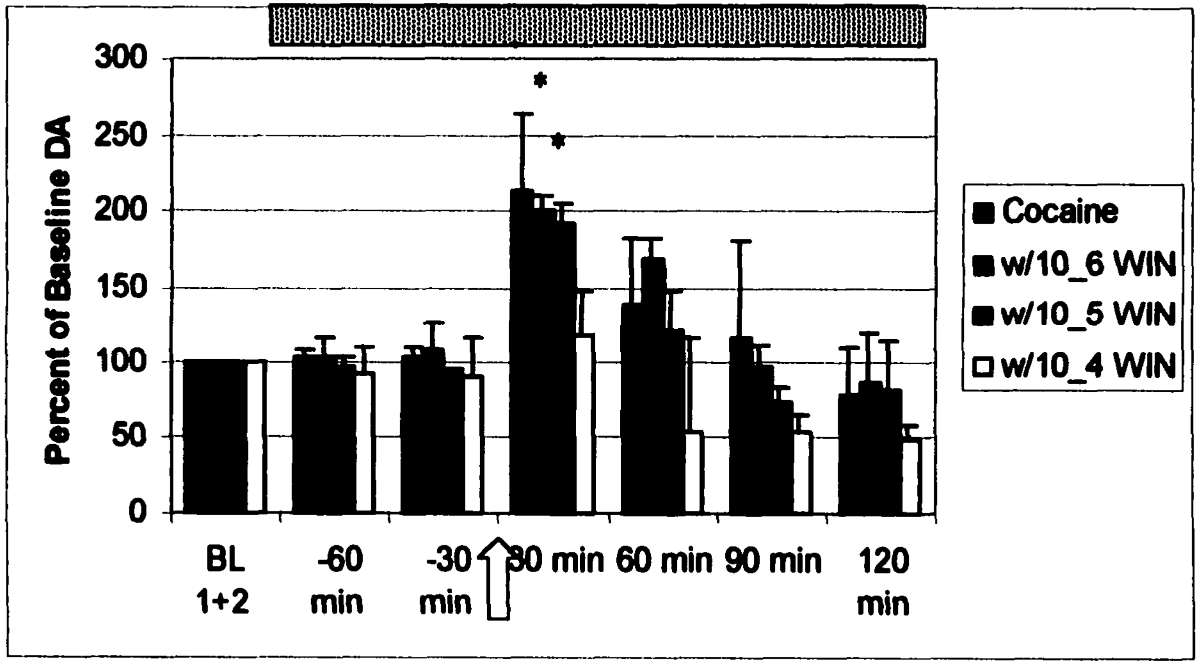

FIG.15 Effect of the NK-1 receptor antagonist **WIN 51,708** on cocaine evoked release of DA from the striatum of intact and freely moving rats. **WIN 51,708** (10^{-4}, 10^{-5}, and 10^{-6} M) was perfused locally through the microdialysis probe for 180 minutes (as indicated by the rectangle). administration of compound cause a dose-dependent decrease in cocaine evoked dopamine release Arrow indicates time of systemic cocaine administration. Dopamine levels are expressed as percent of baseline. *P < .05 compared to baseline by two factor analysis of variance with repeated measures and Fishers post-hoc analysis

SUBSTANCE P
PEPTIDE
ANTAGONIST

D-Arg^1- D-Pro^2-Lys-Pro-Gln-Gln- D-Trp^7-Phe-D-Trp^9-Leu-Leu^{11}

FIG.16 Molecular structure of substance P receptor peptide antagonist

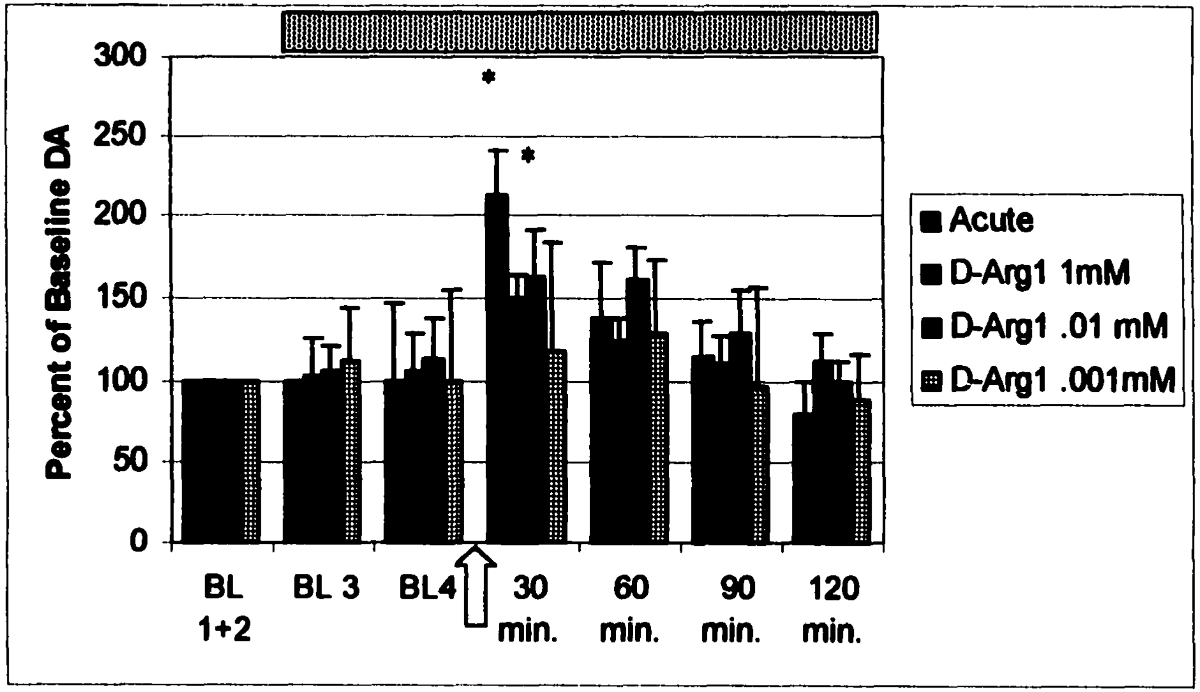

FIG.17 Effect of the NK-1 peptide receptor antagonist **Arg^1, D-Pro^2, D-Trp^{7,9,} Leu^{11}** on the cocaine evoked release of DA from the striatum of intact awake and freely moving rats. Three different doses of **Arg^1, D-Pro^2, D-Trp^{7,9,} Leu^{11}** (10^{-3}, 10^{-5}, 10^{-6}) were perfused intracranially through the microdialysis probe for 180 minutes (as indicated by the rectangle). And the highest concentration compound reduced cocaine evoked dopamine release significantly in the first 30 minutes post injection. Arrow indicates administration of systemic cocaine. Dopamine levels are expressed as percent of the average baseline dopamine release. *P.05 compared to baseline by two factor ANOVA with repeated measures and Fishers post-hoc analysis.

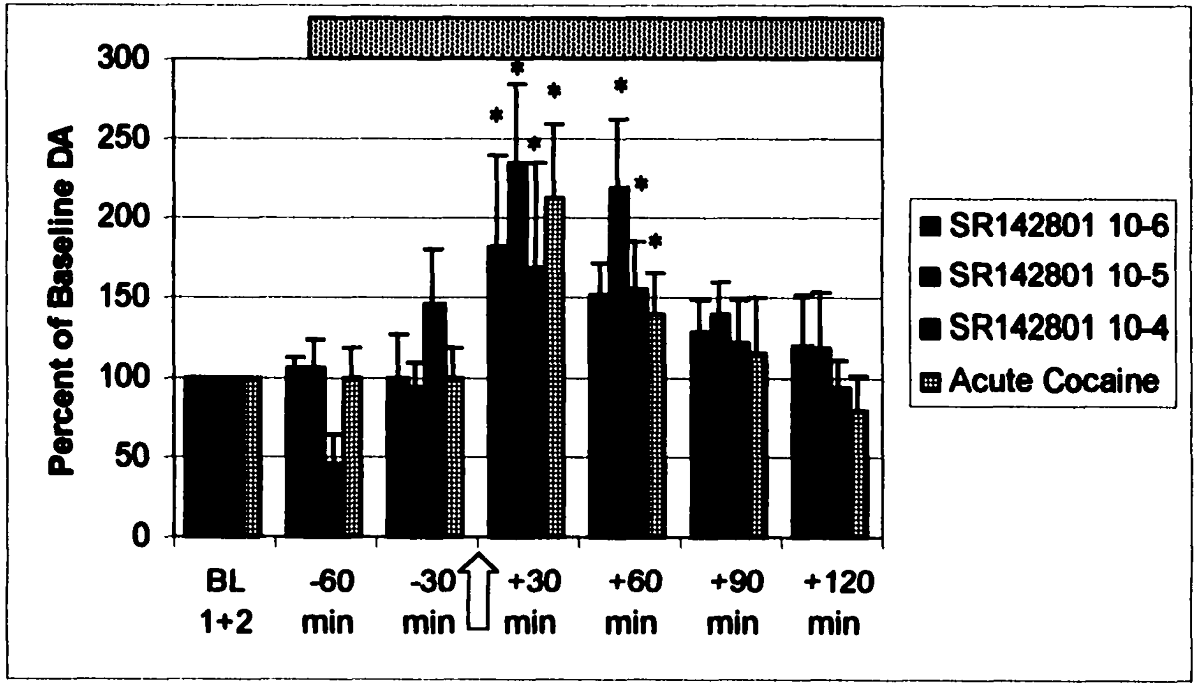

FIG.18 Effect of the NK-3 non-peptide receptor antagonist **SR142801** on the cocaine evoked release of DA from the striatum of intact, awake and freely moving rats. **SR142801** (10^{-4}, 10^{-5}, and 10^{-6} M) was perfused locally through the microdialysis probe for 180 minutes (as indicated by the rectangle). Arrow indicates administration of systemic cocaine. Dopamine levels are expressed as percent of the average of the first three baseline samples collected. Administration of the compound had no significant effect on cocaine evoked dopamine release at any time point.*P.05 compared to baseline by two factor ANOVA with repeated measures and Fishers post-hoc analysis.

