Université de Sherbrooke

Time course and regional specificity of neurochemical changes in the rat brain following intra-accumbal and intra-striatal injections of 6,7-ADTN (2-amino-6,7-dihydroxy-1,2,3-tetrahydronaphthalene)

par

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Mémoire présenté à la Faculté de Médecine en vue de l'obtention

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LIST OF ABBREVIATIONS

5-HT	serotonin
5-HIAA	5-hydroxyindoleacetic acid
ADTN	2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene
AM	amygdala
DA	dopamine
DOPAC	dihydroxyphenylacetic acid
GABA	y-aminobutyric acid
HPLC	high performance liquid chromatography
HVA	homovanillic acid
MAO	monoamine oxidase
NA	nucleus accumbens
NT	neurotensin
PFC	prefrontal cortex
ST	striatum
SN	substantia nigra
VTA	ventral tegmental area

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Résumé

La schizophrénie est une maladie qui affecte jusqu'à 1% de la population de l'Amérique de Nord. La pathologie de la schizophrénie est souvent expliquée par des théories dopaminergiques. Les théories suggèrent que la schizophrénie serait causée par une activité excessive de la dopamine dans les régions mésolimbiques. Tous les antipsychotiques sont des antagonistes de la dopamine au cerveau. Ces drogues ont leur activité dans des régions dopaminergiques (l'accumbens et striatum). Les antipsychotiques atypiques ont une plus haute affinité pour les recepteurs 5-HT_2 et causent moins d'effets extrapyramidaux. Ce projet contient deux différentes études sur les changements dopaminergiques et sérotonergiques provoqués par un agoniste dopaminergique. On voulait examiner le cours temporel des changements, les effets neurochimiques dans des régions à distance de l'accumbens, et déterminer la spécificité des effets en étudiant les conséquences neurochimiques d'une stimulation dopaminergique dans le striatum. La première étude a examiné les effets de 12.5 µg de 6,7 ADTN (un analogue rigide de la dopamine) injecté dans le noyau accumbens sur la dopamine, HVA, DOPAC, sérotonine, et 5-HIAA dans les régions suivantes: cortex préfrontal, noyau accumbens, striatum, amygdale, substance noire, et le aire tegmentaire ventrale. Les changements neurochimiques étaient examinés à un niveau contrôle, 60, et 120 minutes après injections. La deuxième étude a examiné les mêmes changements neurochimiques dans les mêmes régions du cerveau sauf que l'injection de 6,7 ADTN était dans le striatum. Brièvement, après injection dans le noyau accumbens il y avait une augmentation de la dopamine dans l'amygdale et la substance noire. Ces deux régions sont impliquées dans les symptômes négatifs de la schizophrénie. Une augmentation de la sérotonine avait une effet inhibiteur sur la dopamine dans le cortex préfrontal et le striatum. Après injections de la 6,7 ADTN dans le striatum il y a une énorme augmentation de l'activité dopaminergique dans le cortex qui n'est pas inhibée par la sérotonine. L'activité dopaminergique était augmentée aussi dans le noyau accumbens et l'amygdale avec des changements significatifs du niveau de sérotonine. La substance noire avait une diminution de la sérotonine qui n'est pas accompagnée par une augmentation de l'activité dopaminergique. Ces résultats suggèrent que le rôle de la sérotonine au niveau de la substance noire n'est pas inhibiteur. En conclusion, la stimulation des régions terminales dopaminergiques mésostriées et mésolimbiques cause des changements neurochimiques répandus et à long terme dans le cerveau. La stimulation d'une région n'affecte pas seulement cette région mais produit aussi des changements importants dans d'autres régions du cerveau. Les interactions entre le cortex préfrontal et les systèmes dopaminergiques sous-corticaux pourraient nous aider à mieux comprendre les maladies impliquant la dopamine.

Time Course and Regional Specificity of Neurochemical Changes in the Rat Brain Following Intra-accumbal and Intra-striatal Injections of ADTN

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ABSTRACT

Schizophrenia is a disease that is estimated to affect up to 1% of North America's population. The dopaminergic theories used to explain the etiology of schizophrenia suggest that excessive dopaminergic transmission in major dopamine terminal areas such as the nucleus accumbens and striatum leads to the positive and negative symptoms associated with the disease. Also, antipsychotic medications are assumed to establish their affect within both the accumbens and striatum. More recently, serotonin has come to play a larger role in the drug therapy of schizophrenia through the use of atypical antipsychotics. These new therapeutic compounds have a high affinity for 5-HT_2 receptors and cause fewer extrapyramidal side-effects. This experiment contains two different but conjoined studies that examine dopaminergic and serotonergic changes induced by a dopamine agonist. The first study examined the effects of 12.5 μg of 6,7-ADTN bilaterally injected into the nucleus accumbens on neurochemical changes, specifically dopamine, HVA, DOPAC, serotonin and 5-HIAA, within the prefrontal cortex, nucleus accumbens, striatum, amygdala, substantia nigra, and ventral tegmental area. The neurochemical changes were examined 60 and 120 minutes after drug injection. The second study examined the same neurochemical changes using the same time course within the same brain regions only following injections of ADTN into the striatum. Neurochemical analysis was done by high performance liquid chromatography. This study found that neurochemical changes were not localized to only the sites of injection. In fact neither site of injection showed any remarkable neurochemical changes at either time point in comparison to control levels. Overall, after injection of 6,7 ADTN into the accumbens there was an increase in dopamine functional activity (DA/HVA ratio) in the amygdala and substantia nigra and an increase in dopamine turnover (DA/DOPAC ratio) in the amygdala. Increased serotonin had an inhibitory effect on dopamine in the prefrontal cortex and striatum. After injection of 6,7 ADTN into the striatum results showed an enormous increase in dopamine functional activity within the PFC that cannot be inhibited by the concomittant increase in serotonin. There was also an increase in dopamine functionality in both the accumbens and amygdala which was accompanied by significant changes in serotonin levels. The substantia nigra showed significant decreases in serotonin. These changes were not accompanied by any increase in dopamine turnover or functionality suggesting that serotonin does not have an inhibitory role within the substantia nigra. In conclusion, dopaminergic stimulation of the nucleus accumbens and striatum causes widespread and long term changes in their major afferent and efferent regions. Future studies should examine the interaction between the prefrontal cortex and the subcortical dopaminergic system to develop a better understanding of behavioral problems related to dopamine transmission.

Introduction

Of the many biological theories concerning the etiology of schizophrenia, the most widely accepted is related to disturbances in dopaminergic transmission. Specifically, there is a discrete and persistant dysfunction within the mesolimbic dopamine (DA) system of patients with schizophrenia. The ventral tegmental area is the departure point of the DA dysfunction which projects to the nucleus accumbens (NA) and the amygdala (AM). The NA is involved in psychomotor behavior and the AM is implicated in mood and emotions (Woodruff et al., 1977; Tamminga et al., 1988; Bachneff, 1991; Costall et al., 1991). Much of the main support comes from the observation that therapeutically useful antipsychotic drugs (haloperidol, clozapine, or chlorpromazine) act on the dopamine system (Moghaddam and Bunney, 1990; Kandel et al., 1991; Costall et al., 1991; Vankammen, 1991; Wise and Heffner, 1991; Reynolds, 1992). The principle effect of antipsychotic medication is to try and regulate the overall level of dopamine present in the brain of patients with schizophrenia (Lee et al., 1988; Nemeroff and Bissette, 1988; Tamminga et al., 1988; Costall et al., 1991; Grace, 1991; Kandel et al., 1991; Vankammen, 1991). The administration of dopamine antagonists results in improvement of many of the symptoms commonly associated with schizophrenia such as : odd ideation, unusual perceptual experiences, delusional thinking, and hallucinations (Nemeroff and Bissette, 1988; Cleghorn et al., 1991; Davis et al., 1991; Vankammen, 1991; Carson and Butcher, 1992; Seeman, 1992). Attenuation of these symptoms can also be achieved by administration of dopamine synthesis inhibitors, dopamine storage depletors, and dopamine autoreceptor agonists (Tamminga et al., 1988; Nemeroff and Bissette, 1988; Costall et al., 1991; Fields et al., 1991; Lyon, 1991; Wise and Heffner, 1991). Chronic administration of haloperidol decreases dopamine turnover in the

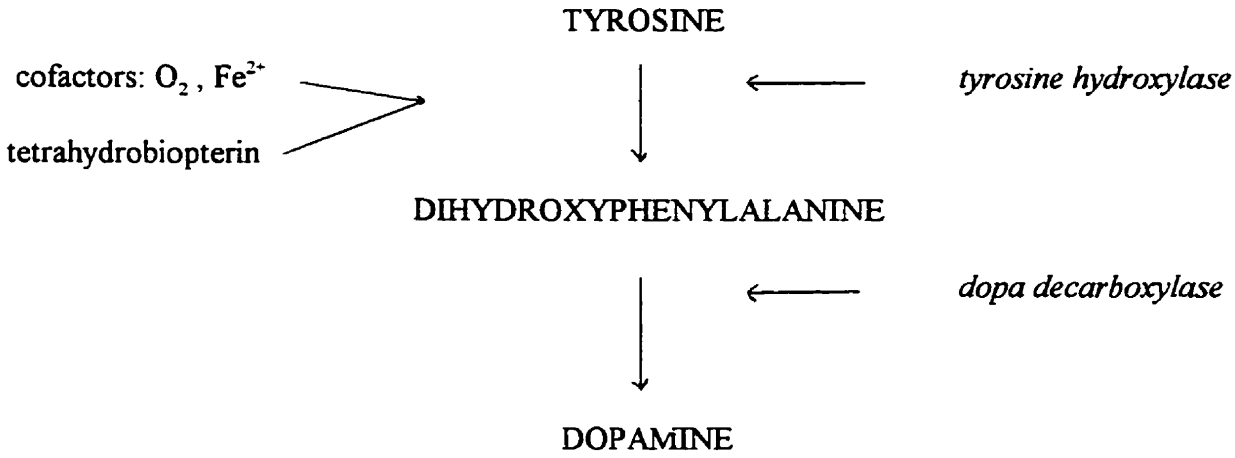
prefrontal cortex and striatum and short term clozapine administration increases the extracellular concentration of dopamine in the nucleus accumbens and prefrontal cortex (Hernandez et al., 1990; Moghaddam and Bunney, 1990). Before entering into the specifics of the neurochemical changes it is important to review some fundamentals of dopaminergic transmission within the central nervous system.

1. Dopamine

Dopamine (3,4-dihydroxyphenylethylamine) is one of three neurotransmitters known as the catecholamines along with norepinephrine and epinephrine. The catecholamines are monoamines, only one (NH_2) amine group , and each contains a catechol nucleus. This nucleus is composed of a benzene ring with two attached hydroxyl groups. Dopamine is the precursor of norepinephrine and can be found in both the peripheral and central nervous system.

Persson and Waldeck (1970) were one of the first to suggest the interaction between dopamine and norepinephrine neurons in the brain. Their results showed that NE synthesis can be inhibited by blocking dopamine B-hydroxylase, the enzyme that converts DA to NE, with FLA-63. Alpha-methyltyrosine can inhibit NE synthesis by acting on tyrosine hydroxylase which is the first enzyme to act on tyrosine. Results showed that the levels of NE decreased more rapidly using FLA-63. Alpha-methyltyrosine only decreased NE levels rapidly if apomorphine, a DA agonist which reduces DA turnover, was pre-administered. This experiment clearly illustrated the interactive nature of DA and NE brain neurons.

1.1 Dopamine Synthesis



Tyrosine is a dietary amino acid that is actively transported out of the blood stream and taken across the blood brain barrier and into the catecholaminergic neurons (Brown and Gershon, 1993). The level of tyrosine in the brain is continously high so there is always enough substrate for tyrosine hydroxylase, the rate limiting enzyme of dopamine synthesis. There are four major factors that regulate its activity; **(1.)** dopamine will compete with tetrahydrobiopterin for binding sites on the enzyme and act as its own inhibitor, **(2.)** the availability of tetrahydrobiopterin **(3.)** presynaptic dopamine autoreceptors, and **(4.)** the rate of impulse flow through the dopamine pathways in the central nervous system (Dipaolo, 1994). Dopa decarboxylase, the second major enzyme, is very active with a high turnover rate so the level of L-DOPA free in the brain at any time is extremely small. (see Table 1 for sites of pharmacological modulation of DA synthesis and catabolism).

1.2 Dopamine release and uptake

A nerve impulse arrives at the terminal button of a dopaminergic neuron and causes a calcium-dependent release of dopamine. The nerve impulse also activates tyrosine hydroxylase by stimulating the phosphorylation of tyrosine hydroxylase. This increases its affinity for the cofactor tertrahydrobiopterin which is essential for dopamine synthesis. The release of dopamine is modulated by presynaptic autoreceptors that function as a negative feedback loop within the dopamine system (Nemeroff and Bissette, 1988; Dipaolo, 1994). The quantity of dopamine released depends on the rate and firing pattern of dopamine neurons. Dopamine terminals have high affinity uptake sites which maintain a pool of releasable state transmitter and participate in the termination of DA action. These uptake sites are membrane carriers that can transport dopamine in either direction depending on the concentration gradient (Brown and Gershon, 1993). The carrier will actively pump dopamine back into the terminal button.

1.3 Dopamine metabolism

Dopamine is converted to DOPAC (dihydroxyphenylacetic acid) by monoamine oxidase (MAO), oxidation takes place within the neuronal and glial mitochondria. Extraneuronal metabolism by catechol-o-methyltransferase (COMT) followed by MAO produces homovanillic acid (HVA) (Brown and Gershon, 1993; Dipaolo, 1994). COMT acts only on substrates with a catechol nucleus and is found mostly in the cytoplasm. MAO controls the level of dopamine within the neurons by degrading any free DA and degrading reuptaken DA before it can be reinvesiculated. An increase in HVA is considered a good indicator of the functional activity of dopamine neurons in the brain (Wise and Heffner, 1991).

Cass et al. (1993) studied the metabolism of locally injected DA in the striatum and the nucleus accumbens. Results showed that **(1)** reuptake by a DA transporter was the major removal system of DA from extraneuronal space **(2)** 6-hydroxydopamine, a chemical neurotoxin that selectively destroys catecholaminergic neurons, lesions or preinjection of cocaine or nomifensine increased the time course of injected DA in both the striatum and nucleus accumbens but the effect was stronger in the accumbens and **(3)** the accumbens was more sensitive to uptake inhibitors because it contained fewer DA transporters.

Table 1

Pharmacological modulation of DA synthesis and catabolism

DRUGS	EFFECTS
SYNTHESIS INHIBITORS	
- ex: AMPT blocks tyrosine hydroxylase - ex: carbidopa blocks dopa decarboxylase	blocks synthesis of dopamine
REUPTAKE INHIBITORS	
- ex: cocaine or amphetamines	prevents reuptake of dopamine from high affinity uptake sites in the synaptic cleft
RECEPTOR BLOCKERS	
- postsynaptic	blocks dopamine transmission and neuronal feedback loops
- presynaptic	increases dopamine synthesis and release
RECEPTOR AGONISTS	
- postsynaptic ex: apomorphine or bromocriptine	mimics dopamine transmission and triggers neural feedback loops: antiparkinson effect
- presynaptic	decreases dopamine synthesis and release
RECEPTOR ANTAGONISTS	
- ex: haloperidol, chlorpromazine, thioridazine	antipsychotic / tranquilizer
CATABOLISM INHIBITORS	
-ex: pargyline or iproniazid (blocks MAO)	increases synaptic concentration of dopamine

1.4 Dopamine pathways in the brain

There are two major dopamine pathways in the brain known as the long length systems (Almaric and Koob, 1993). These long length systems link the substantia nigra and ventral tegmental area with their target areas. They form functionally selective afferents to the basal ganglia (ex: striatum and nucleus accumbens) which are paralled by functionally selective efferents. The classical division of these pathways are;

A9 cells of the substantia nigra	---------->	striatum	NIGROSTRIATAL
A10 cells of the VTA	---------->	limbic system	MESOCORTICOLIMBIC

The characteristics of the two major dopamine pathways are very similar. Both pathways have autoreceptors and have the capacity for high affinity dopamine transport. Pharmacologically, both tracts respond to dopaminergic agonists by decreasing synthesis, turnover, and catabolism and to DA antagonists by increasing synthesis, catabolism and turnover. In recent studies it has become clear that there is a great deal of anatomical overlap (Fallon, 1988; Gerfen, 1992) but for the sake of clarity these divisions will be described independently

1. Nigrostriatal

This group of axons projects from the A9 cells located in the zona compacta of the substantia nigra (SN) to the striatum and caudate putamen (Deutch et al., 1988; Anderson and Reiner, 1991). The striatum receives all the major inputs to the basal ganglia from both the substantia nigra and neocortical areas. The dopaminergic circuitry of the basal ganglia is set up in parallel gangliar-thalamo-cortical projections which process send back information to the cortex (Almaric and Koob, 1993). This pathway is associated with behavior inititation, stereotypy and motor function.

2. Mesolimbic

This pathway is made principally of cell bodies from the A10 area of the ventral tegmental area, joins the medial forebrain bundle and then projects to the different components of the limbic system, the frontal cortex and also to the amygdala (Nauta et al., 1978; Berger et al., 1991; Gerfen, 1992). The projections follow two major axon routes; **(1.)** an axon bundle crosses the SN and VTA and then joins the medial forebrain bundle and **(2.)** an axon bundle runs dorsally and joins the medial forebrain bundle within the hypothalamus (Deutch et al., 1988; Lemoal and Simon, 1991). Fallon (1988) examined the projections leaving the SN and VTA and found abundant axon collateralization within the projections from the medial SN and lateral VTA. This creates overlap between two major dopaminergic pathways and allows the SN and VTA to project to different forebrain areas simultaneously (Deutch et al., 1988; Fallon, 1988).

The dopaminergic pathways project to their target areas in a topographically organized manner. This means that medial dopamine neurons project to medial forebrain structures and lateral dopamine neurons project to lateral forebrain structures. Almaric and Koob (1993) examined all the afferent and efferent connections of the dopamine pathways and found that the nucleus accumbens, striatum, and amygdala were major target zones of dopaminergic projections and that these regions collectively formed the center for integrating sensory information in order to perform behavioral acts.

The dopaminergic pathways consist of two types of dopamine neurons. The first is spontaneously active with a slow and consistent firing rate which is easily affected by dopaminergic antagonists. The second is a non-spontaneously firing neuron that is hypoexcitable and is less affected by antagonists (Grace, 1988). The control of dopaminergic activity in the

functioning of the normal brain is done by three means. **(1.)** Through the long loop system which gives feedback from the terminal regions of both dopaminergic pathways to modulate dopamine cell activity so that if too much dopamine is released the long loop will act in order to decrease dopamine release into the synapse (Bunney, 1988). **(2.)** The short loop involves autoreceptors located presynaptically on the cell body or terminal button of the dopaminergic neurons. These autoreceptors will inhibit the synthesis and release of dopamine (Bunney, 1988). **(3.)** The last control of dopaminergic activity is the release of cotransmitters, like neurotensin which can inhibit dopamine release at the autoreceptor level. Cotransmitters act on presynaptic receptors to prevent the release of dopamine (Bunney, 1988; Nemeroff and Bissette, 1988).

Where the dopaminergic pathways lead is critical to the discussion of the relationship between psychotic symptoms and dopamine. The neuroanatomy of schizophrenia will be discussed in more detail later but briefly, the nigrostriatal system originates in the substantia nigra and projects to the striatum and other areas of the basal ganglia and is directly implicated in the control of motor movement (Nemeroff and Bissette, 1988; Costall et al., 1991; Davis et al., 1991). This could implicate the involvement of dopamine in the motor dysfunctions seen in schizophrenia such as catatonia. The mesocorticolimbic system leads from the ventral tegmental area which connects the limbic system to the cortex, and projects to the nucleus accumbens, lateral septum, and the amygdala all of which are involved in affective, motivational, and motor experiences (Costall et al., 1991; Davis et al., 1991). The nucleus accumbens receives projections from both the ventral tegmental area and amygdala which are implicated in motor activity (Yim and Mogenson, 1982; 1988). The amygdala has a projection to the striatum, within the nigrostriatal pathway, which contributes to the motor aspects of emotive responses which is often

lacking in schizophrenics (Yim and Mogenson, 1988). The areas of the brain associated with the affective and motor dysfunctions of the brain (the striaum, nucleus accumbens and amygdala) are the same areas that are heavily innervated by dopaminergic neurons.

1.5 Dopamine Receptors

This review of dopamine receptors will mostly be limited to the D_1 and D_2 types which are better functionally delineated than the D_3 - D_5 receptors. Dopamine receptors are seven transmembrane domain G-protein receptors with about 400 amino acids (Civelli et al., 1991; Strange, 1991, Odowd, 1993). These receptors have a molecular weight of 50000 Kd which is glycosylated with oligosaccharide chains that have a mass around 20000 Kd. The D_1 receptors have a short third intracellular loop and a long carboxy tail which is ideal for G_s stimulation since a major interaction site between the receptor and the G protein is the third loop. The D_2 receptors have a long third intracellular loop and a short carboxy tail which is suited for G_i activation (Strange, 1993). The ligand binding site is created when the transmembrane helices bundle together and form a cavity where the ligand binds via an interaction with amino acid side chains. In common, both the D_1 and D_2 receptors have conserved aspartic acid residues in the second and third transmembrane domains. In the second domain, aspartic acid maintains the shape of the protein and in the third domain aspartic acid provides the counter ion for cationic amine head binding. In the fifth transmembrane domain both D_1 and D_2 have two serine residues which interact with the catechol hydroxy groups of dopamine (Strange, 1993).

The D_2 presynaptic receptor is called an autoreceptor because it acts as its own negative feedback mechanism to decrease the release and actions of dopamine. DA autoreceptor stimulation decreases DA synthesis, turnover, and dopaminergic cell firing. Low doses of

dopamine agonists decrease motor activity and striatal dopamine release due to the activation of the DA autoreceptor (Almaric and Koob, 1993). This decreases the release of dopamine and decreases the stimulation of postsynaptic D_2 receptors which attentuates motor activity (Wood and Altar, 1988).

The striatum, both the dorsal and ventral portions, contain D_1 and D_2 receptors (Sokoloff et al., 1990; Sunahara et al., 1991; Sokoloff and Schwartz, 1995). These regions of the ST are implicated in emotion, behavior, and mood and are the characteristics most affected in cases of schizophrenia where there is a presumed overstimulation of dopamine receptors (Cleghorn et al., 1991; Seeman, 1992). The striatum, both the dorsal and ventral portions, contain D_1 and D_2 receptors. The majority of antipsychotic drugs achieve their effect through an interaction with D_2 receptors. The extrapyramidal motor side effects of these drugs are assumed to arise from the inhibition of D_1 and D_2 receptors (Strange, 1991; Gainetdinov et al., 1996). The majority of antipsychotic drugs achieve their effect through an interaction with D_2 receptors

Most of the functional parameters of the newer dopamine receptors are not fully understood and are still classifed as D_1-like and D_2 - like (Civelli et al., 1991; Strange, 1993). In recent years the pharmacological differentiation and anatomical location between D_3 and D_4 have become more defined. Both D_3 and D_4 are similar to the D_2 receptor which stimulates a G_i protein which inhibits the activation of adenylate cyclase (Caine and Koob 1993). The homology between the entire D_2 and D_3 receptor is 52% but the homology of the the transmembrane domains, where the ligand binds, is 75% (Sokoloff et al., 1990). The anatomical expression of D_3 and D_4 is more discreet than D_2 which is highly expressed in all dopamine projection fields. The D_3 receptor is localized to the ventral striatal complex, the shell of the nucleus accumbens and to a lesser extent

in the hippocampus and temporal lobe (Sokoloff et al., 1990; Caine and Koob, 1993; Sokoloff and Schwartz, 1995). The D_4 receptor is located in the frontal cortex, amygdala, and hippocampus (Sokoloff et al., 1990; Caine and Koob, 1993; Sokoloff and Schwartz, 1995).

