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Effects of 5-HT Receptor-related Compounds in Behavioural Tests of Mesolimbic Dopamine Activity

by

Kee-Chan Ahn

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science in Pharmaceutical Sciences

Faculty of Pharmacy & Pharmaceutical Sciences

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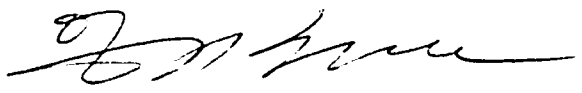

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Effects of 5-HT Receptor-related Compounds in Behavioural Tests of Mesolimbic Dopamine Activity" by Kee-Chan Ahn in partial fulfillment of the requirements for the degree of Master of Science in Pharmaceutical Sciences.

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ABSTRACT

The effects of 5-HT related compounds injected into brain on locomotor activity and intracranial self-stimulation (ICSS) mediated by the mesolimbic dopamine (DA) system were investigated. Microinjections of 8-OH-DPAT (8-hydroxy-2-dipropylaminotetralin), a 5-HT_{1A} receptor agonist, into the dorsal raphé nucleus (DRN) resulted in hypoactivity while administration into the median raphé nucleus (MRN) increased total activity. An interaction between dose and time was observed with both raphé nuclei. With ICSS of the ventral tegmental area (VTA), administration of 8-OH-DPAT into either of these raphé nuclei decreased reward thresholds. In addition, systemic administration of WAY 100635 [N-(2-(4-(2-methoxyphenyl)-1-piperazyl)ethyl)-N-(2-pyridinyl)cyclohexane carboxamide], a 5-HT_{1A} receptor antagonist, blocked the ICSS reward enhancing effects of 8-OH-DPAT in both raphé nuclei. Microinjection of TFMPP [4-(3-trifluoromethylphenyl)piperazine], a 5-HT agonist, into the nucleus accumbens (NAS) increased reward thresholds. Under equivalent conditions, another 5-HT agonist, RU 24969 [5-methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1H-indole], had no significant effect in the NAS. At this NAS site, administration of amphetamine decreased reward thresholds. The results with 8-OH-DPAT demonstrate that there are differential functional roles for the ascending serotonergic projections from the DRN and the MRN in the rat brain. The involvement of the 5-HT_{1A} receptor in these effects was confirmed by the antagonism of effects of 8-OH-DPAT by WAY 100635. The differential effects of TFMPP and RU 24969 may reflect the relative lack of pharmacological specificity of

these compounds. Further work with more selective drugs will be necessary to elucidate the mechanism of TFMPP effects in the NAS.

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ABBREVIATIONS

%	percentage
µg	microgram(s)
µl	microlitre(s)
µm	micrometer(s)
5-CT	5-carboxamidotryptamine
5-HIA	5-hydroxyindoleacetaldehyde
5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine
5-HTP	5-hydroxytryptophan
5-HTQ	trimethylserotonin
6-OHDA	6-hydroxydopamine
8-OH-DPAT	8-hydroxy-2-dipropylaminotetralin
AC	adenylate cyclase
ADH	aldehyde dehydrogenase
ANOVA	analysis of variance
AO	aldehyde oxidase
AP	anterior and posterior
APZ	amperozide

AR	aldehyde reductase
ATP	adenosine triphosphate
cAMP	cyclic adenosine monophosphate
CNS	central nervous system
COMT	catechol O-methyltransferase
CRF	continuous reinforcement
CSF	cerebrospinal fluid
C-ter.	C-terminal
DA	dopamine
DAG	diacylglycerol
DC	direct current
DOB	4-bromo-2,5-dimethoxyamphetamine
DOI	4-iodo-2,5-dimethoxyamphetamine
DOPA	3,4-dihydroxyphenylalanine
DOPAC	3,4-dihydroxyphenylacetic acid
DRN	dorsal raphé nucleus (or nuclei)
DV	dorsal and ventral
EPS	extrapyramidal symptoms
g	gram(s)
GABA	γ-aminobutyric acid

h	hour(s)
HVA	3-methoxy-4-hydroxyphenylacetic acid
Hz	hertz
ICSS	intracranial self-stimulation
IP	intraperitoneal
IP_3	inositol triphosphate
kg	kilogram(s)
log	logarithm
LSD	lysergic acid diethylamide
M50	frequency that maintained half-maximal response rates
MAO	monoamine oxidase
max	maximum
MDMA	3,4-methylenedioxy-N-methylamphetamine
MFB	medial forebrain bundle
mg	milligram(s)
min	minute(s) or minimum
ML	medial and lateral
ml	millilitre(s)
mm	millimeter(s)

mM	millimolar
MRN	median raphé nucleus (or nuclei)
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NAS	nucleus accumbens
ng	nanogram(s)
p	probability
PFC	prefrontal cortex
PPHT	2-(N-phenylethyl, N-propyl)-amino-5-hydroxytetralin
RMAX	maximal number of responses at a single frequency
RU 24969	5-methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1H-indole
SCRNC	serotonin club receptor nomenclature committee
SEM	standard error of mean
SN	substantia nigra
STR	striatum
TFMPP	4-(3-trifluoromethylphenyl)piperazine
TM	transmembrane
TRES	total number of responses per session

VTA	ventral tegmental area
WAY 100635	N-(2-(4-(2-methoxyphenyl)-1-piperazyl)ethyl)-N-(2-pyridinyl)cyclohexane carboxamide

INTRODUCTION

1. General Introduction

Since major neurochemicals such as serotonin (5-hydroxytryptamine, 5-HT) and dopamine (DA) were first identified in the central nervous system (CNS) in the early 1950s (Gaddum, 1953; Twarog and Page, 1953; Bedard *et al.*, 1969; Carlsson, 1987), enormous strides have been made towards understanding the anatomy, behavioural functions and clinical importance of neurons utilizing these neurotransmitters. It is true that much of the early research on neurotransmission in the CNS focused on these monoamines. Psychopharmacology has been helped greatly by advances, both technical and theoretical, in other areas of pharmacology. It can be said that the 1960s was the decade of the synapse, the 1970s the decade of receptor, and the 1980s the decade of post-receptor intra-neuronal mechanisms. These decades of research were triggered by the increasing recreational use of psychoactive drugs such as lysergic acid diethylamide (LSD), mescaline and marijuana, and by the discovery that chlorpromazine and reserpine could alleviate some of the symptoms of schizophrenia. Reserpine was shown to cause monoamine neurotransmitters to leak from their synaptic vesicles into the cytoplasm of the presynaptic cell, where they are broken down by enzymes (Carlsson *et al.*, 1957 & 1958). This finding represented one of the earliest pieces of evidence of an interaction between a psychoactive drug and a putative neurotransmitter.

A psychopharmacological drug action is one in which behavioural change is brought about by an exogenous chemical. There is intense interest in determining the

mechanism involved at the molecular level. The binding affinity between drugs and the receptors at which they act is determined by forces underlying the intermolecular interaction between ligand molecules and amino acid residues of receptor proteins. After binding to receptors, many drugs tend to modify a second messenger system, leading to signal transduction which is required to cause cellular responses and behavioural effects. An alternate mechanism for this stage of signal transduction is direct alteration of membrane ion channels.

Observed behavioural effects of peripheral drug administration represent the empirical outputs of complicated interactive mechanisms through the central and peripheral nervous systems. Generally, manipulations of DA neurotransmission by agonists that stimulate DA receptors induce motor activation and repetitive or stereotyped behaviour (Beninger, 1983). Thus, locomotor activity measurements have often been used as behavioural tests of DA receptor antagonism for potential antipsychotic drug action. In addition, self-stimulation paradigms such as electrical brain self-stimulation or drug self-administration in laboratory animals have been used as models for studying DA involvement in brain reward mechanisms (Ettenberg *et al.*, 1981). Nakahara *et al.* (1992) revealed *in vivo* evidence that drugs of abuse like psychomotor stimulants, opiates, nicotine and ethanol, which show rewarding properties, increase extracellular DA preferentially in the nucleus accumbens (NAS) as compared with the striatum (STR), two important terminal projection sites of forebrain DA pathways. Kuhar *et al.* (1991) have reviewed the evidence for DA hyperactivity as a basis for the reinforcing properties of cocaine in the mesolimbic system of the brain.

Schizophrenia is one of the most common psychiatric disorders. Over the last 30 years, DA hyperactivity in forebrain has been proposed as a basis for understanding schizophrenia (Farde, 1997). This hypothesis has been followed by the recently evolved concept of dysfunctional integration between cortical and subcortical DA activity (Willner, 1997). Moreover, interest in a possible role of monoamine neurotransmitters other than DA in the pathogenesis of schizophrenia is increasing, especially in the case of 5-HT. If 5-HT plays a role in schizophrenia (Ohuoha *et al.*, 1993; Breier, 1995), then DA and 5-HT systems cannot be considered separately when describing the disease (Kahn and Davidson, 1993; Kapur and Remington, 1996). Both are anatomically closely connected (Törk, 1991) and functionally highly interactive (Kelland *et al.*, 1990). Kahn and Davidson (1993) stated that at least some of the clinical effects of neuroleptics, conventional antipsychotics producing significant neurological side effects, may be due to an alteration of DA and 5-HT interactions.

5-HT receptors may exist as hetero-receptors in DA pathways such as the mesolimbic system. In this system 5-HT receptors are at presynaptic sites on non-5-HT-containing neurons and regulate the activity of those neurons. Thus, 5-HT release can modulate DA activity by binding to the hetero-receptors on the presynaptic elements of DA neurons. Consequently, 5-HT receptor-related compounds can be expected to modulate DA activity (See section 4. 5-HT-DA interactions).

The studies in this thesis investigated the role of 5-HT receptors in regulating the activity of a forebrain dopaminergic pathway, the mesolimbic system. Increased

activity in this DA system at the level of the nucleus accumbens (NAS) has been considered as a principle factor implicated in the etiology of schizophrenia (Weinberger, 1987; Farde, 1997). Thus, this pathway has been identified as a target for many antipsychotic drugs (Crow *et al.*, 1975). The study of interactions between DA and 5-HT, by increasing our knowledge of the regulatory mechanisms in the mesolimbic system, may contribute to our understanding of the mechanisms of antipsychotic drug action.

In the present experiments, the mesolimbic pathway from the ventral tegmental area (VTA) to the NAS was investigated by the administration of 5-HT related compounds. Some of these compounds were administered locally into 5-HT cell body areas such as the dorsal raphé nucleus (DRN) and the median raphé nucleus (MRN) to modulate the mesolimbic DA system. Other compounds were microinjected into the NAS. Finally, systemic administration was used as a route for testing antagonism of 5-HT receptors.

To obtain a thorough understanding of the potential interactions between DA and 5-HT, it is essential to describe the individual neurotransmitter systems, focusing on their pathways and receptors. The following sections provide a brief review of DA and 5-HT neural systems.

2. Dopamine

Dopamine (DA) was first identified in brain about 40 years ago (Carlsson *et al.*, 1958). There has been enormous interest in understanding the anatomical, behavioural and clinical importance of neurons using this catechol-monoamine as a neurotransmitter substance. DA is found in the central nervous system (CNS) and at some ganglia in the autonomic nervous system. Most of our knowledge of the functional role of DA relates to the CNS.

2.1. DA Synthesis and Catabolism

DA is made from the amino acid L-tyrosine, which is hydroxylated by the enzyme tyrosine hydroxylase to 3,4-dihydroxyphenylalanine (L-DOPA). Tyrosine is taken up into dopaminergic neurons from plasma by an active-transport process found on the nerve membrane. Tyrosine hydroxylase is found in the cytoplasm and on cell membranes of catecholamine neurons, and utilizes a pteridine cofactor, ferrous ions and oxygen. The rate-limiting step in the synthesis of DA is the conversion of tyrosine into L-DOPA by tyrosine hydroxylase. Under normal conditions, this enzyme is saturated with L-tyrosine, and increases in circulating tyrosine levels do not increase the rate of DA synthesis. L-DOPA is actively taken up into DA neurons or glial cells in the CNS where it is decarboxylated by aromatic L-amino acid decarboxylase (DOPA decarboxylase) to form DA. DOPA decarboxylase is a cytoplasmic enzyme, which requires pyridoxal phosphate (vitamin B6) as cofactor. Most of the DA produced is found in storage granules in which it forms complexes with chromogranins, divalent metal ions and adenosine

triphosphate (ATP). Stored neuronal DA is released into the synaptic cleft by the process of exocytosis in response to action potentials and to drugs. This release is dependent upon an influx of calcium into the neuron (Cooper *et al.*, 1996).

Following release into the synaptic cleft, the biological activity of DA is terminated by a neural uptake system, which is a high-affinity, energy-dependent active transport system. Any DA that is taken up into a neuron or which is not bound in the storage granules is catabolized by monoamine oxidase (MAO) and by catechol O-methyltransferase (COMT). MAO is located mostly on mitochondria, while COMT is a soluble enzyme found in the cytoplasm. A major metabolite of DA is 3,4-dihydroxyphenylacetic acid (DOPAC), which is converted by the actions of COMT into homovanillic acid (HVA, 3-methoxy-4-hydroxyphenylacetic acid) (McIlwain and Bachelard, 1971; Lader, 1980; Cooper *et al.*, 1996).

Figures 1 and 2 illustrate the pathways for synthesis and breakdown of DA, describing multiple enzyme systems and their cofactors.

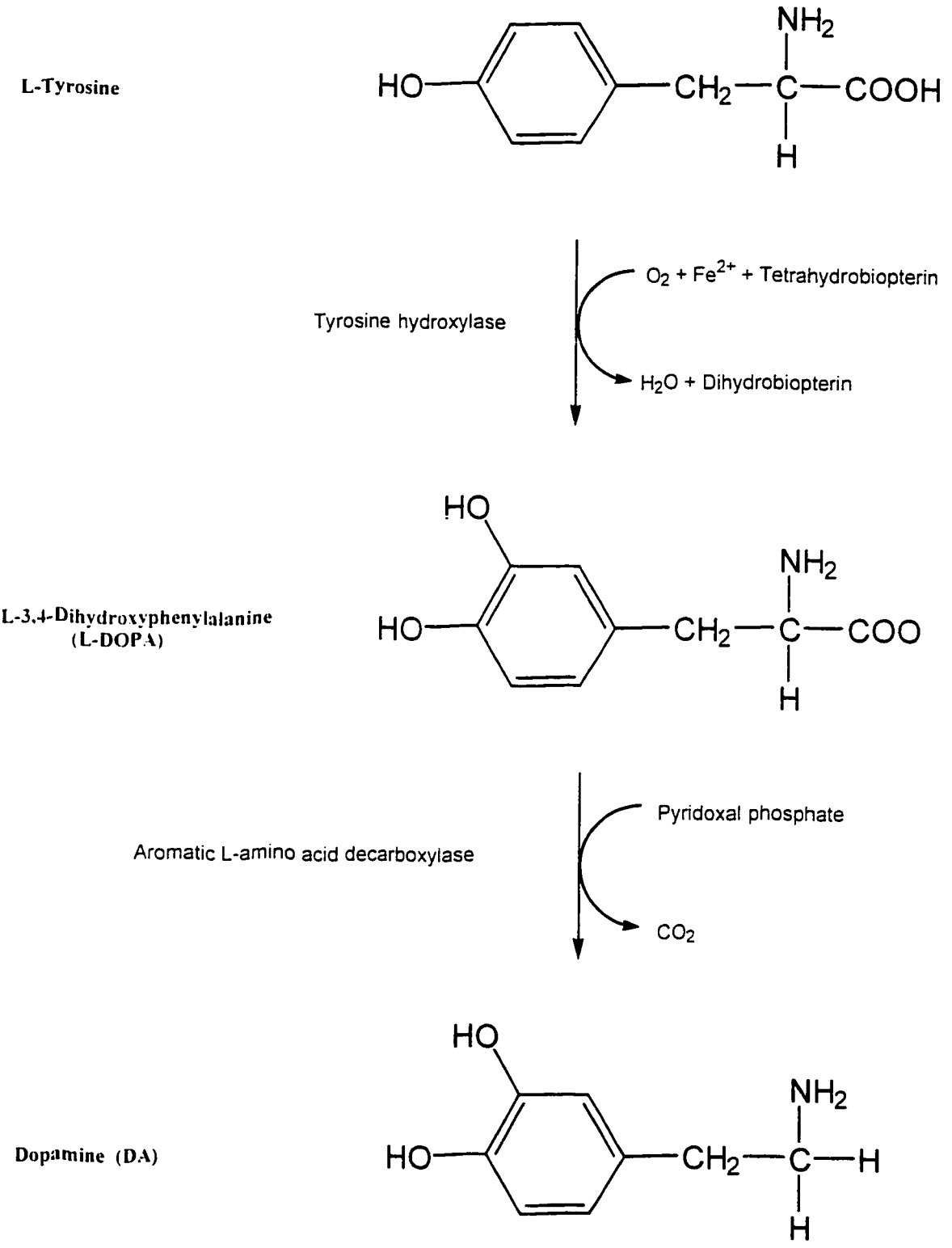


Figure 1. The pathways of dopamine synthesis

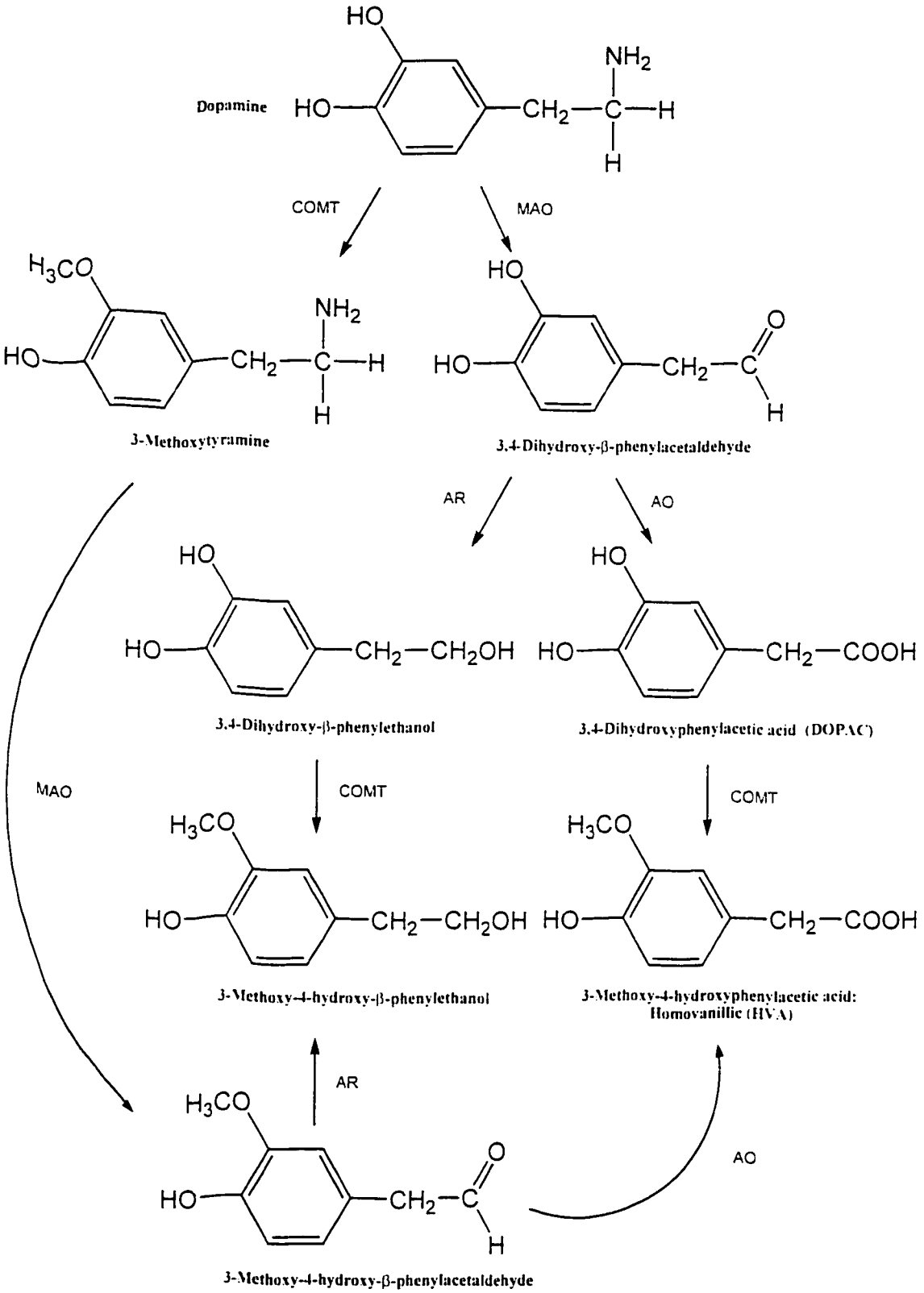


Figure 2. The pathways of dopamine inactivation (COMT, catechol O-methyltransferase; MAO, monoamine oxidase; AO, aldehyde oxidase; AR, aldehyde reductase)

2.2. DA Pathways

Three major neuronal systems (Figure 3) in the brain use DA as a neurotransmitter (Dahlström and Fuxe, 1964): There are the mesocorticolimbic, nigrostriatal and tuberoinfundibular systems.

Mesocorticolimbic System

The neuronal cell bodies (A10) of the mesocorticolimbic system lie in the ventral tegmentum of the midbrain. The axons from these cell bodies terminate in the head of the caudate nucleus, the nucleus accumbens, the olfactory tubercle, the amygdaloid nuclei, the frontal and cingulate cortex. This system is believed to be concerned with cognition, learning, reinforcement, memory and emotion. The mesocorticolimbic DA pathway is thought to be important for the neurochemical etiology of schizophrenia. It has been suggested that excessive DA activity of the mesolimbic system may be associated with positive symptoms such as hallucinations and delusions, and that abnormally low DA activity of the mesocortical system may be a cause of negative symptoms such as alogia, anhedonia and affective flattening (Davis *et al.*, 1991). Many therapeutically important drugs, including antipsychotics, interact with dopamine receptors in the limbic system.

Nigrostriatal System

The neuronal cell bodies (A8/9) of the nigrostriatal system lie in the substantia nigra (SN) of the midbrain and their axons terminate in the caudate

nucleus-putamen complex (neostriatum) (Bedard *et al.*, 1969). This system is associated with integration of incoming sensory stimuli and control of movement (Anden *et al.*, 1966; Lingjaerde, 1994). This pathway forms part of the extrapyramidal system of the basal ganglia. The motor side-effects of antipsychotic drugs, including Parkinson-like effects, are related to DA receptor blockade in the striatum and inactivation of DA neurons in the SN (Gerlach, 1991).

Tuberoinfundibular System

The neuronal cell bodies (A12) of the tuberoinfundibular system lie in the region of the arcuate nucleus of the hypothalamus, bearing short axons which terminate in the median eminence. This system is associated with neuronal control of the hypothalamic-pituitary endocrine system (Cooper *et al.*, 1996). Antipsychotics influence the secretion of hormones (e.g., increased prolactin; Meltzer, 1985) in the pituitary and elsewhere mainly as a result of their blockade of DA receptors in this system (Schatzberg and Nemeroff, 1998).

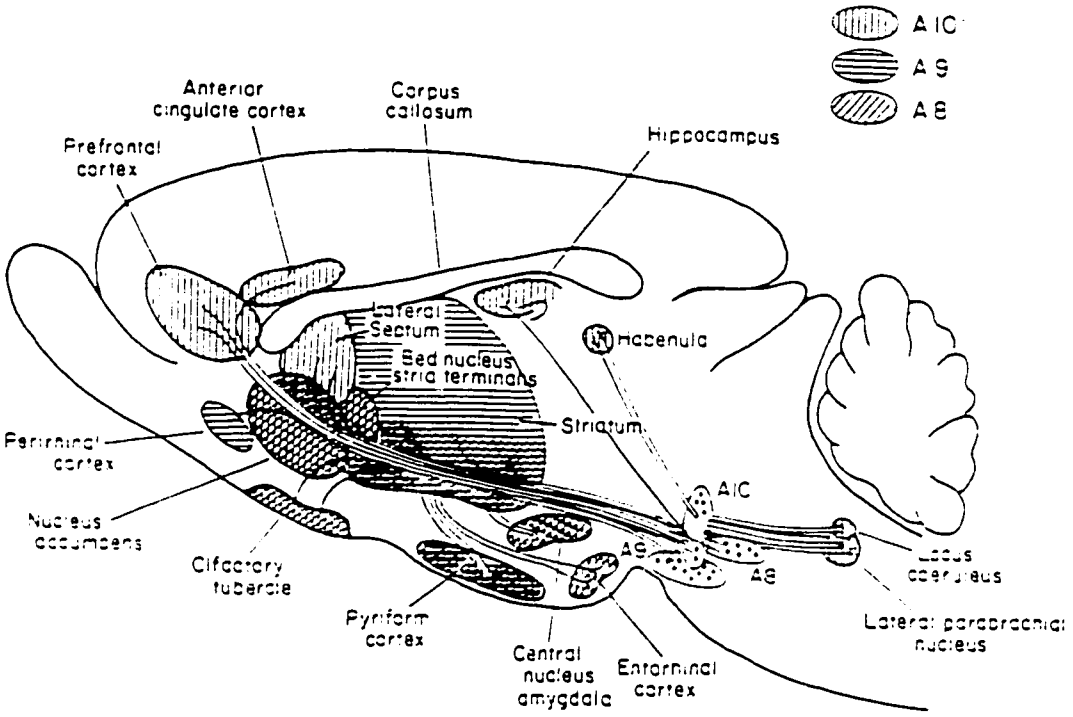


Figure 3. Principal dopaminergic systems in the brain (adapted from Cooper *et al.*, 1996)

2.3. DA Receptors

Several prominent psychiatric and neurological disorders, including schizophrenia, Parkinson's disease and Huntington's chorea are associated with abnormalities of DA neurotransmission. Therefore, DA receptors in the brain represent a major target in the treatment of these disorders.