L733,060

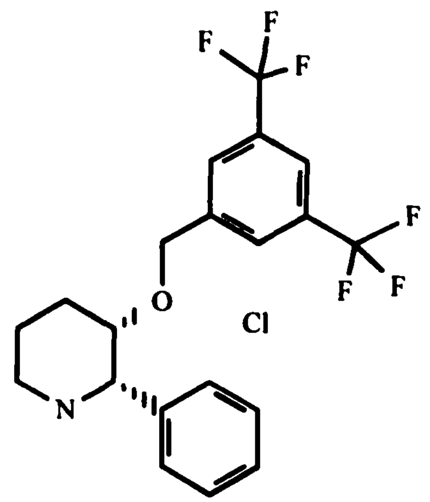

FIG.19 Molecular structure of non peptide antagonist L733,060

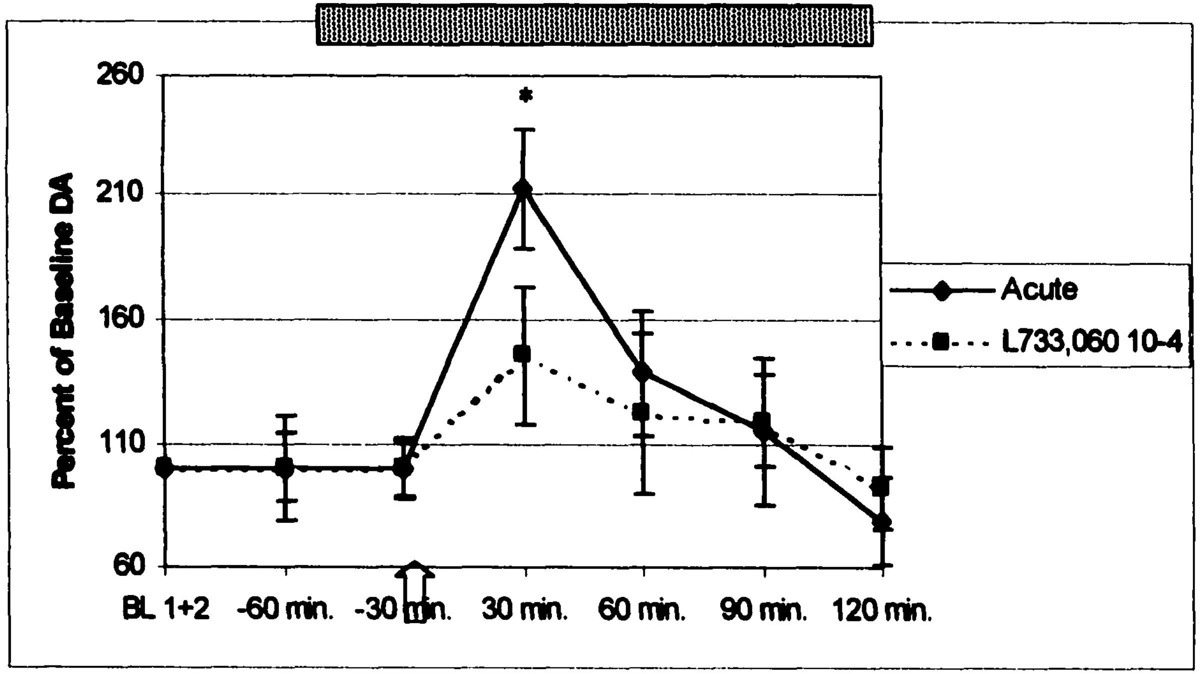

FIG.20 Effect of the NK-1 non-peptide receptor antagonist **L733,060** on the cocaine evoked release of DA from the striatum of intact awake and freely moving rats. A 10^{-4}M **L733,060** aCSF solution was perfused intracranially through the microdialysis probe for 180 minutes (as indicated by the rectangle). Arrow indicates administration of systemic cocaine. Compound significantly reduced cocaine evoked dopamine release. Arrow indicates administration of systemic cocaine.Dopamine levels are expressed as percent of the baseline. *P.05 compared to baseline by two factor ANOVA with repeated measures and Fishers post-hoc analysis.

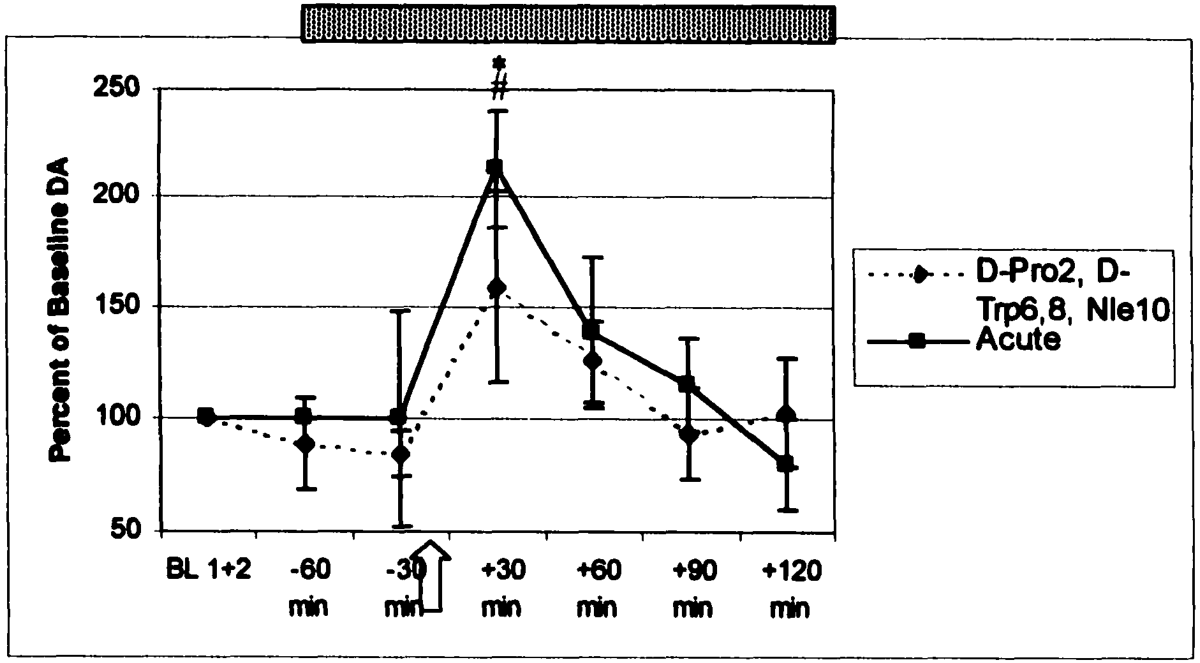

FIG.21 Effect of the NK-3 peptide receptor antagonist **D-Pro^2, D-Trp^{6,8}, Nle^{10}** on the cocaine evoked release of DA from the striatum of intact awake and freely moving rats. **D-Pro^2, D-Trp^{6,8}, Nle^{10}** (10^{-3} M) was perfused locally through the microdialysis probe for 60 minutes prior to and 2 hours post cocaine injection (as indicated by the rectangle). Arrow indicates time of systemic cocaine administration. Compound had no significant effect on cocaine evoked dopamine release. Dopamine levels are expressed as percent of baseline dopamine release. *P.05 compared to baseline by two factor ANOVA with repeated measures and Fishers post-hoc analysis.

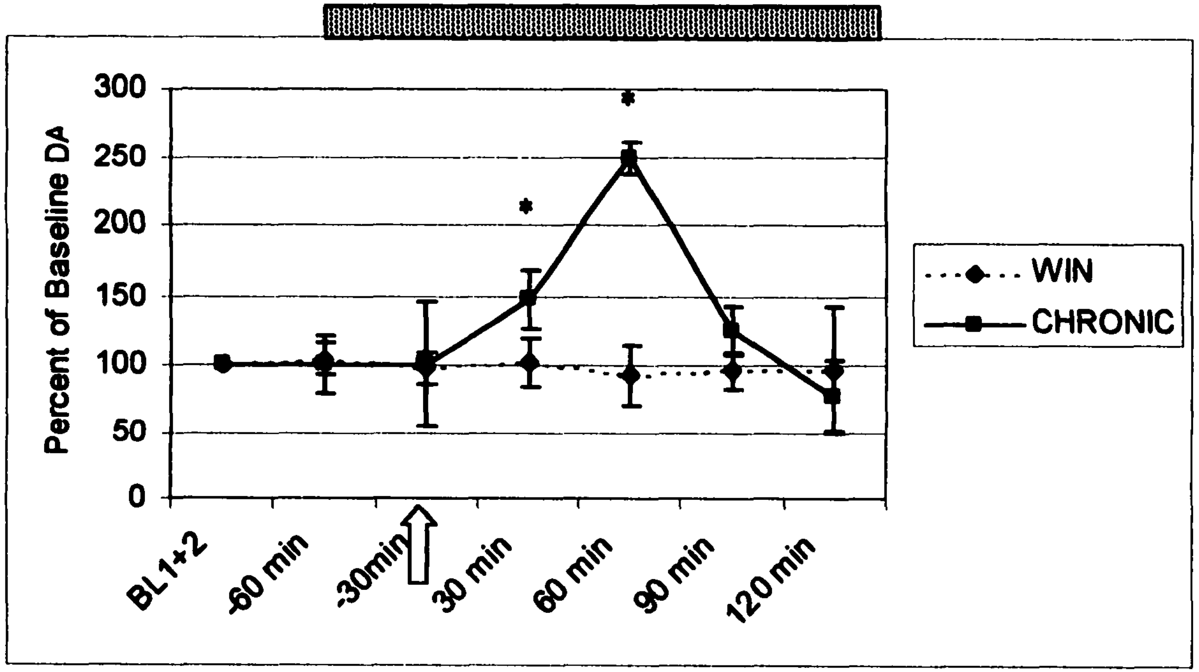

FIG.22 After seven days of treatment with systemic cocaine, the effects of the NK-1 non-peptide receptor antagonist **WIN 51,708** on the cocaine evoked release of DA from the striatum of intact, awake and freely moving rats. A 10^{-4}M aCSF solution was perfused intracranially through the microdialysis probe for 180 minutes (as indicated by the rectangular box) Compound brought cocaine evoked dopamine release down to basal levels. Arrow indicates administration of systemic cocaine. Dopamine levels are expressed as percent of baseline samples. *P.05 compared to baseline by a two factor ANOVA with repeated measures and Fishers post-hoc analysis.

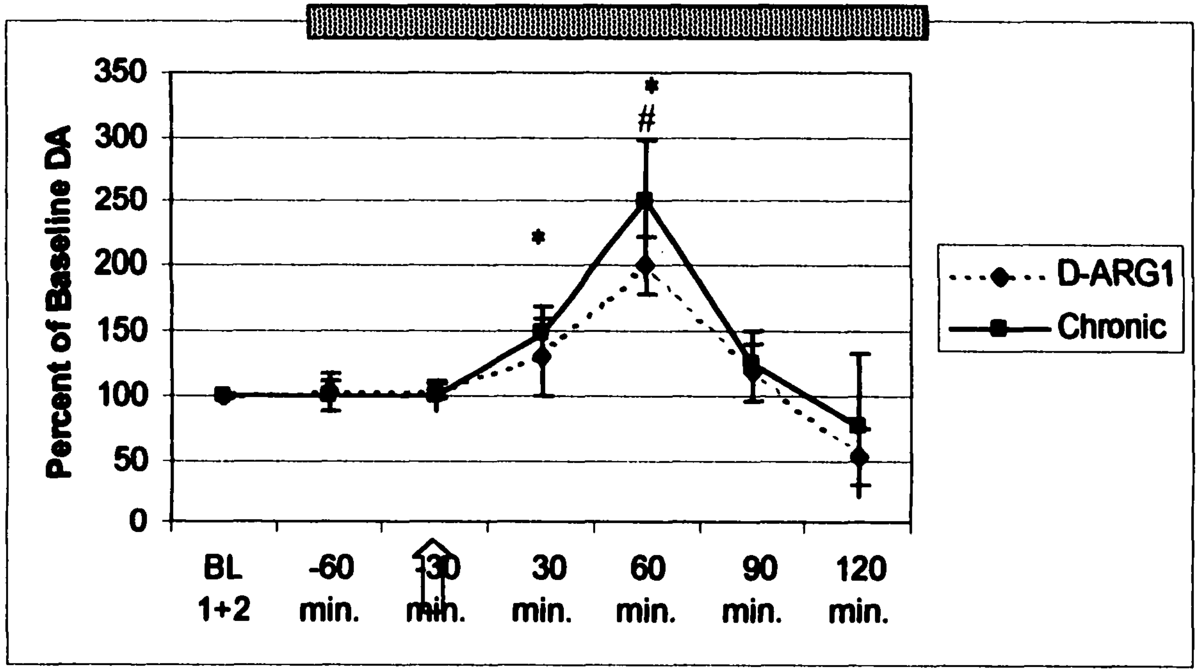

FIG.23 After seven days of treatment with systemic cocaine, the effects of the NK-1 peptide receptor antagonist **Arg^1, D-Pro^2, D-Trp^{7,9} Leu^{11}** on the cocaine evoked release of DA from the striatum of intact, awake and freely moving rats. A 10^{-4}M **Arg^1, D-Pro^2, D-Trp^{7,9} Leu^{11}** aCSF solution was perfused intracranially through the microdialysis probe for 180 minutes (as indicated by the rectangular box). Peptide antagonist had no significant effect on cocaine evoked dopamine release. Arrow indicates administration of systemic cocaine.Dopamine levels are expressed as percent of baseline samples collected. *P.05 compared to baseline by a two factor ANOVA with repeated measures and Fishers post-hoc analysis.