The discrete location of the D3 receptor to the ventral striatal complex in the rat, which receives its primary dopaminergic input from the A10 VTA cells, has led some researchers to speculate on the functionality of the receptor. Sokoloff & Schwartz (1995) summarized the possible roles of the D3 receptor including: inhibition of locomotor activity, cocaine self-administration, tonic activation of neurotensin gene expression and inhibition of striatal dopamine release. No functional data is yet available on possible roles for the D_4 receptor. The discrete location of the D_3 receptor also makes it a better target site for future antipsychotics.

Table 2

Dopamine Receptors Overview

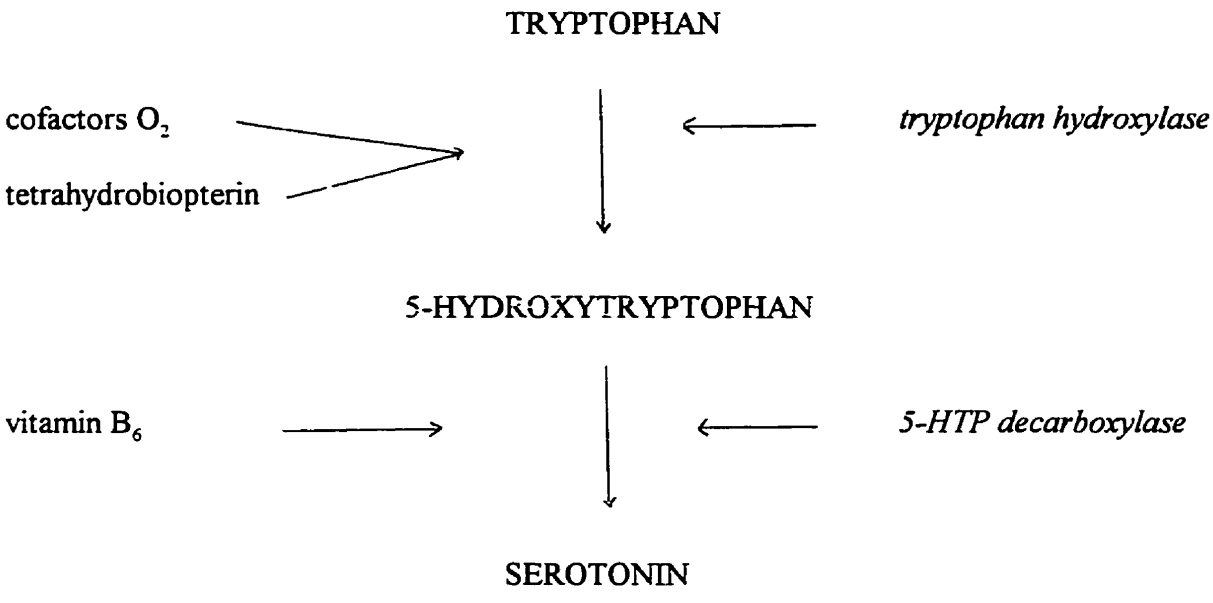
Characteristic	**D_1**	**D_2**	**D_3**	**D_4**
Region	dors. ST NA	ST NA SN VTA	ven.ST NA HIPP	F.CX AM HIPP
Location	postsynaptic	pre+postsynaptic	postsynaptic	unconfirmed
Response	activate G_s increase adenylate cyclase	activate G_i decrease adenylate cyclase	activate G_i decrease adenylate cyclase	activate G_i decrease adenylate cyclase
Agonists	SKF 38393	quinpirole	7 OH DPAT	apomorphine
Antagonists	SCH 23390	sulpiride	unspecified	clozapine

2. Serotonin

Serotonin (5-HT) is a neurotransmitter called an indolealkylamine with widespread influence in the brain and spinal cord through diverse axonal collateralizations. Serotonin is found primarily in the mucous membranes (90%) of the digestive tract and blood platelets (8%). The brain contains only 2% of the body's serotonin with the highest concentration to be found in the pineal gland (VanHoutte, 1991). It is implicated in basic survival functions such as appetite, sexual behavior, aggression, and sleep. 5-HT is also active in cerebral blood flow regulation, sensory processing and pain mechanisms. 5-HT is unable to cross the blood brain barrier therefore brain cells must manufacture their own.

Functional levels of serotonin are used along with dopamine levels to assess possible cases of schizophrenia, depression, anxiety, Alzheimer's disease and some personality disorders (Siever et al., 1991; Nagayama et al., 1991; Levy and Vandekar, 1992; Lesch et al., 1993). There are various methods used to assess circulating levels of 5-HT. Through cerebrospinal fluid analysis, metabolite levels can be determined. 5-HIAA, the serotonergic metabolite, is significantly decreased in a majority of medication free depressed patients (Nagayama et al., 1991; Levy and Vandekar, 1992; Lesch et al., 1993). Plasma tryptophan levels provide an index of the quantity of tryptophan available to be converted into 5-HT. This indicates an overall measure of central 5-HT activation. Rosse et al. (1992) studied the effects of low tryptophan diets as an adjunct to traditional neuroleptic treatment for schizophrenia to try and improve psychotic symptoms.

2.1 Serotonin Synthesis



The first step in synthesis of serotonin is the active uptake of the amino acid tryptophan, which comes from dietary intake, from blood plasma to brain cells. Tryptophan is the primary substrate of 5-HT and cannot be synthesized by the brain. The uptake of tryptophan is done through a high affinity active carrier process open to competition between 5-HT, large neutral amino acids, and branch chain amino acids therefore dietary protein and carbohydrate content can influence brain 5-HT levels (Boadlebiber, 1993; Cowan, 1994). Once tryptophan has entered the brain the next step is the hydroxylation of tryptophan at the 5 position by tryptophan hydroxylase to form 5-hydroxytryptophan (5-HTP). This is the rate limiting step of 5-HT synthesis. Tryptophan hydroxylase is synthesized in neuronal cell bodies and travels down the axon to the nerve terminal. This enzyme requires both molecular oxygen and tetrahydropteridine as cofactors

2.3 Serotonin Metabolism

5-HT is deaminated by monoamine oxidase A (MAO A), which is both intra and extra neuronal, to form 5-hydroxyindoleacetalaldehyde (5-HIA). 5-HIA can be further oxidized by aldehyde dehydrogenase to form 5-hydroxyindoleacetic acid (5-HIAA) or 5-hydroxytryptophol (Hindle, 1994). The NAD^+/NADH ratio in the tissue determines the levels of these two metabolites. The level of 5-HIAA excreted in the brain and cerebrospinal fluid is considered a reliable index of 5-HT synthesis and metabolism (Boadlebiber, 1993).

2.4 Serotonin Pathways in the Brain

The distribution of serotonin neurons in the CNS is clearly demonstrated using the neurotoxin 5,6-dihydroxytryptamine (5,6-DHT). 5,6-DHT has a very high affinity for serotonergic neurons and creates a yellow fluorescence after formaldehyde treatment that can be seen fluorimetrically. The majority of 5-HT neurons project from the raphe nuclei towards the cortical and limbic structures. Serotonin cell bodies located in the raphe nuclei are restricted to areas around the midline of the pons and the upper brainstem with a great deal of overlap in their projection fields (Azmitia and Whitakerazmitia, 1991). The cell bodies are divided into nine nuclei B1-B9. There is no easily defined typography to the serotonergic pathways but, in general, the caudal cell bodies (B1,B2,&B3) project to the spinal cord and medulla with their terminals found in the dorsal and ventral horns of the spinal cord. B4-B9 constitute the ascending serotonergic pathways with B7 and B8 creating 80% of the forebrain's serotonin.The more rostral B4-B9 fibers project to the basal ganglia, thalamus, hypothalamus and limbic system and the intermediate fibers to the cerebral cortex (Azimitia and Whitakerazimitia, 1991). Serotonergic fibers have been traced

to the major limbic pathways such as the medial forebrain bundle, stria medullaris, stria terminalis, fornix, and fasciculus retroflexus. Vertes (1991) examined the projections of the dorsal raphe nucleus and found that the majority ascended from the medial forebrain bundle (See section on major limbic fiber pathways). The dorsal raphe sends projections to the ventral tegmental area, substantia nigra and select regions within the striatum and amygdala.

2.5 Serotonin Receptors

There are two major subtypes of serotonin receptors 5-HT_1 and 5-HT_2 which mediate inhibition and excitation respectively (Peroutka, 1990; Bonate, 1991; Radja et al., 1991; Lesch et al. 1993). All serotonin receptors are part of the G protein family that is characterized by seven transmembrane domains (Julius, 1991). 5-HT_1 can be further subdivided into 5-HT_{1A} and 5-HT_{1B}. The 5-HT_{1A} receptors, found in the hippocampus and the raphe nuclei, are autoreceptors which inhibit adenylate cyclase and are located on both neurons and astrocytes (Radja, 1991; Boess and Martin, 1994). In the hippocampus 5-HT_{1A} receptors hyperpolarize CA1 pyramidal cells by opening K^+ channels. In the raphe nuclei they mediate the inhibition of the nuclei through a calcium activated potassium conductance which slows the normally pacemaker- like activity of these cells. 5-HT_{1B} acts as a release modulator and can be found in the substantia nigra and the globus pallidus (Radja, 1991). 5-HT_2 receptors are located in layer IV of the cortex and these receptors initiate phosphonositide turnover and membrane depolarization. These receptors mediate 5-HT induced excitation of facial motor neurons. 5-HT_{1A} agonists are considered anxiolytics and 5-HT_2 antagonists are considered atypical antipsychotics (Fuller et al., 1991;

Glennon et al., 1991; Levy and Vandekar, 1992). 5-HT_3 receptors are directly linked to a cation channel.

Table 3

Pharmacological modulation of 5-HT synthesis and catabolism

DRUGS	EFFECTS
SEROTONERGIC NEUROTOXIN	
- 5,6 dihydroxytryptamine	destruction of 5-HT cells
- PCA (p-chloroamphetamine)	inhibits tryptophan hydroxylase & MAO
RECEPTOR AGONISTS	
- LSD	inhibits receptors
- a-methyltryptamine	stimulates receptors
- psilocin	inhibits receptors
RECEPTOR ANTAGONISTS	
- methysergide	blocks postsynaptic receptor
- cinanserin	
- lysergic acid diethylamide	
SYNTHESIS INHIBITORS	
- PCPA	blocks synthesis of 5-HTP & 5-HT
- a-methyl 5-HTP	blocks 5-HTP decarboxylase
REUPTAKE INHIBITORS	anxiolytics
- fluoxetine	blocks reuptake of synaptic 5-HT
- chlordiazepoxide	
- diazepam	
- chlorimipramine	antidepressant
MAO INHIBITORS	antidepressant
- iproniazid	
- pargyline	

3.0 Serotonin - Dopamine Interaction

The relationship between dopamine and serotonin has been elucidated throughout many different types of studies. If the relationship had to be summarized then 5-HT would best be characterized as a modulatory transmitter with inhibitory effects on dopaminergic activation (Siever et al., 1991). There are established reciprocal connections between DA and 5-HT in the substantia nigra and the striatum and there are ascending serotonergic projections going from the dorsal raphe to the forebrain. Specifically, these projections travel to the substantia nigra and also to the caudate-putamen.

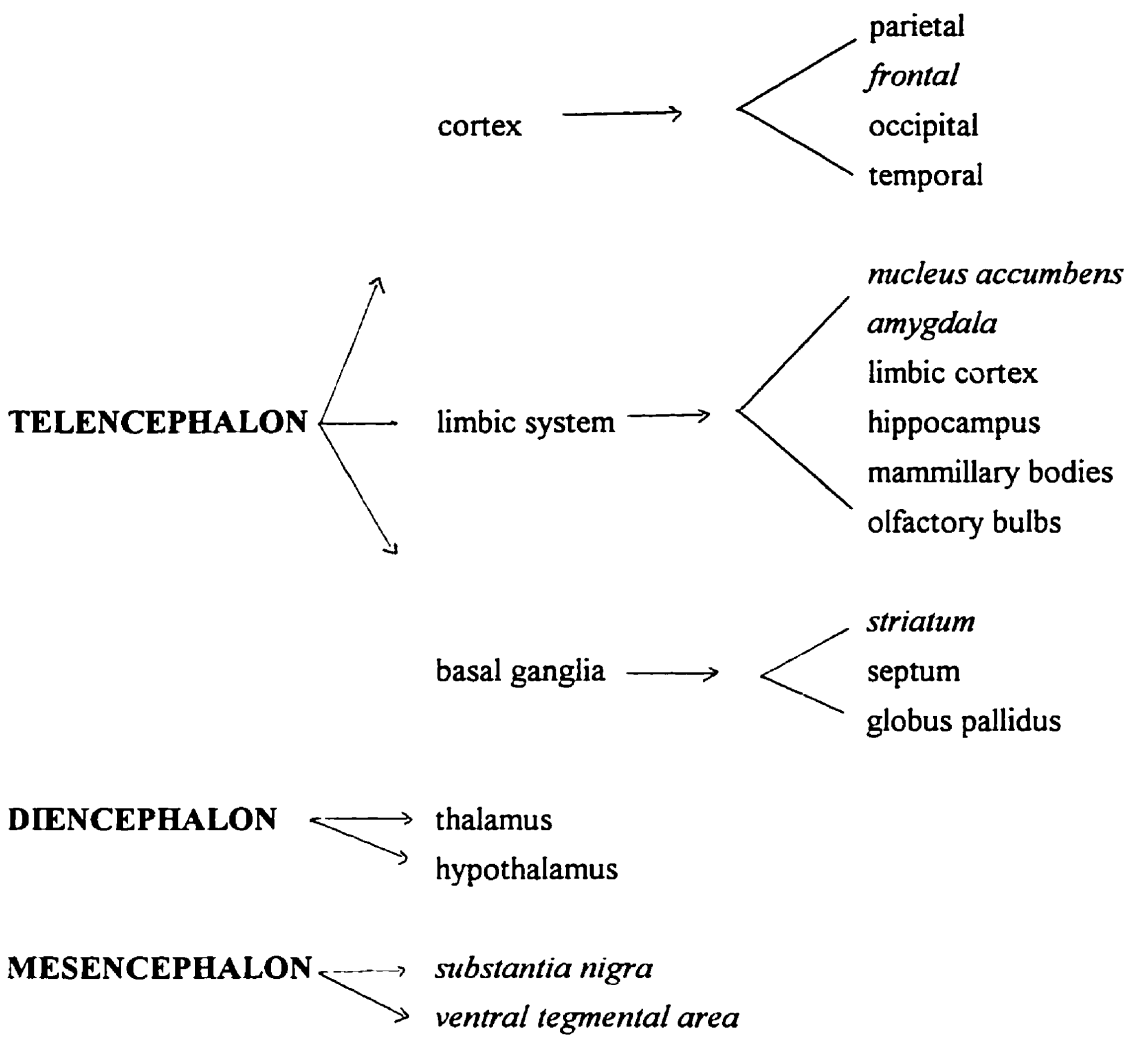
Lappalainen et al.(1991) found that acute administration of SCH 23390 (D_1 receptor antagonist) increases DA metabolism and also has a minor blocking effect on the 5-HT_2 receptor. Kelland et al. (1993) examined the responsiveness of nigrostriatal dopamine neurons to systemic administration of 5-HT. Their results showed that 5-HT 1A (autoreceptor) agonists increased the firing rate of slow firing dopamine neurons and if the serotonergic fibers were depleted using the neurotoxin 5,7-dihydroxytryptamine there was no observed increase in firing rate. Their tentative conclusion was that these normally slow firing DA neurons are under an inhibitory influence by 5-HT which is inhibited by the 5-HT agonist causing an increase in firing rate.

Recent research on schizophrenia is not just focusing on the dopamine system alone. Recent results and the increasing interest in atypical neuroleptics suggest that both dopamine and serotonin dysfunctions are involved in the etiology of schizophrenia. Atypical neuroleptics (such as sulpiride and clozapine) are drugs which still primarily act by blocking dopamine receptors but which have a set of pharmacological properties different from the traditional neuroleptics (Tamminga, 1983). Deutch et al. (1991) found that 5-HT was implicated in the actions of atypical

antipsychotics which are generally more associated with the dopamine system. Clozapine, considered to be an atypical antipsychotic, acts through multiple receptor mechanisms like. Specifically, cloazapine binds with great affinity to D_2 receptors and also binds to a high ratio of 5-HT_2 receptors. Levy and Vandekar (1992) conducted an extensive review of serotonergic antipsychotics. 5-HT_2 antagonists perform as atypical antipsychotics and appear to have a slight affinity for the D_2 receptor. Rosse et al. (1992) examined the effects of a low tryptophan diet along with antipsychotic medication in a group of schizophrenics and found that the diet improves symptoms of psychosis and the antipsychotics appear to offer protection against the depressive effects of the low tryptophan diet. Research over the past ten years has consistently shown that 5-HT does have an influence on the activity of dopamine.

Table 4

Anatomy of the rat brain



4.0 Anatomy of the Rat Brain

The areas in italics of figure 1 represent the brain regions that will be examined in detail within this study. The mesencephalon will be the first area examined with specific regard to the substantia nigra and ventral tegmental area because they are the key projection zones of the nigrostriatal and mesocorticolimbic dopamine projection pathways. The telencephalon will be next

with the focus on the prefrontal cortex, basal ganglia, and limbic system with an emphasis on the striatum and nucleus accumbens.

4.1 Mesencephalon

This region of the brain, also known as the midbrain, is tube shaped and connects the forebrain with the hindbrain (Smith and van der Kooy, 1985). The midbrain is divided into two eminences by the tectum. The midbrain contains both the ventral tegmental area and the substantia nigra.

4.1.1 Ventral Tegmental Area

This area of the brain is composed of a mass of multipolar cells that are located ventrally to the medial longitudinal bundle and are adjacent to the tegmentum. The VTA acts as a relay station on a pathway that runs from the hypothalamus to the motor nuclei of the hindbrain (Bowsher, 1975). The descending fibers spread caudally and dorsally over the tegmentum and raphe nucleus and continue caudally until the locus coeruleus (Zeman, 1963). The ascending fibers project to the thalamus and hypothalamus via the medial forebrain bundle. Along the way some VTA fiber project on to the fasciculus retroflexus and travel to the lateral habenular nucleus (Nauta et al., 1978). Within the thalamus the VTA fibers pass through the nucleus reuniens, ventromedial nucleus, and mediodorsal nucleus (Swanson, 1982). The ascending and descending connection between these brain regions is the medial forebrain bundle which itself receives fibers from the VTA (Domesick, 1988).

The afferents originate mostly from the limbic structures such as the nucleus accumbens and amygdala (Nauta et al., 1978). There are two pathways from the VTA to the nucleus accumbens **(1.)** via the medial forebrain bundle and **(2.)** the dorsal way through the fasciculus retroflexus and the stria medullaris (Phillipson and Griffiths, 1985). The efferents of the VTA are called the mesolimbic and mesocortical pathways. The major efferents project to the nucleus accumbens, striatum, frontal cortex, septum, amygdala, and the substantia nigra pars compacta (Phillipson and Griffiths, 1985; Domesick, 1988). Within the amygdala, the anterior portion receives VTA efferents coming from the striatum and the central and lateral portions receive direct VTA fibers (Swanson, 1982; Domesick, 1988).

4.1.2 Substantia Nigra

This area is a broad thick band that extends forward into the hypothalamus becoming thicker and rounder as it extends (Bowsher, 1975). The substantia nigra is divided into two regions:

1. the pars compacta dorsal layer which contains dopamine neurons
2. the pars reticulata ventral layer which contains GABAergic neurons

The SN projects to structures involved in the performance of motor tasks and acts as a relay station for striatal influences on motor activity (Smith and Bolam, 1991; Conde, 1992). Afferents to the SN arise from the striatum, nucleus accumbens, frontal cortex, tectum, tegmentum, and brain stem (Conde, 1992; Deniau et al., 1994). The SN is a major output center of the basal ganglia involved in the expression of striatal functions and creates the link between the striatum and areas outside the basal ganglia (Smith and Bolam, 1991). The core of the nucleus

accumbens sends projections to the SN then the fibers travel to the motor nuclei of the thalamus and on to the prefrontal cortex (Deniau et al., 1994). Efferents from the SN include the striatum, globus pallidus, mediodorsal nucleus of the thalamus, and prefrontal cortex. The SN along with the VTA and the retrorubral field project fibers that are rich in dopamine through the basal forebrain to inhibit these acetylcholine rich areas of the brain (Zaborsky et al.,1991).

4.2 Telencephalon

Within this region of the brain the focus will be on the prefrontal cortex, the limbic system, and the basal ganglia. The prefrontal cortex receives and sends projections to the dopaminergic targets of the brain. The limbic system contains both the amygdala and nucleus accumbens. The anatomical description of the basal ganglia will focus mostly on the striatum.

4.2.1 Prefrontal Cortex

The prefrontal cortex is part of the motor system. Within the frontal cortex as a whole there is a hierarchy where the neural impulses travel from the PFC to the premotor cortex and on to the frontal cortex (Zeman, 1963). The PFC is formed of neurons that have a nonspecific influence on motor control and movement. The key role of the PFC is the temporal organization of behavior. In response to sensory information, the performance of a complex behavior must be organized in order to produce a correct behavioral sequence. Therefore, some motor impulses must be excited and others inhibited to produce a temporally correct behavior (Almaric and Koob, 1993). The PFC receives projections from the anteromedial, pulvinar, and dorsomedial nuclei of the thalamus, the substantia nigra, the ventral tegmental area, and the tegmentum (Bowsher,

1975; Beckstead, 1979; Berger et al., 1991). There are indirect reciprocal connections with the nucleus accumbens, striatum, and amygdala, and SN (Bunney and Aghajanian, 1976; Beckstead, 1979;). The PFC makes direct synaptic contact with the DA neurons of the VTA (Bachneff, 1991; Murase et al., 1993).

Within the PFC are different neuronal groupings that encode for specific behaviors. The rostral PFC encodes for sensory cues, the orbital area encodes for reward and punishment as well as gustatory and olfactory input, and the caudal PFC encodes for anticipitory motor action (Zaborsky et al., 1991). The dopaminergic neurons of the PFC inhibit subcortical DA neurons therefore low DA activity in the PFC can lead to the development of schizophrenic symptoms (Bachneff, 1991; Deutch, 1993; Murase et al., 1993). Disruption of the dopaminergic system in the PFC results in increased DA responsiveness in the NA (Davis et al., 1991; Deutch, 1993; Deniau et al., 1994).