DA receptors are classified into two main groups: D_1-like and D_2-like DA receptors (Table 1). D_1-like DA receptors include the D_1 and D_5 DA receptor subtypes (Sunahara *et al.*, 1990 & 1991), which are positively coupled to adenylyl cyclase and function at postsynaptic and terminal presynaptic sites (Civelli *et al.*, 1993). Three main types of D_2-like DA receptors have been identified: D_2, D_3 and D_4 DA receptor subtypes, which function as autoreceptors on the cell bodies of DA-containing neurons and as postsynaptic receptors. These receptors are negatively coupled to adenylyl cyclase (Civelli *et al.*, 1993; Kalivas, 1993).

While D_1 and D_2 receptors are widely expressed in many neural systems, the novel D_3 and D_4 receptors appear to be concentrated within parts of the limbic system, and the D_5 receptor has a limited and unusual distribution (Table 2). In brain regions such as the SN and VTA, high levels of $D_{2\text{-like}}$ DA receptors, but not $D_{1\text{-like}}$ DA receptors, are detected. D_1 and D_2 DA receptors have the highest densities in the neostriatum (caudate) while D_3 DA receptors are very abundant in parts of the NAS and the islands of Calleja. The hippocampus is one of the few regions of the brain that have been found to express all five subtypes of the DA receptors. The cortex expresses D_1 DA receptors at relatively low levels.

Table 1. The classification of dopamine receptor subtypes (summarized from data or reviews by Gingrich and Caron, 1993; Meador-Woodruff, 1994; Seeman, 1995; Cooper *et al.*, 1996; Neve and Neve, 1997).

Class	Subtypes	Transductional Characteristics	Structural Characteristics	Drugs Acting on the Receptors
$D_{1\text{-like}}$	D_1	G-protein coupled Stimulate AC ↑ cAMP	7 TM. No Intron. Long C-ter. Rat 446 amino acids Human 446 amino acids	SKF 38393 SKF 89615 SKF89626
	D_5	No G-protein coupled Mainly ↑ IP_3 / DAG Stimulate AC	7 TM. No Intron. Long C-ter. Rat 475 amino acids Human 477 amino acids	B-HT 920 SCH 23390 BW 737C
$D_{2\text{-like}}$	$D_{2(short)}$	G-protein coupled Inhibit AC ↓ cAMP	7 TM. Introns. Short C-ter. Rat 415 amino acids Human 414 amino acids	Quinpirole Bromocriptine Sulpiride
	$D_{2(long)}$		7 TM. Introns. Short C-ter. Rat 444 amino acids Human 443 amino acids	
	$D_{3(short)}$	G-protein coupled Inhibit AC ↓ cAMP	7 TM. Introns. Short C-ter. Rat 446 amino acids Human 400 amino acids	7-OH-DPAT UH 232
	$D_{3(long)}$			
	D_4 (at least 8 subtypes)	G-protein coupled Inhibit AC ↓ cAMP	7 TM. Introns. Short C-ter. Rat 385 amino acids Human 387 amino acids	Clozapine

Table 2. Neuroanatomical distribution and abundance of dopamine receptors in brain (summarized from data or reviews by Snyder, 1990; Gingrich and Caron, 1993; Meador-Woodruff, 1994; Seeman, 1995; Neve and Neve, 1997). Abundance is noted by high (+++), medium (++), low (+) and doubtful (?) according to ligand binding affinity to receptors.

Class	Subtypes	Distribution and Abundance
$D_{1\text{-like}}$	D_1	Caudate putamen (+++). Nucleus accumbens (+++). Olfactory tubercle (+++). Amygdala (+++). Cortex (++). Septum (+). Hippocampus (+). Hypothalamus (+). Thalamus (+). Cerebellum (+)
	D_5	Hippocampus (++). Parafascicular nucleus of thalamus (++). Mammillary bodies (++). Anterior pretectal nuclei (++). Hypothalamus (−). Striatum (?). Cortex (?)
$D_{2\text{-like}}$	D_2	Caudate putamen (+++). Substantia nigra pars compacta (+−−). Ventral tegmental area (+++). Zona incerta (+++). Nucleus accumbens (+−−). Globus pallidus (+++). Olfactory tubercle (+++). Amygdala (+). Septum (+). Hippocampus (+). Cortex (+). Hypothalamus (+). Thalamus (−). Cerebellum (+)
	D_3	Nucleus accumbens (+++). Islands of Calleja (+++). Substantia nigra (++). Ventral tegmental area (++). Olfactory tubercle (++). Ventral pallidum (++). Caudate putamen (+). Amygdala (+). Septum (+). Hippocampus (+). Cortex (+). Hypothalamus (+). Thalamus (+), Cerebellum (+)
	D_4	Hypothalamus (++). Thalamus (++). Frontal cortex (++). Amygdala (+). Hippocampus (+). Olfactory bulb (+), Striatum (+). Nucleus accumbens (+). Substantia nigra (?). Ventral tegmental area (?)

3. 5-Hydroxytryptamine (5-HT)

5-HT (serotonin), a neurotransmitter in the CNS and in the myenteric plexus of the gut, was discovered about 60 years ago (Gaddum, 1953; Erspamer, 1963; Page, 1976). High concentrations of 5-HT are found in the enterochromaffin cell system of the gastrointestinal tract and in blood platelets. 5-HT is an indole-monoamine, and many features of its synthesis, storage, release and inactivation are similar to the processes occurring in tissues which synthesize the other monoamines.

3.1. 5-HT Synthesis and Catabolism

5-HT is synthesized from the aromatic amino acid L-tryptophan, which is actively taken up into 5-HT neurons by means of a carrier mechanism for large neutral amino acids. L-tryptophan is hydroxylated to 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan hydroxylase. Tryptophan hydroxylase is only found in the cytoplasm of 5-HT neurons and is the rate-limiting enzyme in the synthesis of 5-HT. This enzyme requires molecular oxygen and pteridine cofactor for its activity. 5-HTP is decarboxylated in the cytoplasm to 5-HT by the non-specific enzyme aromatic L-amino acid decarboxylase. 5-HT storage mechanisms have many features in common with catecholamine storage processes. 5-HT is believed to be bound in a granular complex with chromogranins, divalent metal ions and adenosine triphosphate (ATP). Stored neuronal 5-HT is released into the synaptic cleft by the process of exocytosis in response to action potentials and to drugs. This release is dependent upon an influx of calcium into the neuron (Cooper *et al.*, 1996).

Any 5-HT in the neuron but not present in storage vesicles is converted into inactive metabolites by the enzyme monoamine oxidase (MAO). MAO is a mitochondrial enzyme, which oxidatively de-aminates 5-HT into 5-hydroxyindoleacetaldehyde (5-HIA), which is then converted by the actions of an aldehyde dehydrogenase into 5-hyroxyindoleacetic acid (5-HIAA), the major inactive metabolite of 5-HT (McIlwain and Bachelard, 1971; Lader, 1980; Cooper *et al.*, 1996).

Figure 4 and 5 illustrate the pathways of synthesis and breakdown of 5-HT, describing multiple enzyme systems and their cofactors.

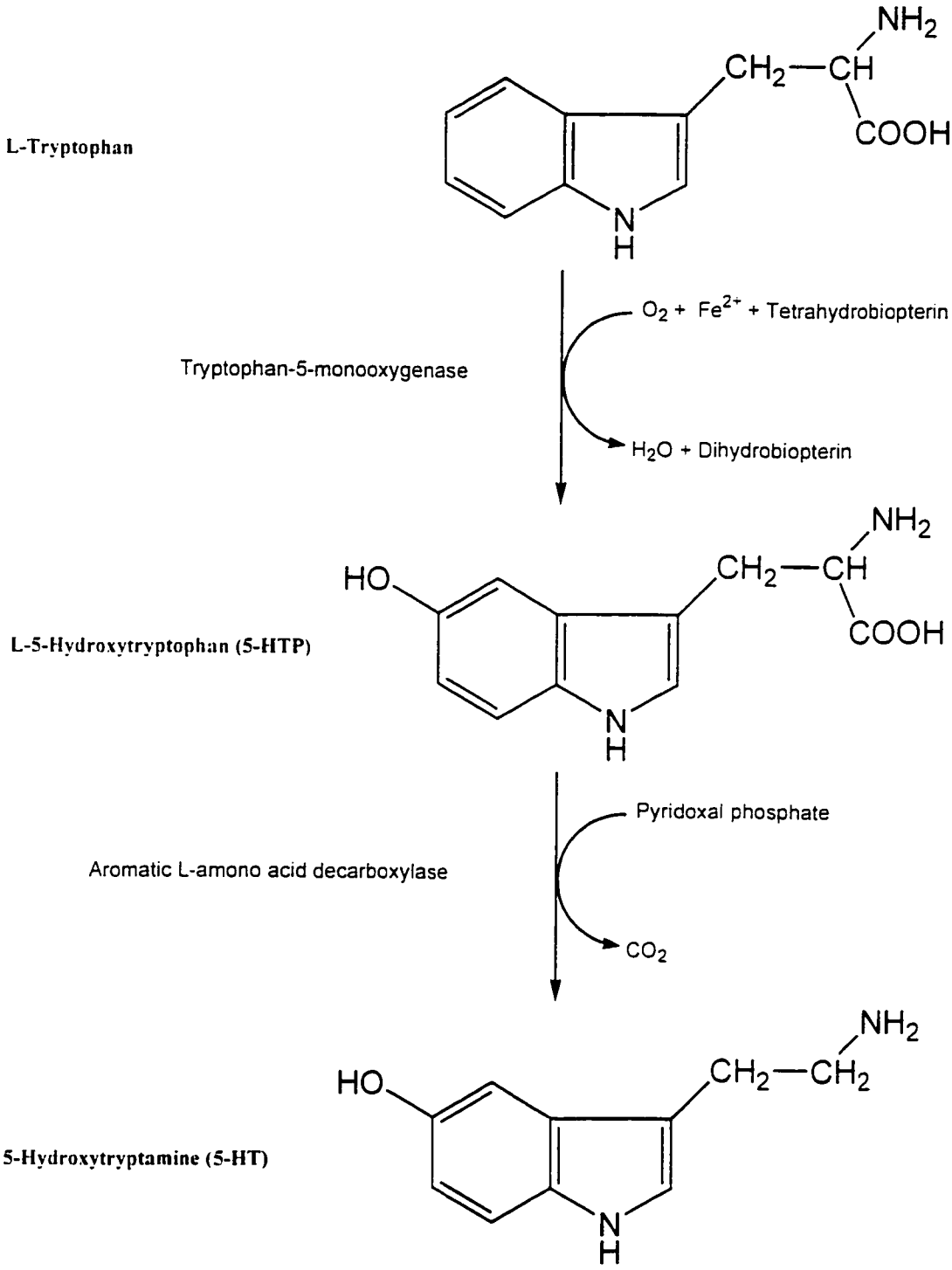


Figure 4. The pathways of 5-HT synthesis

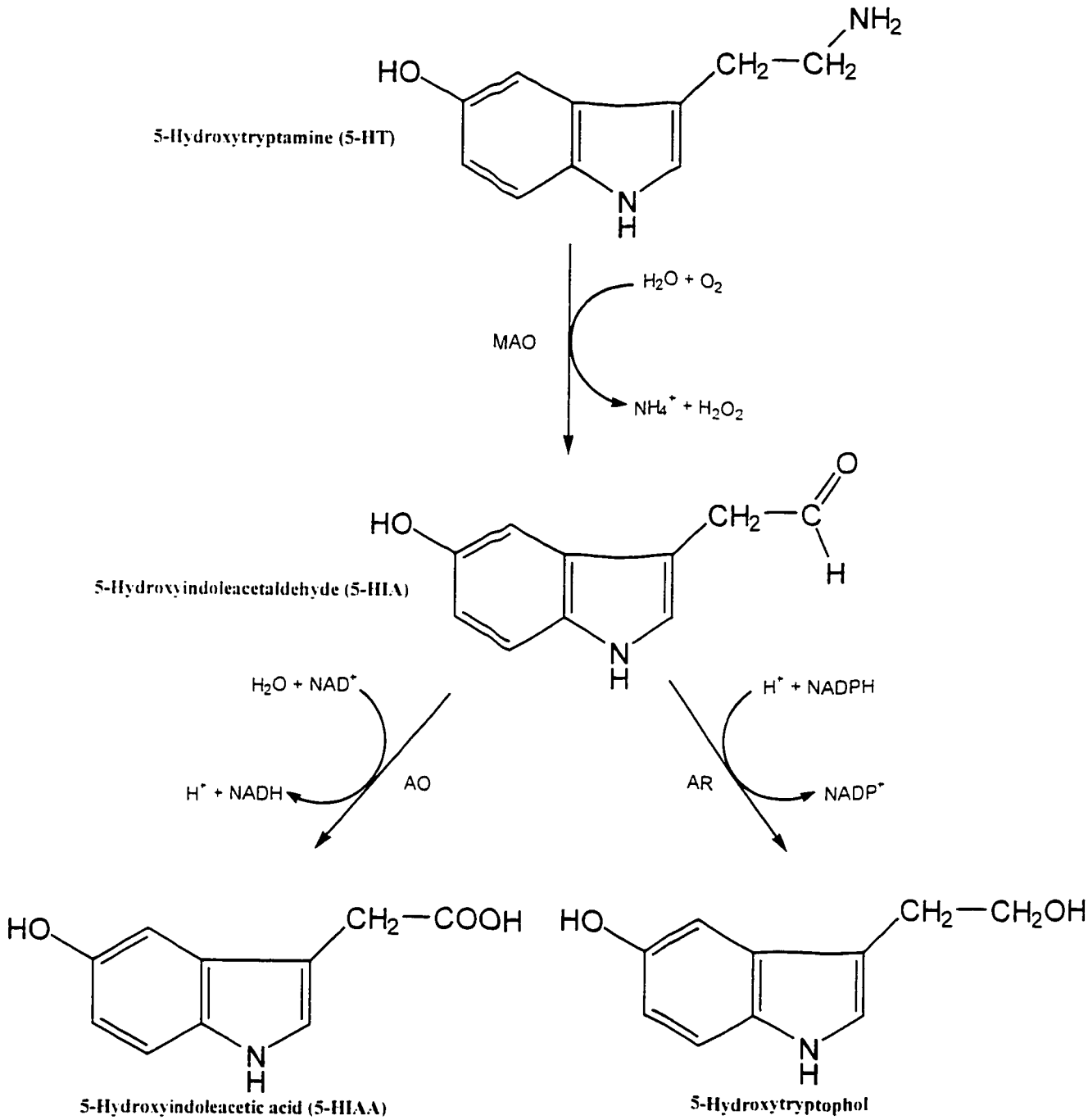


Figure 5. The pathways of 5-HT inactivation (MAO, monoamine oxidase; AO, aldehyde oxidase; AR, aldehyde reductase)

3.2. 5-HT Pathways

The 5-HT system is one of the most diffusely organized projection systems of the brain (Figure 6). All 5-HT cell bodies in the brain are located in midline (or raphé) nuclei (B1 - B9) (Törk. 1991). The major target regions of the 5-HT projections are in the forebrain and in the spinal cord.

Rostral Ascending System

The rostral part of 5-HT system consists of the caudal linear nucleus (B8), the dorsal raphé nucleus (B6 & B7), the median raphé nucleus (B5 & B8) and nucleus pontis oralis (B8 & B9). Two anatomically distinct types of ascending 5-HT projections have been identified: the transtegmental and periventricular systems (Parent *et al.*, 1981).

The transtegmental system is the pathway where most of the neuronal cell bodies (B6 & B7) lie in the dorsal raphé nucleus (DRN) of the brain stem and to a lesser extent (B8) in the median raphé nucleus (MRN); their fibers converge into the VTA of the rostral midbrain.

The periventricular system is the pathway where the neuronal cell bodies (B7) lie in the rostral pole of dorsal raphé nucleus (DRN) of the brain stem and terminate mainly in the periventricular hypothalamus, arcuate nucleus and internal layer of the median eminence.

Both ascending 5-HT systems merge in the medial forebrain bundle (MFB) area of caudal hypothalamus. From that area, these ascending projections follow several limbic pathways to reach distant territories of innervation. The striatum derives its 5-HT afferents mainly from the DRN (B6 & B7), and the hippocampus from the MRN (B5 & B8) (Lorens & Guldberg, 1974; Azmitia & Segal, 1978; Kreiss and Lucki, 1994; López de Pablo *et al.*, 1996). The amygdala receives a 5-HT projection from the DRN (Azmitia and Segal, 1978). The central nucleus of the amygdala is an important nucleus that receives inputs via the basal and medial nucleus from the lateral nucleus and projects to a variety of hypothalamic and brainstem target areas (Davis, 1994; Pitkänen *et al.*, 1997).

Caudal Descending System

The caudal part of the 5-HT system consists of the raphé pallidus nucleus (B1), the raphé obscurus nucleus (B2) and the raphé magnus nucleus (B3). 5-HT pathways from these midline regions in the brain stem innervate the medulla and the spinal cord. These descending projections of the raphé nuclei modulate spinal sensory and motor neurons.

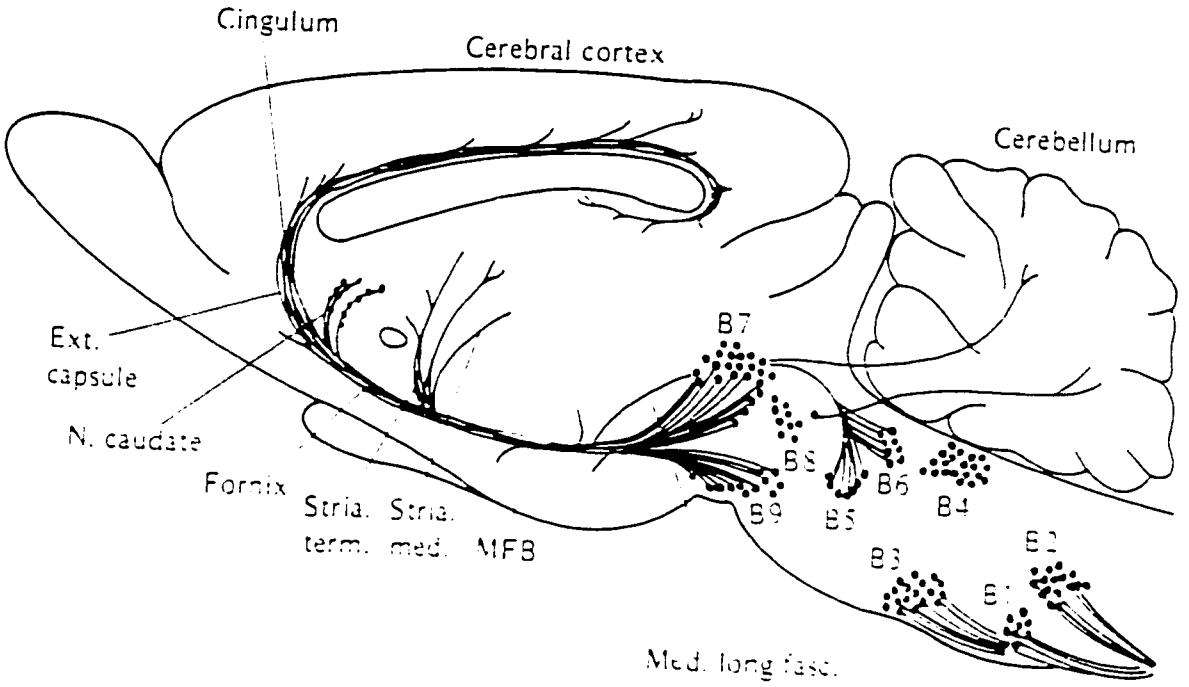


Figure 6. Principal serotoninergic systems in the brain (adapted from Cooper *et al.*, 1996)

3.3. 5-HT Receptors

The development of gene cloning techniques led to the discovery of many new 5-HT receptor subtypes. In response to the increasing complexity of 5-HT receptor classification, the Serotonin Club Receptor Nomenclature Committee (SCRNC) proposed a new system according to operational, transductional and structural characteristics (Humphrey *et al.*, 1993; Hoyer *et al.*, 1994). At present, at least eight subtypes of 5-HT receptors in brain tissue have been defined and characterized on the basis of development of molecular biology (Table 3). In the present classification, the known 5-HT_{1C} and 5-HT_2 receptors were renamed as 5-HT_{2C} and 5-HT_{2A}, respectively. In this thesis, the term 5-HT_2 receptors will describe results for which specificity for the 5-HT_{2A} and 5-HT_{2C} subtypes is undetermined. For some new 5-HT receptors, such as 5-ht_{5A}, 5-ht_{5B}, and 5-ht_6, there is limited information concerning operational, transductional and structural characteristics. For this reason, the lower case appellation is presently used to define these gene products (Kenakin *et al.*, 1992; Martin and Humphrey, 1994).

Great interest has focused on 5-HT_{1A}, 5-$HT_{1B/D}$, 5-HT_{2A} (formerly 5-HT_2), 5-HT_{2C} (formerly 5-HT_{1C}) and 5-HT_3 receptors as targets for antipsychotic drug action (Gerlach, 1991; Kahn *et al.*, 1993). 5-HT_{1A} somatodendritic, 5-$HT_{1B/D}$ and 5-HT_3 presynaptic autoreceptors are associated with a negative feedback mechanism that limits 5-HT neuronal activity. In addition, it is likely that the 5-HT_{1A}, 5-$HT_{1B/D}$ and 5-HT_3 receptors also act as postsynaptic 5-HT receptors in some brain regions. 5-HT_2 receptors appear to be postsynaptic receptors that serve to potentiate serotonergic activity (Mansour *et al.*, 1995).

In the VTA there is a relatively high density of 5-HT_{1B} and 5-HT_{2A} receptors (Hellendall *et al.*, 1993; Kalivas, 1993; Boess and Martin, 1994). In the VTA and SN of rats, the 5-HT_{1B} and 5-HT_2 receptor have been associated with a pharmacological or physiological function (Kalivas, 1993). The NAS also has moderate amounts of 5-HT_{1B} and 5-HT_3 receptors, and a lower density of 5-HT_{2C} receptors (Bruinvels *et al.*, 1993; Ohuoha *et al.*, 1993; Boess and Martin, 1994). 5-HT_3 receptors are present in the NAS (Boess and Martin, 1994) as well (Table 4). The amygdala has a relatively high density of 5-HT_{1A}, 5-HT_{2C} and 5-HT_3 receptors (Boess and Martin, 1994).

5-HT_{1A} receptors play a critical role in mediating inhibition of cell firing in the midbrain raphé nuclei (Dourish *et al.*, 1986; Chen *et al.*, 1992). In a number of studies, 8-hydroxy-di-N-propylamino-tetralin (8-OH-DPAT), a 5-HT_{1A} agonist, has been used for investigating behavioural activity involving the 5-HT system (Hillegaart, 1990; Higgins and Elliott, 1991; Montgomery *et al.*, 1991; Hogg *et al.*, 1994; Fletcher *et al.*, 1995). Microinjection of 8-OH-DPAT into the DRN resulted in increased social interaction but did not change the motor activity of rats tested in bright light, indicating an anxiolytic response (Hogg *et al.*, 1994). At lower doses, 8-OH-DPAT activates somatodendritic 5-HT receptors while at higher doses, this drug stimulates postsynaptic 5-HT receptors (Tricklebank *et al.*, 1984; Dourish *et al.*, 1988; Montgomery *et al.*, 1991). Therefore, it is thought that the effects of this drug depend on the light level and dose. Moreover, 8-OH-DPAT is a potent facilitator of male rat sexual behaviour (Fernández-Guasti and Rodríguez-Manzo, 1997). WAY100635, a 5-HT_{1A} antagonist, has no intrinsic influence on the dorsal

raphé cell firing in vivo, whilst antagonising the inhibition of firing induced by 8-OH-DPAT (Jones and Haskins, 1991; Mundey *et al.*, 1996). Administration of 5-HT_{2C} receptor agonists can influence locomotion, feeding behaviour, body temperature, and hormone secretion and produce anxiogenic effects (Quattrone *et al.*, 1981; Wozniak *et al.*, 1989; Kennett *et al.*, 1989). From post mortem analysis of the brains of schizophrenics, increased numbers of 5-HT_2 receptors have been observed in the NAS and ventral putamen whereas reduced numbers of 5-HT_2 receptors have been observed in the prefrontal cortex (PFC). Densities of 5-HT_{1A} and 5-HT_2 receptors are increased in the posterior cingulate, temporal cortices and hippocampus (Joyce, 1993; Ohuoha *et al.*, 1993). Therefore, altered 5-HT activity can be considered directly or indirectly as an etiological factor in schizophrenia.