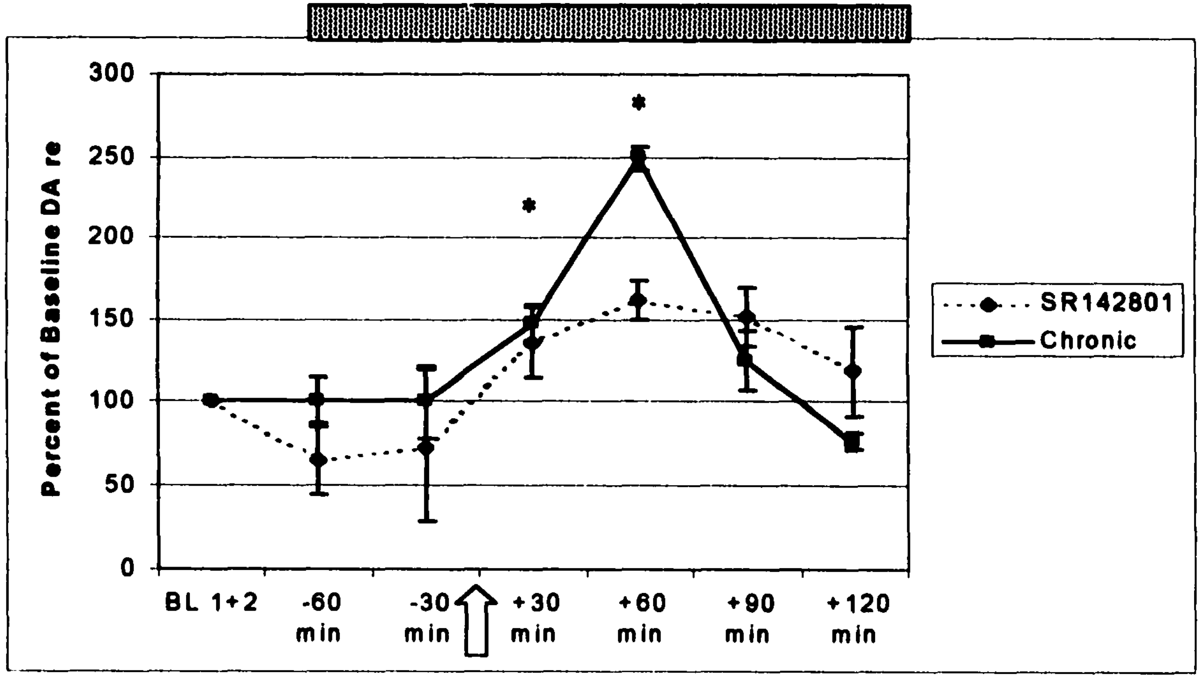

FIG.24 After seven days of treatment with systemic cocaine, the effects of the NK-1 peptide receptor antagonist **SR142801** on the cocaine evoked release of DA from the striatum of intact, awake and freely moving rats. A 10^{-4}M aCSF solution was perfused intracranially through the microdialysis probe for 180 minutes (as indicated by the rectangular box) Although there was a non-significant reduction in dopamine efflux, the compound had no significant effect on cocaine evoked dopamine release. Arrow indicates time of systemic cocaine administration. Dopamine levels are expressed as percent of the average of the first three baseline samples collected. *P.05 compared to baseline by a two factor ANOVA with repeated measures and Fishers post-hoc analysis.

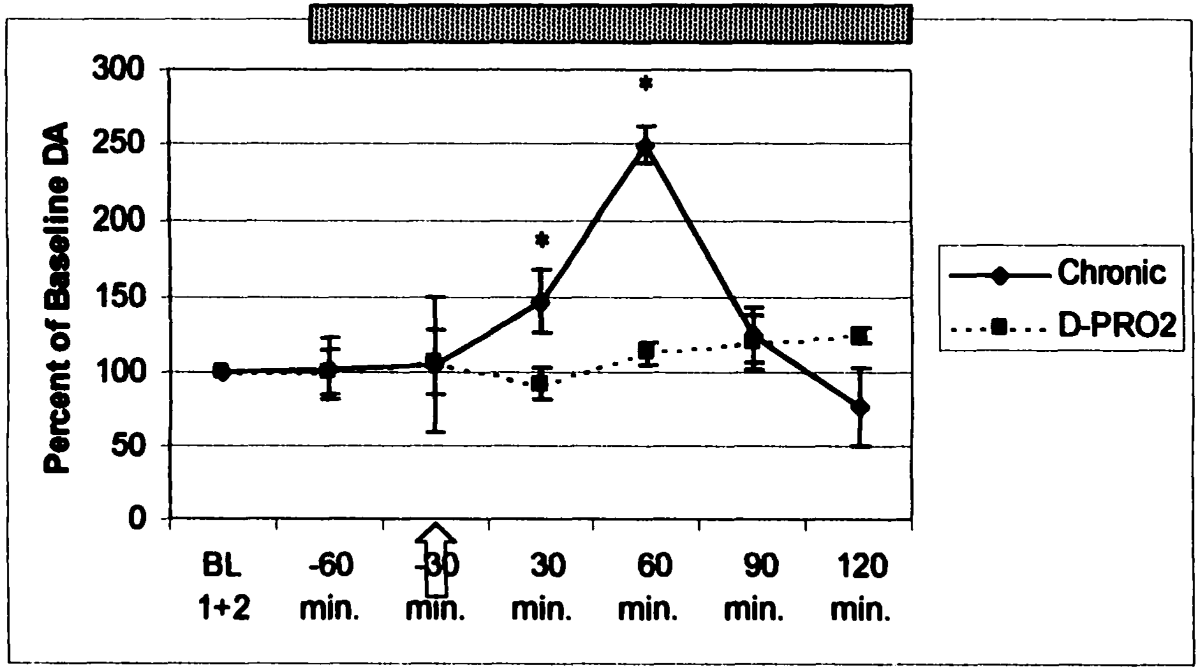

FIG.25 After seven days of treatment with systemic cocaine, the effects of the NK-1 peptide receptor antagonist **D-Pro^2, D-Trp^{6,8}, Nle^{10}** the cocaine evoked release of DA from the striatum of intact, awake and freely moving rats. A 10^{-3}M aCSF solution was perfused intracranially through the microdialysis probe for 180 minutes (as indicated by the rectangle). Administration compound reduced cocaine evoked dopamine release to basal levels. Arrow indicates time of systemic cocaine administration. Dopamine levels are expressed as percent of baseline dopamine release. *P.05 compared to baseline by a two factor ANOVA with repeated measures and Fishers post-hoc analysis.

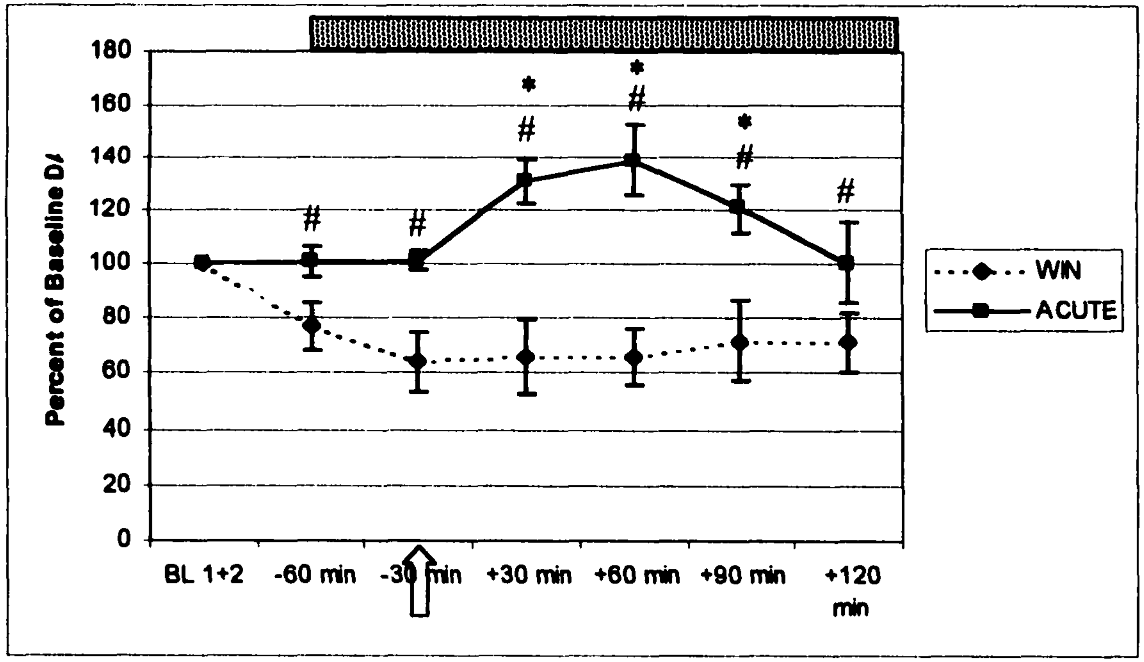

FIG.26 Effect of the NK-1 non-peptide receptor antagonist **WIN 51,708** on the cocaine evoked release of DA from the nucleus accumbens of intact awake and freely moving rats. A 10^{-4}M aCSF solution was perfused intracranially through the microdialysis probe for 180 minutes (as indicated by the box) Arrow indicates administration of systemic cocaine. Arrow indicates time of systemic cocaine administration Compound significantly reduced cocaine evoked dopamine release to below basal levels. Dopamine levels are expressed as percent of baseline dopamine release. *,# $P<.05$ compared to baseline by a two factor ANOVA with repeated measures and Fishers post-hoc analysis in animals treated with cocaine alone(*) and 10^{-4}WIN51,708(#).

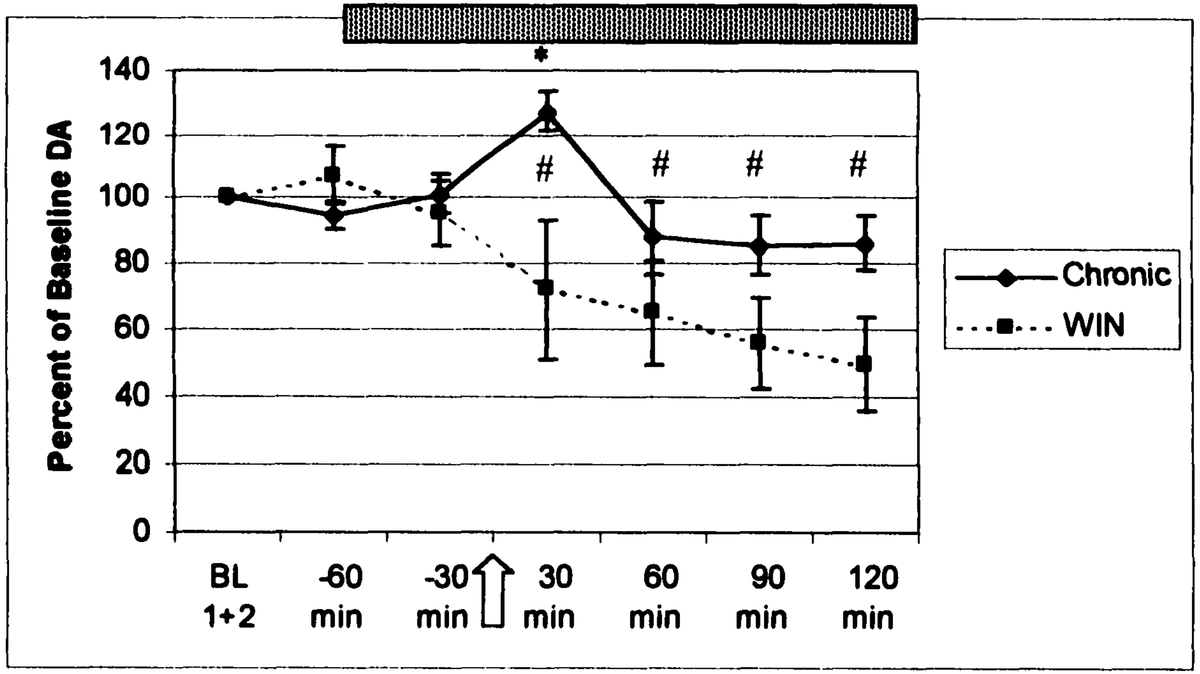

FIG.27 After seven days of treatment with systemic cocaine, the effects of the NK-1 peptide receptor antagonist **WIN 51,708** the cocaine evoked release of dopamine from nucleus accumbens of intact, awake and freely moving rats. A 10^{-4}M aCSF solution was perfused intracranially through the microdialysis probe for 180 minutes (as indicated by the rectangle). Compound significantly reduced cocaine evoked dopamine release to below basal levels. Arrow indicates time of systemic cocaine administration. Dopamine levels are expressed as percent of baseline dopamine release. *,# P<.05 compared to baseline by a two factor ANOVA with repeated measures and Fishers post-hoc analysis in animals treated with cocaine alone(*) and 10^{-4}WIN51,708(#).