4.2.2 Limbic System

The limbic system is a grouping of brain regions once called the rhinecephalon because these regions were thought to be involved in olfactory functions. Gradually these regions became the limbic system on the basis of their location in the telencephalon and anatomical connections called " le lobe limbique" by Broca (Beckstead, 1979). The limbic system stands between the neocortex, hypothalamic-pituitary axis, and the sensory systems (Kotter and Meyer, 1993). The limbic system includes the hippocampus, mammillary bodies, olfactory bulbs, limbic cortex, amygdala, and nucleus accumbens. The latter two will be described in greater detail further on.

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The hippocampal formation consists of the hippocampus proper, located on both sides of the hippocampal sulcus, and the dentate gyrus, located on the floor of the hippocampal sulcus and the back of the corpus callosum (Isaacson, 1974; Smith and van der Kooy, 1985). The hippocampus is implicated in the integration of data that creates the feeling of emotion through activation of the higher cortical areas (Isaacson, 1974). The mammillary bodies, a collection of nuclei, are found on the ventral surface of the brain and mark the posterior limit of the hypothalamus (Isaacson, 1974; Smith and van der Kooy, 1985). The two main efferent pathways of the bodies are the mammillothalamic tract which projects to the anterior nucleus of the thalamus and the mammillotegmental tract which projects to the dorsal tegmentum (Isaacson, 1974). Lesions of these pathways can lead to behavioral deficits in active avoidance, spatial discrimination tasks, and possibly the motivation to perform (Isaacson, 1974; Smith and van der Kooy, 1985). The olfactory nerve fibers carry stimuli from the nose which project to the olfactory bulbs which then project to the olfactory tract. The smell sensory pathway travels from the olfactory bulbs to the lateral olfactory stria, which passes through the surface of the amygdaloid nucleus, and on to the sensory cortex. The amygdaloid nucleus has two major efferent fiber pathways the stria terminalis and the diagonal band. The limbic cortex constitutes the cortex of the cingulate gyrus and was originally believed to be involved in the integration of olfactory sensations (Smith and van der Kooy, 1985). The neocortical areas of the frontal, parietal, temporal, and occipital lobes project afferents to the limbic lobe. The anterior nucleus of the thalamus also projects to this cortex which then relays the information to the hypothalamus (Smith and van der Kooy, 1985). A majority of efferent fibers from the cingulate gyrus then project to the

hippocampus. From the hippocampus the efferent fiber bundle becomes the fornix. Listed below are the major limbic fiber pathways delineating all the routes and connections.

4.2.1 Major Limbic Fiber Pathways

There are 7 major limbic pathways which are briefly described below.

Fornix : This pathway is composed of the converging fibers of the alveus and fimbria hippocampi. The fornix receives fibers from the septum and cingulate gyrus and then runs in a semi-circle to the base of the diencephalon. The fornix then sweeps in a curve through the septal area. The fibers terminate in the septal area, preoptic region, and the hypothalamus.

Stria Medullaris: This fiber bundle is formed at the interior plane of the thalamus and terminates in the habenular nuclei.

Stria Terminalis: This pathway is a bilateral arch that collects in the dorsal region of the amygdaloid complex around the caudal end of the internal capsule and then follows the course of the optic tract. The dorsal portion of the stria terminalis divides into a retrocommissural bundle and a supracommissural bundle. The supracommissural fibers innervate the nucleus accumbens.

Anterior Commissure: This fiber tract runs horizontally between the lateral septal nucleus and the preoptic area of the hypothalamus.

Medial Forebrain Bundle: This pathway forms the longitudinal association system of the hypothalamus. It runs in an almost straight course from the olfactory tubercle through the mamillary bodies into the mesencephalon crossing the lateral regions of the hypothalamus. The MFB connects the parts of the hypothalamus with each other. The fibers go from the preoptic,

lateral, anterior, and posterior hypothalamus to the midbrain, pons, cerebellum, medulla oblongata, and spinal cord.

Diagonal Band of Broca: This band is a mass of fibers with its own nucleus that extends dorsoventrally underneath the cortex to the preoptic area of the hypothalamus. The band then becomes less compact and fibers can be traced to the anterior amygdala and to the medial forebrain bundle.

Cingulum: This tract extends underneath the cortex of the cingulate gyrus and spreads diffusely into the hippocampus. It also contains fibers from the frontal cortex.

4.2.4 Amygdala

The amygdala (AM) is a set of nuclei located at the base of the temporal lobe between the pyriform cortex and the lateral border of the hypothalamus. The anterior amygdala merges into the hypothalamus and the cortex at the level of the rhinal fissure (Zeman, 1963; Bowsher, 1975). The dorsal roof is provided by the caudate-putamen and partly by the globus pallidus and posteriorly, the stria terminalis leaves the AM which separates the AM from the hypothalamus (Canteras et al., 1992). There is a cortico-cortical connection that projects from the auditory/visual association area to the entorhinal cortex and then on to the AM. The AM and the amygdalar-hippocampal transition zone provide a main input to the nucleus accumbens (Phillipson and Griffiths, 1985). The AM also projects over the entire dorsal striatum (Jayaraman, 1985; Canteras et al., 1992). The AM receives a major cholinergic excitatory input from the ventral pallidum which continues on to the neocortex; these fibers are presumed to be involved in stimulus reward associative learning (Zaborsky et al., 1991).

5. Nucleus Accumbens

The nucleus accumbens is a rostral gray mass that constitutes a large portion of the ventral striatum as an extension of the head of the caudate nucleus (Powell and Leman, 1976). The NA extends from the bed nucleus of the stria terminalis to the anterior olfactory nucleus (Pennartz et al., 1994). It is considered to be part of the limbic system and is implicated as an interface between the limbic and extrapyramidal systems (Powell and Leman, 1976; Pennartz et al., 1994). Behaviorally the NA is thought to contribute to reward mechanisms, reinforcement, and locomotor activity (Jones et al., 1980; Jolicoeur et al., 1985; Koob, 1992; Almaric and Koob., 1993). Behavior related to the nucleus accumbens will be examined in detail later.

The NA receives afferents from the septum, amygdala, substantia nigra, ventral tegmental area, thalamus, prefrontal cortex, hippocampus, and brainstem (Swanson and Cowan, 1975; Powell and Leman, 1976; Williams et al., 1977; Pennartz et al., 1994). From the thalamus the NA receives afferents from specific nuclei such as: parataenialis nucleus, nucleus reuniens, mediodorsal nucleus, nucleus gelatinosus, and the anteromedial nucleus (Phillipson and Griffiths, 1985). The entire NA receives afferents from the ventral pallidum and these fibers continue on to the VTA which then projects them to the mediodorsal nucleus of the thalamus and to the prefrontal cortex (Phillipson and Griffiths, 1985).

The NA sends off major efferent fibers to the lateral hypothalamus through the medial forebrain bundle. Efferents also project to the striatum, septum, dorsomedial nucleus, anteromedial nucleus, nucleus reuniens, amygdala, substantia nigra (pars compacta & pars reticulata), VTA, cingulum, ventral pallidum, and the globus pallidus (Swanson and Cowan, 1975;

Powell and Leman, 1976; Williams et al., 1977; Pennartz et al., 1994). The efferents of the NA are very similar to the connections of the caudate-putamen with the exception of the projections to the hippocampus and amygdala (Swanson and Cowan, 1975). Data on afferent and efferent fibers indicate that most interconnections are between the NA and other limbic system structures and the non-limbic structures innervated fall within the extrapyramidal motor system (Swanson and Cowan, 1975; Powell and Leman, 1976; Williams et al., 1977; Almaric and Koob, 1993)

The core and shell of the NA differ along several characteristics such as afferent connections, efferent connections, projection fields, and levels of neuropeptides (Pennartz et al., 1994). Zahm and Heimer (1990) studied two transpallidal pathways that originate in the NA one from the core and the other from the shell. The core of the NA projects to the dorsolateral part of the ventral pallidum and then continues on to the SN and subthalamic nuclei. The shell of the NA projects to the ventromedial ventral pallidum and terminates in the VTA. It was also found that there is minimal overlap between these two pathways. The interest in this finding was that the dorsolateral ventral pallidum is closely related to the globus pallidus, which is also innervated by the NA core. This demonstrated a close relationship between the NA core and striatum since the striatum also has the same pallidal projection pathway (Smith and Bolam, 1991).

Meredith et al. (1992) studied the morphological differences between the neural projections leaving the core and shell of the NA. The purpose of the study was to determine if the differences in neurochemical distribution were due to differences in circuitry, neuronal excitability, or synaptic interactions between the core and shell. Results indicated that the striato-midbrain neurons in the shell have fewer dendritic branches and terminal segments than those in the core and there was a larger percentage of spontaneous activity in the shell. Since branch density will

affect the chances of synapses forming by incoming afferents, the suggestion is that the core, which has more neuronal branches, is better suited to receive incoming information (Zahm and Brog, 1992). Meredith et al. (1993) also reported that the shell receives inputs mostly from the PFC, hippocampus, medial VTA, and paraventricular nucleus of the thalamus whereas the core of the NA receives most of its inputs from the PFC, lateral VTA, medial SN, and mediodorsal nucleus of the thalamus. In return the shell, with a much wider projection field, projects to the ventral pallidum, VTA, hypothalamus, and SN. The core of the NA projects to the globus pallidus and substantia nigra which is a more limited projection field.

Overall, there are three major NA target areas (Pennartz et al., 1994).

1. NA - VENTRAL PALLIDUM - SUBSTANTIA NIGRA/VENTRAL TEGMENTAL AREA

The core projects to the ventral pallidum and then to the SN. The shell projects to the ventral pallidum and then to the VTA (Deniau et al., 1994). This pathway shows the close relationship of the NA to the two major dopamine pathways. The NA can affect the striatopallidal system which in turn affects the PFC and the generation of motor activity (Zahm and Heimer, 1990).

2. NA - VENTRAL PALLIDUM - MEDIODORSAL THALAMIC NUCLEI - PFC

The mediodorsal nucleus is the connection center for the prefrontal - striatopallidal - thalamic loop. This nucleus is involved in somatosensory and motor activities (Deniau et al., 1994).Within this pathway there are ventral pallidal feedback projections to the NA so that it may act as its own stimulation inhibitor (Beckstead, 1979).

3. NA - LATERAL HYPOTHALAMUS - PREOPTIC AREA

The lateral hypothalamus and preoptic area are implicated in aggressive behavior, sexual behavior, body temperature and feeding. In order to explain the multiple behavioral functions associated with the NA there must be a coordination of cortical, thalamic, and amygdalar projections. The neurons within the NA have a low spontaneous firing rate. In order to cause a neuronal discharge a visual or auditory stimulus must be presented to intiate motor activity (West and Michael, 1990). This discharge is generated by the collective firing of the cortical, thalamic, and amygdalar afferents which contain parallel loops between them to coordinate near simultaneous activation (Groenewegen et al., 1993).

6. Basal Ganglia

The caudate-putamen, nucleus accumbens, and striatal areas of the olfactory tubercle are all considered part of a larger striatal complex which interfaces with the entire cortex and allows the cortex access to the basal ganglia (Zahm and Brog, 1992). The net output of the basal ganglia has the effect of inhibiting its targets. The level of inhibition depends on the activity of the SN and globus pallidus. The striatum receives widespread inputs from the entire cortex and has very few outputs (McGreer et al., 1987). DA in the striatum controls extrapyramidal motor functions. The basal ganglia affect motor activity via two distinct striatopallidal pathways (Flaherty and Graybiel, 1993; 1994).

1. a direct route from the striatum to the internal segment of the globus pallidus and on to the SN. The SN then activates thalamocortical pathways through a disinhibition mechanism which projects to the frontal cortex.

2. an indirect route from the striatum through the external globus pallidus which inhibits motor activity by modulating the SN disinhibitory processes.

The basal ganglia receive inputs from the neocortex. These inputs are then filtered through the basal ganglia and are subsequently projectioned to the frontal cortex and the brainstem (Jayaraman, 1985). The basal ganglia consists of the striatum, globus pallidus, and caudate-putamen complex. GABA disinhibition of dopamine controls most of the activity in the basal ganglia (Emson et al., 1993). DA innervation of the ventral striatum however is not as uniform as suspected. Voorn et al. (1986) found that DA distribution was highly compartmentalized due to either the cytoarchitecture of the ventral striatum or the distributional patterns of the neurotransmitter.

7. Striatum

Most cortical afferents project to the striatum. The striatum then projects to the major gangliar output structures, such as the globus pallidus and SN. In return, via the pallidothalamic and nigrothalamic pathways, the ST indirectly influences the cerebral cortex (McGreer et al., 1987; Gerfen, 1992; Flaherty and Graybiel, 1994). The dorsal and ventral striatum are implicated in parallel projections that leave the cortex and project to the basal ganglia and then on to the thalamus and back to the cortex (Meredith et al., 1993). The interneurons of the striatum create feedforward and feedback loops that integrate striatal output (Groenewegen et al., 1993; Meredith et al., 1993). The interneurons also act as specific targets for both cortical and thalamic input. From the ST the fibers then continue on to the ventral pallidum, SN, and thalamus (Groenewegen et al., 1993).

The ST is divided into the dorsal striatum which is the caudate-putamen and the ventral striatum which includes the nucleus accumbens and the ventral part of the caudate-putamen. The dorsal striatum is organized into patch and matrix compartments. These compartments are based on neurochemical distribution and input/output connections (Joel and Weiner, 1994). The patch compartments of the ST innervate dopamine neurons of the SN and basal ganglia and the matrix innervates the GABA neurons of the GP and SN and is involved in striatothalamocortical circuitry.

Within the ST there are three major projections:

1. Corticostriatal

This projection involves the motor ST (lateral caudate-putamen) which is innervated by the primary motor cortex (including the PFC), the associative ST (central ST) which receives input from the PFC and anterior cingulate, and the limbic ST which receives input from the prelimbic cortex, hippocampus, NA, and amygdala (Beckstead, 1979).

2. Striatonigrothalamic

This pathway is innervated by the ventral globus pallidus and associative striatum and projects to the SN. From the SN the projection continues to the mediodorsal nucleus of the thalamus (Joel and Weiner, 1994).

3. Striatopallidothalamic

This projection is innervated by the dorsal caudate-putamen, globus pallidus, and the entire striatum. This projection continues on to the ventral anterior nucleus of the thalamus (Jayaraman, 1985; Groenewegen et al., 1993).

Flaherty and Graybiel (1994) examined the input - output organization of the ST and found that there is a divergence/reconvergence pattern in the outputs from the cortex. In the matrix division of the ST, particular compartments will receive inputs from the same regions of the cortex and then project to a specific region of the globus pallidus. The outputs from the cortex are separated and organized at the striatal level and then sent on to the ventral pallidum where the cortical outputs are brought back together. The pallidum itself is able to process different sets of corticostriatal inputs and distribute them to appropriate outflow targets (Smith and Bolam, 1991). The divergence of outputs may help to create an associative network in the striatum that would be implicated in sensorimotor learning.

Within the ST the glutamatergic projections come from the cortex and the thalamus. There are two main neurochemical populations in the ST which will be discussed in detail later. **1.** GABA - Substance P - dynorphin projections from the ST to the SN called the nigrothalamocortical and **2.** GABA - enkephalin projection from the ST to the globus pallidus called the corticostriatopallidal (Emson et al., 1993). These two neuronal families do not overlap and the interneurons of the ST provide the links.

There is some overlap in the striatal projections to the thalamus and to the frontal cortex. The ventroanterior and mediodorsal nuclei both innervate overlapping areas of the PFC through the striatopallidal pathway and striatonigral pathway respectively (Beckstead, 1979; Joel and Weiner, 1994; Deniau et al., 1994). Jayaraman (1985) conclusively demonstrated the overlap using a retrograde tracing technique. The thalamic nuclei connected to the limbic system and frontal cortex do project to the ventral striatum and the thalamic nuclei associated with the motor system do have projections to the dorsal striatum.

8. Neurochemistry

There are seven basic striatolimbic neurochemical systems in the brain: 1. dopamine 2. serotonin 3. GABA 4. neurotensin 5. enkephalins 6. acetylcholine and 7. glutamate. Within this study the focus of the research was on the dopaminergic and serotonergic systems. This section is designed to outline the basic neurochemistry of the nucleus accumbens and striatum with respect to the DA and 5-HT systems.

Yim and Mogenson (1988) extensively examined the role of dopamine in the NA. Their studies have shown that DA acts presynaptically in the NA and its primary role is to inhibit neuronal firing. D_2 receptor activation decreases neuronal firing in response to hippocampal and amygdalar stimulation and the D_1 receptor decreases neuronal firing in response to PFC and thalamic stimulation. However, the role of dopamine is not exclusively inhibitory because, depending on the size of the action potential, DA can have an inhibitory effect on both GABAergic inhibitory and excitatory post-synaptic potentials. (Meredith et al., 1993).

The VTA - NA dopaminergic pathway has also been researched in considerable detail and studies have shown that activation of VTA neurons leads to an increased release of dopamine and dopamine metabolites within the NA (Phillips et al., 1992; Pennartz et al., 1994). Studies have also examined the role of DA in behaviors associated with the NA-VTA stimulation and found that dopamine is the key player in a behavioral outcome. Dopaminergic agonists increase or improve behavioral responding in learned situations whereas dopaminergic antagonists slow down responding in reinforcement paradigms (Roberts et al., 1980; Koob, 1992). The behavioral effects seen relate to the areas of the brain innervated by dopamine.

The assumed role of dopamine in the striatum is that dopamine reduces lateral inhibition between neurons which then opposes the filtering of incoming cortical activity (Salamone, 1992). Behaviorally, this lateral inhibition would translate into DA modulating the ability of sensory, associative and affective processes to influence complex motor functions such that coomplex motor functions would be dissociated from comlpex stimulus processes (Salamone, 1992). Dopamine would also decrease striatal output to the ventral pallidum and onwards to the thalamocortical pathways. Striatal DA functioning mediated by the D_1 and D_2 receptors can cause a decrease of spontaneous firing activity of the NA - ventral pallidal neurons (Mogenson et al., 1988). DA input will also excite ST neurons that coexpress GABA/Substance P. These neurons project from the ST to the SN. However, DA input will inhibit ST neurons that coexpress GABA/ENK and project to the globus pallidus (Meredith et al., 1993; Angulo and McEwen, 1994). Behaviorally, the dorsal striatal, mostly caudate-putamen, dopaminergic system plays a role in eating behavior, skilled responses, and motor activation (Salamone, 1992; Saigusa et al., 1993).

The ventral striatal dopaminergic system, which includes the nucleus accumbens, acts in reward and stimuli related behaviors (Robbins and Everitt, 1992). For example, in a conditioned visual discrimination task (learning to distinguish between visual stimuli in order to receive a reward), a lesion within the nucleus accumbens results in a loss of behavioral extinction. This means the behavior would not cease even long after the reward was removed (Robbins and Everitt, 1992). However, lesions within the dorsal striatum had no effect on behavioral extinction.

The PFC has dopaminergic connections to both the ST and NA. Meredith et al.(1993) have shown that DA can decrease the excitatory inputs from the PFC, AM and thalamus and can also decrease the spontaneous activity of neurons in the nucleus accumbens. The PFC has a direct DA projection to the NA with lateral projections to both the VTA and SN (Bunney and Aghajanian, 1976; Beckstead, 1979; Bachneff, 1991; Murase et al., 1993). Studies have conclusively shown that changes in PFC dopamine turnover will result in changes in NA dopamine turnover (Phillipson and Griffiths, 1985). Garris and Wightman (1994) examined extraneuronal dopamine in the PFC, NA, ST, and AM through stimulation of the ascending fibers of the medial forebrain bundle. Results showed that these regions had different rates of DA release and reuptake. The PFC and AM had the same rates of release and reuptake and were implicated in a long range transfer of chemical information through extraneuronal transmission. Extraneuronal transmission involves the quantity of dopamine left in the synapse after neuronal firing. Some brain regions, such as the PFC, are more " release dominated" and will allow a larger quantity of DA to remain in the synapse than a " uptake dominated" region like the accumbens. In the NA, increased frequency of stimulation led to an increased level of DA not due to an increase in firing rate but rather to a lack of time between stimulations leading to an overloading of the DA carriers involved in reuptake. Therefore, there was an elevated level of extraneuronal DA that was free to diffuse and act on other receptors.

In the NA the synaptic buttons that release glutamate originate in the PFC (Godukhin, 1984). This excitatory amino acid induced release of DA appears to be modulated by glutamatergic afferents from the frontal cortex since frontal cortex ablation causes an increase in DA release within the NA. Glutamate injected into the PFC increases burst firing of DA cells in

the VTA, enhances DA release in the NA, and will block the spontaneous activity of DA neurons in the VTA (Murase et al., 1993).

Neurotensin, often described as an endogenous neuroleptic, appears to interact with DA at the accumbal level which stimulates behavioral outcomes. The highest levels of neurotensin in the brain are found in the nucleus accumbens, substantia nigra, medial prefrontal cortex and amygdala (Stowe and Nemeroff, 1991; Kasckow and Nemeroff, 1991). NT appears to have a direct interaction with the dopamine system at the anatomical, physiological, and behavioral level (Kasckow and Nemeroff, 1991; Stowe and Nemeroff, 1991; Merchant and Dorsa, 1993). As a member of the neuropeptide family with the enkephalins, neurotensin is part of the control mechanisms over motor activity and stereotypy that result from DA imbalances in the nigrostriatal pathway (Angulo and McEwen, 1994). NT has been shown to block behaviors associated with dopaminergic activation (Kalivas et al., 1983; Jolicoeur et al., 1985; 1993). Drumheller et al. (1990) found that i.c.v. injection of neurotensin increases the concentration of dopamine and its metabolites in the projection fields of the dopaminergic pathways of the brain, the nigrostriatal and mesolimbic. Neurotensin increases the turnover of dopamine in the ST, NA, AM, VTA, and SN (Kasckow and Nemeroff, 1991). NT is colocalized with many mesencephalic DA neurons within the SN and VTA and NT receptors have been found to be closely associated with areas such as the NA and ST containing dopamine cell bodies and fibers (Kalivas et al., 1983). Merchant and Dorsa (1993) found increased gene expression coding for neurotensin in the striatum and nucleus accumbens shell after administration of atypical antipsychotics.

The enkephalins are part of the larger group of neuropeptides that are implicated in the functioning of the ST and NA. Within the ST, neuroleptics cause elevated levels of enkephalin as

do 6-OHDA lesions, suggesting that DA functions as an inhibitor of ENK coexpression (Stinus et al., 1992). ENK containing fibers and DA fibers have a similar distribution and are closely associated in the NA and VTA (Stinus et al., 1992). Enkephalin analogues injected into the VTA increase extracellular dopamine levels in the NA and can cause an increase in motor activity (Kalivas and Duffy, 1990). Behaviorally, opiates injected directly into the VTA cause an increase in hyperactivity, sniffing, licking, and rearing and this increase is reversed by injecting naloxone or a dopamine antagonist (Stinus et al., 1992). Koob (1992) and colleagues have demonstrated that there is a strong opioid pathway within the ST and NA that involves the interaction of DA, GABA, and the enkephalins . Rats will self-administer drugs that have a high potential for abuse, such as cocaine, heroin or amphetamines, directly into both the NA and VTA. DA is heavily implicated in this self-administration pathway because 1) 6-OHDA lesions of the NA decrease the reinforcing properties of cocaine and amphetamines and 2) dopamine release is increased while the rats are self-administering (Roberts et al., 1980; Koob, 1992).