Table 3. The classification of 5-HT receptor subtypes (summarized from data or reviews by Middlemiss and Tricklebank, 1992; Shen *et al.*, 1993; Bruinvels *et al.*, 1993; Boess and Martin, 1994; Peroutka, 1994; Martin and Humphrey, 1994; Saudou and Hen, 1994).

Class	Subtypes	Transductional Characteristics	Structural Characteristics	Drugs Acting on the Receptors
5-HT$_1$	5-HT$_{1A}$	G-protein coupled Mainly inhibit AC ↓ cAMP K^+ outflux	7 TM No intron	R(+)-8-OH-DPAT 5-CT R(+)-UH-301 S(-)-Pindolol NAN-190 WAY100.635 LY206130
	5-HT$_{1B}$ (5-HT$_b$)			5-CT Isamoltane
	5-HT$_{1D\alpha}$			Sumatriptan GR127935
	5-HT$_{1D\beta}$			
	5-HT$_{1E}$ (5-HT$_{1E\alpha}$)			RU-24969 Methiothepin
	5-HT$_{1F}$ (5-HT$_{1E\beta}$)			Sumatriptan Methiothepin

5-HT$_2$	5-HT$_{2A}$ (5-HT$_2$)	G-protein coupled ↑ IP_3 / DAG Cl^- influx	7 TM Introns & Exons	α-Methyl-5-HT (±)-DOI R(−)-DOI (±)-DOB Ketanserin Ritanserin Spiperone MDL 100.907
	5-HT$_{2B}$ (5-HT$_{2F}$)			α-Methyl-5-HT (±)-DOI SB204741 SDZ SER-082
	5-HT$_{2C}$ (5-HT$_{1C}$)			α-Methyl-5-HT (±)-DOB Mesulergine Ketanserin TFMPP
5-HT$_3$		Ion channel gated ↑ Na^+ / K^+ conductance Ca^+ influx	4 TM	2-Methyl-5-HT 5-HTQ Zacopride Ondansetron Tropisetron

5-HT$_4$		G-protein coupled ↓ K^+ conductance Activate AC	7 TM	SC-53116 SDZ 205.557
5-ht$_5$	5-ht$_{5A}$	Unknown	7 TM	LSD
	5-ht$_{5B}$	Not G-protein coupled		None known
5-ht$_6$	5-ht$_6$	G-protein coupled Activate AC	7 TM One intron & Exons	LSD 5-CT Methiothepin Amoxipine
5-HT$_7$		G-protein coupled Activate AC	7 TM Two introns & Exons	LSD Clozapine

Table 4. Neuroanatomical distribution and abundance of 5-HT receptors in the rat brain (summarized from data or reviews by Middlemiss and Tricklebank, 1992; Shen *et al.*, 1993; Bruinvels *et al.*, 1993 & 1994; Boess and Martin, 1994; Peroutka, 1994; Martin and Humphrey, 1994; Saudou and Hen, 1994). Abundance is noted by high (+++), medium (++), low (+) and doubtful (?) according to ligand binding affinity to the receptor.

Class	Subtypes	Distribution
5-HT$_1$	5-HT$_{1A}$	Amygdala (+++). Olfactory tubercle (+++). Hippocampus (+++). Thalamus (+++). Raphé nuclei (+++) (higher in the DRN than the MRN). Cortex (+++). Septum (+++). Substantia nigra (?)
	5-HT$_{1B}$ (5-HT$_b$ or 5-HT$_{1D\beta}$)	Substantia nigra (+++). Globus pallidus (+++). Dorsal subiculum (+++). Olfactory tubercle (++). Caudate nucleus (++). Nucleus accumbens (++). Ventral tegmental area (++). Cortex (+). Raphé nuclei (?) (higher in the MRN than the DRN)
	5-HT$_{1D}$ (5-HT$_{1D\alpha}$)	Hippocampus (+++). Olfactory tubercle (++). Caudate nucleus (++). Nucleus accumbens (++). Cortex (+). Substantia nigra (+). Globus pallidus (+). Striatum (?). Hypothalamus (?). Dorsal raphé (?)
	5-HT$_{1E}$ (5-HT$_{1E\alpha}$)	Cortex (+++). Caudate putamen (+++). Claustrum (+++). Substantia nigra (+). Hippocampus (+). Interpeduncular nucleus (+)
	5-HT$_{1F}$ (5-HT$_{1E\beta}$)	Cortex (++). Hippocampus (++). Thalamus (++). Striatum (+). Hypothalamus (+). Raphé nuclei (+)

5-HT$_2$	5-HT$_{2A}$ (5-HT$_2$)	Cortex (+++). Nucleus accumbens (+++). Claustrum (+++). Olfactory tubercle (+++). Caudate putamen (++). Striatum (++). Hippocampus (+). Amygdala (+). Hypothalamus (+).
	5-HT$_{2B}$ (5-HT$_{2F}$)	Cortex (+). Caudate nucleus (?). Hippocampus (?). Nucleus accumbens (?). Striatum (?)
	5-HT$_{2C}$ (5-HT$_{1C}$)	Choroid plexus (+++). Hippocampus (++). Cortex (++). Amygdala (++). Basal ganglia (+). Substantia nigra (+). Olfactory system (+). Raphé nuclei (+). Nucleus Accumbens (+). Hypothalamus (+). Caudate putamen (+)
5-HT$_3$		Cortex (+++). Hippocampus (+++). Amygdala (++). Nucleus accumbens (+). Striatum (+). Olfactory tubercle (+). Hypothalamus (+)
5-HT$_4$		Interpeduncular nucleus (+++). Hippocampus (+). Substantia nigra (+). Olfactory tubercle (+). Striatum (+). Nucleus accumbens (+). Globus pallidus (+). Septum (+)
5-ht$_5$	5-ht$_{5A}$	Hippocampus (+++). Hypothalamus (+++). Cortex (+). Thalamus (+). Striatum (+). Amygdala (+). Olfactory bulb (+)
	5-ht$_{5B}$	Hippocampus (+++). Habenula (+++). Dorsal raphé nucleus (+). Cortex (+)
5-ht$_6$	5-ht$_6$	Striatum (+++). Olfactory tubercle (++). Cortex (++). Amygdala (++). Hippocampus (+). Hypothalamus (+)
5-HT$_7$		Thalamus (+++). Hypothalamus (+++). Hippocampus (+++). Cortex (++). Striatum (++). Olfactory system (++). Raphé nuclei (+). Amygdala (+). Putuitary (?)

4. 5-HT-DA Interactions

The interactions between the 5-HT and DA systems are of particular interest because of the therapeutic success of atypical antipsychotic drugs such as clozapine (Clozaril®), risperidone (Risperdal®), olanzapine (Zyprexa®) and quetiapine (Seroquel®). Understanding the interaction between 5-HT and DA and its therapeutic implications is particularly timely because additional new antipsychotic medications (e.g., sertindole and ziprasidone) with 5-HT-DA interaction profiles are being tested in clinical trials (Gerlach and Peacock, 1995). There are two possibilities for direct 5-HT / DA interactions: 1) The 5-HT system can regulate the DA system. 2) The DA system can regulate the 5-HT system. In fact, it is likely that the two neurotransmitters co-regulate their function throughout the brain (Kahn and Davidson, 1993).

It is necessary to consider the anatomy and physiology of the DA and 5-HT systems in order to see the possibility of their direct interaction. The ascending DA projections from the VTA to the NAS and from the SN to the striatum have been popular foci of studies concerning antipsychotics because these pathways are the major sites of brain interactions among DA-, 5-HT- and GABA (γ-aminobutyric acid)-containing neurons.

4.1. Serotonergic Regulation of Dopaminergic Function

It has been suggested from previous neurochemical and psychopharmacological data that 5-HT projections tonically or phasically inhibit mesolimbic and nigrostriatal DA activity (see the following examples). These

findings indicate that 5-HT autoreceptor agonists and postsynaptic antagonists might increase DA activity (Muramatsu *et al.*, 1988). Many studies support the inhibitory action of 5-HT on DA transmission in both the mesolimbic and the nigrostriatal system. For example, DOPAC and DA levels were markedly increased in the NAS but slightly enhanced in the striatum after electrolytic lesions of the DRN (Hervé *et al.*, 1979). Electrolytic lesions of the MRN induced a similar effect on DOPAC and DA levels in the NAS following electrolytic lesion of the DRN, but reduced DOPAC and DA levels in the frontal cortex (Hervé *et al.*, 1981). In another study, electrolytic lesions of the MRN, but not the DRN, significantly increased locomotor activity (Jacobs *et al.*, 1974).

Many previous studies also indicate that 5-HT has a predominantly inhibitory effect on neurons in the SN. For example, electrical stimulation of the MRN suppressed the activity of spontaneously firing single DA neurons in the SN (Dray *et al.*, 1976). Fibiger and Miller (1977) revealed that stimulation of the DRN inhibited the unit activity of cells in the SN, but stimulation of the MRN induced no consistent effects upon SN cell-firing. Another study demonstrated that electrical stimulation of the DRN selectively inhibited the firing rate of slowly firing DA neurons in the SN, and intravenous administration of 5-HT_{1A} agonists such as 8-OH-DPAT increased the firing rate of these neurons (Kelland *et al.*, 1990). This inhibition of nigrostriatal activity by the DRN stimulation is in accord with the reduced somatodendritic excitability caused by DA release in the nigrostriatal dendrites (Trent and Tepper, 1991). 5-HT liberated from the raphé-nigral terminals facilitates DA release from nigrostriatal dendrites, resulting in a local autoreceptor-

mediated reduction in somatodendritic excitability. In addition, DA release in the nigrostriatal dendrites is associated with facilitation of a dendritic calcium conductance by 5-HT (Nedergaard *et al.*, 1988). The striatum receives an input arising almost exclusively from the DRN while both the DRN and, to a lesser extent, the MRN innervate the SN (Soubrie *et al.*, 1984). Indeed, relatively recent studies (Kreiss and Lucki, 1994; López de Pablo *et al.*, 1996) have supported the above differential projections between the DRN and the MRN with their findings that the DRN and the MRN are mainly associated with the striatum and the hippocampus, respectively. Thus, the DRN may regulate DA activity in the striatum. However, there are arguments that electrolytic lesions of the MRN increase striatal DA, suggesting 5-HT inhibitory function through the MRN in DA activity of striatum (Dray *et al.*, 1976). It has been proposed that inhibition of DA release in the striatum may be mediated by $5HT_2$ receptors (Ennis *et al.*, 1981; Muramatsu *et al.*, 1988).

On the other hand, some other neurochemical and electrophysiological studies indicate that 5-HT may have an excitatory action in the mesolimbic and nigrostriatal system. Microinjection of 5-HT into the VTA resulted in a significant elevation in perfusate levels of the DA metabolites DOPAC and HVA in the NAS (Guan and Mcbride, 1989). 5-HT-induced increases in dialysate DA in the NAS may be associated with $5\text{-}HT_{2A/C}$ and $5\text{-}HT_3$ receptor subtypes (Chen *et al.*, 1991; Parsons and Justice Jr., 1993). An intracellular recording study demonstrated that 5-HT acts on $5HT_{2A/B/C}$ receptors to increase firing in a large proportion of DA-containing cells in the VTA (Pessia *et al.*, 1994). Benloucif *et al.* (1993) supported

a specific receptor-mediated role for 5-HT in the facilitation of DA release in the striatum, suggesting the involvement of 5-HT_1 (probably 5-HT_{1B}, Galloway *et al.*, 1993) and 5-HT_3 receptors. Moreover, partial 5-HT_4 receptor antagonists such as ICS 205930 (mainly 5-HT_3 receptor antagonist) decreased the facilitation of DA release, which supports a possible involvement of 5-HT_4 receptors (Benloucif *et al.*, 1993). The possibility of indirect involvement of 5-HT_4, but not 5-HT_3 receptors, in enhanced DA release of striatum has also received some support from *in vivo* microdialysis and pharmacological studies (Bonhomme *et al.*, 1995; De Deurwaerdère *et al.*, 1997). A microdialysis study with the DA uptake blocker, nomifensine, has also suggested that striatal DA uptake sites may be involved in the DA-releasing action of 5-HT (De Deurwaerdère *et al.*, 1996). The DA synthesis increased by 3,4-methylenedioxymethamphetamine (MDMA) depends both on 5-HT_2 receptor stimulation and DA efflux (Huang and Nichols, 1993). These data are consistent with an excitatory role for endogenous 5-HT in the stimulation of striatal DA release and metabolism (Yadid *et al.*, 1994).

4.2. Dopaminergic Regulation of Serotonergic Function

In 6-OHDA-lesioned rats, DA receptor agonists such as apomorphine or SKF-38393 inhibited the expression of 5-HT_{2A} receptors in the striatum, abolishing increased 5-HT_{2A} mRNA labeling (Laprade *et al.*, 1996). The data from this study suggest that 5-HT_{2A} receptors, regulated by DA, play an important role in the control of motor activity by DA and 5-HT in the basal ganglia. The negative control of DA receptors (probably D_1) on the expression of 5-HT_{2A} receptors may be

considered as a homeostatic mechanism to balance the effects of DA and 5-HT on motor activity. Neonatal dopaminergic lesions with 6-OHDA increased 5-HT and 5-HIAA levels in the rat striatum (Sivam, 1995), suggesting a dopaminergic influence on striatal 5-HT metabolism. In a recent study, the $D_{2/3/4}$ DA receptor agonist quinpirole attenuated expression of striatal levels of the neuronal marker c-*fos* induced by 5-HT (Cook and Wirtshafter, 1998). However, in another study, the functional regulation of 5-HT release from the hippocampus by DA receptors was positive (Matsumoto *et al.*, 1996). Apomorphine and (±)-PPHT [(±)-2-(N-phenylethyl, N-propyl)-amino-5-hydroxytetralin], a $D_{2/3/4}$ receptor agonist, facilitated 5-HT release in the hippocampus and this facilitation was blocked by the D_2 antagonist sulpiride but not by the D_1 antagonist R(+)-SCH-23390. Thus, the D_2 receptor seems to mediate the facilitation of 5-HT release in the hippocampus. Ferré and Artigas (1993) described a DA D_2 receptor-mediated regulation of extracelluar 5-HT concentrations in the DRN. In their microdialysis studies, intra-DRN infusion of various DA agonists induced a dose-dependent increase in the extracellular concentration of 5-HT in the DRN, thereby increasing somatodendritic 5-HT_{1A} autoreceptor stimulation in this nucleus (Ferré and Artigas, 1993; Ferré *et al.*, 1994). Another dose-dependent increase in 5-HT release induced by MDMA was observed in the striatum and the prefrontal cortex (Gudelsky and Nash, 1996). Moreover, stereotyped behaviours were induced by bilateral microinjection of 5-HT into the striatum and this stereotypy was markedly diminished by 6-OHDA lesions of the striatum (Yeghiayan *et al.*, 1997).

Based on the studies described above, DA may regulate 5-HT activity in the mesocorticolimbic and nigrostriatal systems. The direction of regulation is dependent on various sites in the brain. For example, DA may increase 5-HT activity in the hippocampus (Matsumoto *et al.*, 1996) and the DRN (Ferré *et al.*, 1994), but its effects on 5-HT activity in the striatum is still controversial (Laprade *et al.*, 1996; Gudelsky and Nash, 1996).

5. Antipsychotics

5.1. Pathology of Schizophrenia

Two different types of schizophrenic symptoms have mainly been proposed on the basis of the presence of productive or deficit symptoms (Carpenter *et al.*, 1988). Positive symptoms of schizophrenia involve a syndrome of hallucinations, incoherence of speech, delusion, thought disorders, and incongruity of affect. On the other hand, negative symptoms of schizophrenia involve a syndrome of affective flattening, alogia, anhedonia, attentional impairment, and amotivation accompanied by emotional and social withdrawal (Kaplan and Sadock, 1998).

The simple DA hypothesis of schizophrenia is that schizophrenia results from too high DA activity. However, there appear to be two different disturbances in dopaminergic transmission in schizophrenia (Weinberger, 1987). An increase in DA activity in the mesolimbic component of the dopaminergic system may be related to positive symptoms (Crow, 1980). A decrease in DA activity of the prefrontal area may account for negative symptoms (Robbins, 1991), which do not respond as effectively to many of the antipsychotic drugs. The DA hypothesis of schizophrenia continues to be refined and expanded. Recent by, the DA hypothesis of schizophrenia has evolved into a model of dysfunctional integration between cortical and subcortical dopaminergic activity (Willner, 1997), although the classic concept of increased central dopaminergic transmission has dominated for about 30 years.

Serotonin has lately received considerable attention in schizophrenia studies since the observation was made that the 5-HT-DA antagonists have potent

5-HT-related activities. Specifically, the ratio of drug affinity between 5-HT and DA receptors may be very important for antipsychotic drug actions and side effects (Kapur and Remington, 1996). Other neurotransmitters such as norepinephrine, GABA and glutamate have also implicated in the pathophysiology of schizophrenia. It appears, therefore, that multiple neurotransmitter systems interact in a particular balance of activity levels to regulate the signs and symptoms of schizophrenia (Kaplan and Sadock, 1998)

5.2. Typical Antipsychotics

Chlorpromazine, synthesized by Paul Charpentier in 1950, was introduced for relaxing patients and reducing surgical shock. The drug was reported as an effective drug for the treatment of schizophrenia in 1952. Chlorpromazine stimulated the pharmaceutical industry to develop a number of other phenothiazines. Thioridazine and fluphenazine were developed during this time. The phenothiazine antipsychotics are characterized by a three-ring structure with a six-membered central ring containing a sulfur and a nitrogen atom (Marder and Van Putten, 1995).

Newer types of drugs such as butyrophenones and thioxanthenes were also developed in a series. The butyrophenone antipsychotics are characterized by a substituted phenyl ring, which is attached to a carbonyl group attached by a 3-carbon group to a tertiary amino group. Drugs in this group have a tendency to be potent D_2 antagonists and have minimal anticholinergic and autonomic effects. Haloperidol, a substituted piperidine, is the most commonly used drug from this

class. The thioxanthene antipsychotics have a three-ring structure that is similar to the phenothiazines, but with a carbon substituted for a nitrogen atom in the middle ring. *cis*-Thiothixene is a common antipsychotic and has greater potency than its isomer. Clopenthixol and flupentixol belong to this class as well. Dibenzoxazepine has a three-ring structure with a seven-membered center ring, and loxapine is the only drug from this group currently being used as an antipsychotic. Loxapine has similar structure to clozapine except that an oxygen atom replaces an NH moiety in the middle ring (Kaplan and Sadock, 1998; Marder, 1998).

Although the above-mentioned antipsychotic drugs have been the treatment of choice for schizophrenia for many years, there are very significant limitations with them. These classical antipsychotic drugs, termed neuroleptics, block mainly dopaminergic receptors such as D_2 and D_3 (Gerlach, 1991) in the mesocorticolimbic and nigrostriatal DA systems, causing very serious side effects such as extrapyramidal symptoms (EPS), tardive dyskinesia and akinesia. Seeman *et al.* (1976) reported that the affinity of the traditional antipsychotics for D_2 receptors is highly correlated with their effective clinical dose. Neuroleptic-induced EPS in humans results from occupancy of D_2 receptors in the striatum (Farde *et al.*, 1992). Moreover, typical antipsychotics have limited efficacy against negative symptoms, and many patients freed from their delusions and hallucinations are still unable to resume productive lives due to enduring negative symptoms.

5.3. Atypical Antipsychotics

There has been a search for superior antipsychotics because the typical D_2 DA receptor-blocking drugs (Farde, 1997) often do not result in a remission of negative symptoms and produce serious side effects, including EPS. The DA hypothesis of schizophrenia has recently evolved, although the classic concept of increased central dopaminergic transmission had dominated for about 30 years. The new model of schizophrenia, based on the hypothesis of dysfunctional integration between cortical and subcortical dopaminergic activity (Willner, 1997), is supported by the existence of various DA receptor subtypes and negative feedback systems to control DA activity. On this basis, it has been hypothesized that manipulation of subtypes of DA receptors or (and) other neurotransmitter receptors such as 5-HT receptors may lower the propensity for induction of motor side effects of the typical agents and lead to improved antipsychotic efficacy. Atypical antipsychotic drugs have been developed on the basis of this approach. Actually, the 5-HT-DA antagonists appear to be effective for a broader range of patients with schizophrenia than are the typical DA antagonists. Miller *et al.* (1990) have suggested a "D_1 hypothesis of antipsychotic action" that typical neuroleptics produce their antipsychotic action by directly blocking D_2 receptors, leading to an indirect block of D_1 function and an enhancement of cholinergic function. Thus, the atypical antipsychotics such as clozapine produce antipsychotic action by a direct effect on D_1 receptors and produce few EPS because of their blockade of cholinergic receptors. Moreover, Robertson *et al.* (1994) have proposed that the ability of an

antipsychotic to elevate Fos in dorsolateral striatum shoud be associated with propensity to produce EPS.

Clozapine is the first antipsychotic of the so-called atypical class which has been extensively tested in the clinic. Studies suggest that clozapine may improve negative symptoms as well as positive symptoms through increased turnover of DA in the prefrontal cortex (PFC), an effect not seen with typical antipsychotics (Moghaddam and Bunney, 1990; Moghaddam, 1994). This DA facilitation in the PFC with clozapine may be explained by 5-HT_2 receptor antagonism (Nomikos *et al.*, 1994; Schmidt and Fadayel, 1995). Recently D_4 receptors have received particular attention because clozapine displays a ten-fold higher affinity for D_4 receptors compared to D_2 or D_3 receptors and is effective in treating refractory schizophrenics without the side-effect profile of typical neuroleptics (Liégeois *et al.*, 1998). However, involvement of the D_4 receptor is still controversial as an explanation for pharmacological functions of new antipsychotics, and will probably remain so until specific antagonists of this receptor are developed (Reynolds, 1996a & 1996b). A high affinity ratio for blockade of 5-HT_2 receptors relative to D_2 receptors may explain the atypical profile of clozapine, which at therapeutic doses has 20 to 67 % D_2 occupancy, below the 75 to 80 % D_2 threshold occupancy for EPS (Kapur and Remington, 1996). In addition, clozapine has low affinity for D_1 dopaminergic, but high affinity for α_1 adrenergic, H_1 histaminergic and muscarinic cholinergic receptors. This drug is probably the most effective for severely ill patients, but a second-line drug because of its significant adverse effects such as

agranulocytosis, seizures and anticholinergic effects (Kaplan and Sadock, 1998; Owens and Risch, 1998).

The most widely used 5-HT-DA antagonist, risperidone, has similar functional and pharmacological profiles to clozapine, and has fewer EPS than haloperidol but not clozapine. There are few EPS at low doses of risperidone, but the same level of EPS at high doses as for neuroleptics (Kapur *et al.*, 1995). At doses commonly used, it is not associated with EPS. A growing body of evidence supports its role as a first-line agent for first-break, mildly to moderately ill patients and for severely ill, treatment-refractory patients (Kaplan and Sadock, 1998; Owens and Risch, 1998).

Olanzapine is a effective atypical antipsychotic agent which has antagonist profiles against 5-HT_{2A}, 5-HT_{2C}, D_1, D_2 and D_4 receptors. This drug has a mild, but somewhat different, profile of adverse effects compared to risperidone despite sedation, weight gain, hypotension and constipation (Kaplan and Sadock, 1998; Owens and Risch, 1998).

Sertindole is an effective drug with a favorable profile of adverse effects that are mostly transient. This drug may cause hypotension, sinus tachycardia, nasal congestion and decreased ejaculatory volume, but not anticholinergic symptoms. Its half-life of 3 days makes it ideal for poorly compliant patients (Kaplan and Sadock, 1998; Owens and Risch, 1998).

Quetiapine is an effective agent associated with no increased risk of EPS. Sedation, tachycardia, agitation and weight gain may be caused after administration.

It is required to titrate initial doses so as to avoid orthostatic hypotension (Kaplan and Sadock, 1998; Owens and Risch, 1998).