NEOSTIGMINE BROMIDE

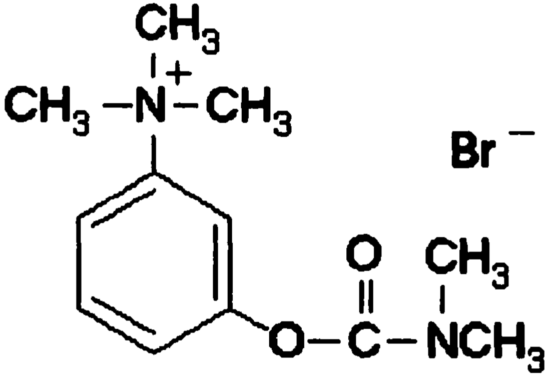

FIG.28 Molecular structure of acetylcholinesterase inhibitor neostigmine.

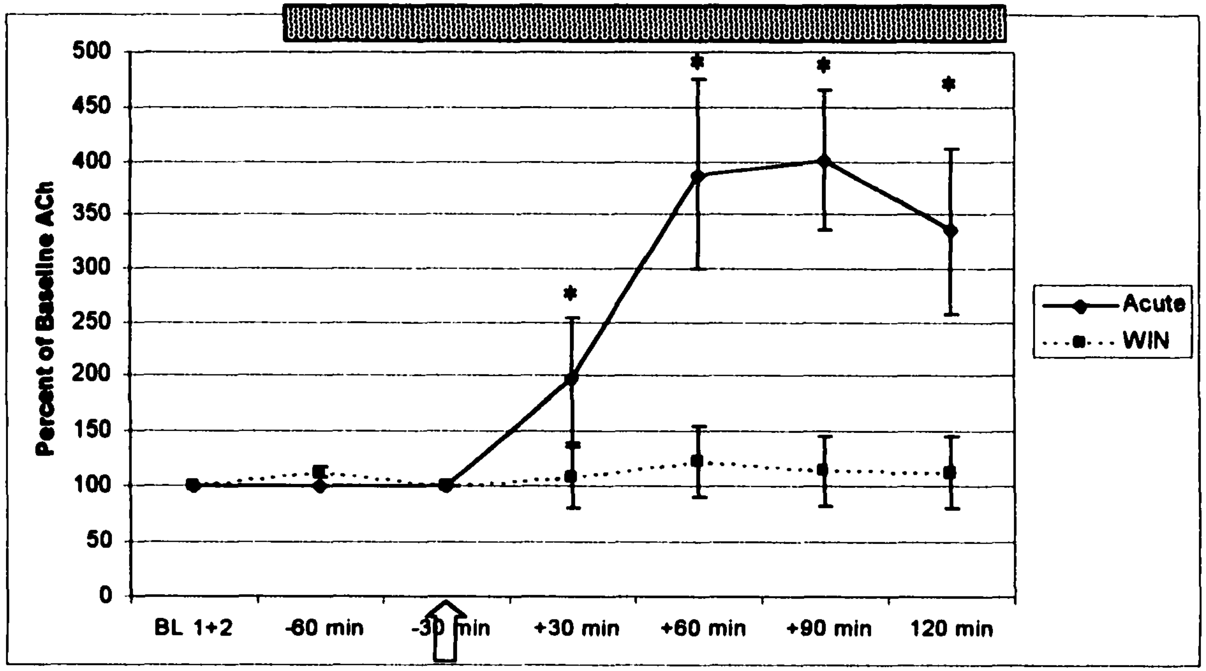

FIG.29 After single treatment with systemic cocaine, the effects of the NK-1 peptide receptor antagonist **WIN 51,708** on cocaine evoked release of Acetylcholine from striatum of intact, awake and freely moving rats. A 10^{-4}M **WIN 51,708** aCSF solution was perfused intracranially through the microdialysis probe for 180 minutes (as indicated by the rectangular box). Administration of compound kept acetylcholine release down to basal levels. Arrow indicates time of systemic cocaine administration. ACh levels are expressed as percent of the baseline average. *P<.05 compared to baseline by a two factor ANOVA with repeated measures and Fishers post-hoc analysis

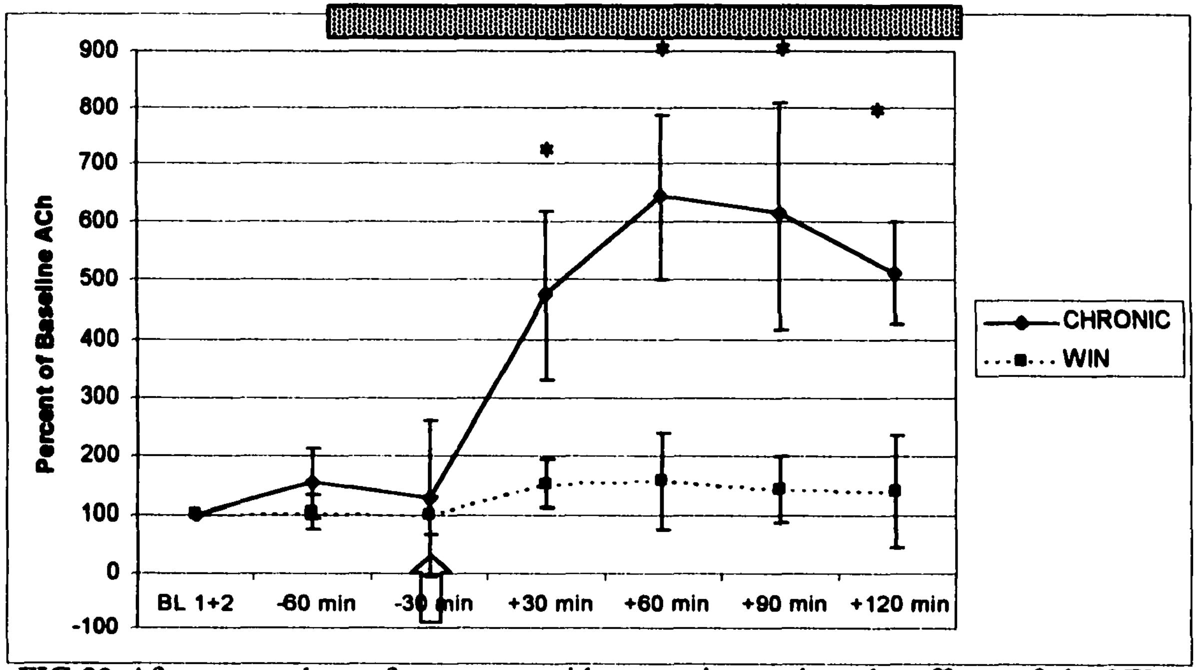

FIG.30 After seven days of treatment with systemic cocaine, the effects of the NK-1 peptide receptor antagonist **WIN 51,708** the cocaine evoked release of Acetlycholine from striatum of intact, awake and freely moving rats. A 10^{-4}M aCSF solution was perfused intracranially through the microdialysis probe for 180 minutes (as indicated by the rectangular box). Administration of compound kept acetylcholine release marginally above basal levels, but not significantly. Arrow indicates time of systemic cocaine administration. ACh levels are expressed as percent of baseline average. *P<.05 compared to baseline by a two factor ANOVA with repeated measures and Fishers post-hoc analysis

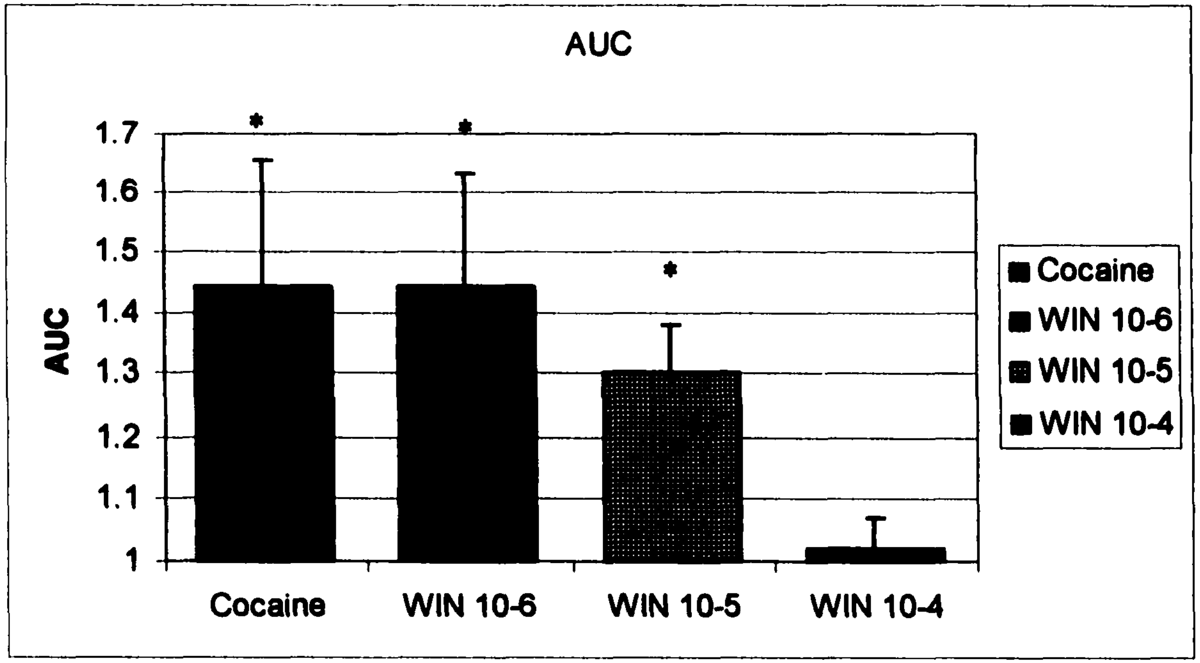

FIG.31 Area Under the Curve. This shows the total release of dopamine over a 4 hour period in all treatment groups. Administration of WIN 51,708 caused a dose dependant decrease in cocaine evoked total dopamine release over a 4 hour time span. Highest concentration of WIN compound administration brought total DA release to basal levels. *P<.05 compared to baseline by a two factor ANOVA and Fishers post-hoc analysis.

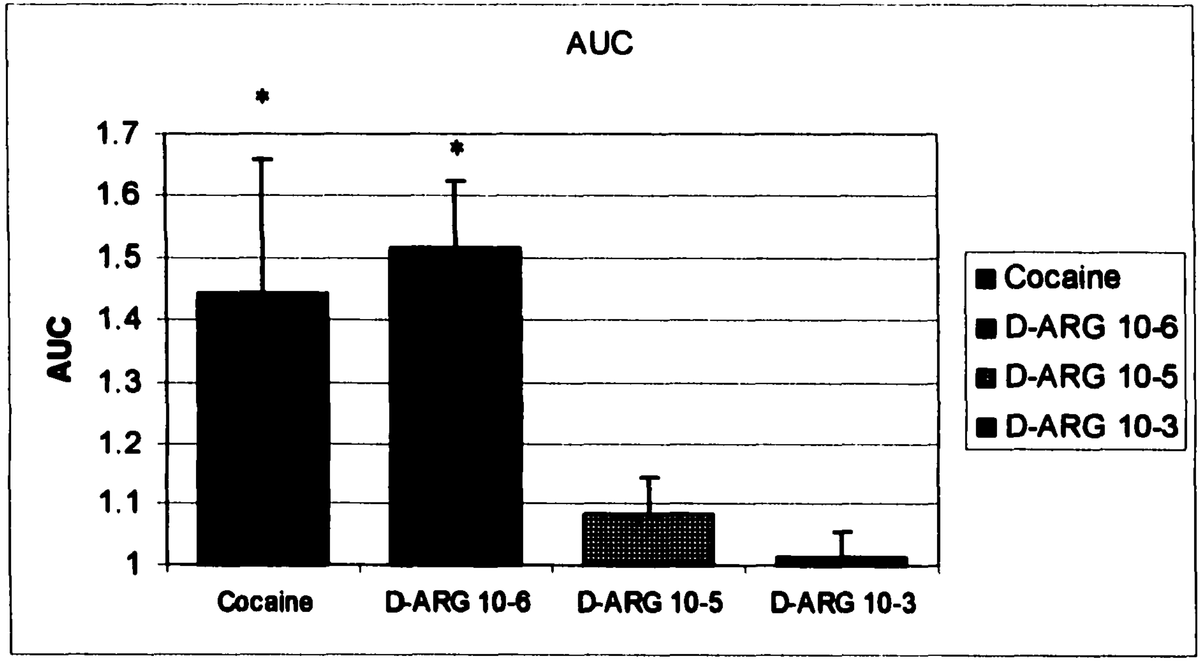

FIG.32 Area Under the Curve. This shows the total release of dopamine over a 4 hour period in all treatment groups. Administration of **Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}** caused a dose dependant decrease in cocaine evoked total dopamine release over a 4 hour time span. Highest concentration of **Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}** brought total DA release to basal levels. *P<.05 compared to baseline by a two factor ANOVA and Fishers post-hoc analysis.