9. Behavior Studies

The NA and ST are parts of the limbic-cortico-striato-pallidal system which are implicated in the performance of many behaviors. The striatum is linked behaviorally with motor activity and stereotypy and the NA with reinforcement, hyperactivity, reward and drug self-administration (Angulo and McEwen, 1994). The NA is like a grouping of neuronal bundles each associated with a specific behavior or function. NA stimulation triggers the appearance of select behaviors that each have their own specific projections.

The suspected role of dopamine in these neuronal groupings of the NA is to weaken competitive action between the ensembles. This is done by a presynaptic decrease of excitatory inputs to these neuronal groupings. In the same presynaptic manner, DA can amplify the input thereby increasing the stimulated behavior or can decrease input to prevent activation of one behavior over another (Pennartz and Lopes da Silva, 1994).

Microinjections of various neurochemicals directly into the NA or one of its major afferents or efferent sites are much researched (Essman et al., 1993). Studies have also been done using electrical stimulation or lesioning of the NA. A common working model which will arise often is the 6-OHDA model. 6-hydroxydopamine is a selective neurotoxin which destroys dopaminergic neurons and is a depletor of tyrosine hydroxylase (Roberts et al., 1980; Koob, 1991). For example, 6-OHDA lesion of the NA blocks the increase in hyperactivity induced by amphetamine and cocaine (Koob et al., 1991). The NA is differentially affected by 6-OHDA. The shell neurons are resistant to the toxin and the core neurons and the caudate-putamen are destroyed (Essman et al., 1993).

Though animal studies cannot reflect all the complexities of human psychosis, administration of central nervous system stimulants such as amphetamine in animals produces a behavioral profile which mimics certain symptoms of schizophrenia such as motor dysfunctions and paranoid or delusional thoughts (Nemeroff and Bissette, 1988; Grace, 1991; Lyon, 1991). The ability of amphetamines to induce a pseudo-psychosis in humans has added a lot of support to the dopamine hypothesis, as has the ability of antipsychotic drugs to reverse the effects of amphetamine psychosis (Nemeroff and Bissette, 1988; Lee, 1988; Cunningham and Kelley, 1992). Amphetamine use has been shown to elicit symptoms such as: excessive startle, aborted and

fragmented behavior, dystonia, ataxia, and apparent hallucinations (Lyon, 1991). Treatment with haloperidol immediately stopped the hallucinatory behavior and motor dysfunctions. Pre-amphetamine injections of antipsychotic drugs has also prevented the development of amphetamine psychosis (Haracz et al., 1993; Leduc and Mittleman, 1995). CNS stimulants like amphetamines act by increasing DA release or inhibiting DA uptake (Lyon, 1991; Koob, 1992). DA releasers have their site of action at DA nerve endings and DA uptake blockers like cocaine and nomifensine increase extraneuronal DA (Wood and Altar, 1988).

9.1 Microinjection Studies with Dopamine

Initial injection studies found that direct accumbal injections of DA increased motor activity in rats (Costall and Naylor, 1975). This effect was also seen microinjecting apomorphine and low doses of amphetamines. These low doses caused an increase in motor activity, sniffing, and rearing and as the dose was increased motor activity and rearing decreased such that only sniffing was left along with licking and biting. 6-OHDA lesions in the VTA prevented this induced hyperactivity.

Pijnenburg et al.(1976) examined the effects of many chemicals microinjected into the NA on motor activity. In its normal state, the NA contains a high level of DA followed by moderately high levels of ACh with low levels of 5-HT (Jones et al., 1980). DA (5 or 10 μg) was injected bilaterally into the NA and caused a strong stimulation of motor activity such as running, rearing, and sniffing. Injections of 5-HT in the same rats produced no effect. Injections of DA metabolites, DOPAC and HVA, also had no effect on motor activity. Pretreatment with the dopamine

antagonist haloperidol reduced motor activity. D-ampheatmine produced hyperactivity and the cholinergic agonist carbachol caused hyperactivty and convulsions.

Similarly, Essman et al.(1993) studied the effects of different DA receptor agonists in the dorsal striatum and NA on locomotor activity. Results sound that neither SKF 38393 (D_1 agonist) or quinpirole (D_2 agonist) was sufficient alone to produce hyperactivity; only a combination of both could induce hyperactivity. D-amphetamine was one of the strongest stimulators of hyperactivity in rats. The D_1 and D_2 receptors activate turning behavior in a specific way. The D_2 specific agonist quinpirole can induce dose dependent turning behavior as can a mixed D_1 D_2 agonist but a D_1 selective agonist like SKF 38393 cannot induce turning behavior in the nucleus accumbens following unilateral injection (Saigusa et al., 1993).

Picrotoxin, a GABA antagonist, injected into the VTA will increase motor activity which can be decreased by injecting GABA into the ventral pallidum (Mogenson et al., 1980). Koob et al. (1991) found that low doses of muscimol, a GABA agonist, caused a dose dependent decrease in motor activity. So it appears that hyperactivity induced by DA stimulation in the NA is mediated by a GABAergic inhibition in the ventral pallidum.

Staunton et al. (1982) discovered that denervation of the DA projection to the NA caused a surprising increase in motor activity after injection of direct dopamine agonists. The conclusion for this unusual finding was that increase motor activity was due to an increase in postsynaptic receptor supersensitivity. Koob et al. (1991) also found that after administration of 6-OHDA within the basal forebrain there was a supersensitivity to apomorphine, amphetamine, and heroin in the NA which caused hyperactivity in rats.

Costall et al. (1991) conducted a unique behavioral study using the 5-HT_3 receptor antagonists ondansetro and zacopride. In this study, amphetamine was directly injected into the NA and hyperactivity developed within about ten minutes. Once there was a significant level of hyperactivity the 5-HT_3 receptor antagonists were directly administered into the NA. Results showed there was a decrease in motor activity back to the baseline level, unlike the more classic neuroleptics which will often decrease motor activity to below normal levels. The 5-HT_3 receptor agonist 2-methyl-5HT was also shown to enhance the increase in amphetamine induced motor activity. A second experiment involving a continuous infusion of DA into the NA for thirteen days showed that the phasic peaks of hyperactivity could be inhibited by antipsychotics, atypical neuroleptics, and by the 5-HT_3 receptor antagonists. Overall, it appears that the 5-HT_3 antagonists can return DA levels to normal in the mesolimbic DA system. This would be accomplished through 5-HT's excitatory effect on the cells of the VTA which would initiate DA cell firing. These 5-HT_3 receptors are not widespread. They are mostly located in the AM, hippocampus, and limbic cortex.

9.2 Microinjection studies with GABA

GABA, the other major player in the mesolimbic dopamine system, has also been used to assess the role of NA in motor activity. $GABA_A$ and $GABA_B$ receptors are located on DA nerve endings in the ST and on DA cell bodies and afferents in the SN (Wood and Altar, 1988). Mogenson et al.(1988) injected the $GABA_A$ receptor antagonist picrotoxin within the hippocampal-accumbal projection and found an increase in motor activity. Baclofen, a $GABA_B$ agonist, injected in the amygdalar-accumbal projection region caused an initial decrease in motor

activity that was followed by a subsequent increase in motor activity. High doses of GABA reduced motor activity and low doses caused an increase in responsiveness to the concentration of GABA reaching the receptors. Pycock and Horton (1979) induced a hyperactive state through intra-accumbal injection of dopamine to study GABA's effects. Results showed that high concentrations of GABA agonists decreased hyperactivity and GABA antagonists increased dopamine's stimulatory effect.

9.3 Microinjection studies with Neurotensin

Neurotensin injections in the VTA caused hyperactivity and increased DA release and DA metabolites in the NA (Kalivas et al., 1983). Neurotensin injected directly into the NA decreased the hyperactivity induced by DA agonists (Gregory et al., 1981). An animal model of Parkinson's disease in rats was developed and tested in a laboratory (Jolicoeur et al., 1991b and 1991c.). A 6-OHDA lesion in the medial forebrain bundle close to the hypothalamus resulted in the appearance of the three main signs: rigidity, hypokinesia, and trembling. Administration of apomorphine and L-Dopa reversed the severity of the three symptoms. Neurotensin was also found to have some anti-parkinsonian effects when injected i.c.v. in that NT appeared to decrease the muscular rigidity and the trembling of rats.

Chronic neuroleptic treatment increases the neurotensin content in both the NA and ST (Kasckow and Nemeroff, 1991). NT, behaviorally and biochemically, shares many of the characteristics of antipsychotic drugs (Jolicoeur et al., 1991, 1993). Jolicoeur et al. (1991 and 1993) have found that neurotensin has a similar pharmacological profile as the atypical

antipsychotics. Both neurotensin and atypical antipsychotics will increase DA turnover, decrease motor activity, and block dopaminergic agonist induced hyperactivity.

Jolicoeur et al. (1983, 1985) examined the effects of NT on behaviors elicited by small doses of dopamine agonists in the NA. ICV administration of NT inhibits penile erection, yawning, and hypoactivity induced by small doses of apomorphine . High doses of dopamine agonists were also examined. I.C.V. administration of NPA (N-n-propylnorapo-morphine) produced hyperactivity and stereotyped behavior. NT injected ICV significantly reduced the hyperactivity but had no effect on stereotypy (Jolicoeur et al., 1982, 1983). Results have also shown that NT decreased the hyperactivity elicited by direct DA administration into the NA. NT injected into the NA also blocked hyperactivity induced by self administration of amphetamine and cocaine in rats (Kalivas and Duffy, 1990).

10. 6,7-ADTN

6,7-ADTN (2-amino-6,7-dihydroxy- 1,2,3,4-tetrahydronaphthalene) is a rigid analogue of dopamine in its extended form and stimulates motor activity for up to fifteen hours when injected into the nucleus accumbens (Woodruff et al., 1977). 6,7-ADTN has no *in vivo* activity if injected peripherally because it cannot cross the blood brain barrier and it is too rapidly metabolized in the periphery (Antonian et al., 1986; Andersen and Jansen, 1990) . Behaviorally, 6,7-ADTN injected directly into the striatum can enhance rotation and sniffing (Antonian et al., 1986).

ADTN binding can be displaced by DA receptor antagonists such as sulpiride, an atypical neuroleptic specific for DA receptors, which binds to the same dopamine receptors but at different sites on the receptor (Woodruff et al., 1984). Westerink et al.(1980) administered a type of

6,7-ADTN precursor to trace the path of ADTN and found **(1)** there is a constant brain level of 6,7-ADTN up to ten hours after administration **(2)** this non-susceptibility to degradation explains its long term activation of motor activity and **(3)** 6,7-ADTN accumulates in DA rich areas of the brain.

Jolicoeur et al.(1985) studied the *in vivo* effects of 6,7- ADTN. ADTN was directly injected into the nucleus accumbens and produced a strong and continuous hyperactivity in rats. Motor activity level was measured immediately before, and 60, 120, 140, 160, and 180 minutes after injection of ADTN. Motor activity level reached peak significance 240 minutes after injection of ADTN. A unique result in this experiment was that the hyperactivity appeared to be suspended, reaching significance 60 minutes after injection but reaching a maximum level 240 minutes post injection. Other dopamine agonists that are less potent have produced increases in motor activity within thirty minutes after injection (Mogenson et al., 1988).

In order to explain Jolicoeur's unusual finding a preliminary experiment was conducted to examine possible neurochemical correlates of the increased motor activity. Analyses revealed that immediately following injections of ADTN, dopamine was increased in the nucleus accumbens, a finding that was incompatible with the behavioral inactivation observed. However, it was also noted that in one terminal region of dopamine fibers, the prefrontal cortex, there was a slight but significant increase in 5-HT, a transmitter known to have an inhibitory effect on motor activity. Further experiments are required to examine in detail the time course and regional specificity of the neurochemical changes in rats following bilateral intra-accumbal and intra-striatal injections of ADTN.

Purpose of the study

Although there is a lot of anatomical, behavioral, and pharmacological data on the dopaminergic systems in the brain, the interaction of dopamine with other neurotransmitters within dopaminergic brain areas is restricted. This study was designed to examine the time course and regional specificity of neurochemical changes in the rat brain after intra-accumbal and intra-striatal injections of 6,7-ADTN. These two regions were chosen for many reasons.

1. The nucleus accumbens and striatum represent the major terminal fields of the mesolimbic and nigrostriatal dopamine pathways.

2. Antipsychotic medications are assumed to establish their effect within the accumbens and striatum to decrease the motor and cognitive dysfunctions so prominent in schizophrenia.

By injecting the dopamine agonist 6,7-ADTN we are hoping to uncover the neurochemical changes in dopamine and serotonin, not only in the accumbens and striatum, but also in their afferents and efferents. The goals of the study are 1) To examine the temporal time course of neurochemical changes within diverse brain regions following a strong pharmacological stimulation of the dopamine receptors in the nucleus accumbens. 2) To examine the specificity of these effects by then examining the neurochemical changes following dopaminergic receptor stimulation in a non limbic region such as the striatum.

Materials and Methods

1 experiment I & II : nucleus accumbens and striatal animals

Male hooded rats obtained from the Canadian Breeding Farm and weighing approximately 250g were used (N=8 per group) They were individually housed in a temperature controlled room having a twelve hour light/night cycle. Experiments were conducted only during the light cycle. Food and water were available ad libitum. Under anaesthesia produced by a ketamine (80mg/kg) and xylazine (12mg/kg) combination, rats were implanted bilaterally with 11mm 23 ga stainless steel indwelling guide cannulae using the following coordinates for the nucleus accumbens: A.P. 1.7, D.V. 5.0, and L ±1.9, so that the tips were resting 2 mm above the nucleus according to the stereotaxic coordinates of Paxinos and Watson (1986). For the striatum the following coordinates were used: A.P. 1.7, D.V. 2.5, and L ±2.5. Cannulae were fixed to the skull with dental cement. Protective stainless steel 30ga stylets were kept in the guide cannulae until the time of injection.

2 Procedure for experiments I and II

After four days of recovery from surgery, animals were randomly assigned to one of three groups 1) a control group that received only saline injections 2) an experimental group that received injections of 6,7-ADTN and was decapitated after 60 minutes and 3) an experimental group that received 6,7-ADTN was was decapitated after 120 minutes. The experimental groups were given either bilateral intra-accumbal or intra-striatal injections of 12.5 µg of 6,7-ADTN (obtained from Sigma Chem Co, USA) and saline (0.9%) for the control group. The preliminary study done in the Jolicoeur lab (unpublished data) showed this dose to be effective in producing the increased motor activity. For preparation of the 6,7-ADTN, 100ml of distilled water was boiled for 15 minutes and then 1 g of sodium bisulphate was added to the water and mixed until

dissolved. 6,7-ADTN was then added and the mixture was removed from light until the drug could cool to room temperature before injection into the brain. Motor activity was measured immediately prior to injection and at 15 minute intervals after injection. Injections were made through a 30 ga injection needle attached to a 50.0 µl Hamilton syringe by PE-20 polyethylene tubing. All injections, 12.5 µg of 6,7-ADTN in a volume of 20 µgl, were made over a three minute period after which the injection needle was kept in place another minute to allow for complete diffusion. At each testing period, motor activity was measured for one minute with a photocell activity apparatus (Lehigh Valley Electronics). The apparatus consisted of a metal circle with a diameter of 24 inches with a wire mesh floor. The inner circle contained 12 photocell detectors placed 4 inches apart. The beams were connected to an automatic counter which records every time there is a photocell interruption. Motor activity counts were recorded as an aid to determine effective cannula placement and pharmacological activity of 6,7-ADTN. Due to the nature of the brain dissection method histological verification of cannula placement is not possible. Animals were sacrificed according to their appropriate post injection times (60 or 120 minutes after 6,7-ADTN injection). The rats were decapitated and the brains were rapidly removed and placed in a dissection block on ice according to the method of Heffner et al. (1980). The following areas were removed for neurochemical analysis after intra-accumbal and intra-striatal ADTN injection: prefrontal cortex, nucleus accumbens, striatum, amygdala, substantia nigra, and ventral tegmental area. Regions were frozen at -80°C until analysis. This study was focused on the underlying neurochemical changes underlying pharmacological stimulation in the accumbens and striatum not the changes in behavior.

3 Neurochemical Analysis for experiment I and II

Tissue samples were weighed on an electronic scale. The tissue was disrupted by means of sonication, for about 20 seconds using the ultrasonic cell disrupter Microson XL, in 400 µl of cold perchloric acid 0.1M $HCLO_4$, for the NA, ST, SN, and VTA and in 1000 µl of cold perchloric acid for the PFC and AM. The brain samples were then centrifuged (refrigerated microlitre centrifuge, model Hermle Z 252 MK purchased from Berthold Hermle AG) at a speed of 15000 rpm for twenty minutes. The clear supernatants were filtered, using cameo microfilters, collected into 1.5 ml Eppendorf tubes and frozen at -80 degrees until needed. The neurochemical analysis was done by high performance liquid chromatography (HPLC) coupled to an electrochemical detector. The HPLC was from Beckman Instruments and had a 110 pump, Beckman model 112, an injector with a 100 µl sample loop, Beckman model 210, and a 10 cm ODS 3 µ reverse phase column, from Chromatographic Sciences Co., to separate the amines. The column eluate was monitored by an electrochemical detector dual electrode model BD 40 from Bioanalytical Systems.

The mobile phase for the neurochemicals consisted of a primary eluting solvent of 100 ml of sodium acetate (0.1M) mixed with 240 µl of sodium octylsulphate and 70 ml of methanol (7% of total mobile phase volume). The total volume was then brought to 1000 ml with distilled water. The pH level of the solution was adjusted to 4.6 with HPLC grade acetic acid. The mobile phase was then filtered through 0.2 µ nylon membranes (Ultipor, Chromatographic Specialities Co.) and degassed for fifteen minutes. Injection volumes for standards and brain sample extracts remained constant at 80 µl with a flow rate of 1.2 ml per minute. All the standard amines and metabolites were purchased from Sigma Chemical. The concentration of the neurochemicals was determined

by comparing sample peak heights to standard peak heights obtained following injection of known levels of DA, DOPAC, HVA, 5-HIAA, and 5-HT and converting them to ng/mg wet weight. Results will be expressed as the ratios of DOPAC/DA, HVA/DA, and 5-HIAA/5-HT found in each region rather than the actual amine concentration. The use of the metabolite/transmitter ratios provides a highly reliable and non-invasive measure of neurotransmitter utilization (Bannon and Roth, 1983; Sharp et al., 1986).

4 Statistical Analysis

Results on ratios of each metabolite to its corresponding amine were analysed by individual one way ANOVAs for each metabolite. Levels of the main factor included in the analysis were controls, animals tested at 60 minutes, and animals tested at 120 minutes. Individual comparisons between controls and each of the treated animal groups (60 and 120 min) were assessed by a Fisher procedure. In all cases differences were considered significant if they had a probability of random occurrence of less than 5 %. Means and standard deviations for data are presented in the appendix.

Results

The results section will be divided into two parts. The first section will cover the results from the intra-accumbal administration of 6,7-ADTN. This section (number 6) will include all the figures related to these experiments. The second results section (number 7) will cover all the results and include the figures related to the experiments after intra-striatal administration of 6,7-ADTN. The figures will be bar graphs of the three metabolite ratios expressed in the control

group, 60 min post-injection experimental group, and the 120 minute post-injection experimental group. Any significant differences will be indicated by an asterisk.

5 EXPERIMENT 1

6,7-ADTN was directly injected into the nucleus accumbens. The following regions were examined for neurochemical changes: PFC, NA, ST, AM, SN, and VTA. The groups examined were 1) a control group that only received saline injections 2) a group decapitated 60 minutes after injection with 6,7-ADTN and 3) a group decapitated 120 minutes after injection with 6,7-ADTN.

5.1 Prefrontal Cortex

Within the prefrontal cortex there was a significant increase in the DOPAC/DA ratio at 60 minutes in comparison to the control ratio, $F(2,27)= 4$, $p<.05$. 120 minutes after 6,7-ADTN injection the DOPAC/DA ratio was not significantly different from the control or 60 min ratios. The HVA/DA ratio showed no significant changes at 60 or 120 min in comparison to the control ratio. There was no significant difference between the control ratio and 60 min ratio for 5-HIAA/5-HT. At 120 minutes there was a significant increase in the 5-HIAA/5-HT ratio in comparison to both the control ratio and the 60 min ratio, $F(2,27)= 6$, $p<.05$.

5.2 Nucleus Accumbens

There were no significant differences between groups in the metabolite ratios of this region. The DOPAC/DA ratio is decreased at both 60 and 120 min but is not significantly different from the control ratio. The HVA/DA ratio is low across all the time points and there is

no fluctuation. In comparison to the control ratio there are changes in the 5-HIAA/5-HT ratios, at 60 min there is a decrease and at 120 min there is an increase, but these changes are not significantly different from the control ratio.

5.3 Striatum

There were no significant differences between the control ratio and the two experimental group ratios for DOPAC/DA. There was an increase in the DOPAC/DA at 60 min that appeared to return to control level by 120 minutes but the change was not enough to reach significance. or HVA/DA ratios. The HVA/DA ratio was low in the control group and remained low at both 60 and 120 min. (see Figure 3) Within the striatum there was a significant difference in the 5-HIAA/5-HT ratio at both 60 minutes and 120 minutes compared to the control level, $F(2,27)= 4$, $p<.05$. There was an enormous increase in the 5-HIAA/5-HT ratio at 60 min followed by a large decrease in the ratio at 120 min.

5.4 Amygdala

Within the amygdala there was a significant increase in the DOPAC/DA ratio at both 60 and 120 minutes in comparison to the control ratio, $F(2,28)= 44$, $p<.05$. The DOPAC/DA ratio increased dramatically, compared to the very low ratio seen in the control group, at 60 min and remained at approximately the same level at 120 min. There was also a significant increase in the HVA/DA ratio at both 60 and 120 min in comparison to the control ratio, $F(2,28)= 5$, $p<.05$. (see Figure 5) The HVA/DA ratio is approximately the same at both 60 and 120 min. There was no significant change in the 5-HIAA/5-HT ratio at either time in comparison to the control ratio.

5.5 Substantia Nigra

There were no significant changes in the DOPAC/DA ratio in either experimental group in comparison to the control ratio. The DOPAC/DA ratio appears constant across all three groups. There was a significant increase in the HVA/DA ratio at both 60 and 120 minutes in comparison to the control ratio, $F(2,27)= 4$, $p<.05$. The HVA/DA ratio is roughly the same at both 60 and 120 min. There was also a significant decrease in the 5-HIAA/5-HT ratio at both 60 and 120 minutes in comparison to the control level, $F(2,27)= 4$, $p<.05$. There is a substantial decrease, compared to the control ratio, in the 5-HIAA/5-HT ratio at 60 min which appears to stay at about the same by 120 min.