Ziprazidone is an additionally effective antipsychotic agent for patients with affective symptoms and for patients with anxiety. This drug is a blocker of reuptake of 5-HT and norepinephrine, and an agonist for 5-HT_{1A} receptors. Adverse effects include sedation, nausea, dizziness and light-headedness, but not weight gain. This is the only 5-HT-DA antagonist suitable for depot formulation (Kaplan and Sadock, 1998).

The putative atypical antipsychotic drug amperozide (APZ) shows high affinity for 5-HT_2 receptors, moderate affinity for α_1-adrenergic receptors and relatively low affinity for D_1, D_2 or 5-HT_{1A} receptors (Haskins *et al.*, 1987; Svartengren and Simonsson, 1990). Nomikos *et al.* (1994), in a study on amperozide, stated that 5-HT_2 receptor antagonism may be of considerable significance for the action of atypical antipsychotic drugs on mesocorticolimbic dopaminergic transmission.

Risperidone (Risperidal®), olanzapine (Zyprexa®) and quetiapine (Seroquel®) have already been used as antipsychotics in Canada. Despite the recent development of several atypical antipsychotics (risperidone, olanzapine, amperozide, ziprazidone, sertindole and quetiapine) (Gerlach and Peacock, 1995), the underlying mechanism for the atypical profile still remains controversial.

6. Hypothesis and Purpose of Proposed Studies

This project focuses on the interaction of 5-HT with the mesolimbic DA pathway of rat brain. This is an area of research that is largely unexplored. The following three studies were undertaken to investigate the effects of 5-HT receptor-related compounds on locomotor activity and VTA self-stimulation:

1) Effects of 8-OH-DPAT administered in the DRN and the MRN on locomotor activity.
2) Effects of 8-OH-DPAT administered in the DRN and the MRN on ICSS of the VTA, and blockade of systemic WAY 100635 on the effects by local 8-OH-DPAT.
3) Effects of TFMPP and RU 24969 administered in the NAS on ICSS of the VTA, and comparison with the effects by amphetamine in the NAS on ICSS of the VTA.

Locomotor activity is associated with mesolimbic and nigrostriatal dopaminergic activity (Koob *et al.*, 1981 & 1984; Nisenbaun *et al.*, 1986). Intracranial self-stimulation of the VTA has also been known to be associated with DA release in the mesolimbic DA pathway (Phillips and Fibiger, 1989; Blaha and Phillips, 1990; Fiorino *et al.*, 1993). Therefore, the modulation of these behaviours by 5-HT receptor-related compounds should increase our understanding of possible interactions between 5-HT and DA. In addition, the present studies may shed

further light on the role of somatodendritic 5-HT_{1A} receptors in locomotor activity and the ICSS rewarding response.

The hypothesis in the present studies is that 5-HT inhibits mesolimbic DA activity. According to the hypothesis, an increased serotonergic tone would inhibit DA release in mesolimbic system that mediates locomotor activity and the ICSS rewarding response. Thus, an increased serotonergic tone may suppress locomotor activity and the ICSS rewarding response. Stimulation of autoreceptors such as 5-HT_{1A} and 5-HT_{1B} receptors could be expected to decrease terminal 5-HT release and promote DA activity while stimulation of postsynaptic receptors such as 5-HT_{2A} and 5-HT_{2C} receptors would be expected to suppress DA activity.

The purpose of the present studies was thus to investigate the behavioural effects of selected 5-HT receptor-related compounds in well-characterized animal models of mesolimbic DA function such as locomotor activity and ICSS of the VTA.

MATERIALS AND METHODS

CHEMICALS AND DRUGS

1. Chemicals used during the present study

   1.1. Potassium Chloride - Fisher Scientific, Fairlawn, NJ, USA

   1.2. Sodium Hydroxide - Fisher Scientific, Fairlawn, NJ, USA

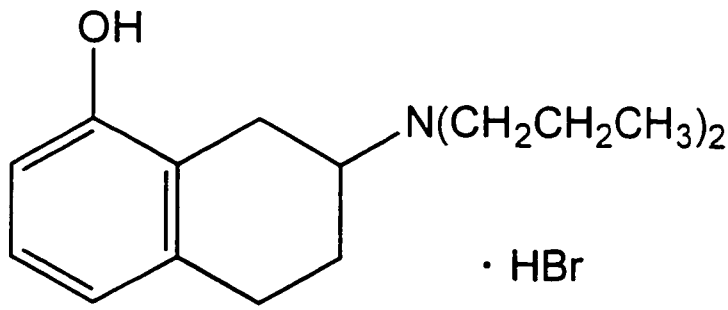
   1.3. Saline, Isotonic, 0.9 % - Fisher Scientific, Fairlawn, NJ, USA

   1.4. Savlon (Chlorhexidine gluconate 1.5 % w/v and cetrimide 15.0 % w/v) - Zeneca Pharma Inc., Mississauga, ON, Canada

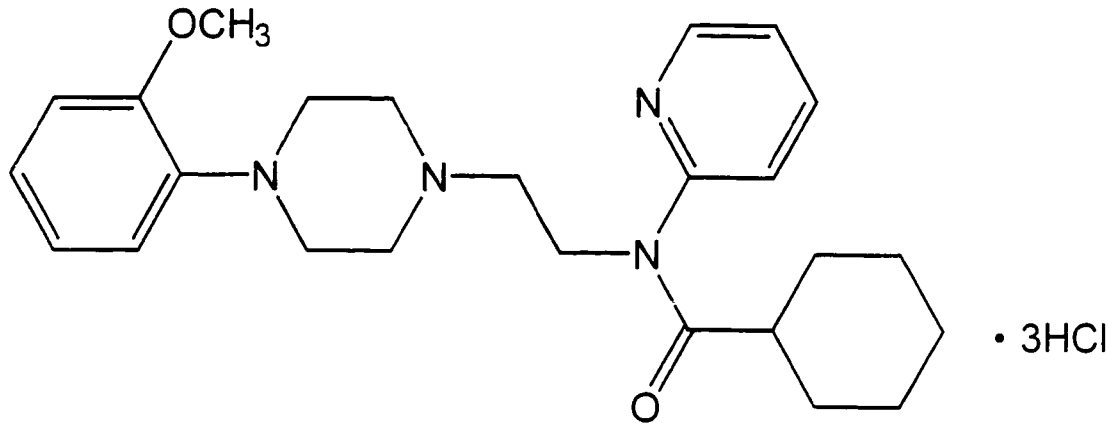
   1.5. Orthodontic Resin power and liquid - The L. D. Caulk Company, Division of Dentsply International Inc., Milford, Delaware, USA

2. 5-HT-Related Drugs used during the present study

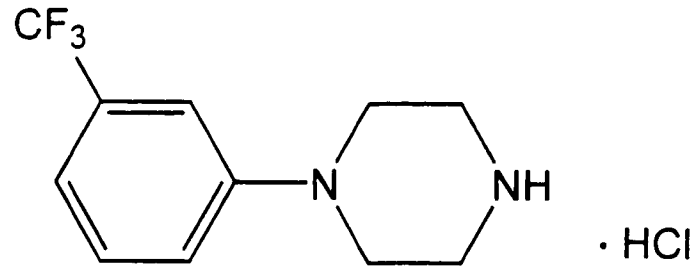
   2.1. 8-OH-DPAT [(±)-8-hydroxy-2-dipropylaminotetralin•HBr] - Research Biochemicals Inc. (RBI), Wayland, MA, USA



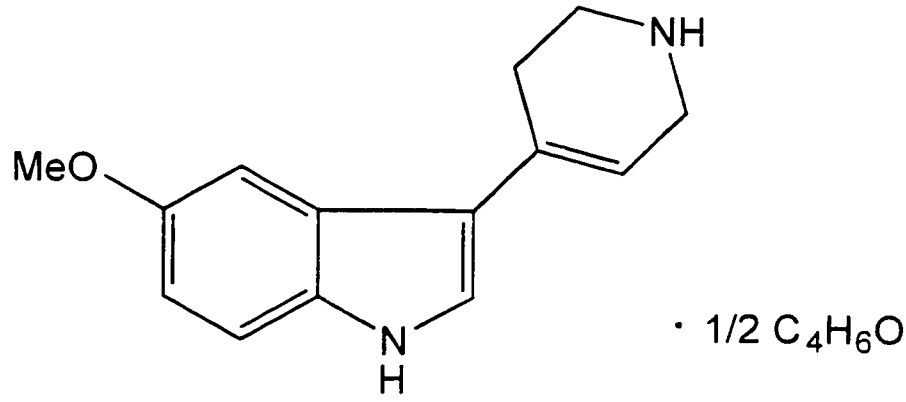
2.2. WAY 100635 [N-(2-(4-(2-methoxyphenyl)-1-piperazyl)ethyl)-N-(2-pyridinyl)cyclohexane carboxamide trihydrochloride] – Wyeth Ayerst. American Home Products Cor.. Philadelphia. Pennsylvania. USA



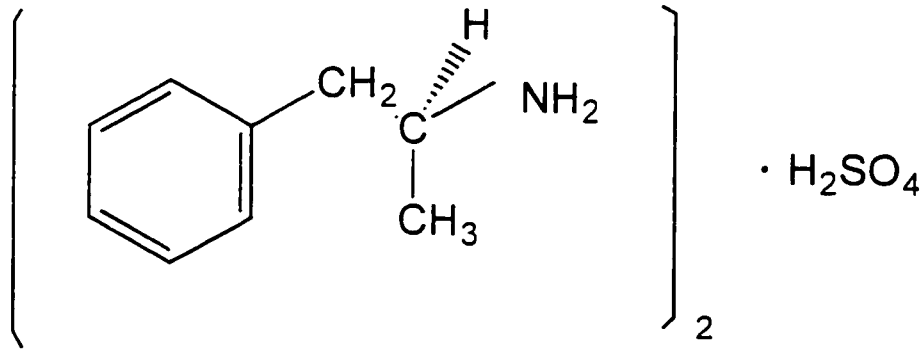
2.3. TFMPP [4-(3-trifluoromethylphenyl)piperazine•HCl] - Research Biochemicals Inc. (RBI). Wayland. MA. USA



2.4. RU 24969 [5-methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1H-indole hemisuccinate] - Roussel-Uclaf, Paris, France



2.5. Amphetamine [S(+)-1-methyl-2-phenylethylamine sulfate] - Research Biochemicals Inc. (RBI), Wayland, MA, USA



INSTRUMENTATION AND APPARATUS

1. Locomotor Activity Monitoring System

1.1. Photocell Box

The activity monitoring system (I. Halvorsen Systems Design, Brownwood, TX, USA) consisted of six open arenas (plexiglass test cages, 17" × 17" × 12") each containing two parallel infra-red grids (12 × 12 diode beams, Infra-red Grid Model 17-12 with vertical sensors). The test cages' sensors were interfaced with a microcomputer system. Three different measures were based on the number of infra-red beam interruptions: 1) Locomotor activity (ambulatory behaviour), corresponding to total number of beam breaks. 2) Rearing, corresponding to the number of upper beam breaks. 3) Consecutive behaviour (stereotyped behaviour) corresponding to two or more consecutive interruptions of the same infrared beam.

1.2. Microcomputer

The behavioural measures were digitally recorded by a computer system (6502 Data Gatherer) for temporal analysis of activity counts.

2. ICSS System

2.1. Modular Test Cage System

Standard operant test chambers (E10-10SF, Coulbourn Instruments, Lehigh Valley, PA, USA) were used, each equipped with a lever, an electrical contact swivel and a grid floor. The experiments were carried out in six operant test chambers (24 × 30 × 29 cm) each equipped with a sound attenuating outer chamber.

2.2. Stimulator and Microcomputer

Gold track slip rings (Stoelting Co., Wood Dale, IL, USA) were used to connect the electrodes to constant current programmable stimulators (I. Halvorsen Systems Design, Brownwood, TX, USA). With these devices, current, frequency and pulse width were under computer control. The control of experiments and recording of behavioural responses were achieved with a microcomputer.

2.3. Oscilloscope

15 MHz Oscilloscopes (B+K Precision 1477, Dynascan, Japan) were used to monitor applied stimulation.

2.4. Monopolar Electrode and Indifferent Electrode

Monopolar nichrome electrodes (bare diameter, 200 μm; length, 2 – 2.5 cm; 1 mm uninsulated tip, Plastics One Inc., Roanoke, VA, USA) were used. A steel screw located in the frontal bone served as a large indifferent electrode.

3. Microinjection System

3.1. Peristaltic Infusion Pump and Microsyringes

Microinjections were carried out with a Bee-Hive MD 1020 pump (Bioanalytical System Inc., Lafayette, IN, USA) using a 10 µl Hamilton syringe (Hamilton Company, Reno, Nevada, USA).

3.2. Tubing

Teflon tubing (PE 10, Fisher Scientific, Fairlawn, NJ, USA) with a capacity of 0.3µl per centimeter and an internal diameter of 0.38 mm was used. A small air bubble was applied as an interface between the artificial CSF and drug solution. The air bubble separated the drug solution from the CSF as a useful indicator of movement of drug solution and pressure in the tubing.

3.3. Guide Cannulae, Stylets and Injection Cannulae

Guide cannulae (22 gauge), stylets (23 gauge, dummy cannulae) and injection cannulae (28 gauge) were made of stainless steel tubing (Plastics One Inc., Roanoke, VA, USA). Each guide cannula-stylet assembly was implanted 1mm over the actual target site in order to reduce target site damage. Each injection cannula was 1 mm longer than the assembly in order to project into the target site.

ANIMALS

Male Sprague-Dawley rats (200 – 250 g) were obtained from Health Sciences Laboratory Animal Services, University of Alberta. Animals weighing 250 – 300 g at the beginning of the experiments were used for this study. They were housed individually at a room temperature of 20 ± 1°C under 12 h light/dark cycle (lights on 7:30 a.m.) with free access to food and water in their home cages. The animals were fed Lab-Blox Feed which contained 4.0 % (min) crude fat, 4.5 % (max) crude fibre and 24 % (min) crude protein (Wayne Feed Division, Continental Grain Co., Chicago, IL, USA). Procedures involving the use of rats were approved by the University of Alberta Health Sciences Animal Welfare Committee and were conducted according to the guidelines established by the Canadian Council on Animal Care.

SURGERY AND CENTRAL IMPLANTATION

Using standard stereotaxic procedures, animals were anesthetized with Somnotol (pentobarbital, 60 mg kg^{-1}, IP) and placed in a stereotaxic frame (Model 900, Kopf Instruments, Tujunga, CA, USA) with the incisor bar set 3.9 mm below the interaural line recommended for the flat skull orientation of adult Sprague-Dawley rats (Paxinos and Watson, 1986). Guide cannulae were implanted 1 mm over the actual target areas and were anchored to the skull by four stainless steel jeweler's screws and dental acrylic.

The cannulae for the DRN and the MRN were implanted at an angle of 30°30° relative to the sagittal and horizontal plane in order to avoid damage to the cerebral aqueduct and the sagittal sinus (Greenshaw, 1997). The cannulae for the shell of NAS were implanted at an angle of 20° from the vertical plane. Stainless steel stylets (23 gauge) were placed in the guide cannulae to prevent the cannulae from clogging since the animals were given intracerebral microinjections at least one week following recovery from surgery. At an angle of 30°30° relative to the sagittal and horizontal plane, some animals were also implanted with a unilateral monopolar stimulating electrode (200 μ bare tip diameter) into the ventral tegmental area (VTA) for ICSS. A large silver indifferent electrode was secured to the frontal bone of the skull. The coordinates used for stereotaxic implantation are shown in Table 5 (based on empirical adjustments of targets derived from Paxinos and Watson, 1986).

Table 5. Stereotaxic surgery coordinates: all angular coordinates were determined in terms of interaural zero (*) or at the bregma reference point starting at skull surface (**). Guide cannulae were aimed 1 mm over real injection sites. Injection cannulae were 1 mm longer than guide cannulae to reach actual target sites. AP, anterior and posterior; ML, medial and lateral; DV, dorsal and ventral.

	AP	ML	DV
Dorsal Raphé Nucleus (DRN)*	− 0.5	− 0.7	+ 4.8
Median Raphé Nucleus (MRN)*	+ 0.8	0	+ 2.6
Ventral Tegmental Area (VTA)*	+ 1.9	− 1.1	+ 2.3
Nucleus Accumbens Shell (NAS)**	+ 1.7	+ 3.5	− 6.6

DRUG ADMINISTRATION

(±)-8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (Research Biochemicals Inc., Natick, MA, USA), 0.1, 1, 2.5 or 5 μg, was microinjected into the DRN or the MRN. For microinjection study into the shell of NAS, N-(3-trifluoromethylphenyl)piperazine (TFMPP) or 5-methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1H-indole (RU 24969) (Research Biochemicals Inc., Wayland, MA, USA), 5 μg relatively, was injected bilaterally. S(+)-Amphetamine, 5 μg, was also injected bilaterally into the shell of NAS as a test for DA receptor activation in this structure. All drugs dissolved in 0.9 % saline were microinjected in a volume of 0.5 μl per site, and controls were given artificial cerebrospinal fluid (CSF) vehicle to control for effects of volume and osmotic pressure. The pump-controlled infusion rate was 0.2 μl min^{-1} and each injection cannula (28 g) was left in place for a further 1 min to allow the drug to diffuse from the cannula tip. N-(2-(4-(2-Methoxyphenyl)-1-piperazinyl)ethyl)-N-2-pyridinyl-cyclohexanecarboxamide (WAY 100635) (Wyeth Ayerst, USA), 0.1 mg kg^{-1}, was injected subcutaneously 5 min after application of 8-OH-DPAT to investigate the blockade of 8-OH-DPAT effects on self-stimulation of the VTA.

All animals were subjected to handling for one hour on each of three habituation day procedures. For each experiment the order of all treatments was randomized for testing using a repeated measures design. One drug dose was tested each week, allowing a one week drug wash-out period between drug administrations.

MOTOR ACTIVITY MEASUREMENTS

Animals were placed individually into a computerised activity monitoring system. Activity was measured over sessions of 30 min duration. Three different measures of motor activity were based on the number of infra-red beam interruptions:

1) Locomotor activity (ambulatory behaviour), corresponding to total number of beam breaks.
2) Rearing, corresponding to number of upper beam breaks.
3) Consecutive behaviour (stereotyped behaviour), corresponding to two or more consecutive interruptions of the same infrared beam.

INTRACRANIAL SELF-STIMULATION

A constant current DC stimulator (connected to each animal by a gold-track slip-ring) provided monopolar stimulation to the VTA. Beginning 1 week after surgery, each rat was trained to lever press on a continuous reinforcement (CRF) schedule, with each response delivering a 1 sec train of cathodal 0.2 ms pulses at 100 Hz. Training sessions of 1 hour duration were continued for at least 7 days. The rate of responding was then determined as a function of current intensity. The intensity that maintained half-maximal rates of responding was determined from a linear regression analysis of the relationship between response rate and current intensity. Following a method of limits procedure (Gallistel and Karras, 1984), the animals were tested daily under conditions whereby the number of pulses per train was systemically varied, initially at the current identified above. Sessions began with animals responding for trains of 160 Hz. At 60 sec intervals the frequency was decreased in 0.1 log steps until the animal ceased to respond, then increased in an equivalent manner until 160 Hz trains were again delivered. At the start of each 60 sec interval, each animal received three trains of stimulation (primes) at the frequency at which stimulation was to be available during that period. This priming stimulation served as a discriminative stimulus to indicate the stimulus characteristics. With this feature of the schedule, manually delivered stimulation is unnecessary at the beginning of the ascending frequency steps. The total number of responses in each 60 sec interval was recorded. With this schedule the session length may vary between 2 and 26 min, i.e. if the animal does not respond then the session will terminate after repeating the first

frequency (160 Hz) bin and if the animal responds at each of the 13 frequencies (160 – 10 Hz) then each frequency bin will be run twice, yielding a maximal session length of 26 minutes. After initial training (15 – 25 sessions) current was adjusted for each animal so that the half-maximal response rate occurred at around the middle of the range of frequencies (50 Hz). Sessions then continued for at least a further ten sessions until responding had stabilized. Dependent variables for the analysis were:

- M50 - the frequency that maintained half-maximal response rates.
- RMAX - the maximal number of responses at a single frequency.
- TRES - the total number of responses per session.

Baseline frequency-response data such as the above were collected from 5 days before every drug administration.

HISTOLOGY

Cannula and electrode placements were verified at the end of the experiments by visual inspection of coronal sections of the brain. Animals were deeply anesthetized before perfusion of the heart with 0.9 % saline followed by 4 % formalin containing 5 % sucrose. The brains were stored in 10 % formalin containing 30 % sucrose for at least 10 days. Frozen 50 μm coronal sections were taken, mounted and stained using a cresyl violet stain. The implantation sites were verified by microscopic examination. Only animals with correct placements were included in the data analysis.

STATISTICS

Data analysis was performed with appropriate factorial repeated-measures analysis of variance (ANOVA). *Post hoc* tests for multiple comparisons were made using Tukey's procedure. Statistical significance was set at the 95 % confidence level (two-tailed probability, $p < 0.05$). For the locomotor activity study, all behavioural results were collected from eight animals per treatment group. In ICSS study, all behavioural results were collected from eleven animals for the DRN, twelve animals for the MRN and ten animals for the NAS per treatment groups, respectively. Data for the study of locomotor activity are expressed as mean ± SEM (Standard Error of Mean) of the total activity, rearing and consecutive counts. Mean ± SEM values for the ICSS study are displayed as percentages of baseline performance for M50, TRES and RMAX, respectively.

RESULTS

Effects of Microinjections of 8-OH-DPAT into the Dorsal and Median Raphé Nucleus on Motor Activity

Following administration of 8-OH-DPAT into the DRN there was a significant dose- and time-dependent decrease in total activity [$F(4,28) = 4.08$, $p < 0.05$ for dose; $F(5,35) = 76.73$, $p < 0.05$ for time]. There was also a significant interaction between drug dose and time effects for the total activity measure [$F(20,140) = 3.12$, $p < 0.05$] and between effects of drug dose and time for rearing [$F(20,140) = 2.53$, $p < 0.05$]. However, there was no significant interaction between drug dose and time effects for consecutive behaviour [$F(20,140) = 1.05$, $p > 0.05$].

By contrast, the intra-MRN microinjection of 8-OH-DPAT caused a significant increase in total activity. The effects of drug dose [$F(4,28) = 4.03$, $p < 0.05$], time [$F(5,35) = 193.73$, $p < 0.05$] and their interaction [$F(20,140) = 2.74$, $p < 0.05$] were significant. An interaction between drug dose and time was also observed with rearing [$F(20,140) = 1.97$, $p < 0.05$], but not with consecutive behaviour [$F(20,140) = 1.46$, $p > 0.05$].

The effects of various doses of 8-OH-DPAT injected into the DRN on the time-course of total activity over the 30-minute test period are shown in Figure 7 and 8. There was a significant decrease in total activity 10 minutes after the administration of both 2.5

and 5 μg of 8-OH-DPAT into the DRN, as compared to CSF-treated control group but not after 0.1 and 1μg treatments. Figure 9 and 10 shows the effects of various doses of 8-OH-DPAT injected into the DRN on rearing for 30 minutes. Rearing was also significantly reduced by 5 μg of 8-OH-DPAT 10 minutes after intracranial microinjection into the DRN. Although the interaction between drug dose and time in consecutive behaviour was not statistically indicated from the above, inspection of the Figure 11 and 12 indicates that 5 μg of 8-OH-DPAT seemed to be effective 15 minutes after microinjection into the DRN.

Contrary to the results from the DRN, total activity in the MRN was increased during a 30 minute test period with a variety of doses (Figure 13 and 14) compared with CSF-treated control group. Total activity in the MRN was significantly increased 10 minutes after the administration of 5 μg of 8-OH-DPAT as compared with CSF-treated control group. Interestingly, rearing was decreased during the first 5 minutes after microinjections of 8-OH-DPAT into the MRN and then went back to CSF-treated control level (Figure 15 and 16). There were no significant changes in consecutive behaviour, which are shown in Figure 17 and 18.

Only rats with cannula placements in the target sites were included in the present data analysis. In Figure 19 are shown actual drug-injection sites of the both raphé nuclei in animals used for motor activity measurements.