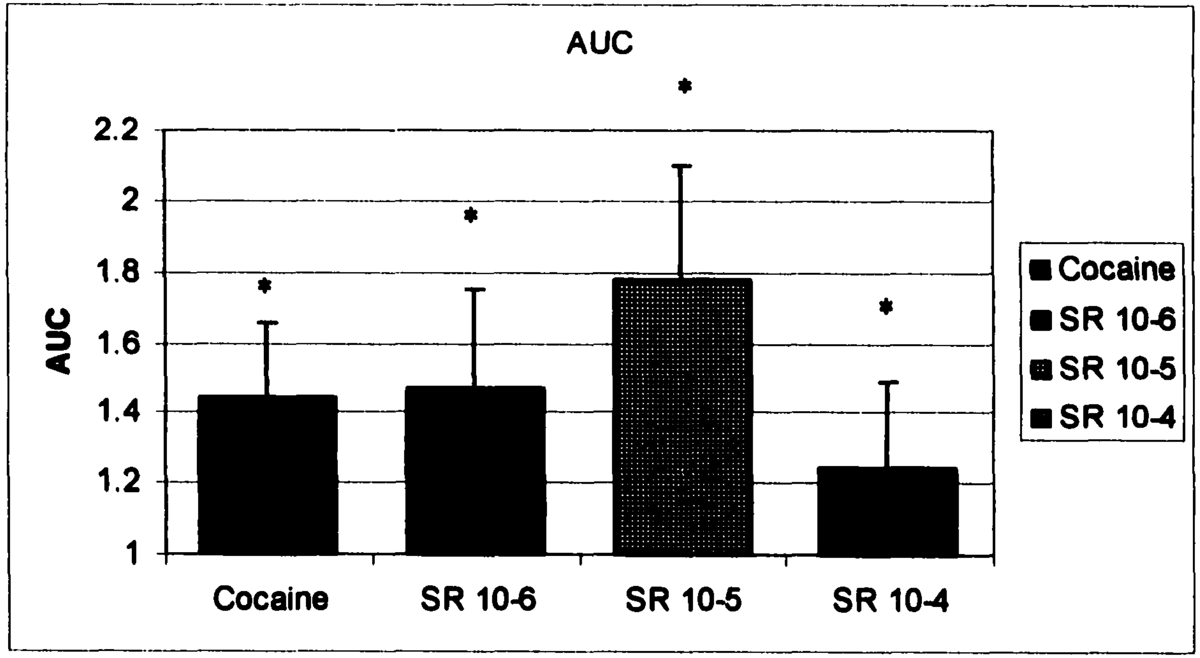

FIG.33 Area Under the Curve. This shows the total release of dopamine over a 4 hour period in all treatment groups. Administration of **SR142801** had no effect on evoked total dopamine release over a 4 hour time span. The 10-5M concentration of **SR142801** actually potentiated total DA release with respect to basal levels. *P<.05 compared to baseline by a two factor ANOVA and Fishers post-hoc analysis.

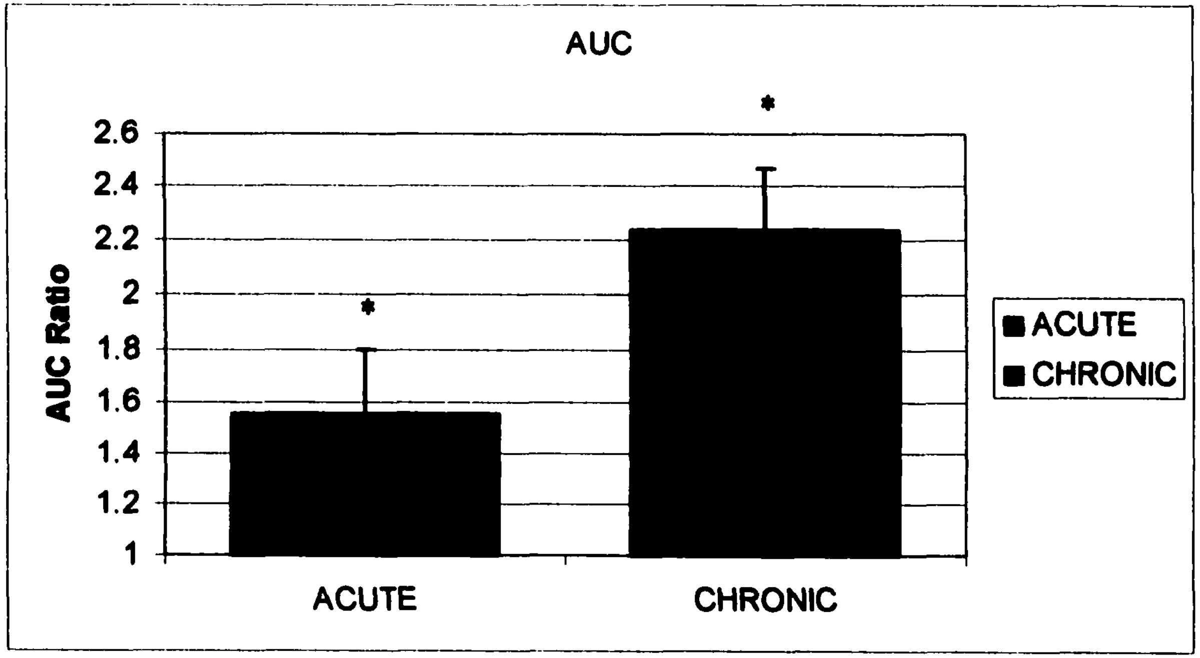

FIG.34 Total DA release comparison in the rat neostriatum. The data here are the mean area under the curve for the 120 minutes following systemic acute and chronic cocaine administration IP (10mg.kg body weight). *$P<0.05$ compared to baseline by 2-way ANOVA.

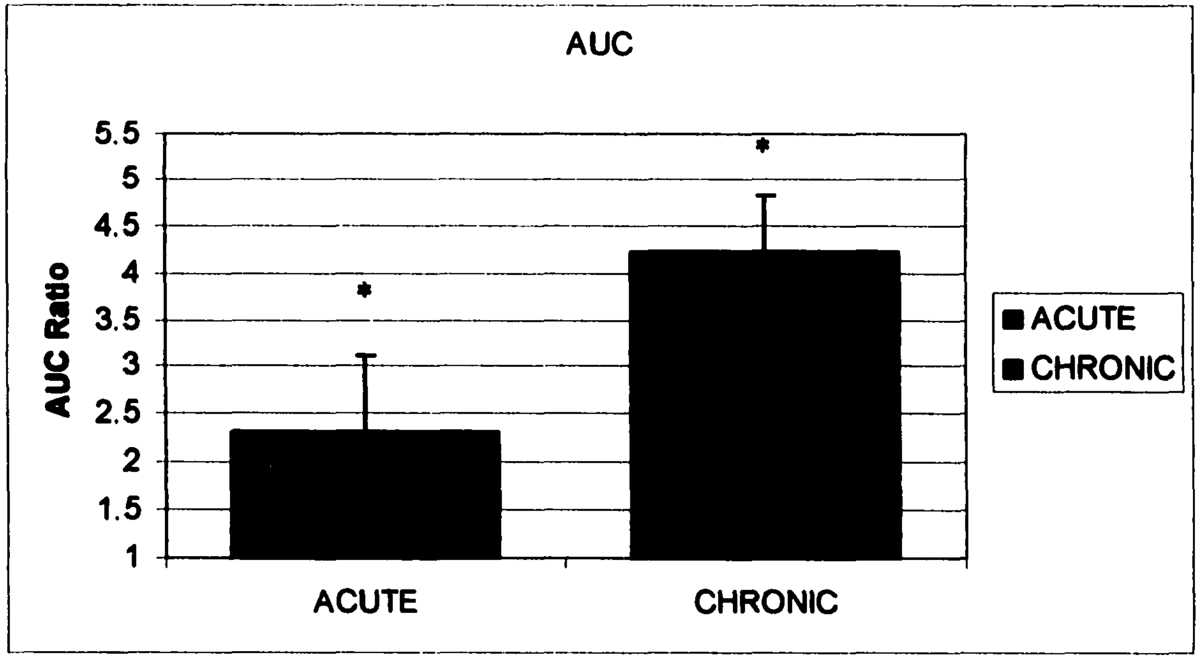

FIG.35 Total ACh release comparison in the rat neostriatum. The data here are the mean area under the curve for the 120 minutes following systemic acute and chronic cocaine administration IP (10mg.kg body weight). *$P<0.05$ compared to baseline by 2-way ANOVA.

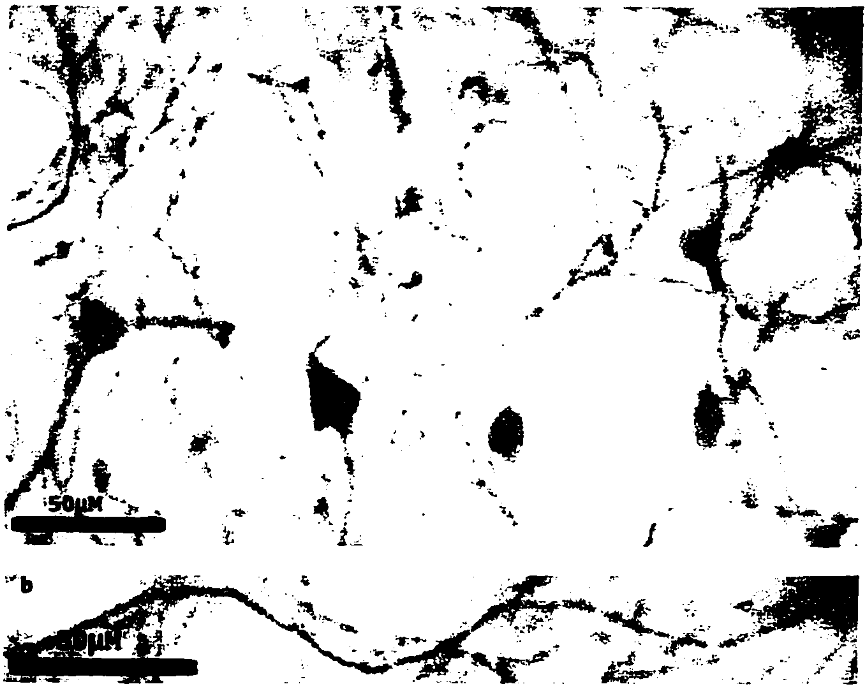

FIG.36 a)digitized light micrograph of striatum showing NK-1 receptor immunopositive neurons. All cells labeled are large aspiny neurons. Neurons

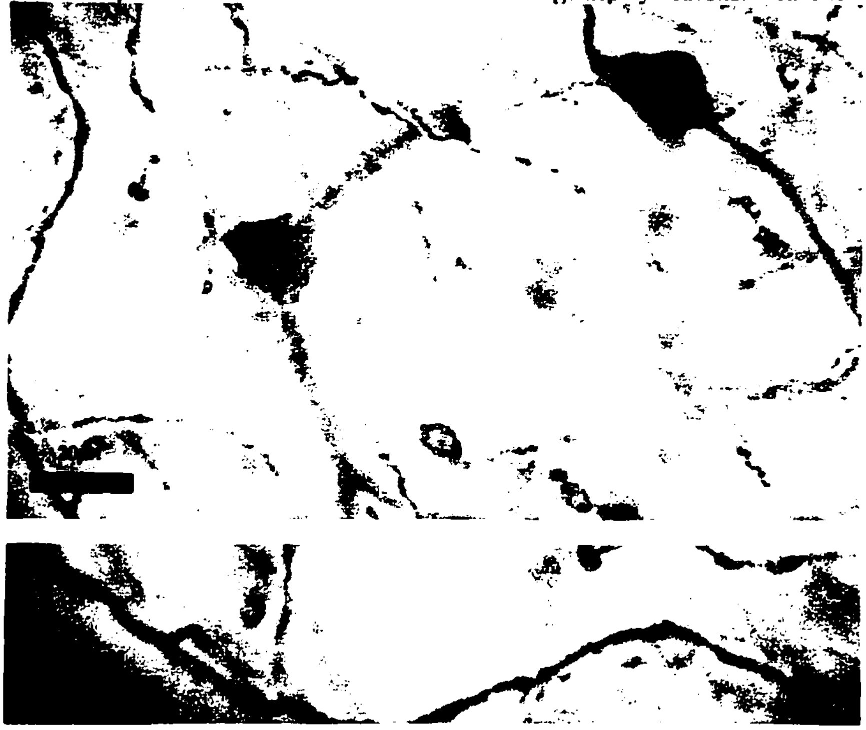

FIG.37a,b)Close-up digitized light micrograph of rat striatum showing NK-1 immunoreactive neurons. All labeled are large aspiny neurons. Neurons were labeled with DAB. All immunoreactivity is on the membrane surface.