5.6 Ventral Tegmental Area

Within the ventral tegmental area there were no significant metabolite ratios in comparison to the control ratio. The DOPAC/DA ratio appears to be constant across the three time points. The HVA/DA ratio in control animals is larger than both the 60 and 120 min ratios but the decrease in HVA/DA ratio is not significant. The 5-HIAA/5-HT ratio is increased at 60 min and is even higher at 120 min but neither of these groups differ significantly from the control ratio or each other. (see Figure 1 through Figure 8 for corresponding bar graphs)

Neurochemical analysis after intra-accumbal stimulation with 6,7-ADTN

This study found that stimulation of the mesolimbic dopamine terminal regions caused long lasting and widespread neurochemical changes within select brain areas.

There are several specific results to discuss concerning select brain regions but there is also an overall point to be raised. Results showed that there were neurochemical changes all over the

brain after intra-accumbal injections of 6,7-ADTN. This is a critical issue because there is often a misunderstanding of what happens when a drug is injected into a specific brain region. As shown within this study, drug injections can have far reaching and long term effects on the neurochemistry of brain regions that can be quite distant from the site of injection. It has already been established by Westerink et al. (1980) that these changes are not due to the diffusion of the drug because ADTN remained at a constant level at the site of injection and was not susceptible to degradation.

The neurochemical analysis of specific brain regions yielded some surprising results (see Tables 5). Looking first at the data obtained from the accumbal injections of 6,7-ADTN results show no significant differences in any of the amine to metabolite ratios within the nucleus accumbens itself. Preliminary studies had shown increased DA levels in the accumbens immediately following ADTN injection but the time points measured within this study were one and two hours post- injection. These later times were used to better delineate the time course of the changes since a pilot study had already looked at earlier time points. There is a visible downward trend from control in the DOPAC/DA ratio at both 60 and 120 minutes post-injection that was not significant. One possible explanation is the large variability between the animals which makes it very difficult to reach significance unless a very large number of animals are included in the study. Within the VTA , which is considered the major center of dopaminergic cells projecting to the accumbens, there is no change within any of the ratios. However, in the SN which is more classically associated with the nigrostriatal pathway, results show an increase in the HVA/DA ratio at both 60 and 120 minutes and a decrease in the 5-HIAA/5-HT ratio at both 60 and 120 minutes. There are two interesting points to be gained from these findings. 1) The

anatomical projections of the dopaminergic pathways are not exclusive to each other and 2) serotonergic activity in the SN, which normally has an inhibitory effect on DA, is decreased thereby increasing the functional activity of dopaminergic neurons which is seen as the elevated HVA/DA ratio. This inhibitory effect of 5-HT activation on dopaminergic activity is also seen in the PFC. At 60 minutes there is an increase in the DOPAC/DA ratio which is gone at 120 minutes probably due to the simultaneous increase in serotonergic activity seen at 120 minutes.

In the ST which is the major efferent of the SN we see no change across the DA ratios but an increase in serotonergic activity at both 60 and 120 minutes after injection of ADTN. As discussed previously, the SN and ST dopamine connection is one of the major dopaminergic pathways in the brain so expectations would be that increased dopaminergic activity seen in the SN at both 60 and 120 minutes would lead to an increase in either DA turnover (the DOPAC/DA ratio) or neuronal functional activity (the HVA/DA ratio) but there was no change. Once again the most likely explanation appears to be the increase in 5-HT activity at both 60 and 120 minutes which would inhibit any increases in the DA ratios.

The AM which constitutes part of the limbic system along with the NA is the only region to show increased DA turnover and functional activity at both 60 and 120 minutes after injection of a DA agonist into the accumbens. There was no change in 5-HT activity at either of the time points in this region.

Overall, after injection of ADTN into the NA results showed increases in DA functional activity in both the AM and SN and an increase in DA turnover in the AM. 5-HT could have played a large role in DA inhibition shown as increase in the 5-HIAA/5-HT ratio within the PFC

and ST. No neurochemical changes were seen within the NA (the injection site) or the VTA (the NA's major afferent.

Table 5

Neurochemical changes in the nucleus accumbens

Increases in amine ratios are indicated by (+) and decreases are indicated by (-). No change is an empty cell.

NUCLEUS ACCUMBENS

	DOPAC/DA		HVA/DA		5-HIAA\5-HT	
	60min	120min	60min	120min	60min	120min
PFC	+					+
NA						
ST					+	+
AM	+	+	+	+		
SN			+	+	-	-
VTA						

Figure 1

Mean metabolite to amine transmitter ratios found in the prefrontal cortex (PFC) presented for the different groups of the experiment: control animals and animals sacrificed at 60 and 120 minutes following ADTN administration into the nucleus accumbens (NA). The DOPAC/DA, HVA/DA, and 5-HIAA/5-HT ratios are illustrated by diagonal stripes, vertical stripes, and open columns respectively. Significant differences ($p<0.05$) between control and experimental animals are indicated by an asterisk.

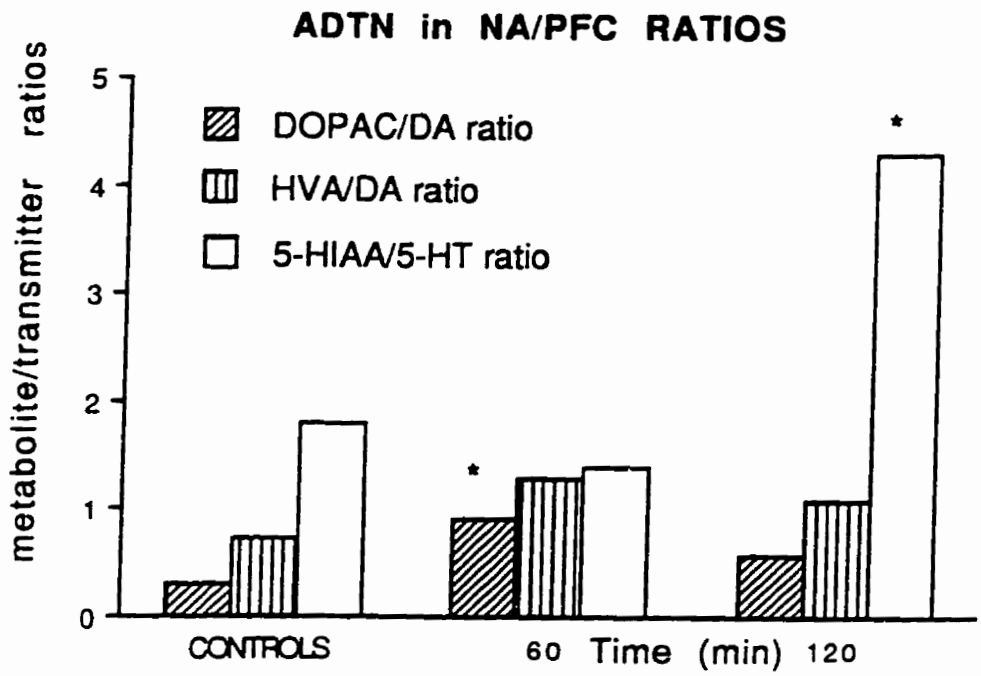

ADTN in NA/PFC RATIOS
metabolite/transmitter ratios
5
4
3
2
1
0
DOPAC/DA ratio
HVA/DA ratio
5-HIAA/5-HT ratio
*
*
CONTROLS
60
Time (min)
120

Figure 2

Mean metabolite to amine transmitter ratios found in the nucleus accumbens (NA) presented for the different groups of the experiment: control animals and animals sacrificed at 60 and 120 minutes following ADTN administration into the nucleus accumbens (NA). The DOPAC/DA, HVA/DA, and 5-HIAA/5-HT ratios are illustrated by diagonal stripes, vertical stripes, and open columns respectively. There are no significant differences between the control and experimental animals.

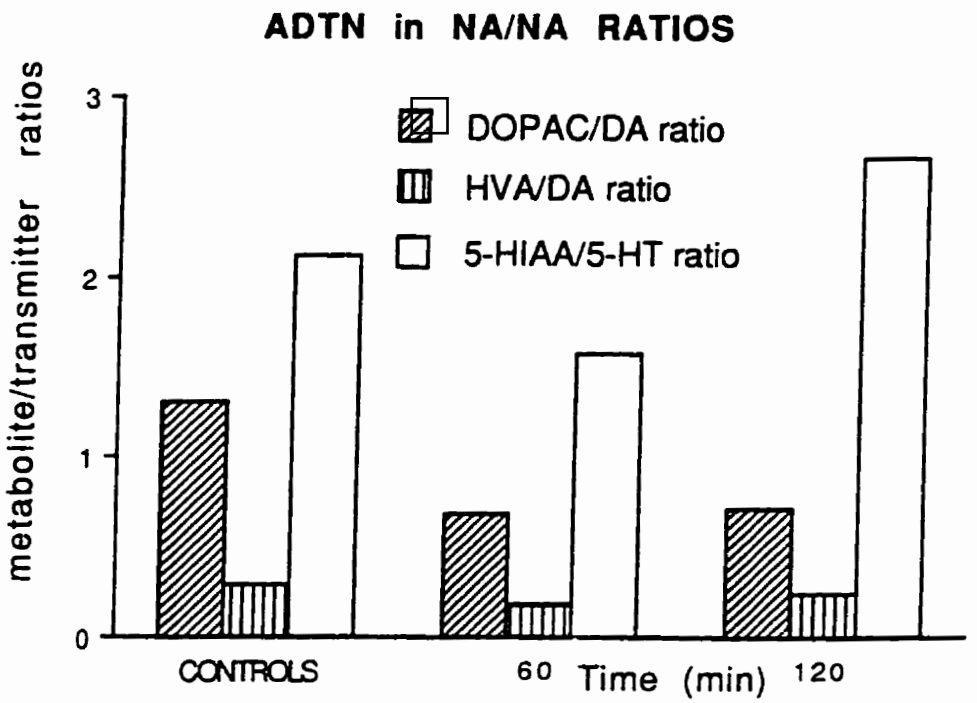

ADTN in NA/NA RATIOS
metabolite/transmitter ratios
3
2
1
0
DOPAC/DA ratio
HVA/DA ratio
5-HIAA/5-HT ratio
CONTROLS
60
Time (min)
120

Figure 3

Mean metabolite to amine transmitter ratios found in the striatum (ST) presented for the different groups of the experiment: control animals and animals sacrificed at 60 and 120 minutes following ADTN administration into the nucleus accumbens (NA). The DOPAC/DA and HVA/DA ratios are illustrated by diagonal stripes and vertical stripes respectively. There are no significant differences between the control and experimental animals.

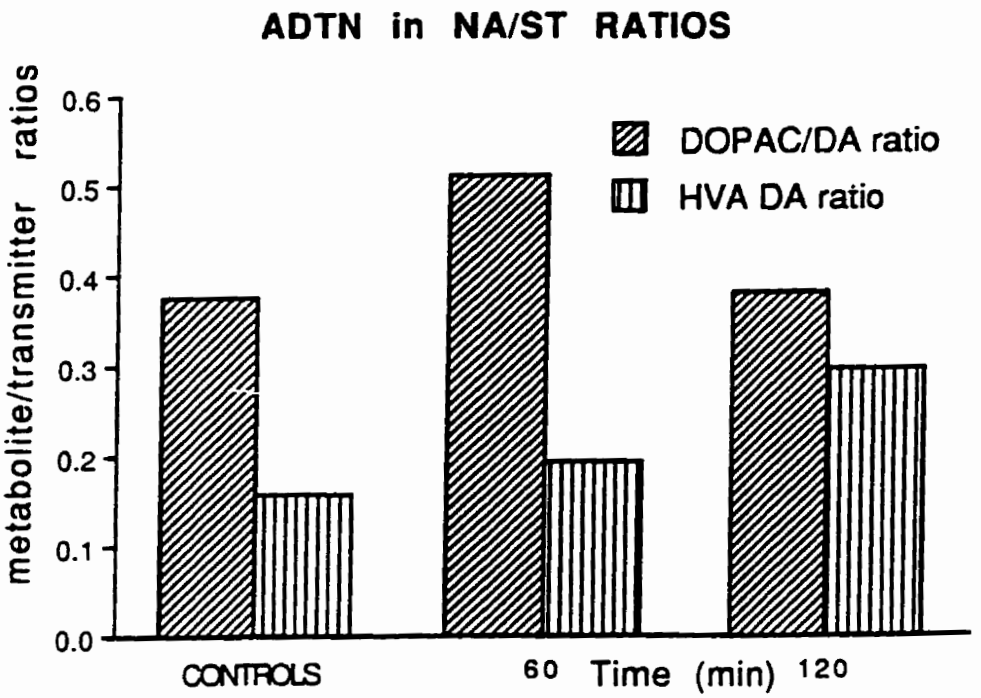

ADTN in NA/ST RATIOS
metabolite/transmitter ratios
0.6
0.5
0.4
0.3
0.2
0.1
0.0
DOPAC/DA ratio
HVA DA ratio
CONTROLS
60
Time (min)
120

Figure 4

Mean metabolite to amine transmitter ratios found in the striatum (ST) presented for the different groups of the experiment: control animals and animals sacrificed at 60 and 120 minutes following ADTN administration into the nucleus accumbens (NA). The 5-HIAA/5-HT ratios are illustrated by open columns. Significant differences ($p<0.05$) between control and experimental animals are indicated by an asterisk.

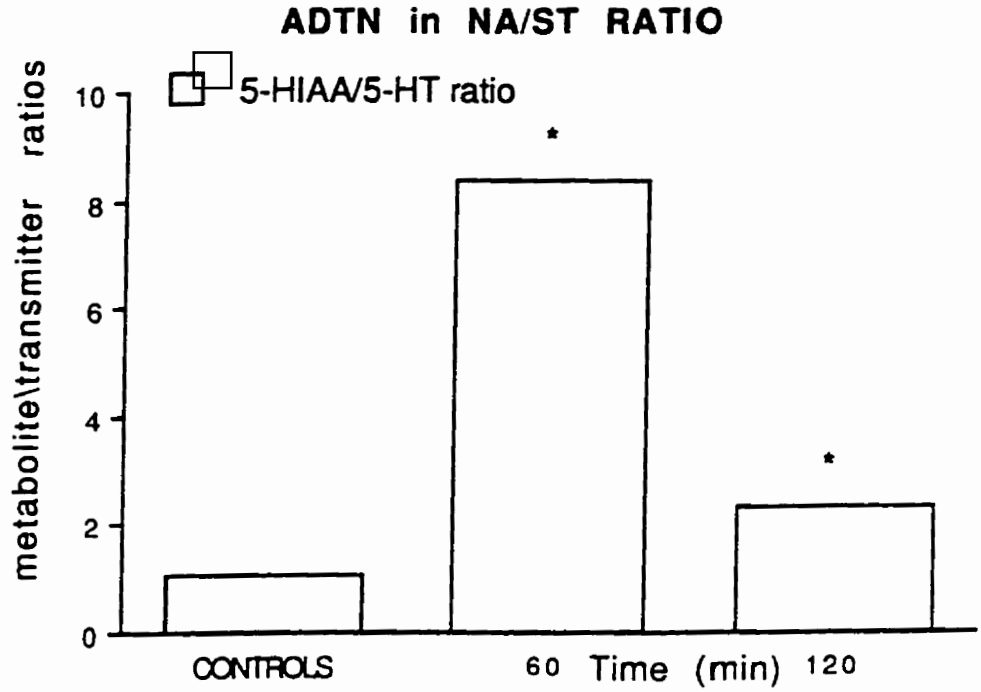

ADTN in NA/ST RATIO
5-HIAA/5-HT ratio
metabolite\transmitter ratios
10
8
6
4
2
0
*
*
CONTROLS
60
Time (min)
120

Figure 5

Mean metabolite to amine transmitter ratios found in the amygdala (AM) presented for the different groups of the experiment: control animals and animals sacrificed at 60 and 120 minutes following ADTN administration into the nucleus accumbens (NA). The DOPAC/DA and HVA/DA ratios are illustrated by diagonal stripes and vertical stripes respectively. Significant differences ($p<0.05$) between control and experimental animals are indicated by an asterisk.

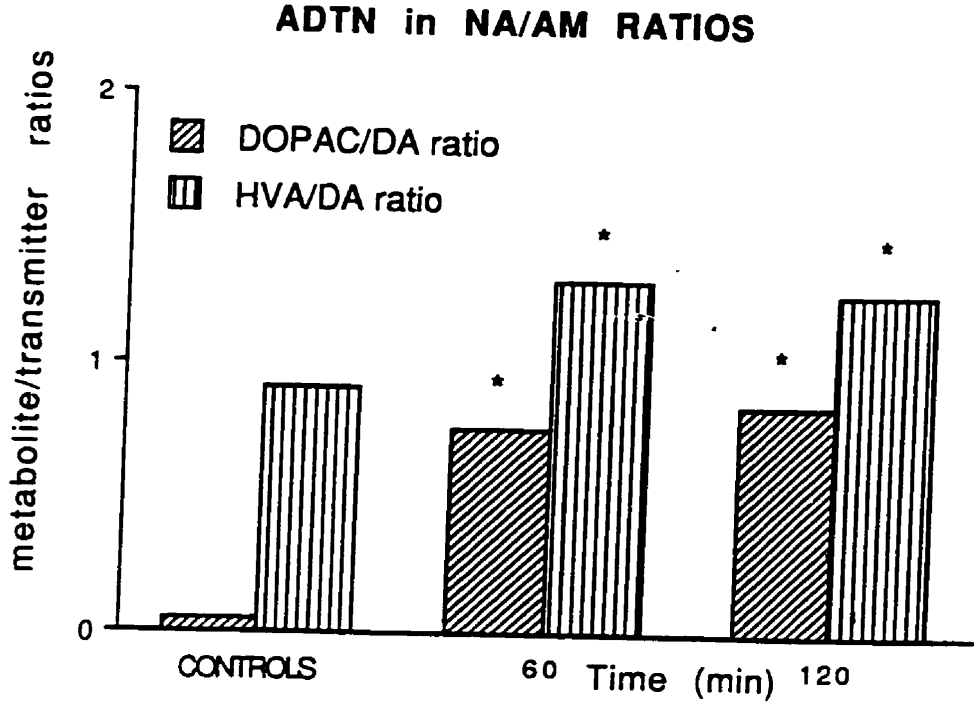

ADTN in NA/AM RATIOS
metabolite/transmitter ratios
2
1
0
DOPAC/DA ratio
HVA/DA ratio
CONTROLS
60
Time (min)
120

Figure 6

Mean metabolite to amine transmitter ratios found in the amygdala (AM) presented for the different groups of the experiment: control animals and animals sacrificed at 60 and 120 minutes following ADTN into the nucleus accumbens (NA). The 5-HIAA/5-HT ratios are illustrated by open columns. There were no significant differences between control and experimental animals.

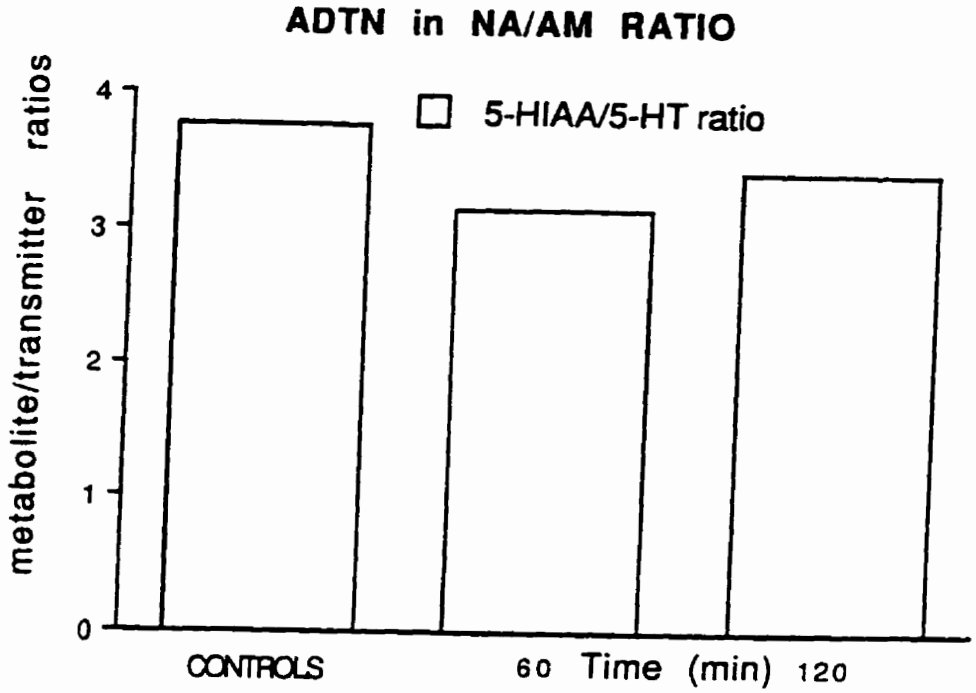

ADTN in NA/AM RATIO
5-HIAA/5-HT ratio
metabolite/transmitter ratios
4
3
2
1
0
CONTROLS
60
Time (min)
120

Figure 7

Mean metabolite to amine transmitter ratios found in the substantia nigra (SN) presented for the different groups of the experiment: control animals and animals sacrificed at 60 and 120 minutes following administration of ADTN into the nucleus accumbens (NA). The DOPAC/DA, HVA/DA, and 5-HIAA/5-HT ratios are illustrated by diagonal stripes, vertical stripes, and open columns respectively. Significant differences ($p<0.05$) between control and experimental animals are indicated by an asterisk.