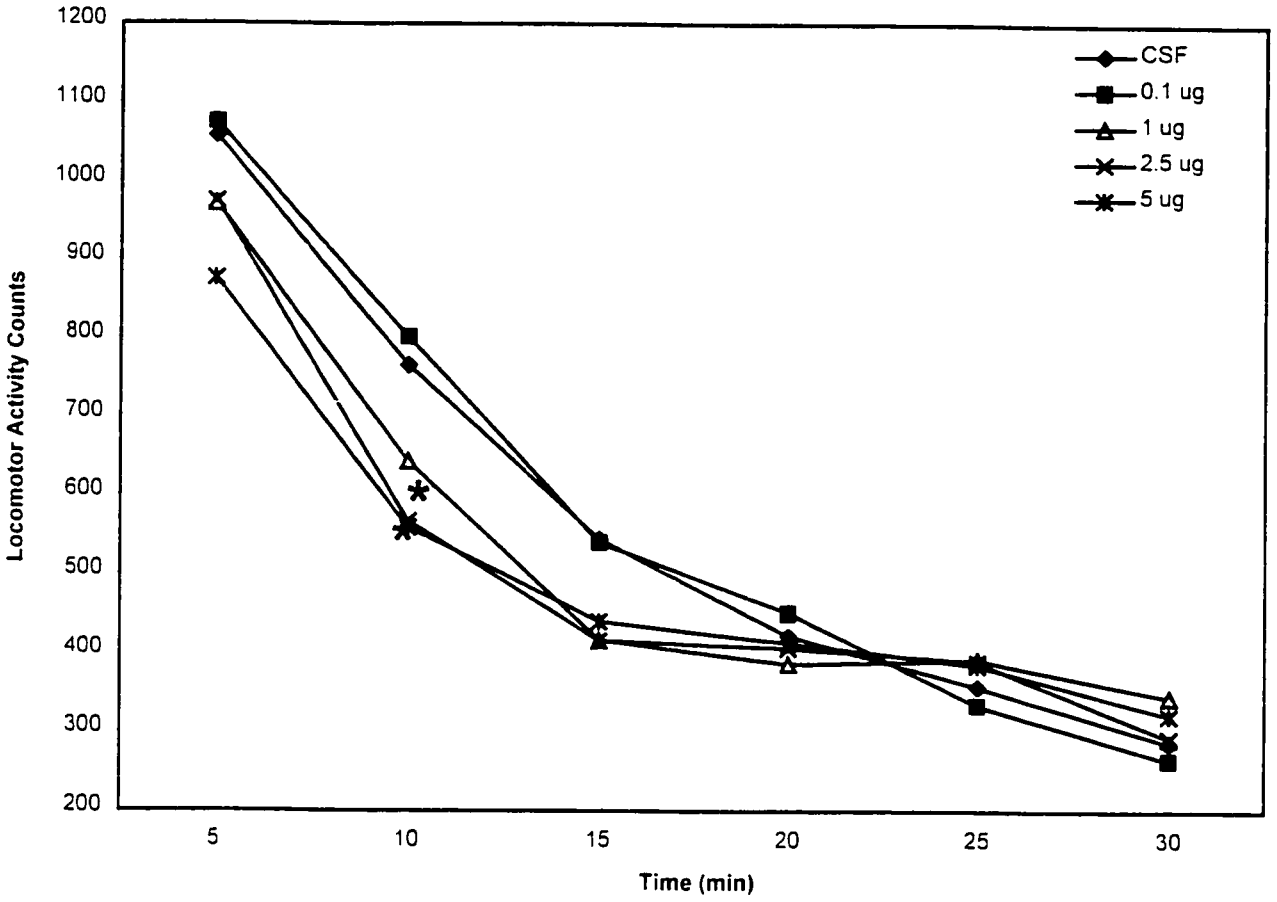


Figure 7. Effects of 8-OH-DPAT injected into the DRN on locomotor activity of rats (n=8) during a 30 min test period. *Effects were significant at $p < 0.05$, ANOVA.

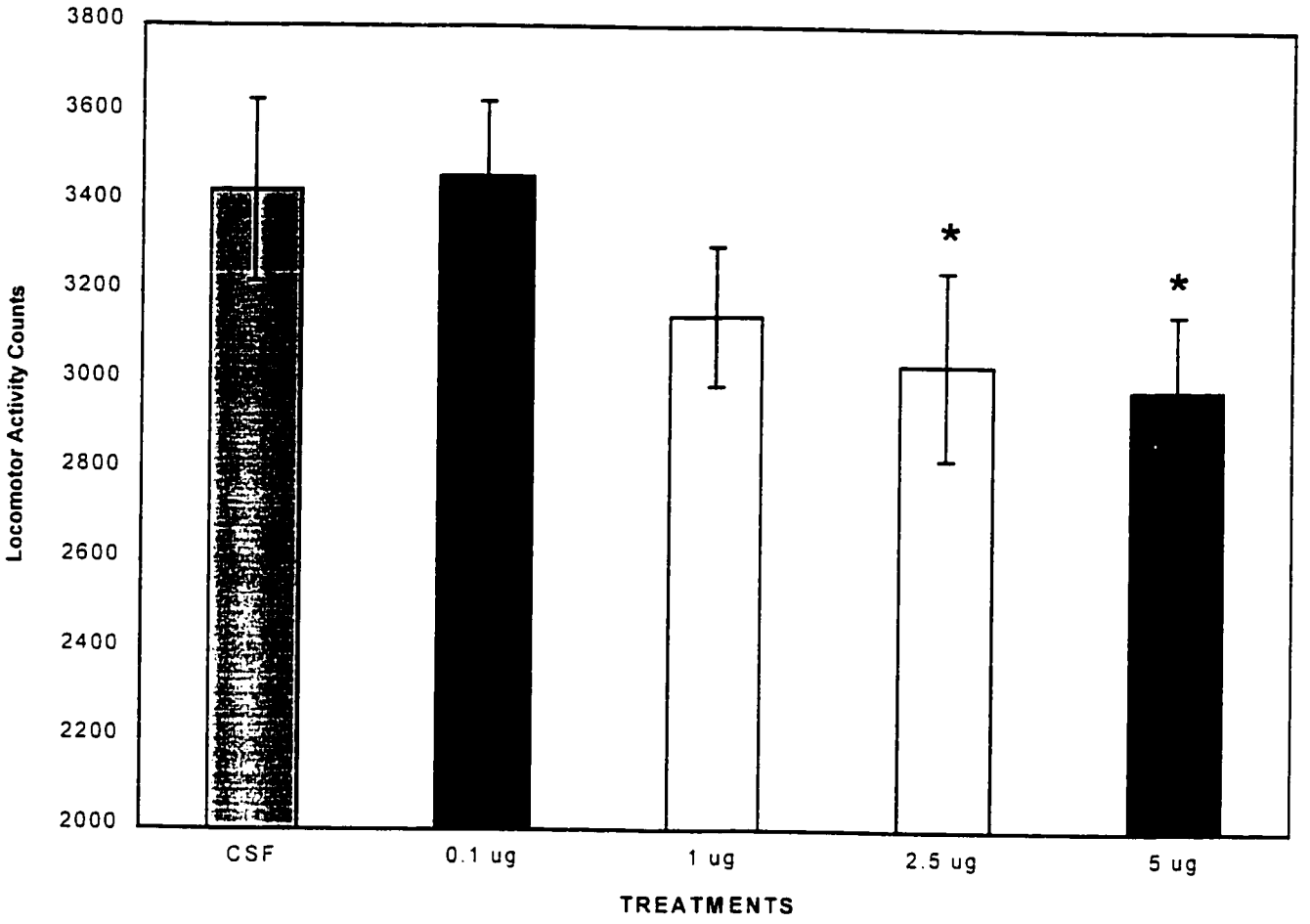


Figure 8. Effects of 8-OH-DPAT injected into the DRN on locomotor activity of rats (n=8) 30 min after microinjections. *Effects were significant at $p < 0.05$, ANOVA.

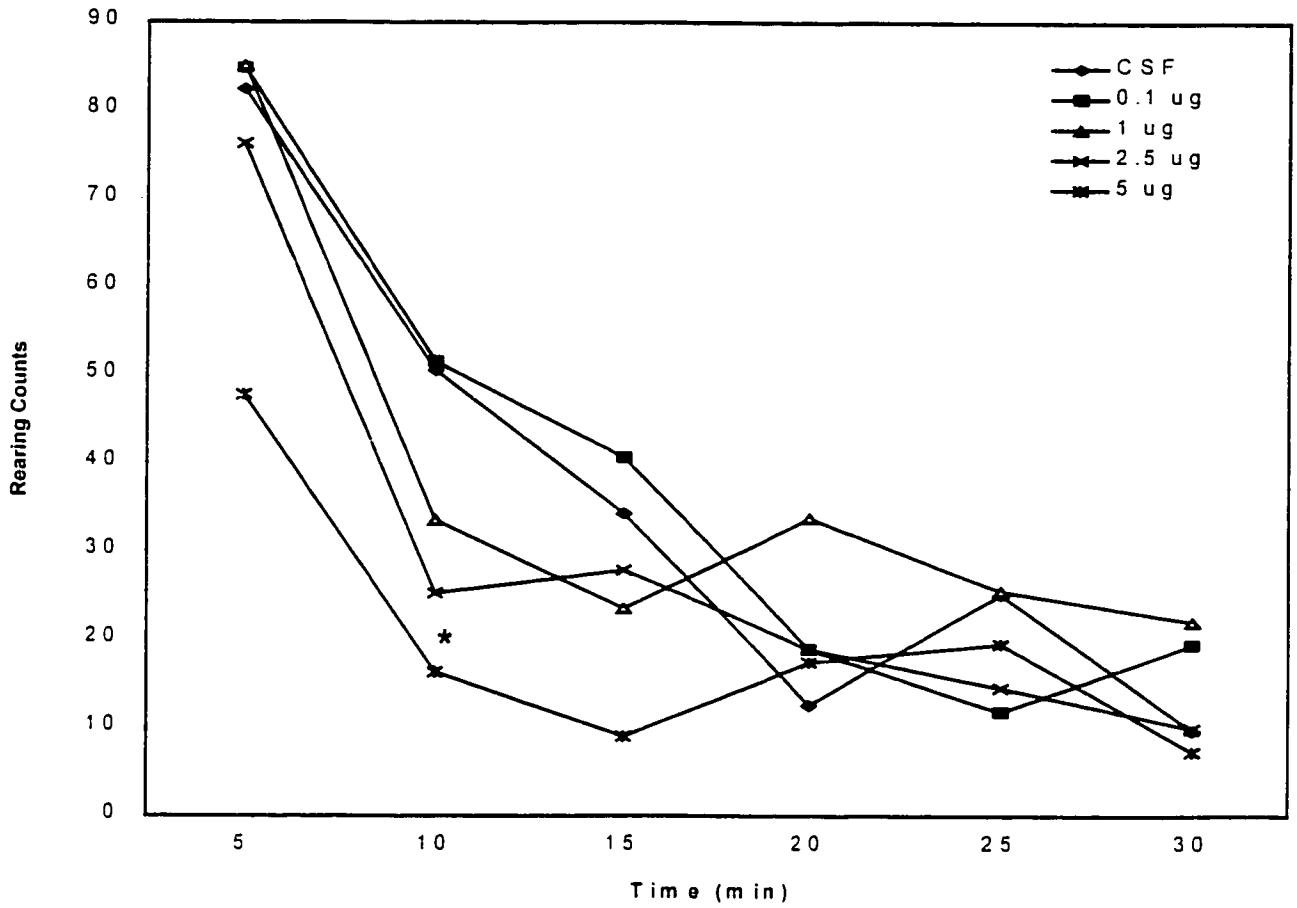


Figure 9. Effect of 8-OH-DPAT injected into the DRN on rearing of rats (n=8) during a 30 min test period. *Effect was significant at $p < 0.05$, ANOVA.

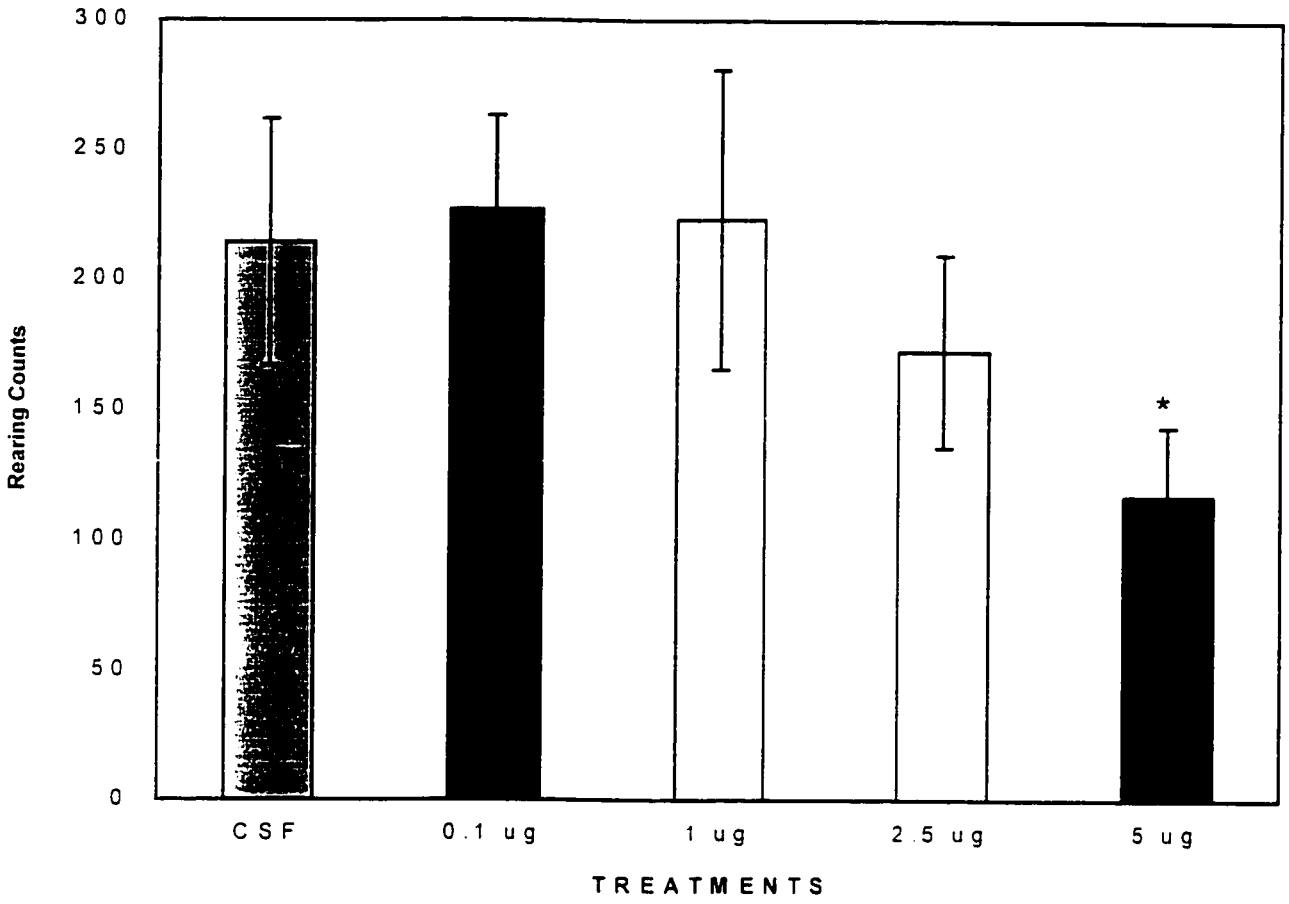


Figure 10. Effect of 8-OH-DPAT injected into the DRN on rearing of rats (n=8) 30 min after microinjections. *Effect was significant at $p < 0.05$, ANOVA.

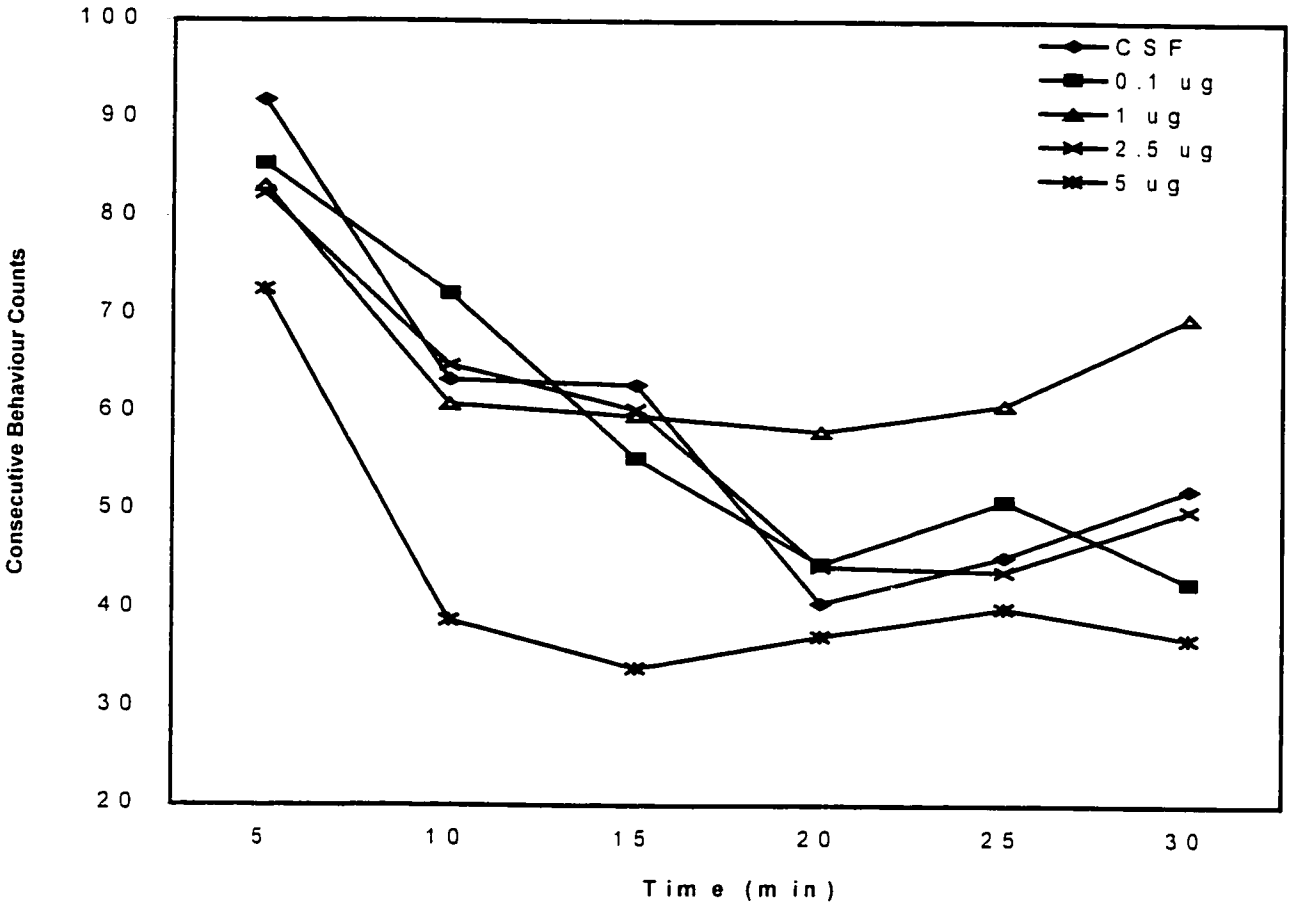


Figure 11. Consecutive behaviour of rats (n=8) during a 30 min test period after microinjections of 8-OH-DPAT into the DRN. There were no significant effects; $p > 0.05$, ANOVA.

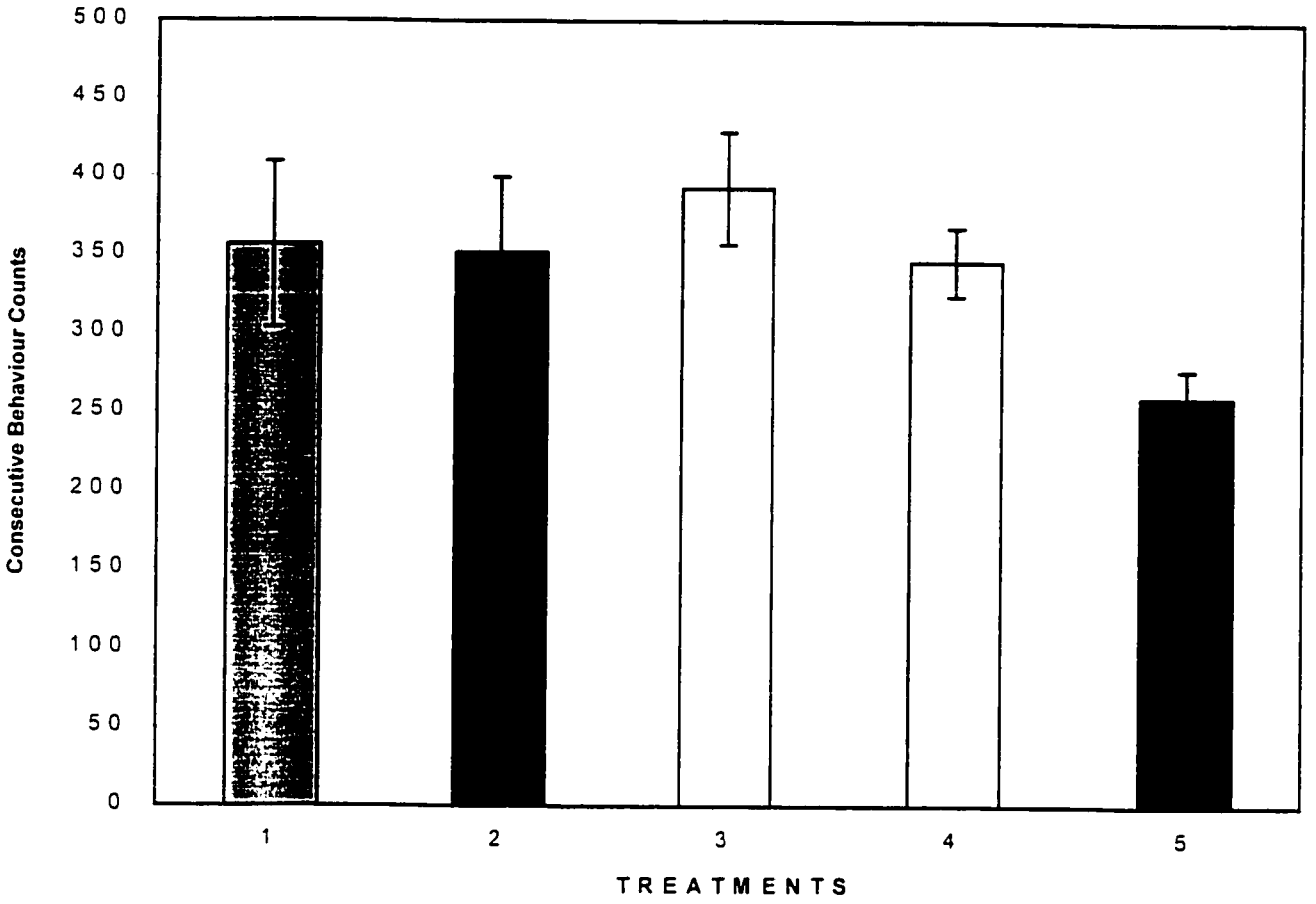


Figure 12. Consecutive behaviour of rats (n=8) during a 30 min test period after microinjections of 8-OH-DPAT into the DRN. There were no significant effects; $p > 0.05$, ANOVA.

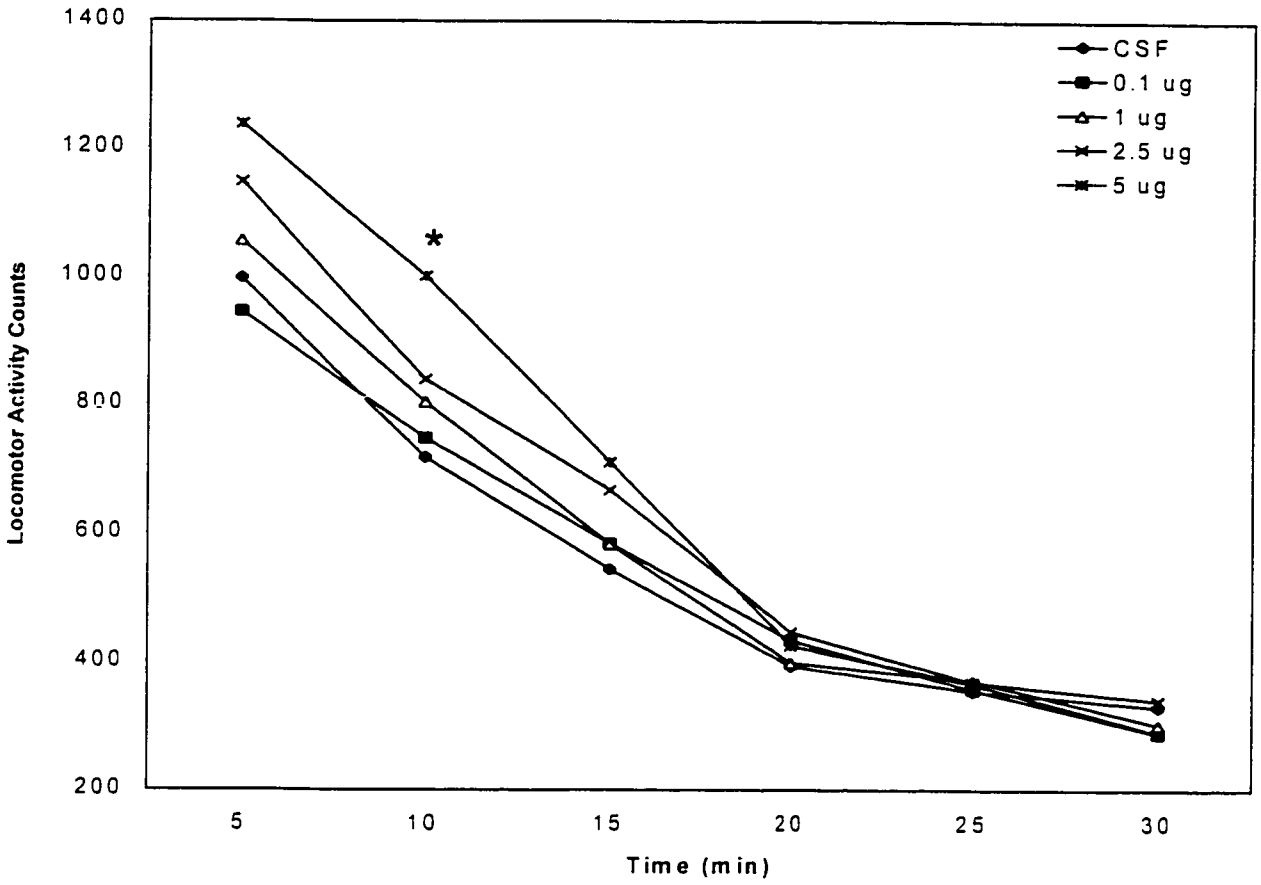


Figure 13. Effect of 8-OH-DPAT injected into the MRN on locomotor activity of rats (n=8) during a 30 min test period. *Effect was significant at $p < 0.05$, ANOVA.