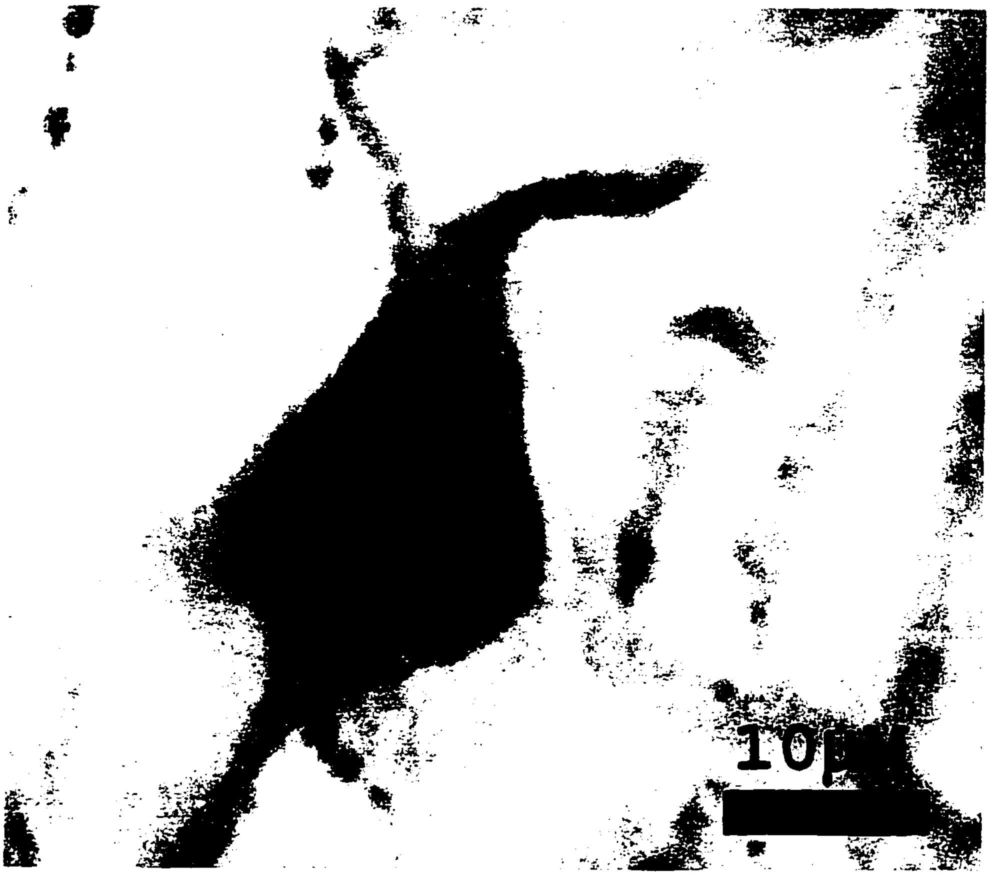

FIG.38 Closeup digital microgragh of NK-1 postivie interneuron with apical dendrites in the rat striatum.

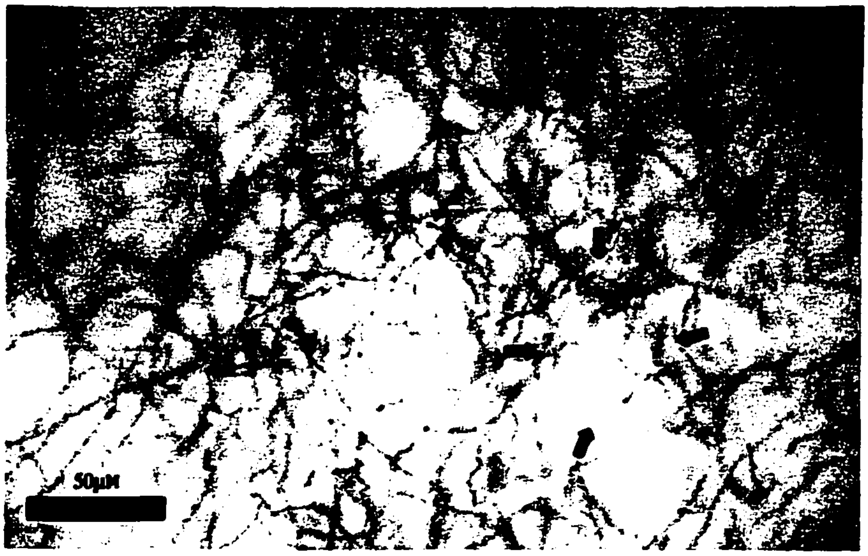

FIG.39 Digital photomicrogragh of DMCpu of rat perfused with 10^{-3}M substance P. Pattern of NK-1immunoreactivity is consistent with receptor internalization. Black arrows indicate border of striosome.

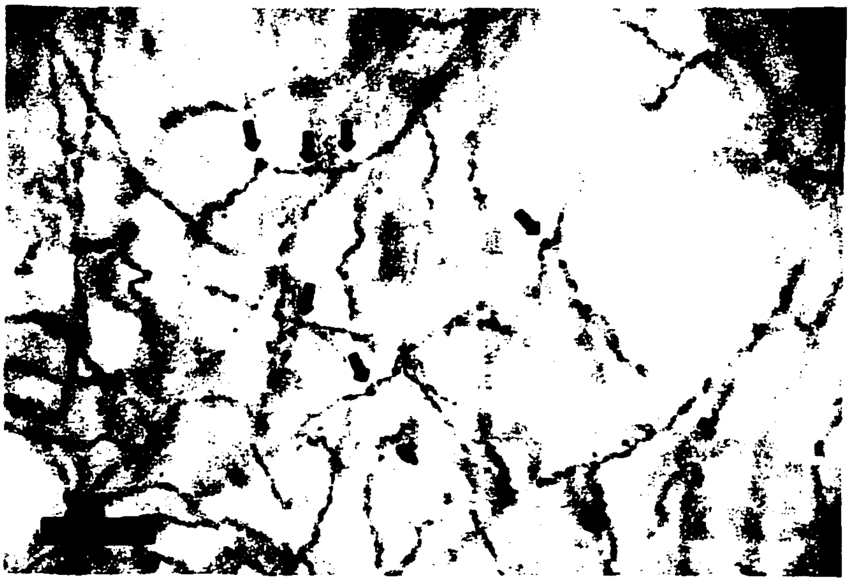

FIG.40 Close-up of digital photomicrogragh of striatum of rat perfused with 10^{-3}M substance P. Pattern of NK-1receptor immunoreactivity is consistent with receptor internalization. Black arrows indicate endosomal bulges on dendrites.

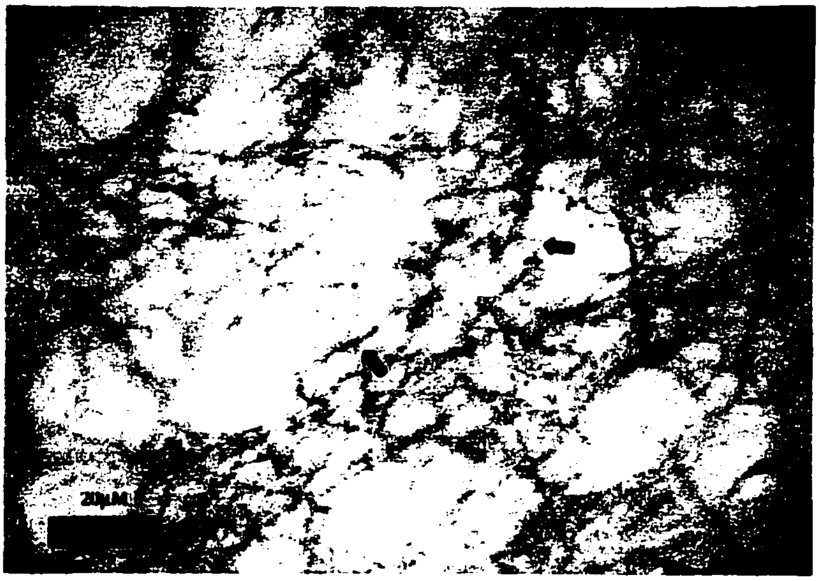

FIG.41 Digital photomicrogragh of striatum of rat injected IP with 10mg/kg cocaine. Pattern of NK-1immunoreactivity is consistent with receptor internalization. Black arrows indicate dendrites with endosomal activity.

FIG.42 Close-up of Digital photomicrogragh of striatum of rat injected IP with 10mg/kg cocaine. Pattern of NK-1immunoreactivity is consistent with receptor internalization. Black arrows indicate border of striosome.

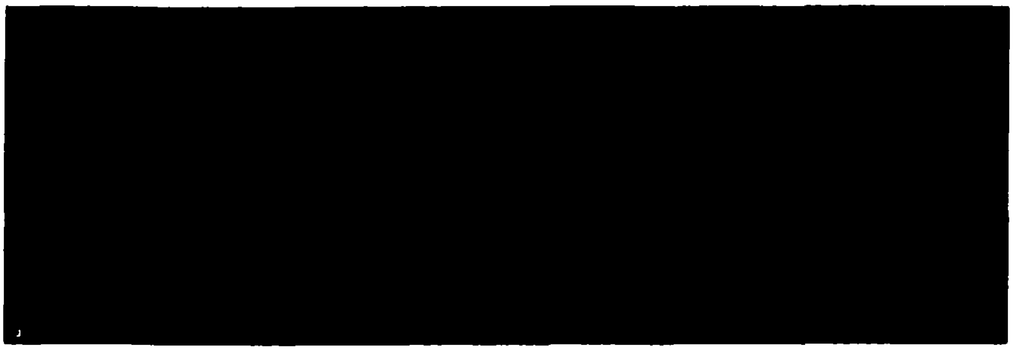

FIG.43 Digitized confocal image of immunofluorescent double labeling showing distribution of choline acetyltransferase and NK 1 receptor immunoreactivity. 60 Micron sections were taken from the medial caudate putamen. Read channel shows distribution of neurokinin 1 receptors green channel shows distribution of ChAt. In the overlay, yellow areas show co-distribution.

FIG.44 Digitized confocal image of effects of substance P infusion. Substance P was administered intracranially for fifteen minutes rats were then sacrificed and tissues removed. Immunofluorescent double labeling showing distribution of choline acetyltransferase and NK 1 receptor immunoreactivity. 60 Micron sections were taken from the medial caudate putamen. Red channel shows distribution of neurokinin 1 receptors, green channel shows distribution of ChAt. In the overlay, yellow areas show co-distribution. Note in the red channel the different distribution of immunoreactivity.