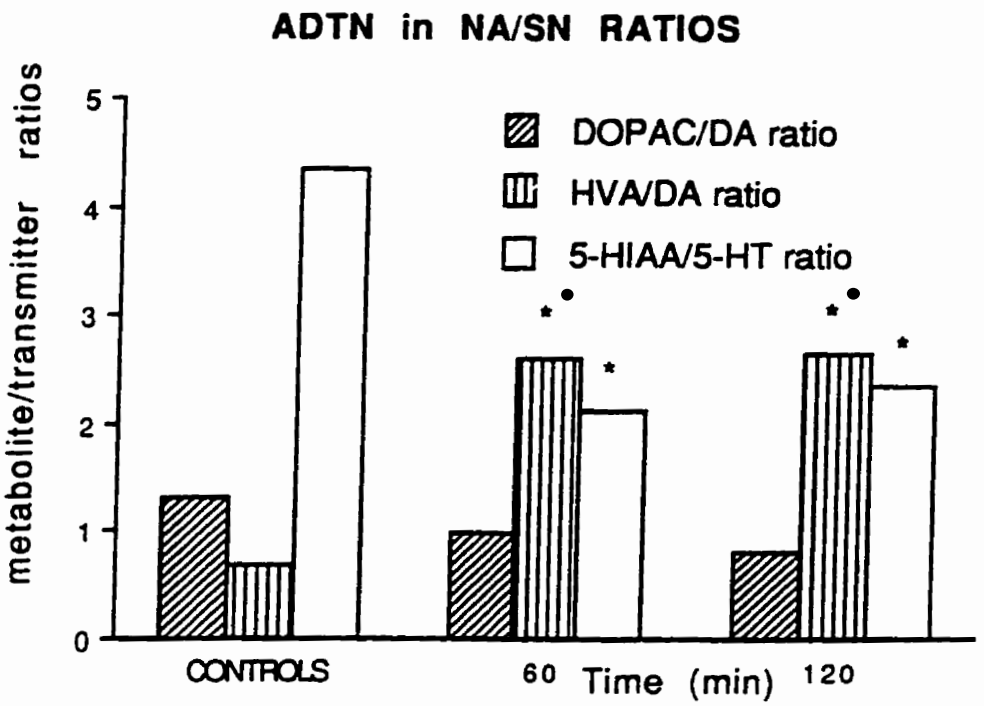
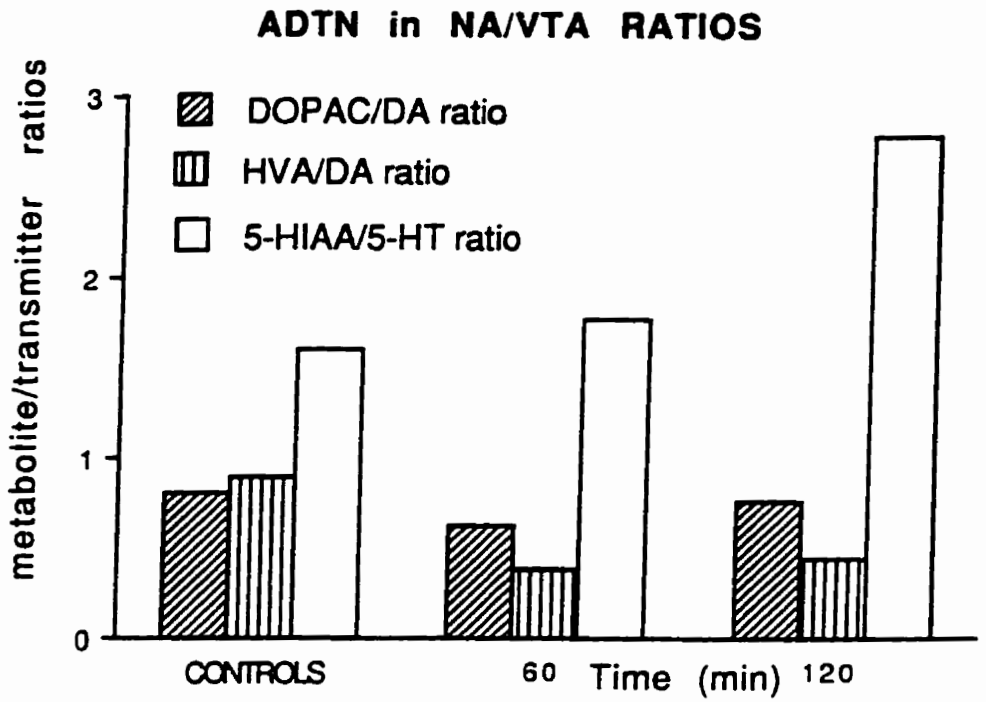

ADTN in NA/SN RATIOS
metabolite/transmitter ratios
5
4
3
2
1
0
DOPAC/DA ratio
HVA/DA ratio
5-HIAA/5-HT ratio
CONTROLS
60
Time (min)
120

Figure 8

Mean metabolite to amine transmitter ratios found in the ventral tegmental area (VTA) presented for the different groups of the experiment: control animals and animals sacrificed at 60 and 120 minutes following ADTN administration into the nucleus accumbens (NA). The DOPAC/DA, HVA/DA, and 5-HIAA/5-HT ratios are illustrated by diagonal stripes, vertical stripes, and open columns respectively. There were no significant differences between the control and experimental animals.


ADTN in NA/VTA RATIOS
metabolite/transmitter ratios
3
2
1
0
DOPAC/DA ratio
HVA/DA ratio
5-HIAA/5-HT ratio
CONTROLS
60
Time (min)
120

6 EXPERIMENT II

6,7-ADTN was directly administered into the striatum. The following regions were removed to examine the neurochemical changes: PFC, NA, ST, AM, SN, and VTA. The times examined were the control group which received only saline injections, the group decapitated 60 minutes after injection with 6,7-ADTN and the group decapitated 120 minutes after injection. The metabolite/transmitter ratios will be presented in bar graphs.

6.1 Prefrontal Cortex

Within the prefrontal cortex there was a significant increase in the DOPAC/DA ratio at 120 minutes in comparison to the control ratio, $F(2,22)= 6$, $p<.05$. There was no significant increase in the DOPAC/DA ratio at 60 min in comparison to control even though there was an increase. The 5-HIAA/5-HT ratio was significantly increased at 120 minutes in comparison to the control ratio, $F(2,22)= 3$, $p<.05$. There was also a very large increase in the 5-HIAA/5-HT ratio at 60 minutes but it was just shy of significance in comparison to the control ratio. There was a significant increase in the HVA/DA ratio at both 60 and 120 minutes after injection in comparison to the control ratio, $F(2,22)= 7$, $p<.05$. This very dramatic increase in the HVA/DA ratio is approximately equivalent at both 60 and 120 min.

6.2 Nucleus Accumbens

Within the nucleus accumbens there was a significant decrease in the DOPAC/DA ratio at both 60 and 120 minutes in comparison to the control ratio, $F(2,23)= 15$, $p<.05$. The decrease appears to be roughly the same at both time points. The 5-HIAA/5-HT ratio was significantly

decreased at 60 minutes in comparison to the control ratio, $F(2,23)= 4$, $p<.05$. At 120 min there was also a decrease in the 5-HIAA/5-HT ratio compared to the control but not enough to reach significance. The HVA/DA ratio was significantly increased at both 60 and 120 minutes in comparison to the control ratio, $F(2,23)= 5$, $p<.05$. At 60 min there is a very dramatic increase in the HVA/DA ratio which decreases by 120 minutes although this decreased level is still much higher than the control ratio.

6.3 Striatum

Within the striatum the DOPAC/DA ratio was significantly decreased at 60 minutes in comparison to the control ratio, $F(2,23)= 3$, $p<.05$. The 120 min ratio also showed a decrease in the DOPAC/DA ratio but not enough to reach significance. The 5-HIAA/5-HT ratios at both 60 and 120 min were decreased from the control ratio but not significant. The HVA/DA ratio was not significant at 60 or 120 minutes even though there was a substantial increase in ratio due to the enormous variability within the experimental groups meatbolite levels.

6.4 Amygdala

Within the amygdala there was a significant increase in the DOPAC/DA ratio at 60 minutes in comparison to the control level, $F(2,23)= 4$, $p<.05$. There was also an increase in the DOPAC/DA ratio at 120 min but it did not reach significance. There was a significant increase in the 5-HIAA/5-HT ratio at 120 min in comparison to the control ratio, $F(2,23)= 3$, $p<.05$. Overall, the 5-HIAA/5-HT trend was an increase from the control level at 60 min that reached significance at 120 min. There were significant changes in the HVA/DA ratio at 60 min in comparison to the control ratio, $F(2,23)= 17$, $p<.05$. The 60 minute group was significantly increased from the control group and by 120 min the HVA/DA ratio had returned back to the control ratio.

6.5 Substantia Nigra

There were no significant changes in the DOPAC/DA ratios across the three groups. The control ratio appeared to be only slightly lower than the ratios at 60 and 120 min. The 5-HIAA/5-HT ratio was significantly decreased at both 60 and 120 minutes in comparison to the control ratio, $F(2,23)= 21$, $p<.05$. Overall, the 60 min 5-HIAA/5-HT ratio was lower than the ratio at 120 min but both were significantly lower than the control. There were no significant changes in the HVA/DA ratios at 60 or 120 min although both were increased in comparison to the control ratio.

6.6 Ventral Tegmental Area

Within the ventral tegmental area there was a significant decrease in the DOPAC/DA ratio at both 60 and 120 minutes in comparison to the control ratio, $F(2,23)= 5$, $p<.05$. The DOPAC/DA ratio is lower at 60 min and increases at 120 min but remains lower than the control ratio at both time points. There were no significant changes in the HVA/DA ratio in comparison to the control level. There was an increase in the HVA/DA ratio at both 60 and 120 min but not enough to reach significance. There was a significant increase in the 5-HIAA/5-HT ratio at 120 minutes in comparison to the control ratio, $F(2,23)= 4$, $p<.05$. Overall, the 5-HIAA/5-HT ratio decreases at 60 min relative to the control ratio and then increases at 120 min to a significant level. (see Figure 9 through Figure 19 for corresponding bar graphs)

Neurochemical changes after intra-striatal stimulation with 6,7-ADTN

Looking now at the results obtained after injection of ADTN into the ST we see again the widespread and varied changes across the various brain regions. Within the ST itself there were

no significant changes other than a decrease in dopamine turnover at 60 minutes which was gone at 120 minutes post-injection. The control level of the 5-HT ratio is high and there is a downward trend 60 and 120 minutes after injection but it was not significant. This apparent decrease in 5-HT activity could account for the apparent increase in the HVA/DA ratio. This increase did not reach significance maybe because the standard deviations were so large and the number of animals so comparatively small that the effect was lost (see appendix 2 for means and standard deviations). The SN which is the other major player along with the ST in the nigrostriatal dopaminergic pathway also shows minimal changes. Looking at the SN, there are no changes within the DA ratios at either time point and a decrease in 5-HT activity at both 60 and 120 minutes. The baseline level of 5-HT activity within the SN is quite high followed by obvious drops at both 60 and 120 minutes which have no effect on DA turnover or functional activity. These results would suggest that 5-HT's normal role within the SN is not inhibitory since large decreases produce no concommitant change in DA ratios.

Within the VTA, the DA cell bodies normally associated with the mesolimbic dopaminergic pathway, there is a decrease in DA turnover at both 60 and 120 minutes. This does not appear to be mediated by the increase in 5-HT activity present at 120 minutes because the initial drop in DA turnover occurs before the increase in the 5-HIAA/5-HT ratio.

The AM results show an increase in DA turnover at 60 minutes which is absent at 120 minutes probably due to the inhibitory effect of the large increase in 5-HT activity by 120 minutes post-injection. The HVA/DA ratio appears to be impervious to the increase in 5-HT suggesting that the functional activity of DA in the AM is not inhibited by 5-HT.

We see similar results in the PFC. There is an upward trend in DA turnover which reaches significance at 120 minutes which is just marginally higher than the level at 60 minutes undoubtedly kept in check by the huge increase in 5-HT activity at both 60 and 120 minutes. Similar to the AM we see that this enormous increase in 5-HT has no inhibitory effect on the functional activity of DA neurons expressed as the HVA/DA ratio

TABLE 6

Neurochemical changes in the striatum

Increases in amine ratios are indicated by (+) and decreases by (-). No change is indicated by an empty cell.

STRIATUM

	DOPAC/DA		HVA/DA		5-HIAA/5-HT	
	60min	120min	60min	120min	60min	120min
PFC		+	+	+		+
NA	-	-	+	+	-	
ST	-					
AM	+		+	+		+
SN					-	-
VTA	-	-				+

Figure 9

Mean metabolite to amine transmitter ratios found in the prefrontal cortex (PFC) presented for the different groups of the experiment: control animals and animals sacrificed at 60 an i20 minutes following ADTN administration into the striatum (ST). The DOPAC/DA and the 5-HIAA/5-HT ratios are illustrated by diagonal stripes and open columns respectively. Significant differences ($p<0.05$) between control and experimental animals are indicated by an asterisk.

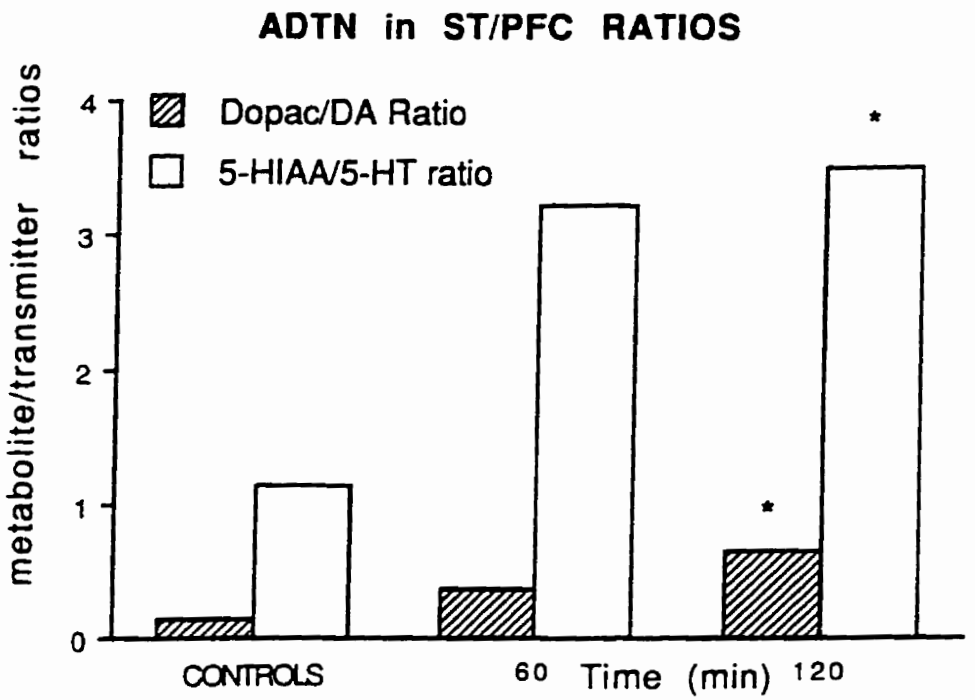

ADTN in ST/PFC RATIOS
metabolite/transmitter ratios
Dopac/DA Ratio
5-HIAA/5-HT ratio
4
3
2
1
0
*
*
CONTROLS
60
Time (min)
120

Figure 10

Mean metabolite to amine transmitter ratios found in the prefrontal cortex (PFC) presented for the different groups of the experiment: control animals and animals sacrificed at 60 and 120 minutes following ADTN administration into the striatum (ST). The HVA/DA ratios are illustrated by vertical stripes. Significant differences ($p<0.05$) between the control and experimental animals are indicated by an asterisk.

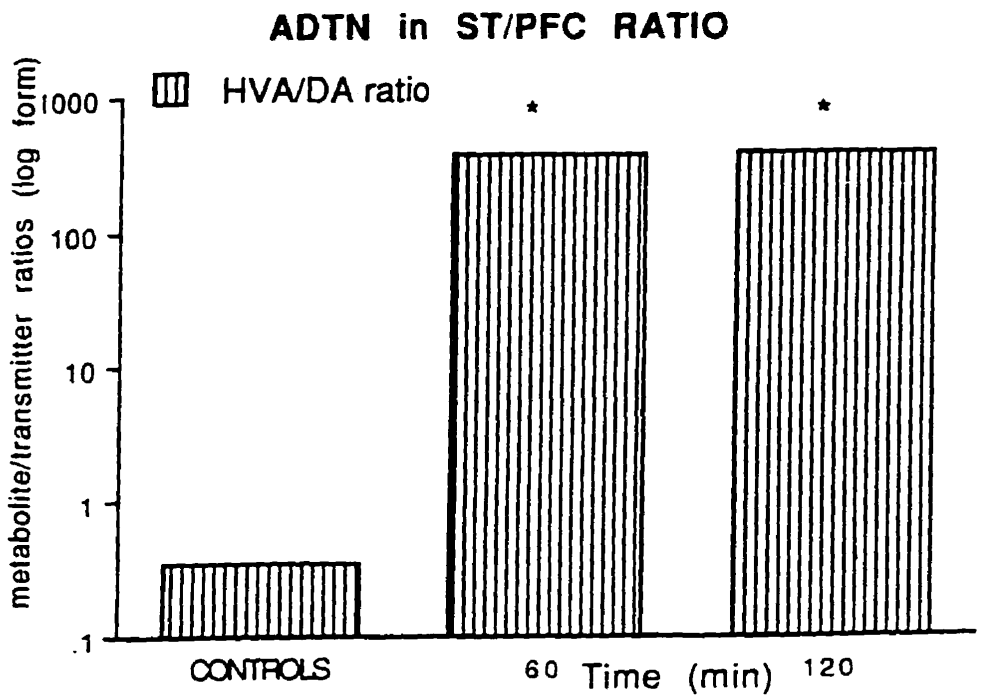

ADTN in ST/PFC RATIO
HVA/DA ratio
metabolite/transmitter ratios (log form)
1000
100
10
1
.1
*
*
CONTROLS
60
Time (min)
120

Figure 11

Mean metabolite to amine transmitter ratios found in the nucleus accumbens (NA) presented for the different groups of the experiment: control animals and animals sacrificed at 60 and 120 minutes following ADTN administration into the striatum (ST). The DOPAC/DA and the 5-HIAA/5-HT ratios are illustrated by diagonal stripes and open columns respectively. Significant differences ($p<0.05$) between control and experimental animals are indicated by an asterisk.

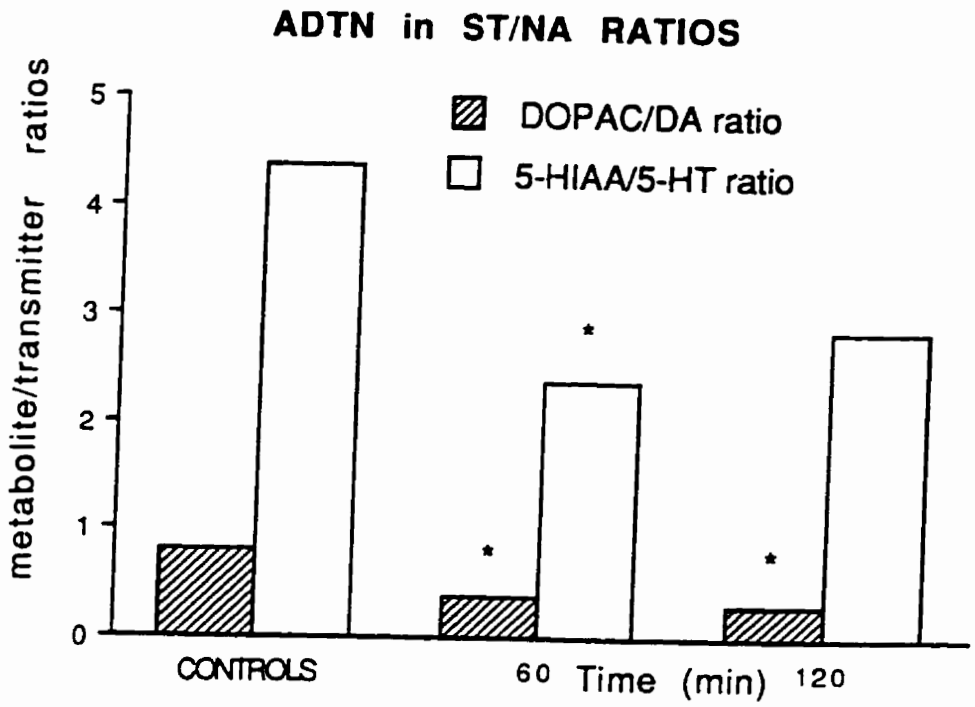

ADTN in ST/NA RATIOS
metabolite/transmitter ratios
DOPAC/DA ratio
5-HIAA/5-HT ratio
0
1
2
3
4
5
*
*
CONTROLS
60
Time (min)
120

Figure 12

Mean metabolite to amine transmitter ratios found in the nucleus accumbens (NA) presented for the different groups of the experiment: control animals and animals sacrificed at 60 and 120 minutes following ADTN administration into the striatum (ST). The HVA/DA ratios are illustrated by vertical stripes. Significant differences ($p<0.05$) between control and experimental animals are indicated by an asterisk.

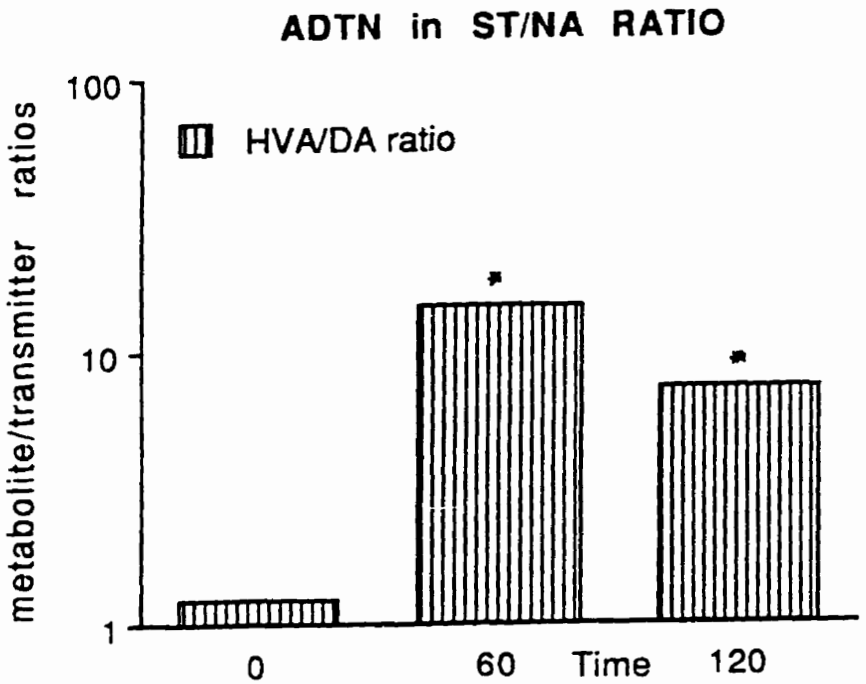

ADTN in ST/NA RATIO
metabolite/transmitter ratios
100
10
1
HVA/DA ratio
*
*
0
60
Time
120

Figure 13

Mean metabolite to amine transmitter ratios found in the striatum (ST) presented for the different groups of the experiment: control animals and animals sacrificed at 60 and 120 minutes following ADTN administration into the striatum (ST). The DOPAC/DA and the 5-HIAA/5-HT ratios are illustrated by diagonal stripes and open columns respectively. Significant differences ($p<0.05$) between control and experimental animals are indicated by an asterisk.