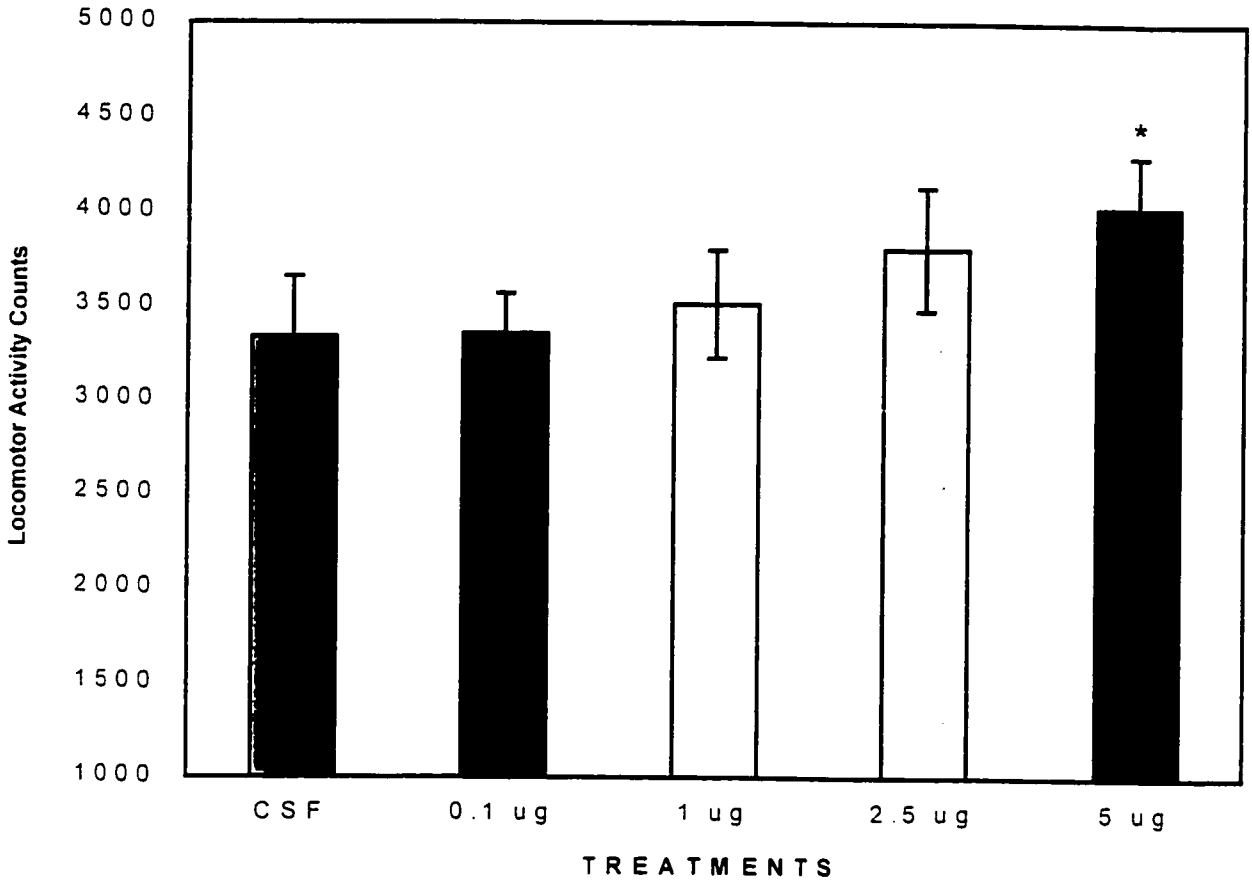


Figure 14. Effect of 8-OH-DPAT injected into the MRN on locomotor activity of rats (n=8) 30 min after microinjections. *Effect was significant at $p < 0.05$, ANOVA.

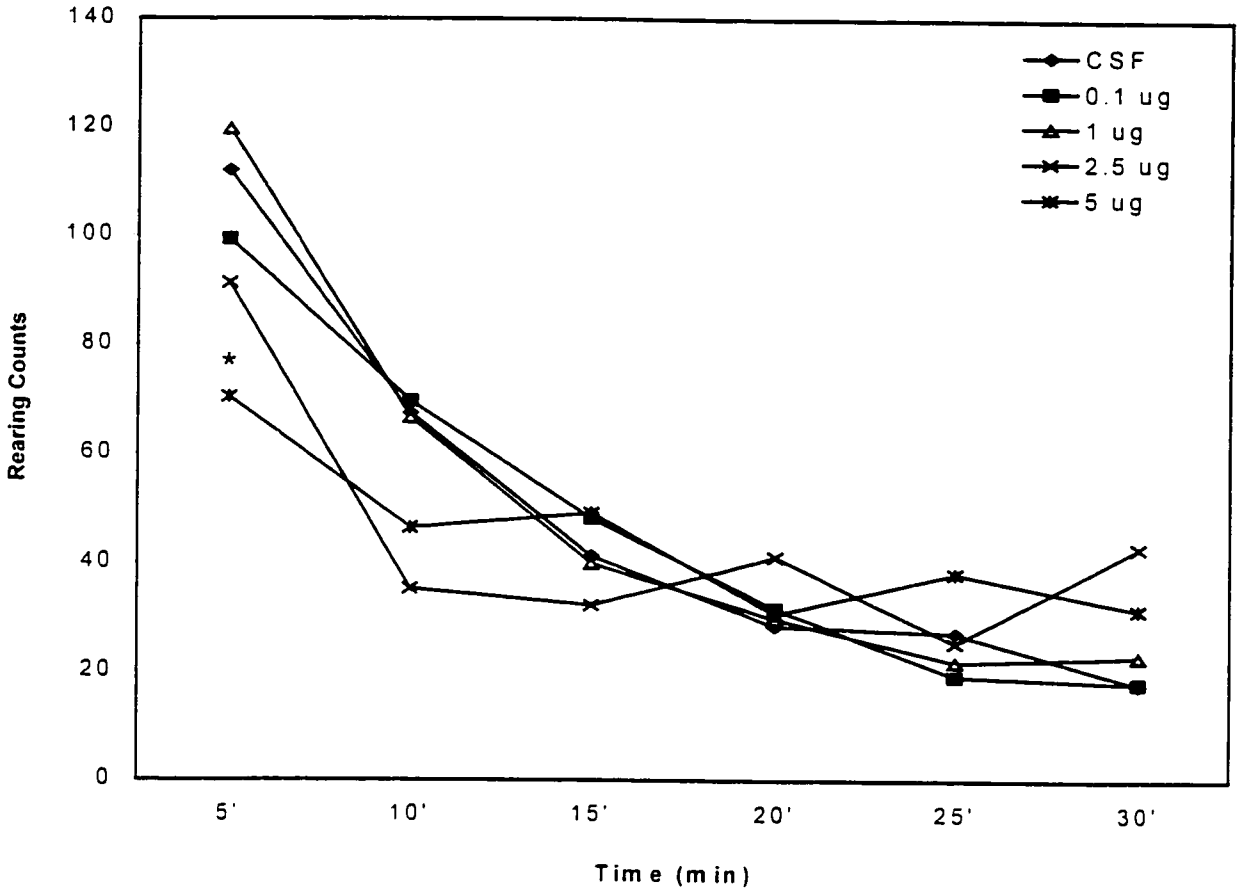


Figure 15. Effect of 8-OH-DPAT injected into the MRN on rearing of rats (n=8) during a 30 min test period. *Effect was significant at $p < 0.05$, ANOVA.

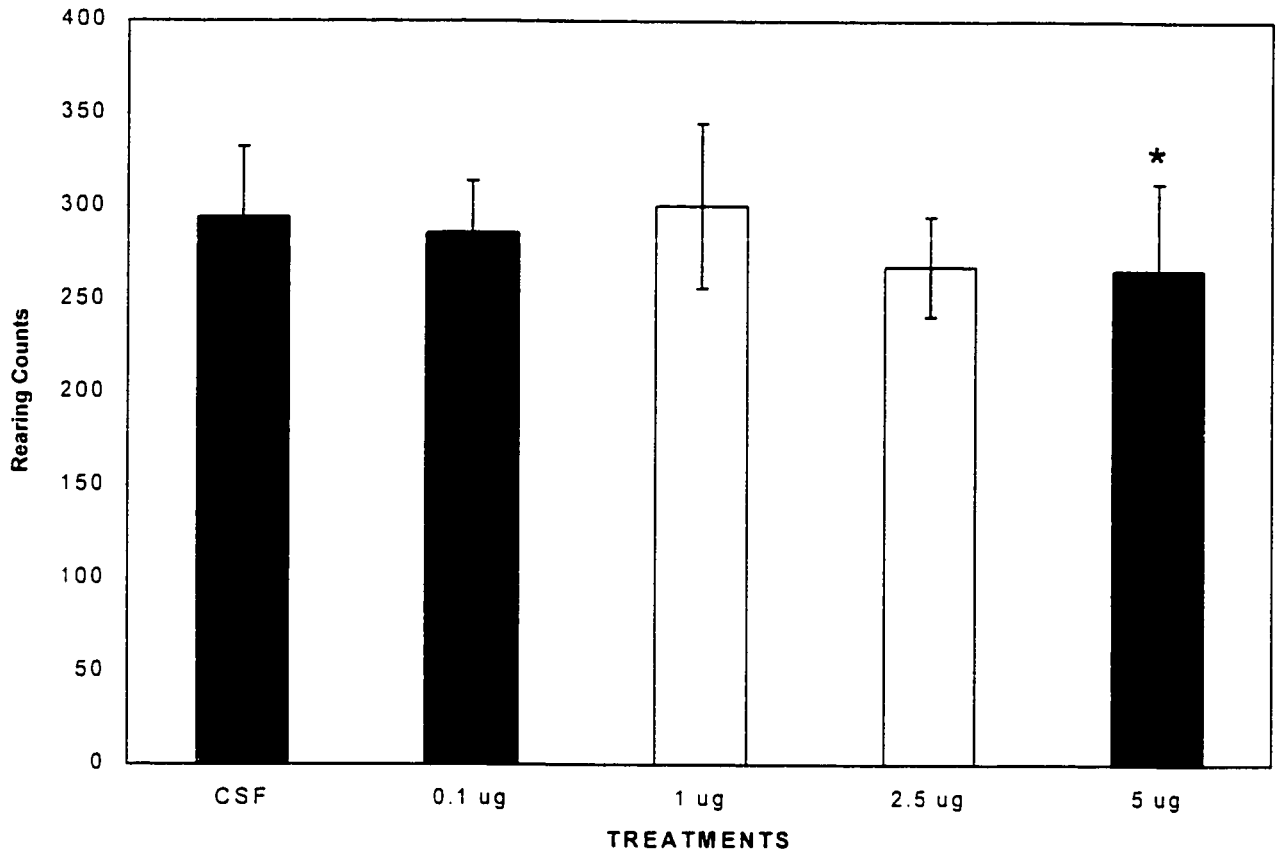


Figure 16. Effect of 8-OH-DPAT injected into the MRN on rearing of rats (n=8) 30 min after microinjections. *Effect was significant at $p < 0.05$, ANOVA.

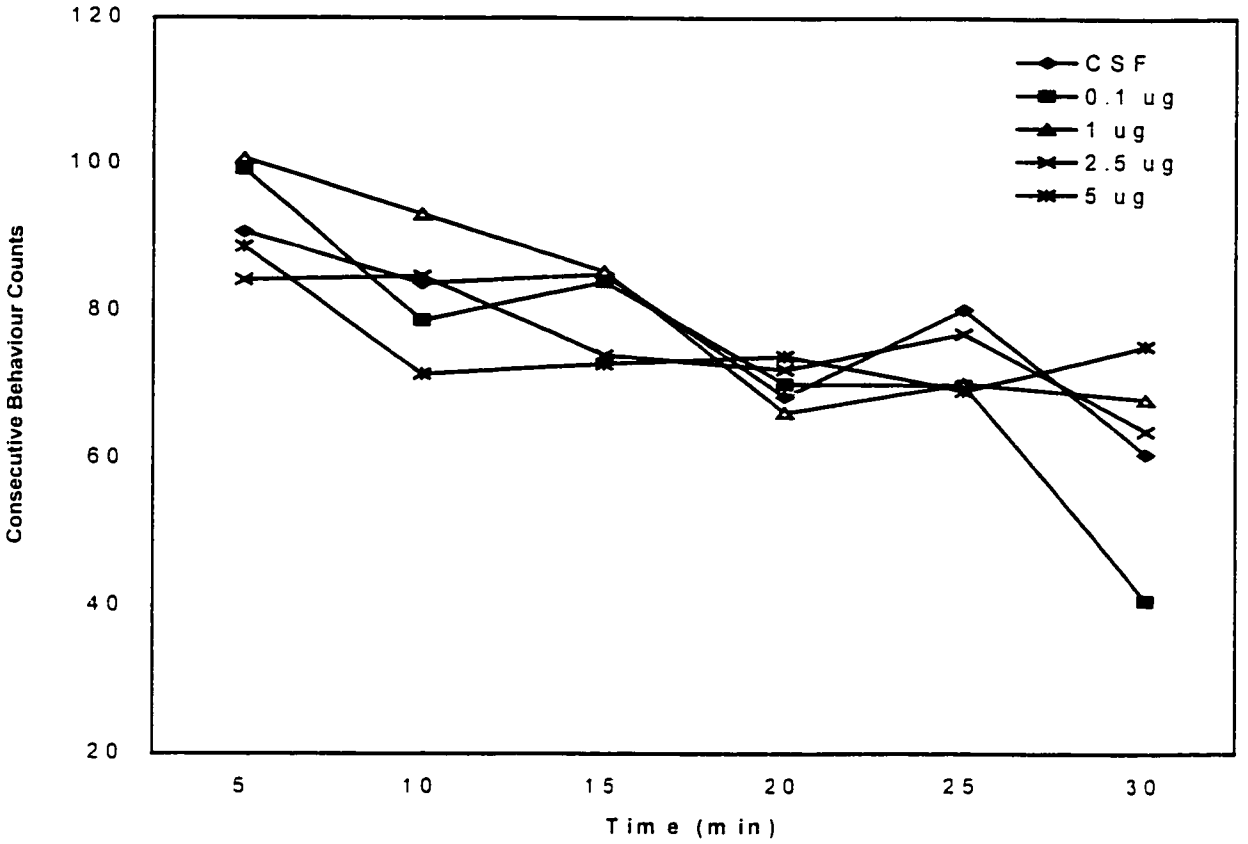


Figure 17. Consecutive behaviour of rats (n=8) during a 30 min test period after microinjections of 8-OH-DPAT into the MRN. There were no significant effects; $p > 0.05$, ANOVA.

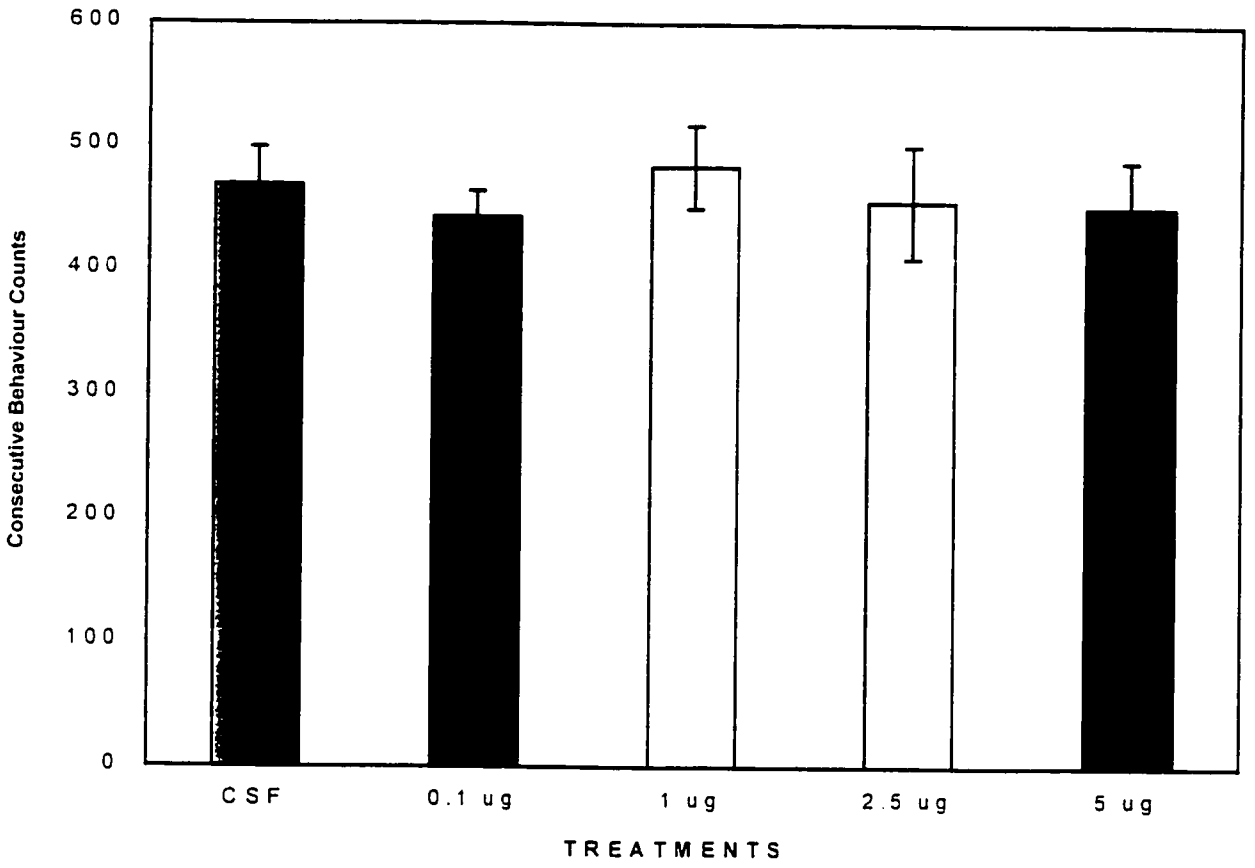


Figure 18. Consecutive behaviour of rats (n=8) during a 30 min test period after microinjections of 8-OH-DPAT into the MRN. There were no significant effects; $p > 0.05$, ANOVA.

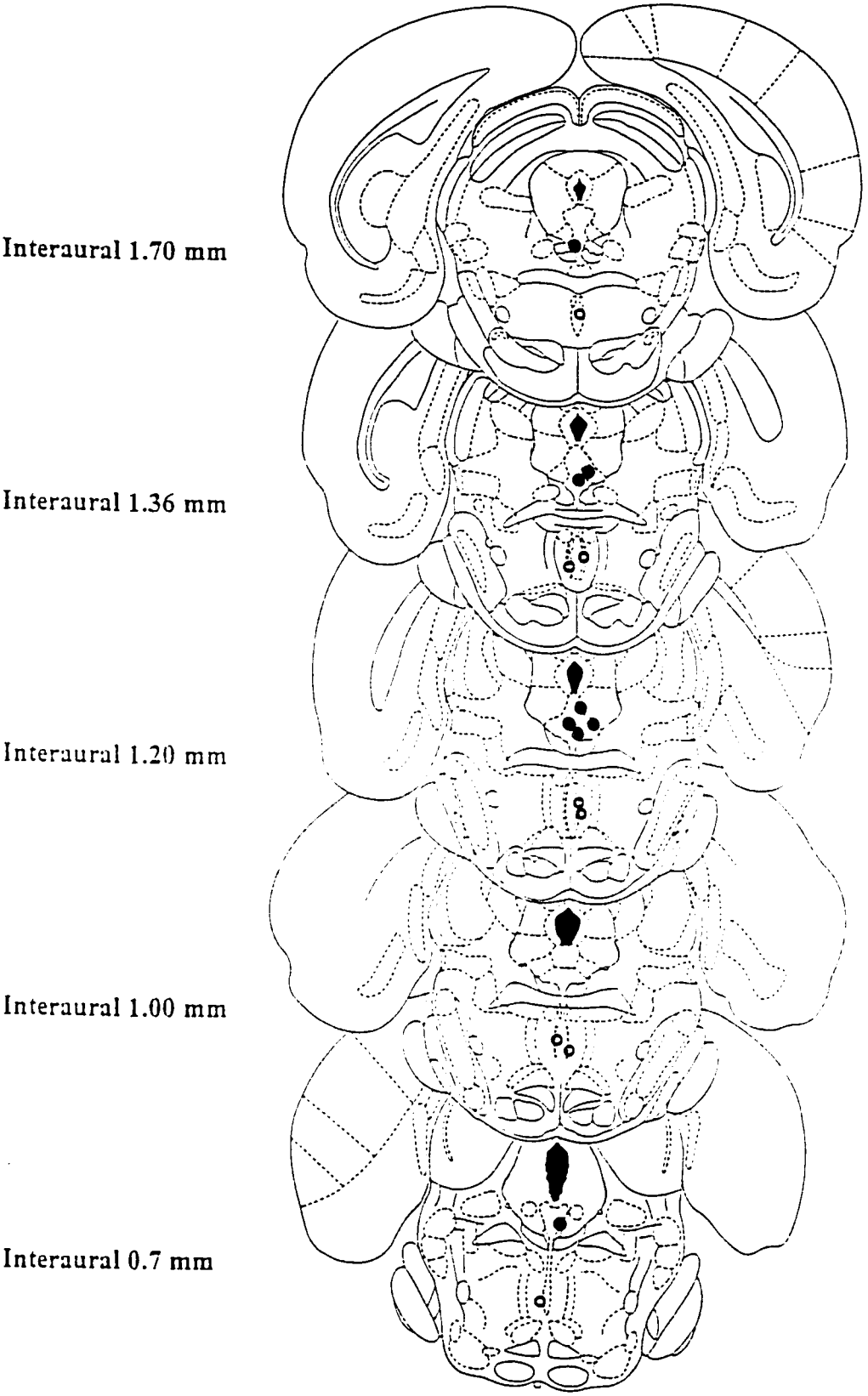


Figure 19. Schematic reconstructions showing the approximate placements of injection sites in the dorsal raphé nuclei (●, n = 8) and the median raphé nuclei (O, n = 8) for rats used for motor activity measurements. The sections were redrawn from the atlas of Paxinos and Watson (1986).

Electrical Self-Stimulation of the Ventral Tegmental Area: Effects of Microinjections of 8-OH-DPAT into the Dorsal and Median Raphé Nucleus

The following data are presented as an average percentage of the baseline performance of each animal. The baseline performance values are presented in Table 6 for the DRN and for the MRN.

DRN injections

The application of 8-OH-DPAT into the DRN induced a significant decrease [$F(2,20) = 10.68$, $p < 0.05$] in the frequency that maintained half-maximal response rates (M50), as illustrated by the data displayed in Figure 20, but there were no effects on the total number of responses per session (TRES), as shown in Figure 21 [$F(2,20) = 2.21$, $p > 0.05$], or the maximal number of responses at a single frequency (RMAX), displayed in Figure 22 [$F(2,20) = 0.12$, $p > 0.05$].