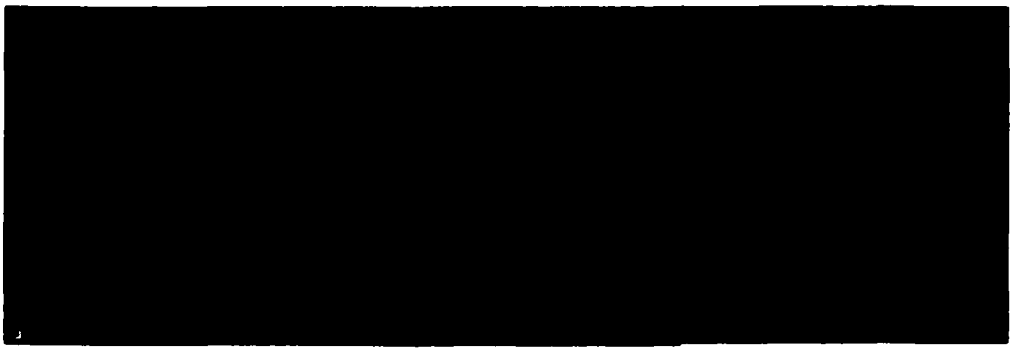

FIG.45 Digitized confocal images of the effects of systemic cocaine administration. cocaine was administered systemically at a dose of 10mg. Kg IP. Rats were then sacrificed and tissues removed. Immunofluorescent double labeling showing distribution of choline acetyltransferase and NK 1 receptor immunoreactivity. 60 Micron sections were taken from the medial caudate putamen. Red channel shows distribution of neurokinin 1 receptors, green channel shows distribution of ChAt. In the overlay, yellow areas show co-distribution. Note in the red channel the different distribution of immunoreactivity. This pattern suggests substance P release.

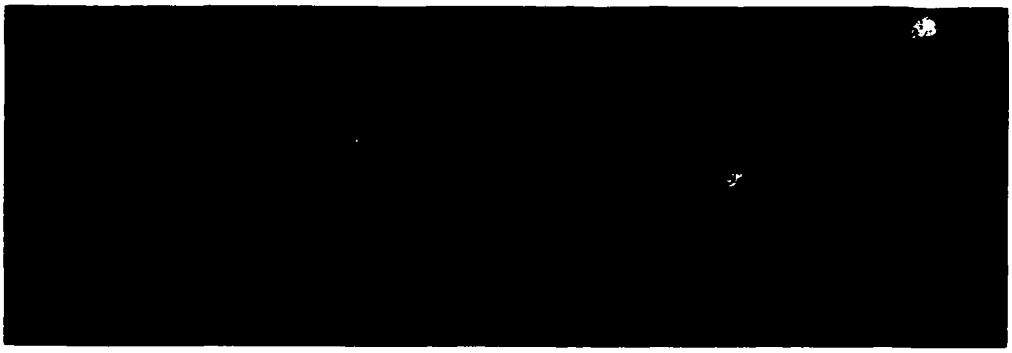

FIG.46 Digitized confocal images of the effects of systemic cocaine administration. Cocaine was administered IP (10mg/kg). fifteen minutes Prior to systemic administration of cocaine the substance P antagonist WIN 57,108 was administered intracranially. Rats were then sacrificed and tissue removed. Immunofluorescent double labeling showing distribution of choline acetyltransferase and NK-1 receptor immunoreactivity. 40 Micron sections were taken from the medial caudate putamen. Red channel shows distribution of neurokinin 1 receptors, green channel shows distribution of ChAt. In the overlay, yellow areas show co-distribution. Note in the red channel the different distribution of immunoreactivity. This pattern suggests that the NK 1 receptor antagonist blocked receptor internalization.

BIBLIOGRAPHY

Acquas E, Di Chiara G (1999) Dopamine D(1) receptor-mediated control of striatal acetylcholine release by endogenous dopamine. Eur J Pharmacol 383:121-127.

Acquas E, Wilson C, Fibiger HC (1997) Nonstriatal dopamine D1 receptors regulate striatal acetylcholine release in vivo. J Pharmacol Exp Ther 281:360-368.

Alberch J, Arenas E, Perez-Navarro E, Marsal J (1993) Control of tachykinin-evoked acetylcholine release from rat striatal slices by dopaminergic neurons. Naunyn Schmiedebergs Arch Pharmacol 348:445-449.

Anderson JJ, Chase TN, Engber TM (1993) Substance P increases release of acetylcholine in the dorsal striatum of freely moving rats. Brain Res 623:189-194.

Anderson JJ, Kuo S, Chase TN, Engber TM (1994) Dopamine D1 receptor-stimulated release of acetylcholine in rat striatum is mediated indirectly by activation of striatal neurokinin1 receptors. J Pharmacol Exp Ther 269:1144-1151.

Anderson KD, Reiner A (1991) Striatonigral projection neurons: a retrograde labeling study of the percentages that contain substance P or enkephalin in pigeons. J Comp Neurol 303:658-673.

Angulo JA, McEwen BS (1994) Molecular aspects of neuropeptide regulation and function in the corpus striatum and nucleus accumbens. Brain Res Brain Res Rev 19:1-28.

Arenas E, Alberch J, Perez-Navarro E, Solsona C, Marsal J (1991) Neurokinin receptors differentially mediate endogenous acetylcholine release evoked by tachykinins in the neostriatum. J Neurosci 11:2332-2338.

Aubert I, Ghorayeb I, Normand E, Bloch B (2000) Phenotypical characterization of the neurons expressing the D1 and D2 dopamine receptors in the monkey striatum. J Comp Neurol 418:22-32.

Bailey DN, Shaw RF (1989) Cocaine- and methamphetamine-related deaths in San Diego County (1987): homicides and accidental overdoses. J Forensic Sci 34:407-422.

Baruch P, Artaud F, Godeheu G, Barbeito L, Glowinski J, Cheramy A (1988) Substance P and neurokinin A regulate by different mechanisms dopamine release from dendrites and nerve terminals of the nigrostriatal dopaminergic neurons. Neuroscience 25:889-898.

Berridge KC, Robinson TE (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Brain Res Rev 28:309-369.

Blanchet F, Gauchy C, Perez S, Soubrie P, Glowinski J, Kemel ML (1998) Distinct modifications by neurokinin1 (SR140333) and neurokinin2 (SR48968) tachykinin receptor antagonists of the N-methyl-D-aspartate-evoked release of acetylcholine in striosomes and matrix of the rat striatum. Neuroscience 85:1025-1036.

Blanchet F, Gauchy C, Perez S, Soubrie P, Glowinski J, Kemel ML (2000) Control by GABA and tachykinins of the evoked release of acetylcholine in striatal compartments under different modalities of NMDA receptor stimulation. Brain Res 853:142-150.

Boix F, Pfister M, Huston JP, Schwarting RK (1994) Substance P decreases extracellular concentrations of acetylcholine in neostriatum and nucleus accumbens in vivo: possible relevance for the central processing of reward and aversion. Behav Brain Res 63:213-219.

Bolam JP, Ingham CA, Izzo PN, Levey AI, Rye DB, Smith AD, Wainer BH (1986) Substance P-containing terminals in synaptic contact with cholinergic neurons in the neostriatum and basal forebrain: a double immunocytochemical study in the rat. Brain Res 397:279-289.

Burrows KB, Meshul CK (1999) High-dose methamphetamine treatment alters presynaptic GABA and glutamate immunoreactivity. Neuroscience 90:833-850.

Calabresi P, Centonze D, Gubellini P, Pisani A, Bernardi G (2000a) Acetylcholine-mediated modulation of striatal function. Trends Neurosci 23:120-126.

Calabresi P, Centonze D, Gubellini P, Marfia GA, Pisani A, Sancesario G, Bernardi G (2000b) Synaptic transmission in the striatum: from plasticity to neurodegeneration. Prog Neurobiol 61:231-265.

Cantrell AR, Catterall WA (2001) Neuromodulation of Na+ channels: an unexpected form of cellular plasticity. Nat Rev Neurosci 2:397-407.

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

Centonze D, Gubellini P, Bernardi G, Calabresi P (1999a) Permissive role of interneurons in corticostriatal synaptic plasticity. Brain Res Brain Res Rev 31:1-5.

Centonze D, Calabresi P, Giacomini P, Bernardi G (1999b) Neurophysiology of Parkinson's disease: from basic research to clinical correlates. Clin Neurophysiol 110:2006-2013.

Chevalier G, Deniau JM (1990) Disinhibition as a basic process in the expression of striatal functions. Trends Neurosci 13:277-280.

Christie MJ, Summers RJ, Stephenson JA, Cook CJ, Beart PM (1987) Excitatory amino acid projections to the nucleus accumbens septi in the rat: a retrograde transport study utilizing D[3H]aspartate and [3H]GABA. Neuroscience 22:425-439.

Ciliax BJ, Nash N, Heilman C, Sunahara R, Hartney A, Tiberi M, Rye DB, Caron MG, Niznik HB, Levey AI (2000) Dopamine D(5) receptor immunolocalization in rat and monkey brain. Synapse 37:125-145.

Contant C, Umbriaco D, Garcia S, Watkins KC, Descarries L (1996) Ultrastructural characterization of the acetylcholine innervation in adult rat neostriatum. Neuroscience 71:937-947.

Dam TV, Martinelli B, Quirion R (1990) Autoradiographic distribution of brain neurokinin-1/substance P receptors using a highly selective ligand [3H]-[Sar9,Met(O2)11]-substance P. Brain Res 531:333-337.

Damsma G, Robertson GS, Tham CS, Fibiger HC (1991) Dopaminergic regulation of striatal acetylcholine release: importance of D1 and N-methyl-D-aspartate receptors. J Pharmacol Exp Ther 259:1064-1072.

De Araujo JE, Huston JP, Brandao ML (2001) Place aversion induced by microinjections of C-fragment of substance P into the dorsal periaqueductal gray of rats is mediated by tachykinin NK(1) receptors. Peptides 22:1447-1452.

de Belleroche JS, Bradford HF (1980) Presynaptic control of the synthesis and release of dopamine from striatal synaptosomes: a comparison between the effects of 5-hydroxytryptamine, acetylcholine, and glutamate. J Neurochem 35:1227-1234.

De Felice LJ, Adams SV (2001) Serotonin and norepinephrine transporters: possible relationship between oligomeric structure and channel modes of conduction. Mol Membr Biol 18:45-51.

Dearry A, Gingrich JA, Falardeau P, Fremeau RT, Jr., Bates MD, Caron MG (1990) Molecular cloning and expression of the gene for a human D1 dopamine receptor. Nature 347:72-76.

DeBoer P, Heeringa MJ, Abercrombie ED (1996) Spontaneous release of acetylcholine in striatum is preferentially regulated by inhibitory dopamine D2 receptors. Eur J Pharmacol 317:257-262.

Deniau JM, Chevalier G (1985) Disinhibition as a basic process in the expression of striatal functions. II. The striato-nigral influence on thalamocortical cells of the ventromedial thalamic nucleus. Brain Res 334:227-233.

DeVane CL (2001) Substance P: a new era, a new role. Pharmacotherapy 21:1061-1069.

Di Chiara G (1995) The role of dopamine in drug abuse viewed from the perspective of its role in motivation. Drug Alcohol Depend 38:95-137.

Elliott PJ, Mason GS, Stephens-Smith M, Hagan RM (1991) Behavioural and biochemical responses following activation of midbrain dopamine pathways by receptor selective neurokinin agonists. Neuropeptides 19:119-126.

Filip M, Thomas ML, Cunningham KA (2000) Dopamine D5 receptors in nucleus accumbens contribute to the detection of cocaine in rats. J Neurosci 20:RC98.

Galarraga E, Hernandez-Lopez S, Tapia D, Reyes A, Bargas J (1999) Action of substance P (neurokinin-1) receptor activation on rat neostriatal projection neurons. Synapse 33:26-35.

Gerfen CR (1991) Substance P (neurokinin-1) receptor mRNA is selectively expressed in cholinergic neurons in the striatum and basal forebrain. Brain Res 556:165-170.

Gerfen CR (1992) The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia. Annu Rev Neurosci 15:285-320.

Gerfen CR, Young WS, 3rd (1988) Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. Brain Res 460:161-167.

Gerfen CR, McGinty JF, Young WS, 3rd (1991) Dopamine differentially regulates dynorphin, substance P, and enkephalin expression in striatal neurons: in situ hybridization histochemical analysis. J Neurosci 11:1016-1031.

Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ, Jr., Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429-1432.

Gifford AN, Johnson KM (1992) Effect of chronic cocaine treatment on D2 receptors regulating the release of dopamine and acetylcholine in the nucleus accumbens and striatum. Pharmacol Biochem Behav 41:841-846.