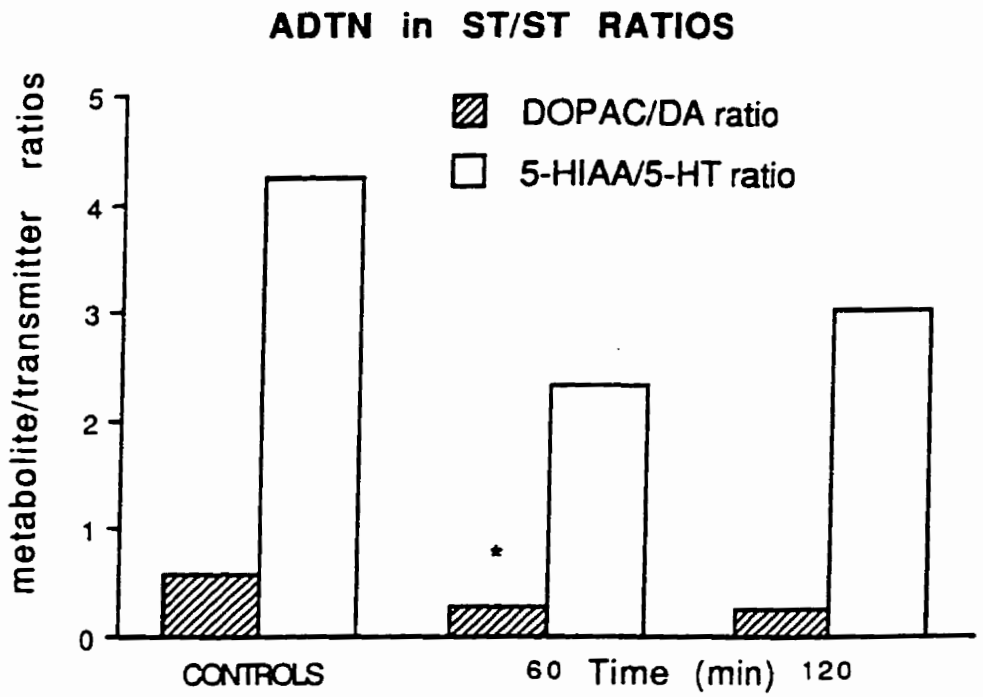

ADTN in ST/ST RATIOS
metabolite/transmitter ratios
5
4
3
2
1
0
DOPAC/DA ratio
5-HIAA/5-HT ratio
*
CONTROLS
60
Time (min)
120

Figure 14

Mean metabolite to amine transmitter ratios found in the striatum (ST) presented for the different groups of the experiment: control animals and animals sacrificed at 60 and 120 minutes following ADTN administration into the striatum (ST). The HVA/DA ratios are illustrated by vertical stripes. There were no significant differences between the control and experimental animals.

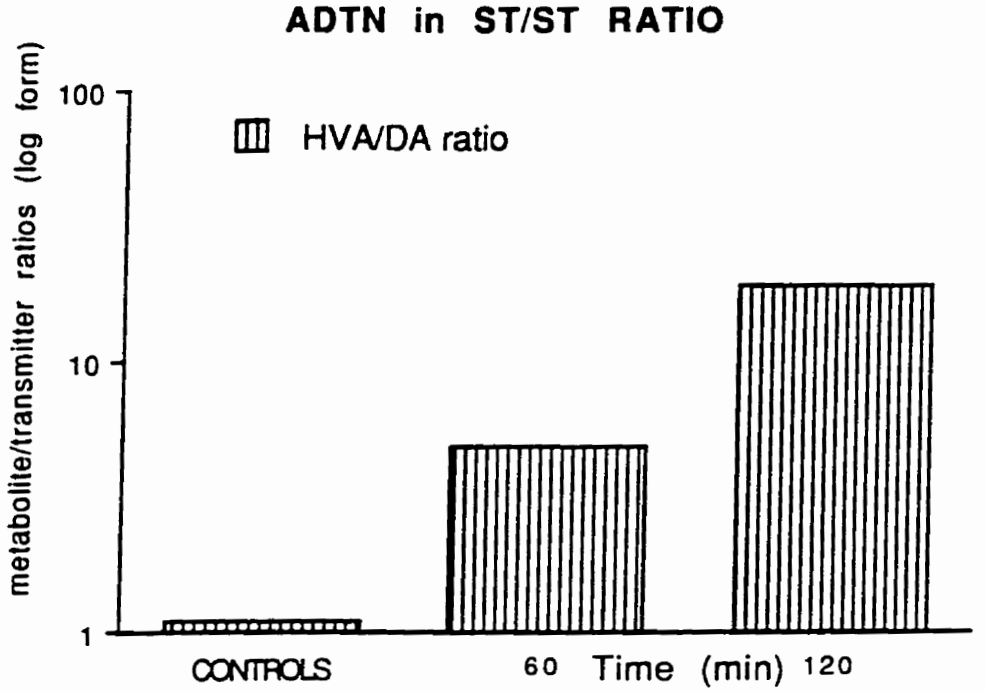

ADTN in ST/ST RATIO
metabolite/transmitter ratios (log form)
100
10
1
HVA/DA ratio
CONTROLS
60
Time (min)
120

Figure 15

Mean metabolite to amine transmitter ratios found in the amygdala (AM) presented for the different groups of the experiment: control animals and animals sacrificed at 60 and 120 minutes following ADTN administration into the striatum (ST). The DOPAC/DA and the 5-HIAA/5-HT ratios are illustrated by diagonal stripes and open columns respectively. Significant differences ($p<0.05$) between control and experimental animals are indicated by an asterisk.

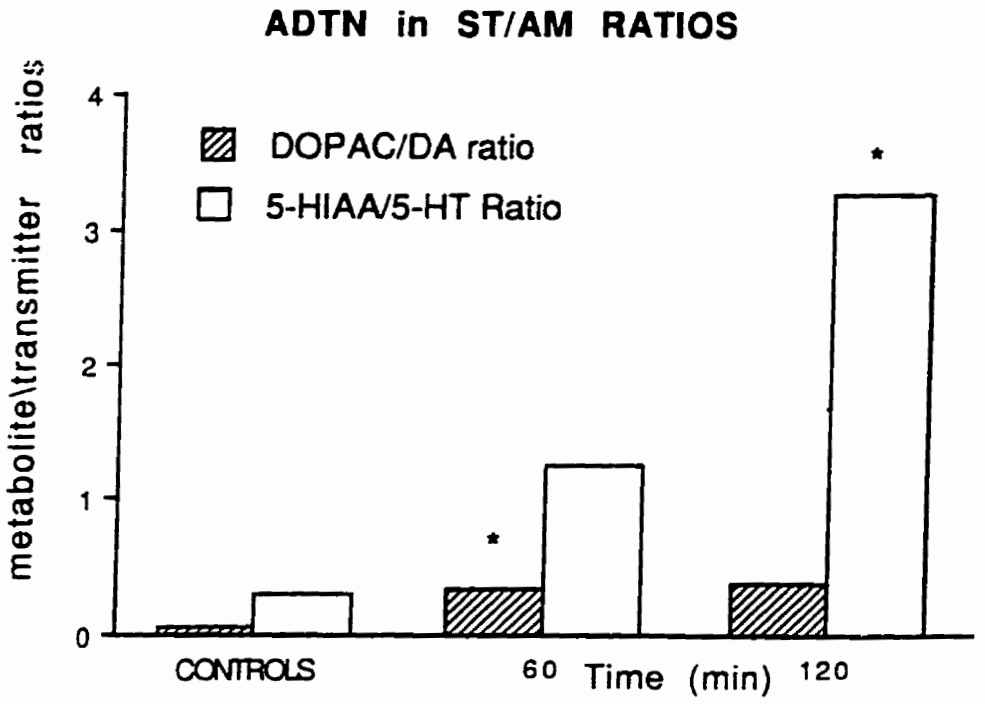

ADTN in ST/AM RATIOS
metabolite\transmitter ratios
4
3
2
1
0
DOPAC/DA ratio
5-HIAA/5-HT Ratio
*
*
CONTROLS
60
Time (min)
120

Figure 16

Mean metabolite to amine transmitter ratios found in the amygdala (AM) presented for the different groups of the experiment: control animals and animals sacrificed at 60 and 120 minutes following ADTN administration into the striatum (ST). The HVA/DA ratios are illustrated by vertical stripes. Significant differences ($p<0.05$) between control and experimental animals are indicated by an asterisk.

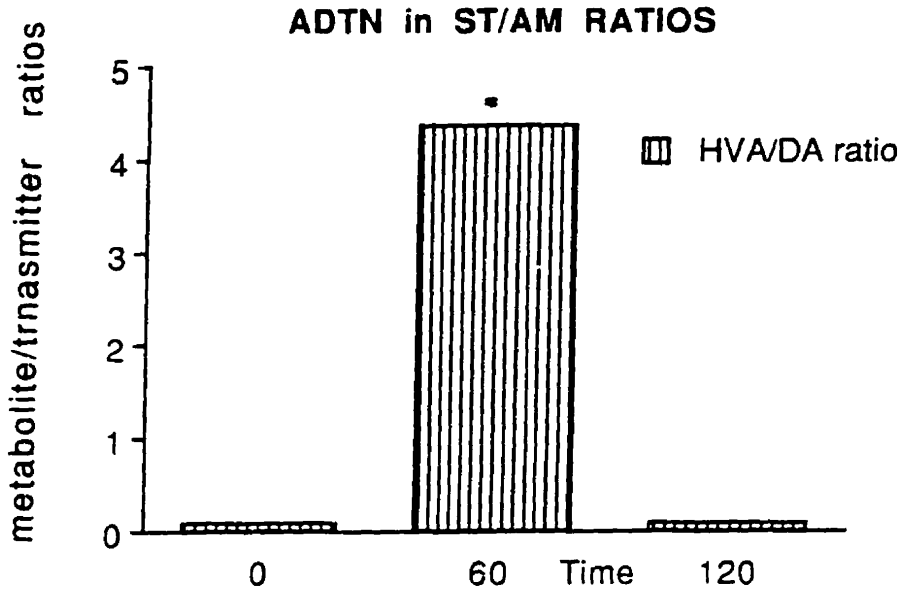

ADTN in ST/AM RATIOS
metabolite/trnasmitter ratios
5
4
3
2
1
0
*
HVA/DA ratio
0
60
Time
120

Figure 17

Mean metabolite to amine transmitter ratios found in the substantia nigra (SN) presented for the different groups of the experiment: control animals and animals sacrificed at 60 and 120 minutes following ADTN administration into the striatum (ST). The DOPAC/DA and the 5-HIAA/5-HT ratios are illustrated by diagonal stripes and open columns respectively. Significant differences ($p<0.05$) between control and experimental animals are indicated by an asterisk.

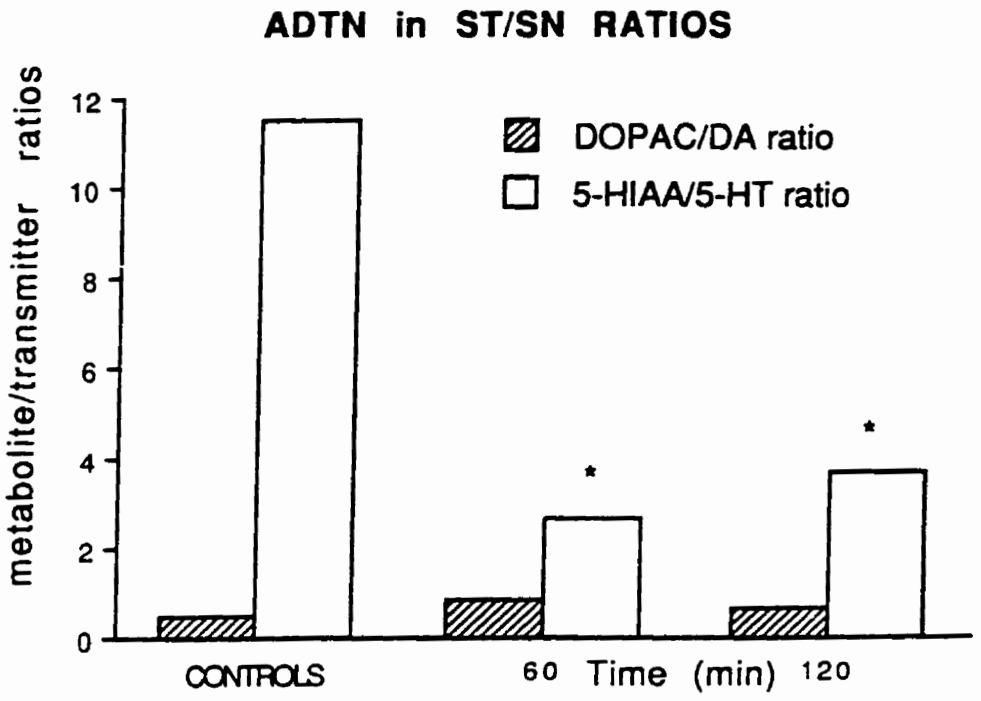

ADTN in ST/SN RATIOS
metabolite/transmitter ratios
12
10
8
6
4
2
0
DOPAC/DA ratio
5-HIAA/5-HT ratio
*
*
CONTROLS
60
Time (min)
120

Figure 18

Mean metabolite to amine transmitter ratios found in the substantia nigra (SN) presented for the different groups of the experiment: control animals and animals sacrificed at 60 and 120 minutes following ADTN administration into the striatum (ST). The HVA/DA ratios are illustrated by vertical stripes. There were no significant differences between control and experimental animals.

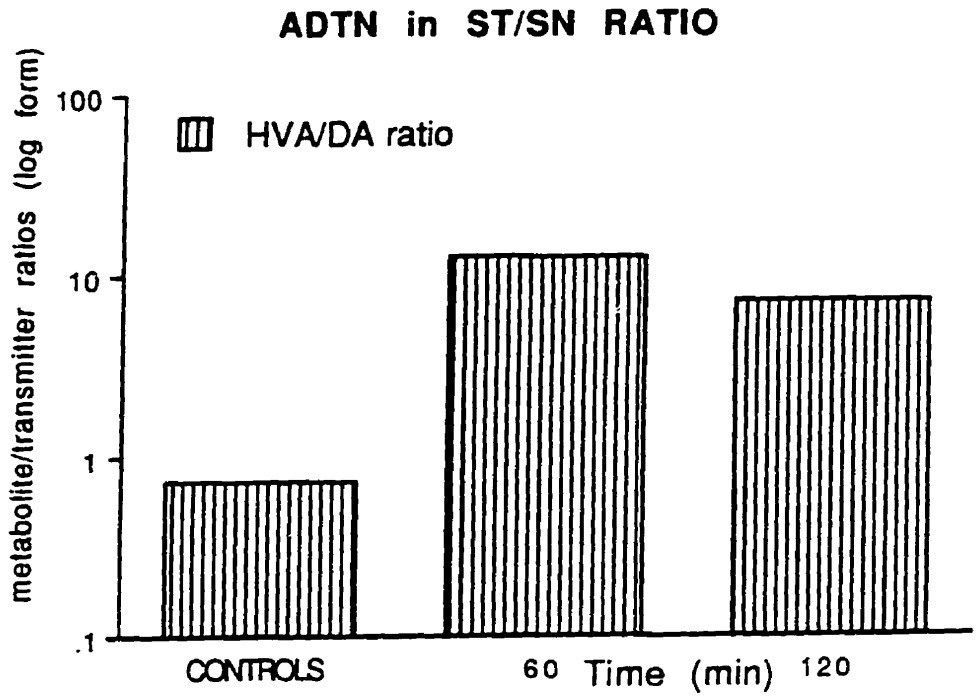
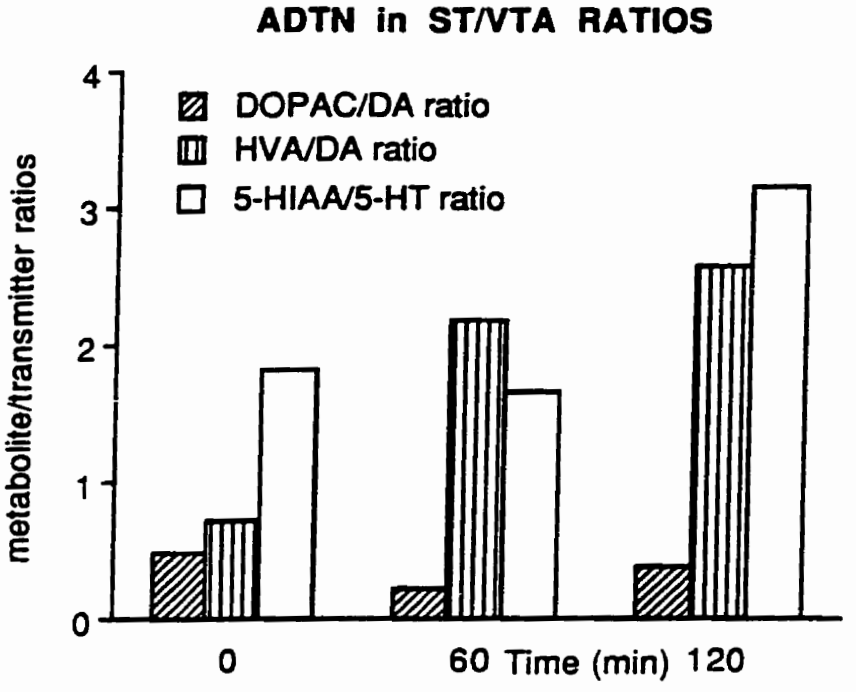

ADTN in ST/SN RATIO
metabolite/transmitter ratios (log form)
100
10
1
.1
HVA/DA ratio
CONTROLS
60
Time (min)
120

Figure 19

Mean metabolite to amine transmitter ratios found in the ventral tegmental area (VTA) presented for the different groups of the experiment: control animals and animals sacrificed at 60 and 120 minutes following ADTN administration into the striatum (ST). The DOPAC/DA, HVA/DA and the 5-HIAA/5-HT ratios are illustrated by diagonal stripes, vertical stripes, and open columns respectively. Significant differences ($p<0.05$) between control and experimental animals are indicated by an asterisk.


ADTN in ST/VTA RATIOS
DOPAC/DA ratio
HVA/DA ratio
5-HIAA/5-HT ratio
metabolite/transmitter ratios
4
3
2
1
0
0
60
Time (min)
120

DISCUSSION

This study examined the time course and regional specificity of neurochemical changes following injection of 6,7-ADTN into the nucleus accumbens and striatum. 6,7-ADTN is a rigid analogue of dopamine which stimulates motor activity when injected into the NA and enhances rotation and sniffing when injected into the striatum (Antonian et al., 1986). Jolicoeur et al. (1985) studied the *in vivo* effects of 6,7-ADTN injected into the nucleus accumbens and showed a strong and continuous hyperactivity in rats. The motor activity reached peak significance 240 minutes after injection. The unusual finding within this study was that motor activity reached significance 60 minutes after injection but appeared suspended and did not reach peak activity until 240 minutes after injection. A preliminary study (Jolicoeur, unpublished data) was conducted to examine the possible neurochemical correlates underlying the increased motor activity. This initial study showed that immediately after injection of 6,7-ADTN in the NA, dopamine was increased in the NA and the PFC showed a slight but significant increase in 5-HT. The present study was designed to better delineate the neurochemical changes following injections of 6,7-ADTN into the NA and to examine the specificity of these changes by then injecting 6,7-ADTN into the ST.

Results showed that following 6,7-ADTN injection into the NA there were no significant changes in any of the metabolite/transmitter ratios at 60 or 120 minutes post-injection. In review, the NA receives afferents from the PFC, AM, SN, and VTA. The NA then projects efferents to the AM, SN, and VTA. The lack of any significant neurochemical changes in the NA could be due to several possibilities. 1) Within the PFC, the significant increase in the DOPAC/DA ratio at 60 minutes may have led to the subsequent activation of the serotonergic system in the PFC which

caused a significant increase in the 5-HIAA/5-HT ratio seen at 120 minutes. 5-HT is assumed to have an inhibitory effect on dopamine therefore a significant 5-HT projection from the PFC to the NA could prevent significant DA changes within the NA (Siever et al., 1991). 2) The other possibility is the significantly increased DA activity seen in the SN may indirectly effect other regions. The increased HVA/DA ratio seen at both 60 and 120 minutes within the SN may have been acting at the autoreceptor level. This would have the effect of decreased DA activity at the SN terminal regions such as the NA and ST (Drumheller et al., 1990; Smith and Bolam, 1991). As shown in table 5, there is decreased DA activity in both the NA and ST.

The VTA, which projects a major afferent to the NA showed no significant metabolite/neurotransmitter ratio changes. Overall, the VTA projects to the NA, ST, AM, and SN and receives, in majority, fibers from the NA and AM (Swanson, 1982). There are a few possible explanations to explain the lack of neurochemical changes within a major accumbal terminal region. 1) The increased DA activity in the SN at 60 and 120 minutes, uninhibited by serotonin which was significantly decreased at both 60 and 120 minutes, may have indirectly caused the lack of change within the VTA by causing decreased DA activity in the VTA terminal regions such as the NA and ST (Nauta et al., 1978; Domesick, 1988). Therefore, due to the strong interconnections between the VTA and both the NA and ST the decreased DA activity, suppressed by DA in the SN acting at the autoreceptor level, affected the VTA through the efferent projections from the NA and ST. 2) Another possible explanation for the lack of VTA change is that the PFC also makes direct synaptic contact with the DA neurons of the VTA (Bachneff, 1991; Murase et al., 1993). Therefore, the increase in serotonergic activity in the PFC

that reaches significance by 120 minutes may have had an inhibitory effect on DA activity within the VTA.

The PFC showed some very interesting changes. In review, the PFC projects to the NA, ST, AM, and SN and receives fibers from the limbic structures such as the NA and AM (Nauta et al., 1978; Phillipson and Griffiths, 1985). As discussed above, the PFC is implicated in the lack of neurochemical changes within both the NA and VTA. At 60 minutes post-injection there is a significant increase in the DOPAC/DA ratio, no change in the HVA/DA ratio and no significant change in the 5-HIAA/5-HT ratio. By 120 minutes there are no longer any DA related changes and there is a significant and substantial increase in the 5-HIAA/5-HT ratio. This increased 5-HT is known to have an inhibitory effect on DA which would then effect projection regions such as the VTA and NA (Siever et al., 1991).

The ST receives a majority of afferents from the cortex and some from the VTA and sends a major projection to the SN (Mcgreer et al 1987; Gerfen, 1992; Flaherty and Graybiel, 1994). Within the ST there were no DA changes at 60 or 120 minutes but there was a significant increase in serotonergic activity at both 60 and 120 minutes. The are several potential explanations: 1) The previously discussed hypothesis of increased DA acting at the autoreceptor level within the SN which would subsequently block increases in DA at the terminal regions. 2) The PFC which projects heavily to the ST could have also influenced DA activity through the increase in 5-HT seen at 120 minutes (Beckstead, 1979). 3) The most likely explanation is the intrinsic increase in 5-HT activity within the ST at both 60 and 120 minutes. This increase may have developed as a response to the direct DA analogue being injected into the NA as a way to maintain

neurochemical homeostasis through the inhibitory effect of 5-HT or through stimulation of 5-HT fibers projecting from the PFC (Mogenson et al., 1988).

The AM showed increases in both DA ratios, DOPAC/DA and HVA/DA, at both 60 and 120 minutes. There were no significant changes in serotonergic activity. The AM projects to the NA and ST and receives projections from the neocortex, VTA, and NA (Phillipson and Griffiths, 1985; Jayaraman, 1985; Canteras et al., 1992). As discussed previously in section 8, the AM is involved in extraneuronal transmission and has a different rate of DA release and reuptake than most regions (Garris and Wightman, 1994). DA stimulation within the NA led to increased DA activity in the AM, through their direct reciprocal connection, which then led to a continued increase in DA activity 60 and 120 minutes later. This continued significant increase could be due to extraneuronal transmission which involves the quantity of neurotransmitter left in the synapse after neuronal firing. The AM is "release dominated" and will allow larger quantities of DA to be left in the synapse for longer than average periods of time. This could explain the long term significant changes seen within the AM (Garris and Wightman, 1994).