MRN injections

Microinjection of 8-OH-DPAT into the MRN also induced a significant decrease in the M50 measure, as illustrated by the data shown in Figure 23 [$F(2,22) = 7.43$, $p < 0.05$], but there were no effects on TRES, see Figure 24 [$F(2,22) = 7.69$, $p > 0.05$] or RMAX, see Figure 25 [$F(2,22) = 4.62$, $p > 0.05$].

5-HT_{1A} receptor antagonism by WAY 100635

The 5-HT_{1A} receptor antagonist WAY 100635 (0.1 mg kg^{-1}) blocked the VTA self-stimulation threshold-reducing effects of 8-OH-DPAT in both the DRN [$F(2,20) = 10.68$, $p < 0.05$] and MRN [$F(2,22) = 7.43$, $p < 0.05$]. These effects are illustrated in Figure 20 for the DRN and Figure 23 for the MRN. Unexpectedly, a significant decrease in TRES after 8-OH-DPAT plus WAY 100635 treatment was observed in the MRN compared with the group, that was treated with 8-OH-DPAT alone (see Figure 24).

Rate-frequency curves are displayed in Figures 26 (DRN) and 27 (MRN) to describe a percentage of maximum response in relation to frequency change (log Hz). The effects of microinjections of 8-OH-DPAT into the DRN and the MRN are illustrated by shifts to the left (Figure 28). Microinjections of WAY 100635 returned those shifts by 8-OH-DPAT up to the levels of the CSF-treated control group (Figure 28).

Only rats with cannula placements in the target sites were included in the present data analysis. In Figure 29 are shown actual drug-injection sites of the both raphé nuclei for VTA ICSS. Electrode-stimulating sites in the VTA were shown in Figure 30 for the rats drug-injected into the DRN and Figure 31 for the rats drug-injected into the MRN.

Table 6. 5-Day baseline VTA self-stimulation performance for rats with DRN or MRN cannulae

Behavioural measures	M50	TRES	RMAX
Rats with DRN cannulae	55 ± 5	294 ± 44	28 ± 3
Rats with MRN cannulae	52 ± 3	321 ± 32	30 ± 3

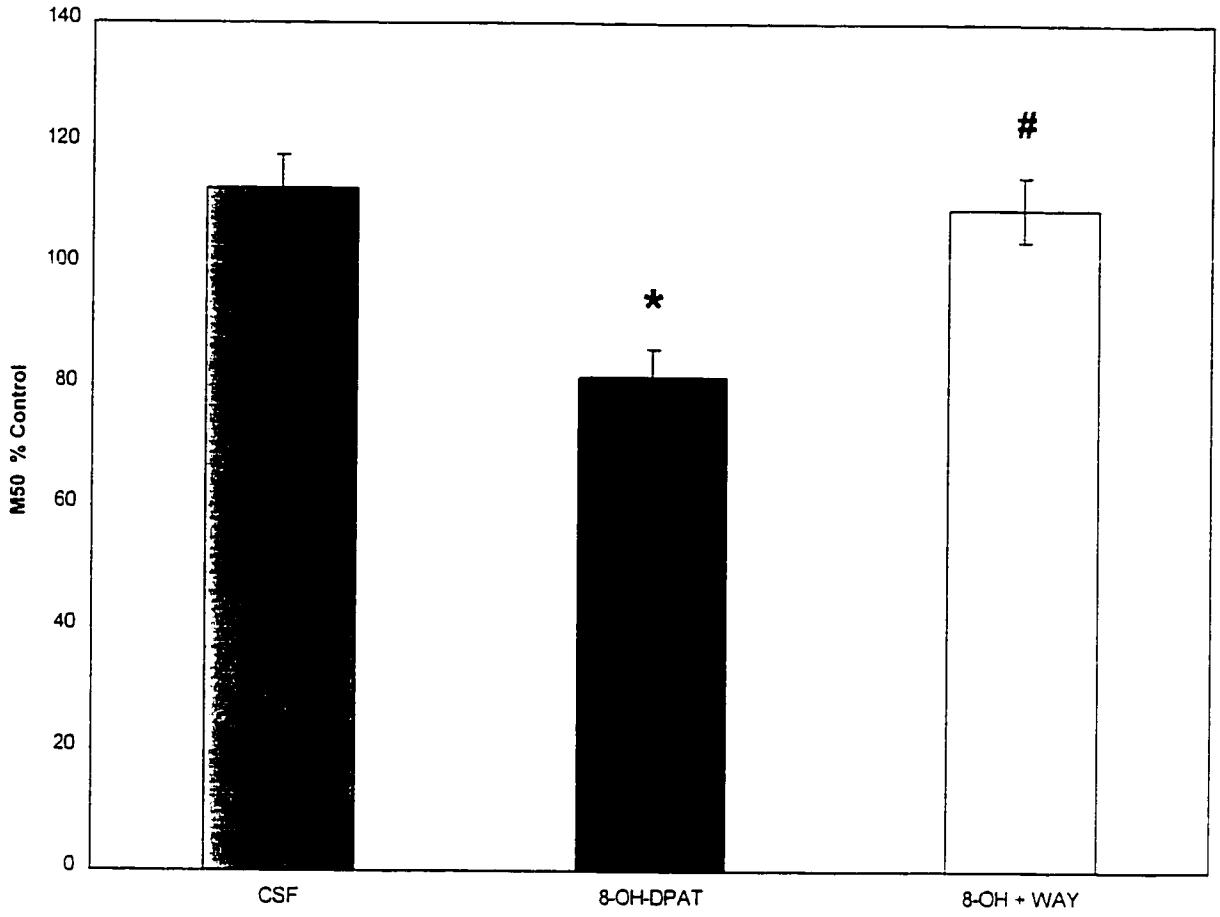


Figure 20. Effects of 8-OH-DPAT (5 μg) injected into the DRN on M50 of rats (n = 11) trained to self-stimulate on a continuous reinforcement schedule of electrical stimulation of the VTA at different stimulation frequencies. Significant differences ($p < 0.05$, ANOVA) between groups were denoted by • (compared with CSF-treated group) and # (compared with 8-OH-DPAT-treated group).

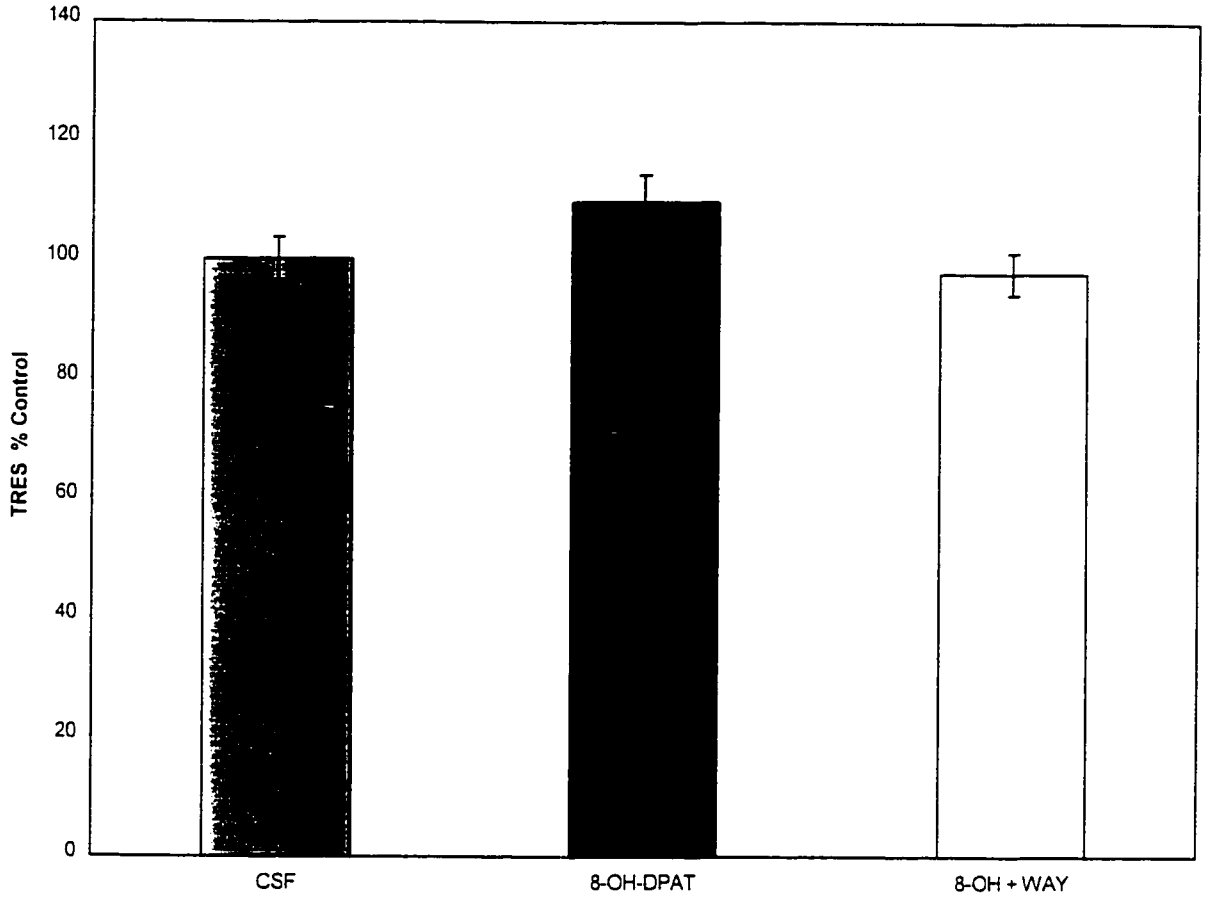


Figure 21. Lack effect of 8-OH-DPAT (5 μg) injected into the DRN on TRES of rats (n = 11) trained to self-stimulate on a continuous reinforcement schedule of electrical stimulation of the VTA at different stimulation frequencies. Significant level, $p < 0.05$, ANOVA

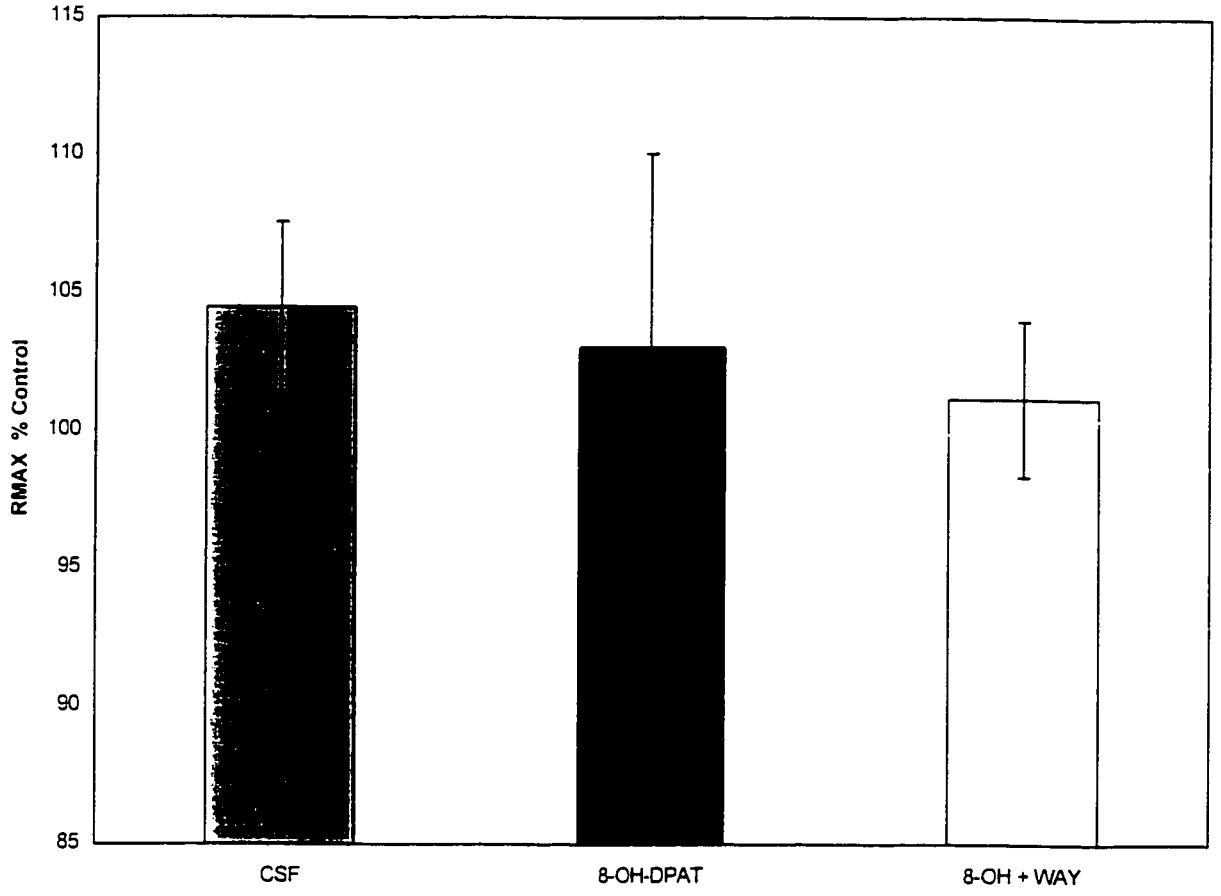


Figure 22. Lack effect of 8-OH-DPAT (5 μg) injected into the DRN on RMAX of rats (n = 11) trained to self-stimulate on a continuous reinforcement schedule of electrical stimulation of the VTA at different stimulation frequencies. Significant level, $p < 0.05$, ANOVA

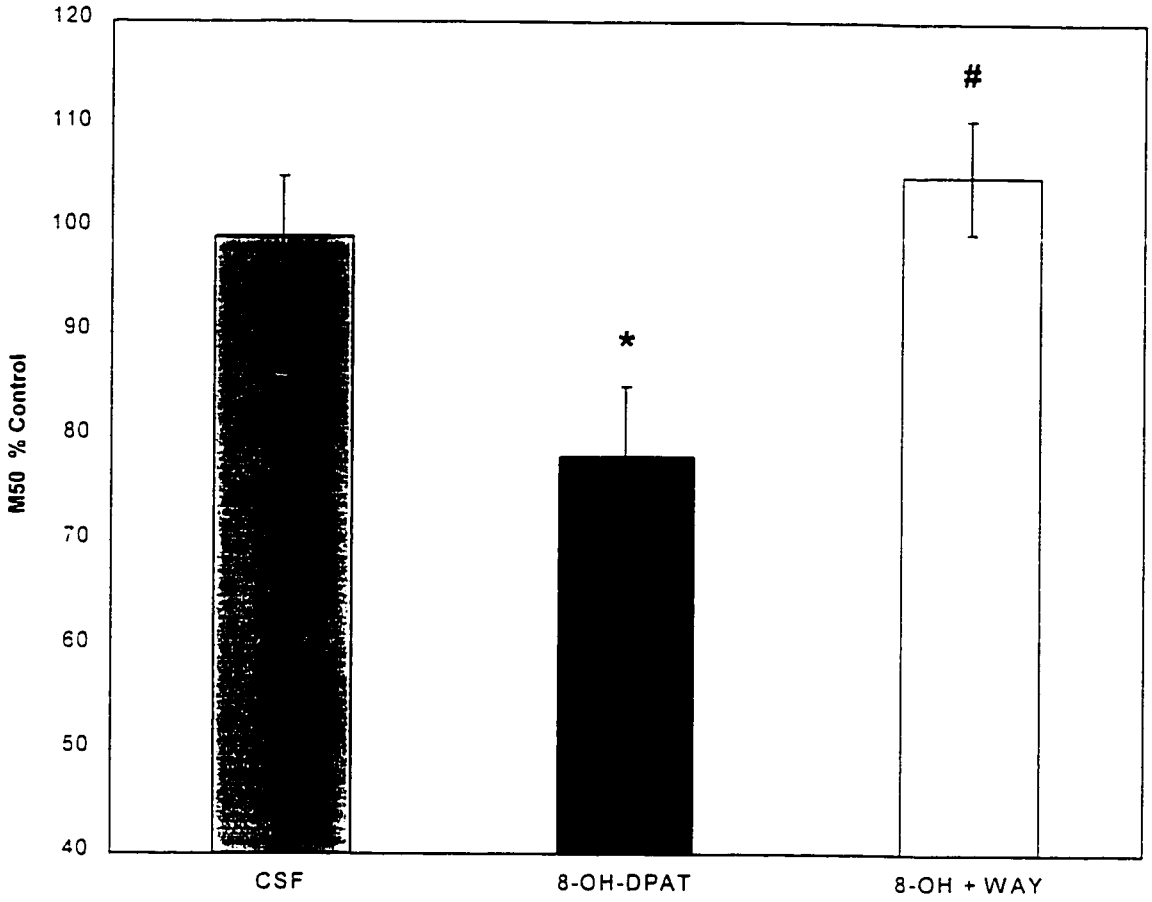


Figure 23. Effects of 8-OH-DPAT (5 μg) injected into the MRN on M50 of rats (n = 12) trained to self-stimulate on a continuous reinforcement schedule of electrical stimulation of the VTA at different stimulation frequencies. Significant differences ($p < 0.05$, ANOVA) between groups were denoted by • (compared with CSF-treated group) and # (compared with 8-OH-DPAT-treated group).

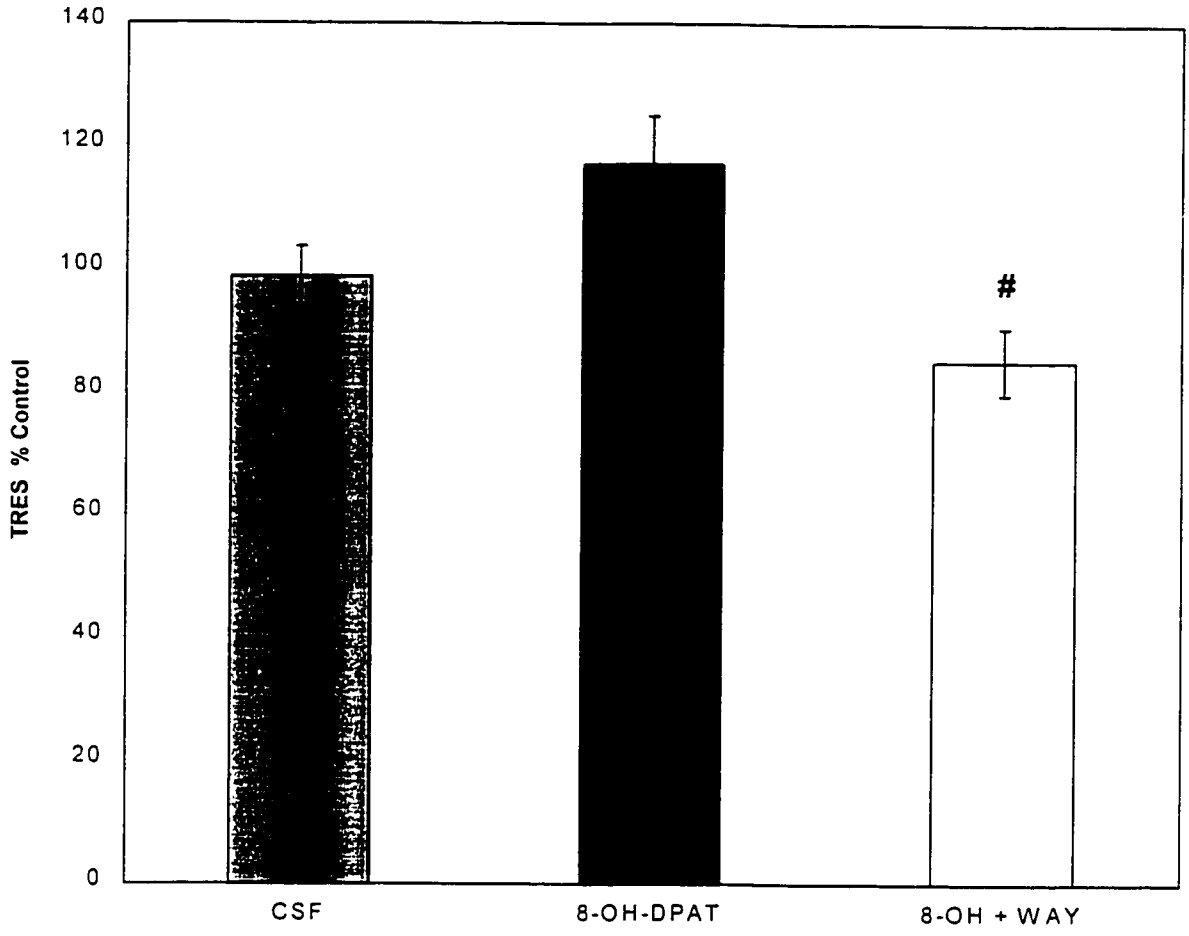


Figure 24. Lack effect of 8-OH-DPAT (5 μg) injected into the MRN on TRES of rats (n = 12) trained to self-stimulate on a continuous reinforcement schedule of electrical stimulation of the VTA at different stimulation frequencies. Significant differences ($p < 0.05$, ANOVA) between groups were denoted by # (compared with 8-OH-DPAT-treated group).

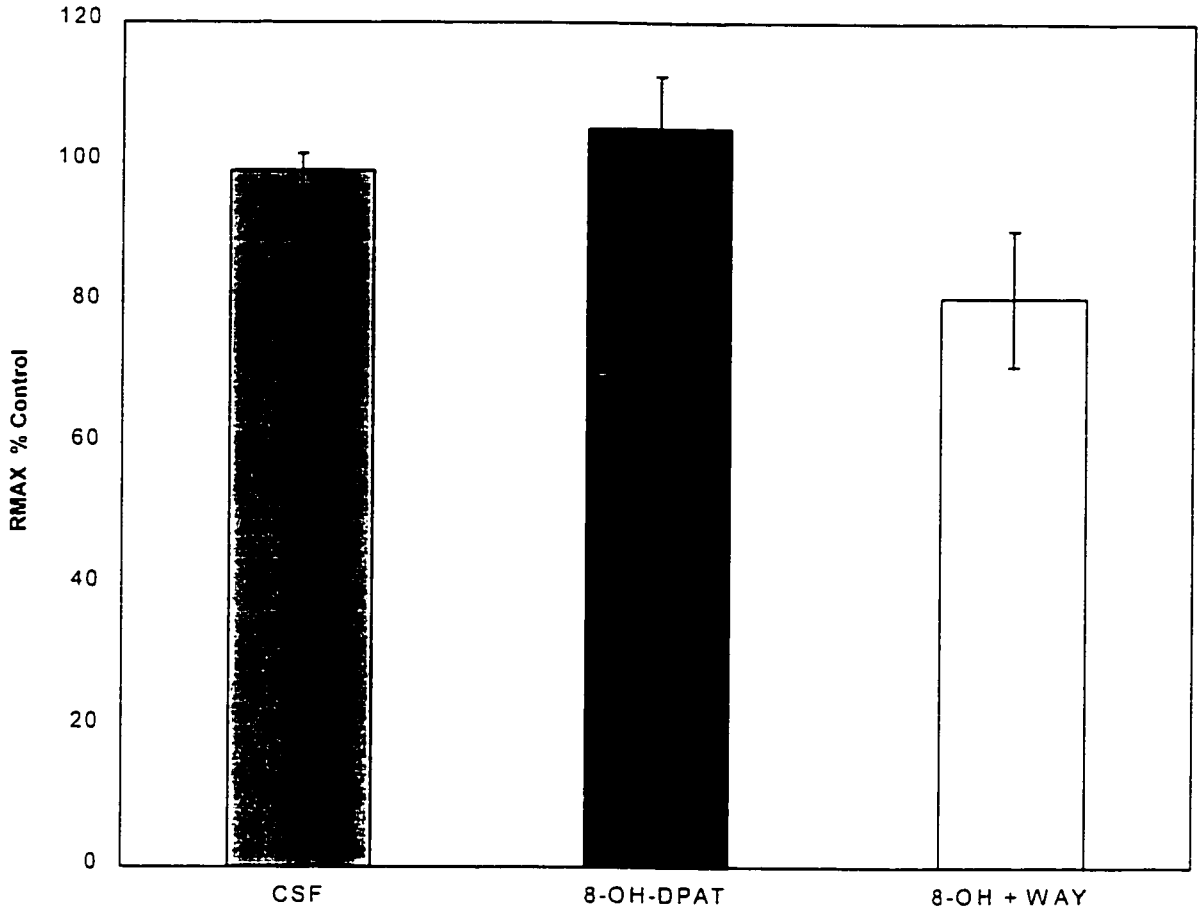


Figure 25. Lack effect of 8-OH-DPAT (5 μg) injected into the median raphé nucleus on RMAX of rats (n = 12) trained to self-stimulate on a continuous reinforcement schedule of electrical stimulation of the VTA at different stimulation frequencies. Significant level, $p < 0.05$, ANOVA