Graybiel AM (1995) Building action repertoires: memory and learning functions of the basal ganglia. Curr Opin Neurobiol 5:733-741.

Gygi SP, Gibb JW, Johnson M, Hanson GR (1993) Blockade of tachykinin NK1 receptors by CP-96345 enhances dopamine release and the striatal dopamine effects of methamphetamine in rats. Eur J Pharmacol 250:177-180.

Hanania T, Johnson KM (1999) Regulation of NMDA-stimulated [14C]GABA and [3H]acetylcholine release by striatal glutamate and dopamine receptors. Brain Res 844:106-117.

Harrison S, Geppetti P (2001) Substance p. Int J Biochem Cell Biol 33:555-576.

Harsing LG, Jr., Zigmond MJ (1998) Postsynaptic integration of cholinergic and dopaminergic signals on medium-sized GABAergic projection neurons in the neostriatum. Brain Res Bull 45:607-613.

Helke CJ, Krause JE, Mantyh PW, Couture R, Bannon MJ (1990) Diversity in mammalian tachykinin peptidergic neurons: multiple peptides, receptors, and regulatory mechanisms. Faseb J 4:1606-1615.

Hooper NM, Turner AJ (1985) Neurokinin B is hydrolysed by synaptic membranes and by endopeptidase-24.11 (enkephalinase) but not by angiotensin converting enzyme. FEBS Lett 190:133-136.

Hyman SE (1996) Addiction to cocaine and amphetamine. Neuron 16:901-904.

Inciardi JA, Surratt HL (2001) Drug use, street crime, and sex-trading among cocaine-dependent women: implications for public health and criminal justice policy. J Psychoactive Drugs 33:379-389.

Izzo PN, Bolam JP (1988) Cholinergic synaptic input to different parts of spiny striatonigral neurons in the rat. J Comp Neurol 269:219-234.

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

Kalivas PW (1985) Interactions between neuropeptides and dopamine neurons in the ventromedial mesencephalon. Neurosci Biobehav Rev 9:573-587.

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

Kalivas PW, Duffy P (1993b) Time course of extracellular dopamine and behavioral sensitization to cocaine. II. Dopamine perikarya. J Neurosci 13:276-284.

Kaneko S, Hikida T, Watanabe D, Ichinose H, Nagatsu T, Kreitman RJ, Pastan I, Nakanishi S (2000) Synaptic integration mediated by striatal cholinergic interneurons in basal ganglia function. Science 289:633-637.

Kebabian JW, Petzold GL, Greengard P (1972) Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain, and its similarity to the "dopamine receptor". Proc Natl Acad Sci U S A 69:2145-2149.

Khan S, Whelpton R, Michael-Titus AT (2000) Substance P modulation of striatal dopamine outflow is determined by M(1) and M(2) muscarinic receptors in male wistar rats. Neurosci Lett 293:179-182.

Koob GF (1996) Drug addiction: the yin and yang of hedonic homeostasis. Neuron 16:893-896.

Koob GF, Le HT, Creese I (1987) The D1 dopamine receptor antagonist SCH 23390 increases cocaine self-administration in the rat. Neurosci Lett 79:315-320.

Kraft M, Noailles P, Angulo JA (2001) Substance P modulates cocaine-evoked dopamine overflow in the striatum of the rat brain. Ann N Y Acad Sci 937:121-131.

Lason W (2001) Neurochemical and pharmacological aspects of cocaine-induced seizures. Pol J Pharmacol 53:57-60.

Le Moine C, Bloch B (1995) D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. J Comp Neurol 355:418-426.

Lee JM, McLean S, Maggio JE, Zamir N, Roth RH, Eskay RL, Bannon MJ (1986) The localization and characterization of substance P and substance K in striatonigral neurons. Brain Res 371:152-154.

Letters HMH (1999) Cocaine abuse and addiction - part II. In: Harv Ment Health Lett, pp 1-4.

Lieb K, Fiebich BL, Berger M (2000) [Substance P receptor antagonists--a new antidepressive and anxiolytic mechanism?]. Nervenarzt 71:758-761.

Lindefors N (1992) Amphetamine and haloperidol modulate preprotachykinin A mRNA expression in rat nucleus accumbens and caudate-putamen. Brain Res Mol Brain Res 13:151-154.

Mantyh PW, Gates T, Mantyh CR, Maggio JE (1989) Autoradiographic localization and characterization of tachykinin receptor binding sites in the rat brain and peripheral tissues. J Neurosci 9:258-279.

Mantyh PW, Rogers SD, Ghilardi JR, Maggio JE, Mantyh CR, Vigna SR (1996) Differential expression of two isoforms of the neurokinin-1 (substance P) receptor in vivo. Brain Res 719:8-13.

Marco N, Thirion A, Mons G, Bougault I, Le Fur G, Soubrie P, Steinberg R (1998) Activation of dopaminergic and cholinergic neurotransmission by tachykinin NK3 receptor stimulation: an in vivo microdialysis approach in guinea pig. Neuropeptides 32:481-488.

Melichar JK, Daglish MR, Nutt DJ (2001) Addiction and withdrawal--current views. Curr Opin Pharmacol 1:84-90.

Michael-Titus AT, Blackburn D, Connolly Y, Priestley JV, Whelpton R (1999) N- and C-terminal substance P fragments: differential effects on striatal [3H]substance P binding and NK1 receptor internalization. Neuroreport 10:2209-2213.

Molitor F, Truax SR, Ruiz JD, Sun RK (1998) Association of methamphetamine use during sex with risky sexual behaviors and HIV infection among non-injection drug users. West J Med 168:93-97.

Nikolaus S, Huston JP, Korber B, Thiel C, Schwarting RK (1997) Pretreatment with neurokinin substance P but not with cholecystokinin-8S can alleviate functional deficits of partial nigrostriatal 6-hydroxydopamine lesion. Peptides 18:1161-1168.

Onn SP, Grace AA (1994) Dye coupling between rat striatal neurons recorded in vivo: compartmental organization and modulation by dopamine. J Neurophysiol 71:1917-1934.

Paulson PE, Robinson TE (1991) Sensitization to systemic amphetamine produces an enhanced locomotor response to a subsequent intra-accumbens amphetamine challenge in rats. Psychopharmacology (Berl) 104:140-141.

Pierce RC, Duffy P, Kalivas PW (1995) Sensitization to cocaine and dopamine autoreceptor subsensitivity in the nucleus accumbens. Synapse 20:33-36.

Pisani A, Bonsi P, Picconi B, Tolu M, Giacomini P, Scarnati E (2001) Role of tonically-active neurons in the control of striatal function: cellular mechanisms and behavioral correlates. Prog Neuropsychopharmacol Biol Psychiatry 25:211-230.

Pollack AE, Wooten GF (1992) D2 dopaminergic regulation of striatal preproenkephalin mRNA levels is mediated at least in part through cholinergic interneurons. Brain Res Mol Brain Res 13:35-41.

Post RM (1980) Intermittent versus continuous stimulation: effect of time interval on the development of sensitization or tolerance. Life Sci 26:1275-1282.

Raevskii KS, Sotnikova TD, Gainetdinov RR (1996) [The brain dopaminergic systems: receptor heterogeneity, functional role and pharmacological regulation]. Usp Fiziol Nauk 27:3-29.

Ricaurte GA, Guillery RW, Seiden LS, Schuster CR, Moore RY (1982) Dopamine nerve terminal degeneration produced by high doses of methylamphetamine in the rat brain. Brain Res 235:93-103.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 18:247-291.

Saria A (1999) The tachykinin NK1 receptor in the brain: pharmacology and putative functions. Eur J Pharmacol 375:51-60.

Schwartz JC, Diaz J, Bordet R, Griffon N, Perachon S, Pilon C, Ridray S, Sokoloff P (1998) Functional implications of multiple dopamine receptor subtypes: the D1/D3 receptor coexistence. Brain Res Brain Res Rev 26:236-242.

Self DW, Nestler EJ (1995) Molecular mechanisms of drug reinforcement and addiction. Annu Rev Neurosci 18:463-495.

Smith AD, Bolam JP (1990) The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. Trends Neurosci 13:259-265.

Standaert DG, Landwehrmeyer GB, Kerner JA, Penney JB, Jr., Young AB (1996) Expression of NMDAR2D glutamate receptor subunit mRNA in neurochemically identified interneurons in the rat neostriatum, neocortex and hippocampus. Brain Res Mol Brain Res 42:89-102.

Steele TD, Hodges DB, Jr., Levesque TR, Locke KW, Sandage BW, Jr. (1996) The D1 agonist dihydrexidine releases acetylcholine and improves cognition in rats. Ann N Y Acad Sci 777:427-430.

Steinberg R, Rodier D, Souiclhac J, Bougault I, Emonds-Alt X, Soubrie P, Le Fur G (1995) Pharmacological characterization of tachykinin receptors controlling acetylcholine release from rat striatum: an in vivo microdialysis study. J Neurochem 65:2543-2548.

Steinberg R, Souilhac J, Rodier D, Alonso R, Emonds-Alt X, Le Fur G, Soubrie P (1998) Facilitation of striatal acetylcholine release by dopamine D1 receptor stimulation: involvement of enhanced nitric oxide production via neurokinin-2 receptor activation. Neuroscience 84:511-518.

Stoessl AJ (1994) Localization of striatal and nigral tachykinin receptors in the rat. Brain Res 646:13-18.

Taguchi K, Atobe J, Kato M, Chuma T, Chikuma T, Shigenaga T, Miyatake T (1998) The effect of methamphetamine on the release of acetylcholine in the rat striatum. Eur J Pharmacol 360:131-137.

Tarazi FI, Campbell A, Yeghiayan SK, Baldessarini RJ (1998) Localization of dopamine receptor subtypes in corpus striatum and nucleus accumbens septi of rat brain: comparison of D1-, D2-, and D4-like receptors. Neuroscience 83:169-176.

Tardiff K, Marzuk PM, Leon AC, Portera L, Hartwell N, Hirsch CS, Stajic M (1996) Accidental fatal drug overdoses in New York City: 1990-1992. Am J Drug Alcohol Abuse 22:135-146.

Testa CM, Standaert DG, Landwehrmeyer GB, Penney JB, Jr., Young AB (1995) Differential expression of mGluR5 metabotropic glutamate receptor mRNA by rat striatal neurons. J Comp Neurol 354:241-252.

Vanderschuren LJ, Schmidt ED, De Vries TJ, Van Moorsel CA, Tilders FJ, Schoffelmeer AN (1999) A single exposure to amphetamine is sufficient to induce long-term behavioral, neuroendocrine, and neurochemical sensitization in rats. J Neurosci 19:9579-9586.

Vassout A, Veenstra S, Hauser K, Ofner S, Brugger F, Schilling W, Gentsch C (2000) NKP608: a selective NK-1 receptor antagonist with anxiolytic-like effects in the social interaction and social exploration test in rats. Regul Pept 96:7-16.

White NM (1996) Addictive drugs as reinforcers: multiple partial actions on memory systems. Addiction 91:921-949; discussion 951-965.

Word CO, Bowser B (1997) Background to crack cocaine addiction and HIV high-risk behavior: the next epidemic. Am J Drug Alcohol Abuse 23:67-77.

Wu YL, Yoshida M, Emoto H, Tanaka M (1999) Psychological stress selectively increases extracellular dopamine in the 'shell', but not in the 'core' of the rat nucleus accumbens: a novel dual-needle probe simultaneous microdialysis study. Neurosci Lett 275:69-72.

Zhang Y, Loonam TM, Noailles PA, Angulo JA (2001) Comparison of cocaine- and methamphetamine-evoked dopamine and glutamate overflow in somatodendritic and terminal field regions of the rat brain during acute, chronic, and early withdrawal conditions. Ann N Y Acad Sci 937:93-120.

Zhou Q, Karlsson K, Liu Z, Johansson P, Le Greves M, Kiuru A, Nyberg F (2001) Substance P endopeptidase-like activity is altered in various regions of the rat central nervous system during morphine tolerance and withdrawal. Neuropharmacology 41:246-253.

Zigmond MJ, Abercrombie ED, Berger TW, Grace AA, Stricker EM (1990) Compensations after lesions of central dopaminergic neurons: some clinical and basic implications. Trends Neurosci 13:290-296.