The SN and the its neurochemical changes have already been thoroughly discussed in relation to other regions. Briefly, the SN receives projections from the ST and NA and projects to the PFC, NA, and ST (Zaborsky, 1991; Conde, 1992; and Deniau et al., 1994). The SN showed no significant changes for the DOPAC/DA ratio but was significant at both 60 and 120 minutes for the HVA/DA ratio and the 5-HIAA/5-HT ratio. Summarized there was an increase in DA activity that was too powerful to be inhibited by the parallel increase in serotonergic activity.

Overall, after injection of 6,7-ADTN into the nucleus accumbens we see no significant changes in either the VTA or NA probably due to excess DA activity in the SN acting at the

autoreceptor level and causing decreased DA activity in the terminal regions. The PFC has an initial increase in DA activity at 60 min which is subsequently decreased as 5-HT activity increases and reaches significance by 120 minutes. This result along with the increased 5-HT activity at both 60 and 120 minutes in the ST provides support for the hypothesis that 5-HT has an inhibitory effect on DA activity. In the AM there is increased DA activity, possibly due to extraneuronal transmission, at both time points which is not hampered by any changes in 5-HT. Finally, in the SN there are large increases in DA activity, causing the decreased DA activity in the terminal regions, which is not inhibited by large parallel increases in 5-HT activity.

Moving on to the second series of experiments, results showed that following intra-striatal injections of 6,7-ADTN there was a significant decrease in the DOPAC/DA ratio in the ST at 60 min which was no longer significant by 120 min. As discussed in the results section there was an apparent increase in the HVA/DA ratio which did not reach significance undoubtedly due to the variance within the groups. Anatomically, the ST is interconnected with the SN, PFC, and VTA (Mcgreer et al 1987; Gerfen, 1992; Flaherty and Graybiel, 1994). The explanation for lack of neurochemical changes within the ST may be related to the SN. The SN, which had high control levels of 5-HT, showed obvious drops in the 5-HIAA/5-HT ratio at both 60 and 120 minutes which had no effect on the turnover or activity of DA within the SN. This result implies that following DA stimulation in the ST the decrease in 5-HT activity within the SN is not sufficient to stimulate DA activity within the SN itself or the ST, its major terminal region (see table 6 for a summary of results).

Within the VTA, the cell bodies normally associated with the NA through the mesolimbic pathway, there is a decrease in DA turnover at both 60 and 120 minutes. This significant decrease

does not appear to be mediated by the increase in 5-HT activity which only reaches significance at 120 minutes long after DA turnover has significantly decreased. It is not a 5-HT mediated decrease in DA activity therefore the answer is in the anatomical connections. The VTA projects to the NA, ST, AM, and SN and receives, in majority, fibers from the NA and AM (Swanson, 1982). A possible explanation is that the VTA decrease in DA turnover is related to its anatomical connections to the NA. DA activity in the NA, expressed as the HVA/DA ratio, is significant at both 60 and 120 minutes and DA turnover, expressed as the DOPAC/DA ratio, is significantly decreased at both 60 and 120 minutes. The interconnection between the VTA and NA may have allowed to the decreased DA turnover in the VTA in order to compensate for the significantly increased DA activity within the NA (Meredith et al., 1992; Deniau et al. 1994).

The AM and PFC share a similar profile of neurochemical changes following DA stimulation in the ST. The AM receives projections from the neocortex, VTA and NA and then projects to both the NA and ST and (Phillipson and Griffiths, 1985; Jayaraman, 1985; Canteras et al., 1992). The AM showed a significant increase in DA turnover at 60 min that was no longer present at 120 minutes. The HVA/DA ratio was significant at both 60 and 120 minutes. It would appear that the significant increase in the 5-HIAA/5-HT ratio at 120 minutes was sufficient to inhibit DA turnover in the AM. However, the HVA/DA ratio was unaffected by the increase in 5-HT activity. This would suggest that DA turnover in the AM is susceptible to inhibition by 5-HT and the level of DA functional activity is not.

In the PFC there is a significant increase in DA turnover at 120 minutes which is just slightly higher than the level at 60 minutes. This minor increase in DA turnover is likely due to the parallel increase in 5-HT activity at 60 minutes which reaches significance at 120 minutes. Similar

to the AM the substantial increase in 5-HT activity appears to have no inhibitory effect on DA functional activity expressed as the HVA/DA ratio. A possible explanation for the increase in DA activity within the PFC may be related to its anatomical connections to the ST. Within the ST, DA has the role of reducing lateral inhibition to oppose incoming cortical activity which would also have the effect of decreased striatal output to its afferent regions (Salamone, 1992). It is possible that due to the significantly decreased DA turnover in the ST there is no inhibition system to keep the PFC in check therefore DA activity levels reach significance at both 60 and 120 minutes.

Overall, intra-striatal injections of 6,7-ADTN produced an increase in ST dopamine turnover at 60 minutes which was gone by 120 minutes and was apparently not affected by the significantly decreased level of 5-HT activity in the SN, a major projection region, at both 60 and 120 minutes. The VTA showed decreased DA turnover at both 60 and 120 minutes which was not affected by the increase in 5-HT activity at 120 minutes in the VTA. These VTA changes may be mediated by changes in the NA, a major VTA projection zone, which shows increased DA activity at both 60 and 120 minutes that returns to the VTA along the parallel path to suppress DA turnover. The PFC and AM share a comparable neurochemical profile. Both the PFC and AM showed significant increases in DA turnover and activity. However, the substantial increase in 5-HT activity appeared to have no inhibitory effect on DA functional activity expressed as the HVA/DA ratio within either the PFC or AM.

One of the goals of this study was to examine the specificity of the neurochemical changes after intra-accumbal injections of 6,7-ADTN by also examining the time course and regional specificity of changes after injection into the ST. One conclusion to be drawn from these two studies is that there is a specificity to the changes seen after injection of 6,7-ADTN into the NA

versus into the ST. After injection of 6,7-ADTN into the nucleus accumbens we see no significant changes in the VTA or NA probably due to excess DA activity in the SN acting at the autoreceptor level which caused decreased DA activity in the terminal regions. After injection into the ST there is no significant change in DA activity within the SN although there is a decrease in serotonergic activity at 60 and 120 minutes after both intra-accumbal and intra-striatal injections of 6,7-ADTN. After injection into the NA, the PFC has an initial increase in DA activity at 60 min which is subsequently decreased as 5-HT activity increases and reaches significance by 120 minutes. The increased serotonergic activity within the PFC at 120 minutes is not capable of inhibiting the significant increases in DA activity and turnover in the PFC following intra-striatal injection. Following accumbal injections, the AM shows increased DA activity at both time points which is not restrained by any changes in 5-HT. However, after intra-striatal injection, neurochemical changes within the AM show that the significant increase in serotonergic activity at 120 minutes was sufficient to inhibit the previously significant increase in DA. The HVA/DA ratio remained unaffected by the increase in 5-HT activity suggesting that DA turnover in the AM is susceptible to inhibition by 5-HT. In conclusion, there is a definite timecourse and a regional specificity to the neurochemical changes unique to both intra-accumbal and intra-striatal injections of 6,7-ADTN.

Recent studies using 6,7-ADTN have are still delineating all of the DA analogues effects on the dopaminergic system both in the brain and the retina (Morgan et al., 1995). Blenau and associates (1996) showed that 6,7-ADTN is a potent sdisplacer of dopamine site binding that did not react at serotonergic binding sites. This would provide support that serotonergic changes seen within this study are not due to a direct effect of ADTN administration but rather a neurochemical

effect initiated by changes in the dopaminergic system. An interesting new study done by Nicola et al. (1996) demonstrated that 6,7-ADTN mimicked the effects of dopamine, cocaine, and amphetamines demonstrated through reduced excitatory synaptic responses elicited by stimulation of prelimbic cortical afferences. This result is especially interesting since an overdose of amphetamines or cocaine is often thought to mimic the symptoms of psychosis. This would lend more validity to using a 6,7-ADTN model as a means to study an underlying neurochemical basis of mental illness.

Recent studies examining the neurochemical changes underlying increased motor activity are expanding beyond the use of DA analogues. Now studies are also examing the effects of pharmacological stimulation within the VTA. Dalia et al. (1996) examined the induction of locomotor activity in the VTA through injection of Dnqx, a glutamate AMPA receptor antagonist. Results demonstrated an induction of hyperactivity with no associated change in the DOPAC/DA level in the NA or the ST. Pan et al. (1996) studied the effects of locally applied amphetamines into the VTA. Results showed a dose-dependently enhanced release of DA in the accumbens and the PFC. Amphetamine induced hyperactivity through injection into the VTA was also used to examine different serotonergic agonists and antagonists. Gillies et al. (1996) examined the effects of 5-HT_3 receptor selective agents on motor activity following injection into both the NA and VTA. They injected the $5HT_3$ selective agonist 2-methyl-5HT and the selective antagonist ondansetron. Results showed that neither spontaneous or amphetamine induced motor activity was changed after injection of 2-methyl-5HT into the NA. The same injections into the VTA produced long lasting increases in motor activity. However, ondansetron caused an inhibition of motor activity. As shown, there are many recent studies that examine the newer 5HT receptor

agonists and antagonists and their effects on established DA related behaviors such as motor activity.

Recent developments in technology using both the PET and CAT scans have also led to some surprising discoveries. The involvement of serotonin in schizophrenia is also new. With the advent of new "atypical" antipsychotics the possibilities for treatment increased dramatically. Even within the context of this study results showed how DA and 5-HT appeared to be in such fine balance with each other. Marcus et al. (1996) examined the effect of typical and atypical psychotics on DA release within the accumbens. Results found that drugs with high 5-HT_2 receptor antagonist activity, such as amperozide and ritanserin, or low doses of clozapine increased DA concentration to a greater extent within the shell of the NA. D_2 receptor antagonists such as haloperidol and raclopide and high doses of clozapine increased DA concentration in the core of the NA. Overall, it would appear that atypical antipsychotics, which are potent 5-HT_2 receptor antagonists, effect DA tansmission exclusively in the shell of the NA. Merchant et al., (1996) also examined the effect of antipsychotic drugs exclusively within the PFC. This study looked at the effects of acute and chronic administration of haloperidol and clozapine on c-Fos messsenger RNA in the PFC. Results showed that haloperidol had no significant effect even after chronic treatment and clozapine administration increased c-Fos expression. Therefore, it would appear that D_3 receptors, associated with the atypical antipsychotics, may enhance c-Fos gene expression. Looking at the most recent studies, it seems unlikely that any future treatments for schizophrenia would focus only on one neurotransmitter without looking at how other systems are affected by both the disease and the drug treatment.

Presently, it appears that the positive symptoms (delusions/hallucinations) respond better to traditional neuroleptic through dopaminergic treatment and the negative symptoms (lack of affect) are better handled through atypical antipsychotics (Carpenter, 1996). Atypical antipsychotics have other advantages over traditional neuroleptics such as minimizing the prominant extrapyramidal side-effects, tardive dyskinesia, and helping the roughly 30% of schizophrenic patients that do not respond to the usual drug therapies (Siever, 1991).

Not all of the neurochemical changes seen can be explained by the simple model of serotonergic inhibition of dopamine (ex: 5-HT's role in the SN after ADTN injection into the ST) but it does provide a context for the analysis of the widespread changes. All of the areas chosen are those implicated in the etiology of schizophrenia therefore the neurochemical analysis was performed under the predominant hypothesis of traditional and atypical antipsychotics. The results obtained suggest that there is a very complicated relationship between these two major transmitters that is affected by 1) site of drug injection 2) afferents and efferents of the injection site 3) time passed between injection and neurochemical analysis 4) and the natural homeostatic balance between them.

In conclusion, this study sets forth the following statements. After intra-accumbens administration of 6,7-ADTN there is a definite timecourse to the neurochemical changes in the major afferent and efferent dopaminergic and serotonergic areas examined. The subsequent study of intra-striatal 6,7-ADTN demonstrated that the neurochemical changes previously seen were specific to the nucleus accumbens as dopaminergic stimulation in the striatum produced a different pattern and timecourse of neurochemical changes. Stimulation of the mesolimbic and mesostriatal dopamine terminal areas caused widespread and long lasting neurochemical changes.

There is a definite strong effect at the level of the prefrontal cortex after intra-striatal dopaminergic stimulation. There was a significant increase in dopamine turnover 120 minutes after injection that was apparently regulated by what appeared to be an immense increase in serotonergic activity at both 60 and 120 minutes after injection. This increase of serotonin had no effect on the level of dopamine neuron functional activity expressed as the HVA/DA ratio. The results suggest a possible interaction where striatal dopamine stimulation causes an increase in dopamine in the prefrontal cortex. This excessive dopamine release within the prefrontal cortex would then be monitored and controlled by the subsequent release of serotonin.

The significance of these results relates to the not yet fully defined relationship between the dopaminergic and serotonergic pathways of the brain and mental illness. At present there is no perfect drug treatment for schizophrenia. So, by discovering and defining other possible target sites for drug treatment such as serotonergic agonists to inhibit the excessive release of dopamine in the prefrontal cortex the possibilities for improved drug treatment that is more selective becomes available. When drugs can be developed that are more selective there is the added benefit of less global side-effects such as tardive dyskinesia and other extrapyrammidal secondary effects.

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Appendix 1: Nucleus Accumbens

means and standard deviations

Prefrontal Cortex

CONDITION	DOPAC	DOPAMINE	HVA	5-HT	5-HIAA
controls	0.05 ±.04	0.15 ±.09	0.04 ±.02	0.08 ±.02	0.09 ±.06
60 minutes	0.02 ±.01	0.05 ±.04	0.05 ±.03	0.06 ±.03	0.09 ±.04
120 minutes	0.04 ±.04	0.08 ±.07	0.04 ±.02	0.08 ±.03	0.15 ±.08

Nucleus Accumbens

CONDITION	DOPAC	DOPAMINE	HVA	5-HT	5-HIAA
controls	4.63 ±1.83	5.53 ±1.97	1.29 ±.62	0.79 ±.38	1.11 ±.39
60 minutes	1.87 ±.92	2.34 ±1.07	0.93 ±.36	0.99 ±.34	0.96 ±.33
120 minutes	2.35 ±.49	3.56 ±1.05	0.63 ±.36	0.51 ±.13	1.44 ±1.06

Striatum

CONDITION	DOPAC	DOPAMINE	HVA	5-HT	5-HIAA
controls	1.97 ±.72	5.07 ±.85	0.88 ±.26	0.44 ±.16	0.41 ±.18
60 minutes	4.19 ±1.66	7.46 ±3.22	1.53 ±.62	0.39 ±.15	2.08 ±1.39
120 minutes	2.40 ±1.14	7.47 ±2.18	0.73 ±.38	0.39 ±.38	3.26 ±1.06

Amygdala

CONDITION	DOPAC	DOPAMINE	HVA	5-HT	5-HIAA
controls	0.01 ±.003	0.16 ±.05	0.14 ±.05	0.12 ±.03	0.42 ±.09
60 minutes	0.10 ±.03	0.15 ±.06	0.17 ±.04	0.15 ±.03	0.48 ±.07
120 minutes	0.11 ±.03	0.13 ±.03	0.16 ±.03	0.14 ±.03	0.50 ±.09

Substantia Nigra

CONDITION	DOPAC	DOPAMINE	HVA	5-HT	5-HIAA
controls	0.20 ±.10	0.20 ±.11	0.16 ±.08	0.20 ±.11	0.41 ±.20
60 minutes	0.06 ±.04	0.05 ±.04	0.15 ±.05	0.18 ±.07	0.44 ±.23
120 minutes	0.11 ±.07	0.11 ±.06	0.31 ±.10	0.30 ±.10	0.52 ±.14

Ventral Tegmental Area

CONDITION	DOPAC	DOPAMINE	HVA	5-HT	5-HIAA
controls	0.18 ±.08	0.27 ±.09	0.13 ±.06	0.31 ±.13	0.54 ±.21
60 minutes	0.21 ±.08	0.27 ±.04	0.13 ±.05	0.44 ±.21	0.78 ±.49
120 minutes	0.09 ±.03	0.15 ±.10	0.07 ±.04	0.37 ±.13	0.58 ±.15

Appendix 2: Striatum

means and standard deviations

Prefrontal Cortex

CONDITION	DOPAC	DOPAMINE	HVA	5-HT	5-HIAA
controls	0.14 ±.12	0.94 ±.49	0.32 ±.15	0.14 ±.04	0.16 ±.10
60 minutes	0.08 ±.07	0.22 ±.16	84.75 ±16.22	0.13 ±.42	0.42 ±.46
120 minutes	0.05 ±.04	0.08 ±.05	32.78 ±13.17	0,09 ±.06	0.33 ±.14

Nucleus Accumbens

CONDITION	DOPAC	DOPAMINE	HVA	5-HT	5-HIAA
controls	1.08 ±.34	1.33 ±.57	1.62 ±.55	0.28 ±.17	1.23 ±.37
60 minutes	1.57 ±.67	4.27 ±1.28	63.84 ±43.73	0.41 ±.38	1.03 ±1.22
120 minutes	1.46 ±.24	4.63 ±1.83	34.19 ±48.01	0.38 ±.15	1.09 ±.54

Striatum

CONDITION	DOPAC	DOPAMINE	HVA	5-HT	5-HIAA
controls	0.83 ±.26	1.44 ±.49	1.59 ±.42	0.32 ±.15	1.34 ±.37
60 minutes	1.34 ±.54	4.93 ±1.41	17.55 ±11.97	0.31 ±.11	0.61 ±.48
120 minutes	1.07 ±.58	4.16 ±3.02	84.83 ±112.81	0.17 ±.11	0.62 ±.30

Amygdala

CONDITION	DOPAC	DOPAMINE	HVA	5-HT	5-HIAA
controls	0.06 ±.03	1.09 ±.37	0.23 ±.10	0.23 ±.12	0.07 ±.04
60 minutes	0.09 ±.05	0.25 ±.13	6.36 ±4.37	0.28 ±.14	0.25 ±.13
120 minutes	0.09 ±.03	0.27 ±.14	0.21 ±.08	0.13 ±.09	0.49 ±.07

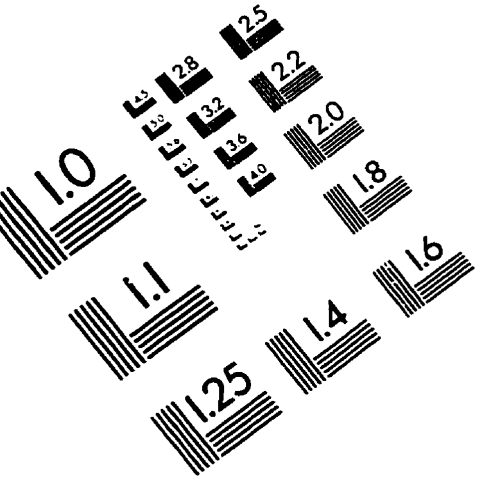
Substantia Nigra

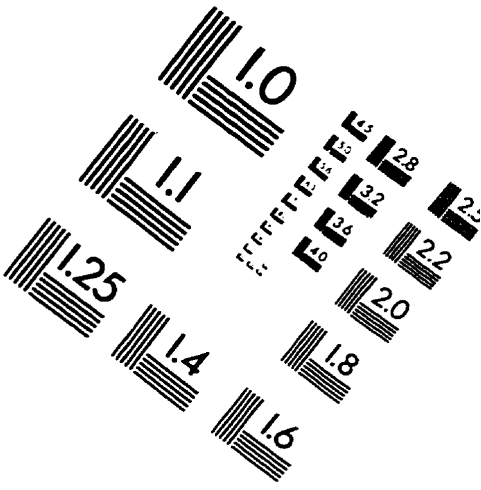
CONDITION	DOPAC	DOPAMINE	HVA	5-HT	5-HIAA
controls	0.18 ±.11	0.37 ±.18	0.27 ±.17	0.16 ±.08	1.90 ±.66
60 minutes	0.08 ±.05	0.10 ±.08	1.23 ±.65	0.20 ±.07	0.54 ±.15
120 minutes	0.15 ±.22	1.87 ±1.82	2.22 ±2.20	0.15 ±.08	0.54 ±.26

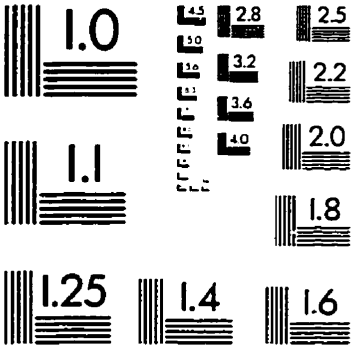
Ventral Tegmental Area

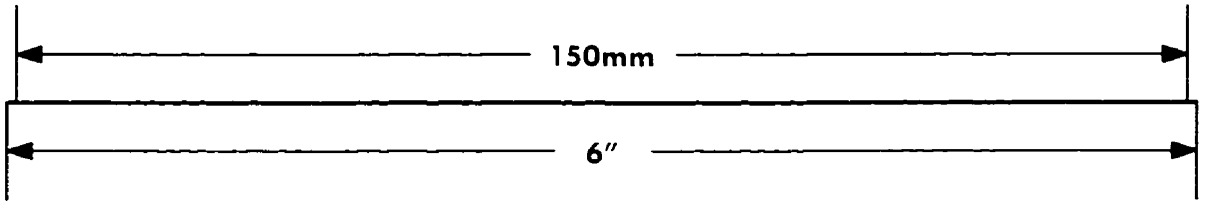
CONDITION	DOPAC	DOPAMINE	HVA	5-HT	5-HIAA
controls	0.20 ±.06	0.41 ±.31	0.30 ±.09	0.29 ±.13	0.52 ±.27
60 minutes	0.13 ±.08	0.58 ±.41	1.28 ±.80	0.29 ±.10	0.47 ±.22
120 minutes	0.17 ±.12	0.44 ±.24	1.14 ±.77	0.21 ±.10	0.67 ±.30

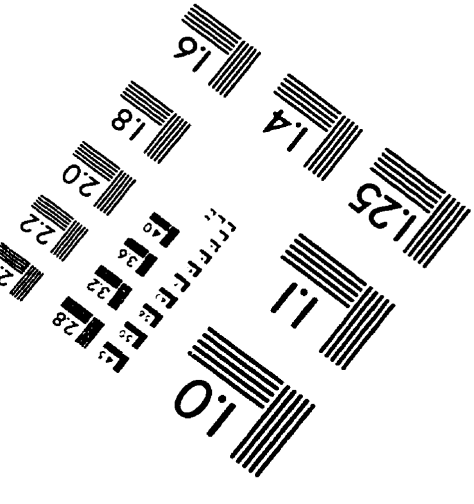
IMAGE EVALUATION TEST TARGET (QA-3)

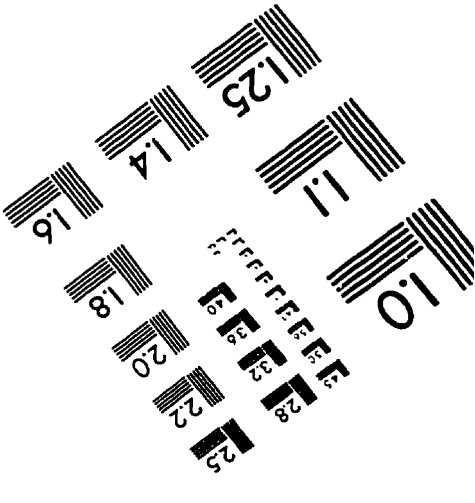












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