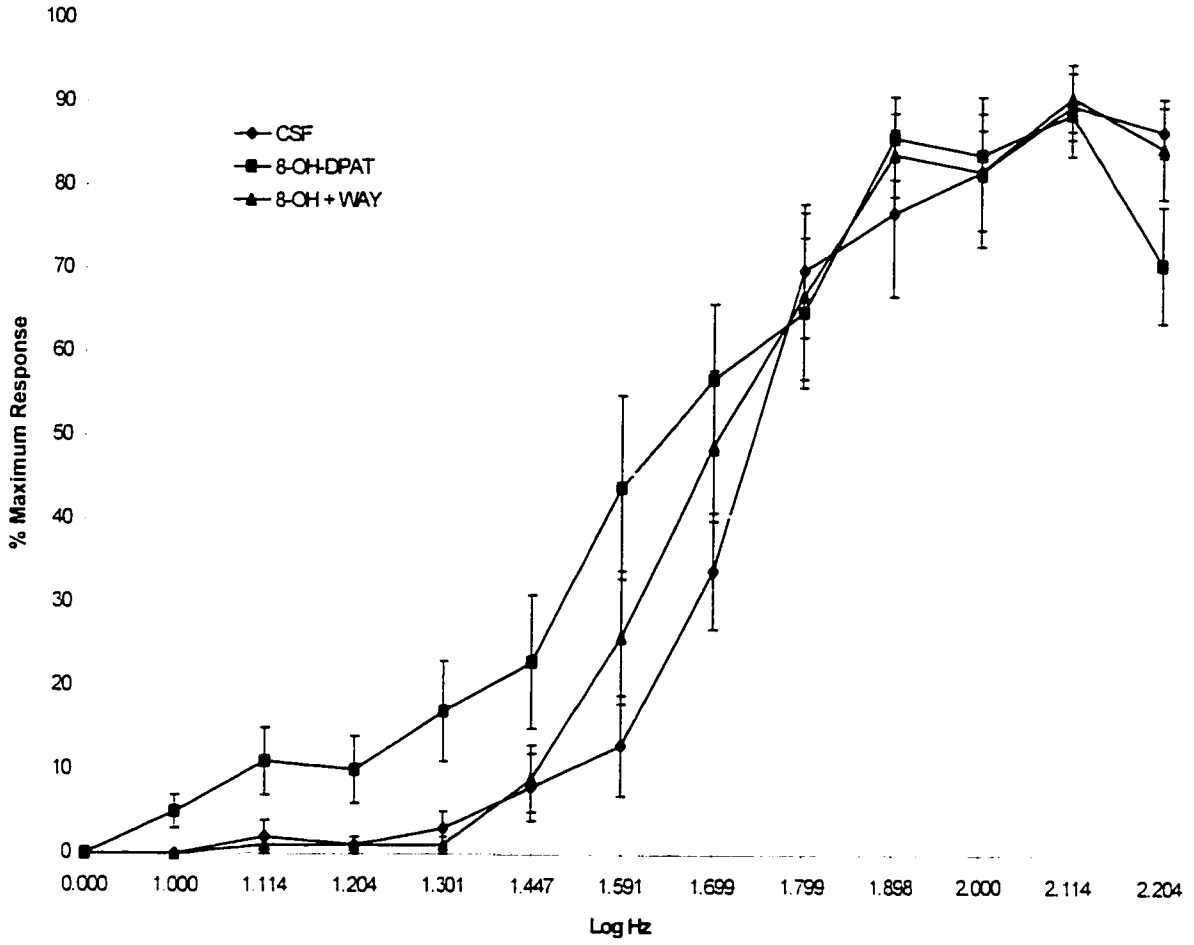


Figure 26. Effects of 8-OH-DPAT (5 μg) injected into the DRN and systemic WAY 100635 (0.1 mg kg^{-1}, sc) on rate-frequency responses for VTA self-stimulation (n = 11).

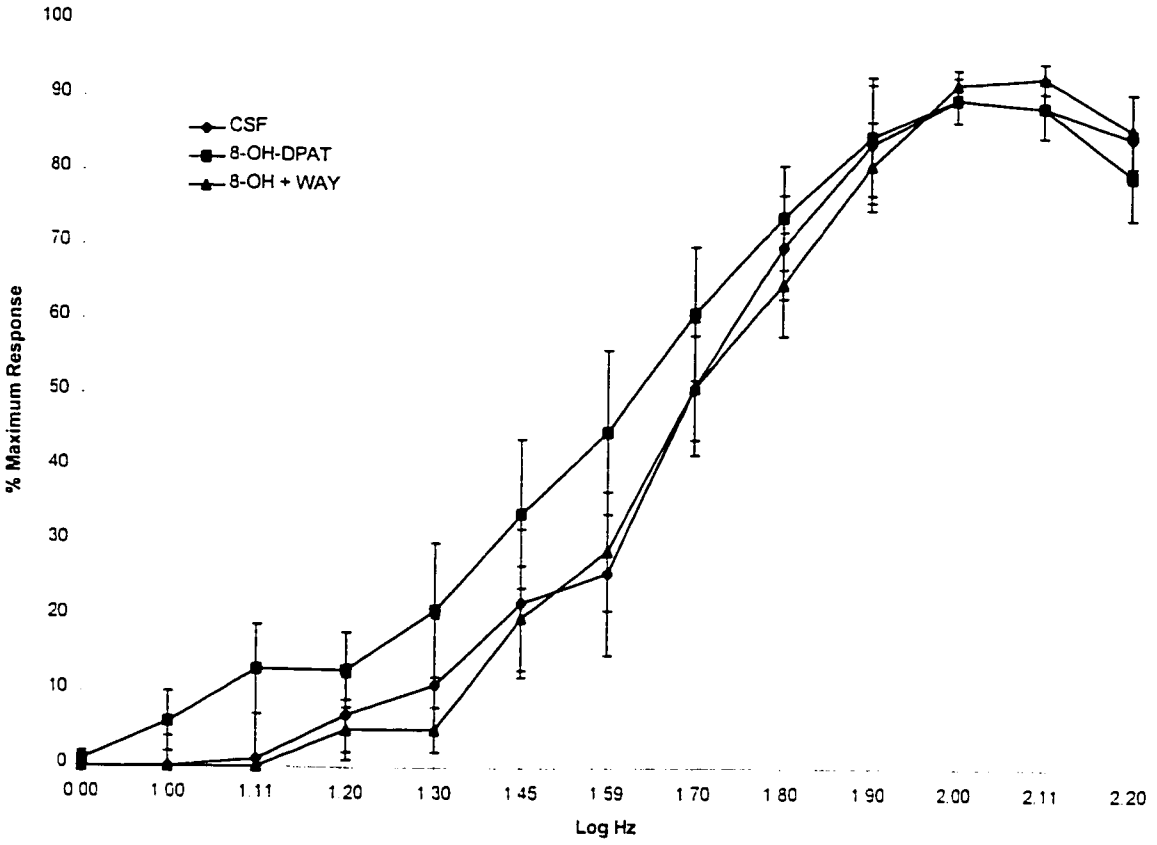
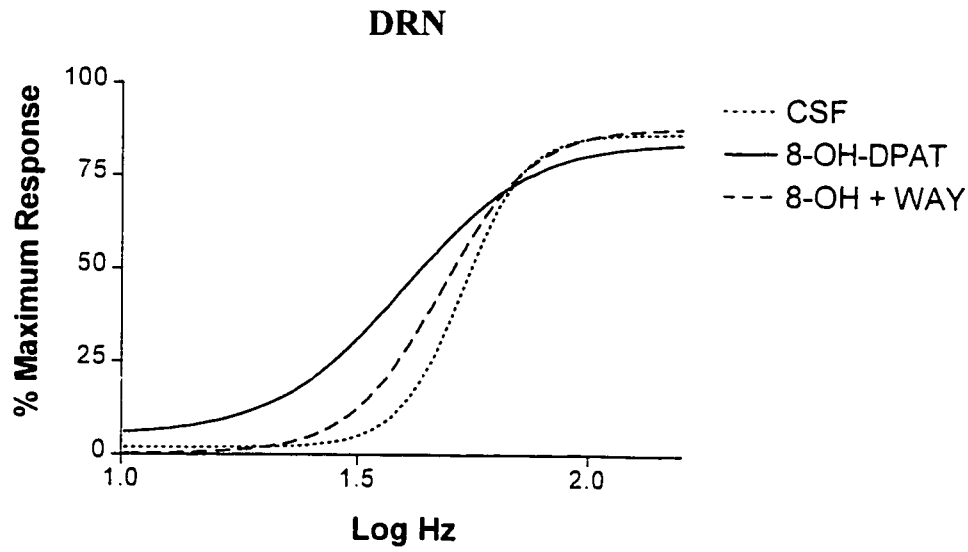


Figure 27. Effects of 8-OH-DPAT (5 μg) injected into the MRN and systemic WAY 100635 (0.1 mg kg^{-1}, sc) on rate-frequency responses for VTA self-stimulation (n = 12).

a.



b.

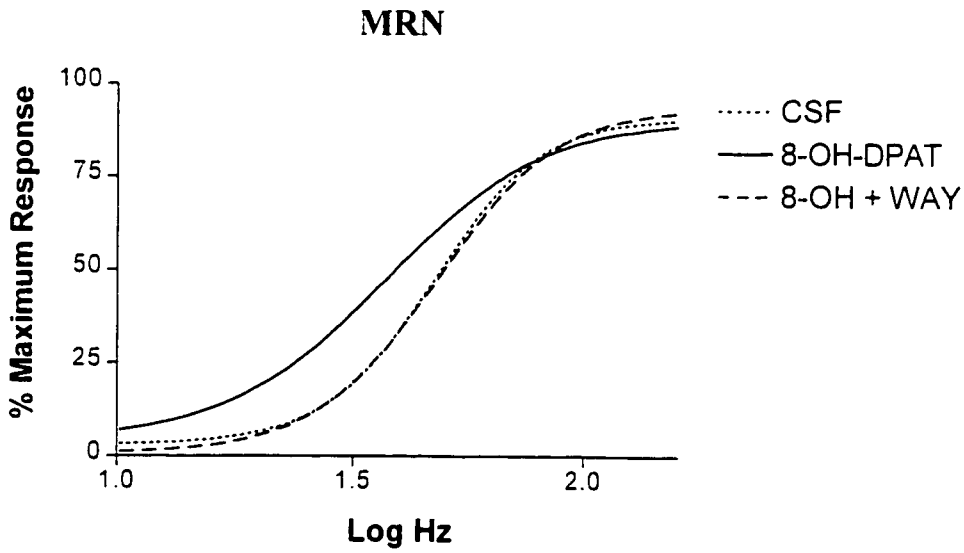


Figure 28. Sigmoidal curves prepared from the rate-frequency curves for the DRN and the MRN, respectively. a) Regression curve for the DRN. b) Regression curve for the MRN.

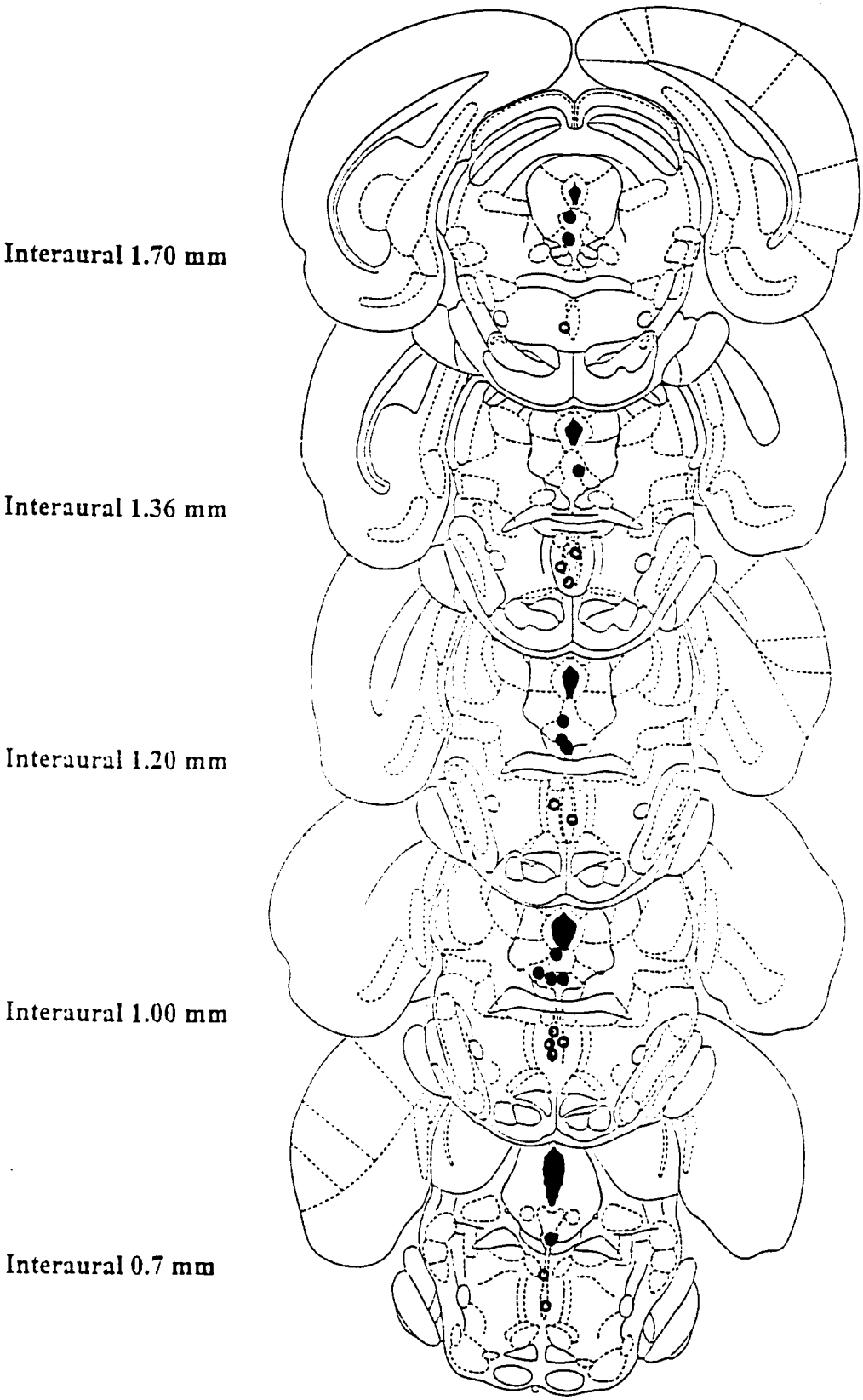


Figure 29. Schematic reconstructions showing the approximate placements of injection sites in the dorsal raphé nuclei (●, n = 11) and the median raphé nuclei (O, n = 12) for rats used for ICSS studies. The sections were redrawn from the atlas of Paxinos and Watson (1986).

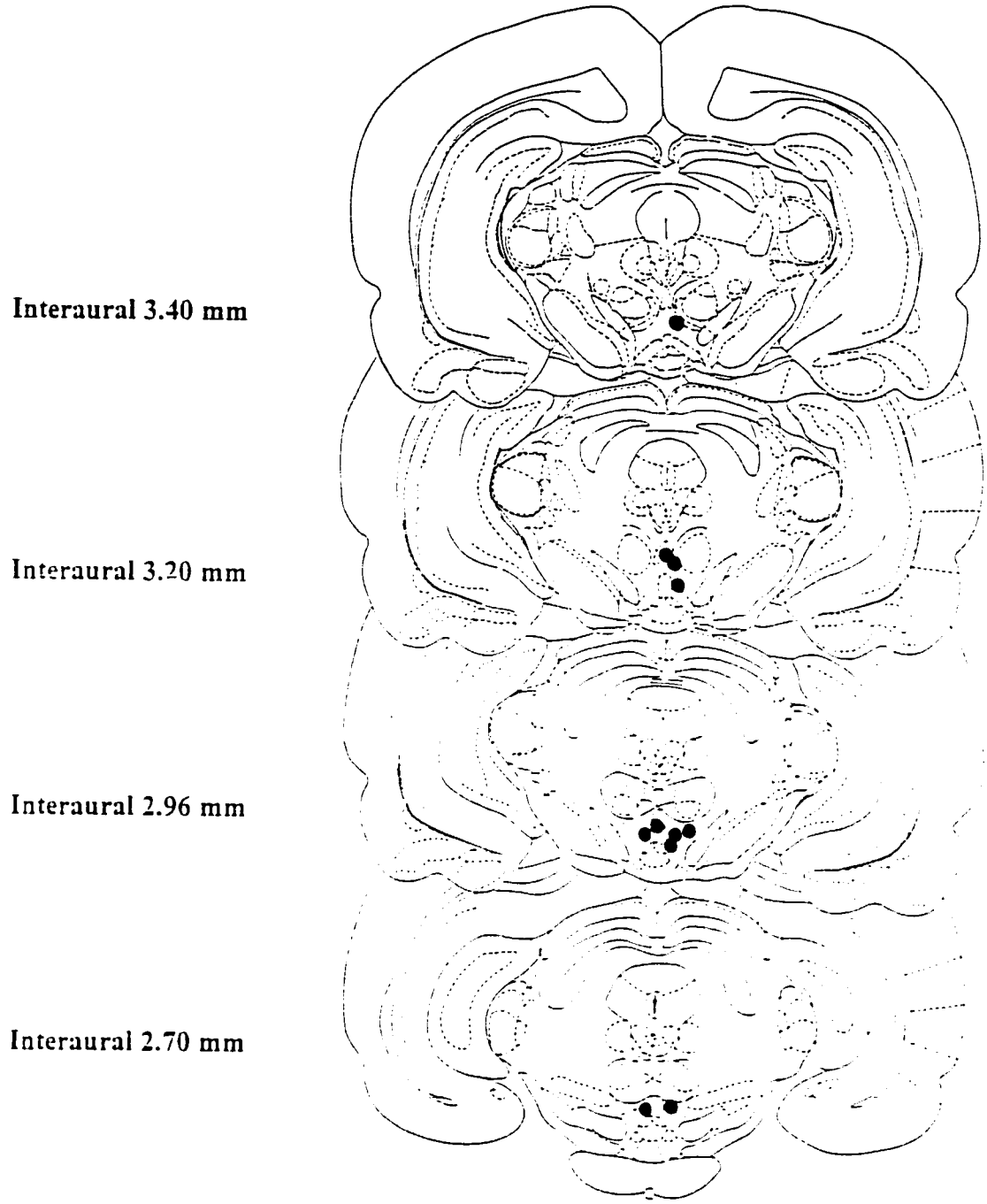


Figure 30. Schematic reconstructions showing the approximate placements of electrode-stimulating sites in the ventral tegmental area (●, n = 11) with the cannulation of the dorsal raphé nuclei. The sections were redrawn from the atlas of Paxinos and Watson (1986).

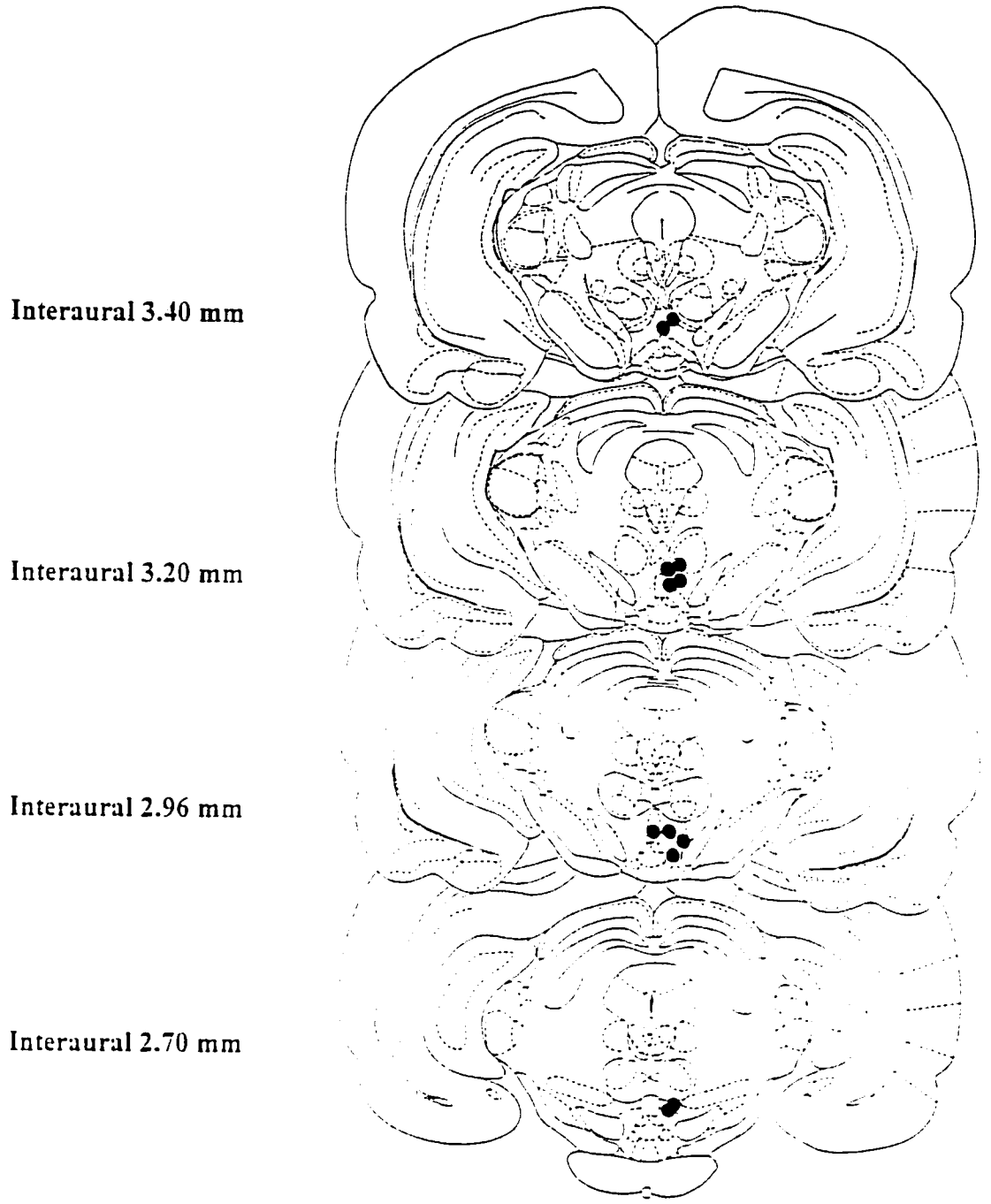


Figure 31. Schematic reconstructions showing the approximate placements of electrode-stimulating sites in the ventral tegmental area (●, n = 12) with the cannulation of the median raphé nuclei. The sections were redrawn from the atlas of Paxinos and Watson (1986).

Electrical Self-Stimulation of the Ventral Tegmental Area: Effects of Microinjections of TFMPP, RU 24969 and (+)-Amphetamine into the Shell of the Nucleus Accumbens

The following data are presented as an average percentage of the baseline performance of each animal, as described for the previous experiment. The baseline performance values are presented in Table 7.

Microinjections of TFMPP into the shell of NAS resulted in a significant increase [$F(3,27) = 27.19$, $p < 0.05$] in the M50 threshold measure, as illustrated by the data displayed in Figure 32. This effect was accompanied by a significant decrease of TRES [$F(3,27) = 12.19$, $p < 0.05$], as seen in Figure 33. However, there was no effect on RMAX [$F(3,27) = 1.01$, $p > 0.05$], as shown by the data displayed in Figure 34. As illustrated by the data in Figures 32 – 34, at the dose (5 µg) used here, microinjection of RU 24969 at this site did not result in any significant effect on M50 [$F(3,27) = 27.19$, $p > 0.05$], TRES [$F(3,27) = 12.19$, $p > 0.05$], or RMAX [$F(3,27) = 1.01$, $p > 0.05$].

Microinjection of (+)-amphetamine (5 µg) into this site induced a significant decrease in M50 [$F(3,27) = 27.19$, $p < 0.05$], and an increase in TRES [$F(3,27) = 12.19$, $p < 0.05$], as illustrated by the data displayed in Figure 32 and 33. The RMAX measure [$F(3,27) = 1.01$, $p > 0.05$] was not affected by amphetamine treatment (see Figure 34).

The rate-frequency curves (Figure 35, 36) are presented to illustrate the change in percentage of maximum response in relation to frequency change (log Hz).

Only rats with cannula placements in the target sites were included in the present data analysis. In Figure 37 are shown actual drug-injection sites of the shell of NAS for VTA ICSS. Electrode-stimulating sites in the VTA are shown in Figure 38 for the rats with drug injected into the shell of NAS.

Table 7. 5-Day baseline VTA self-stimulation performance for rats with NAS cannulae

Behavioural measures	M50	TRES	RMAX
Rats with NAS cannulae	66 ± 4	241 ± 30	56 ± 6

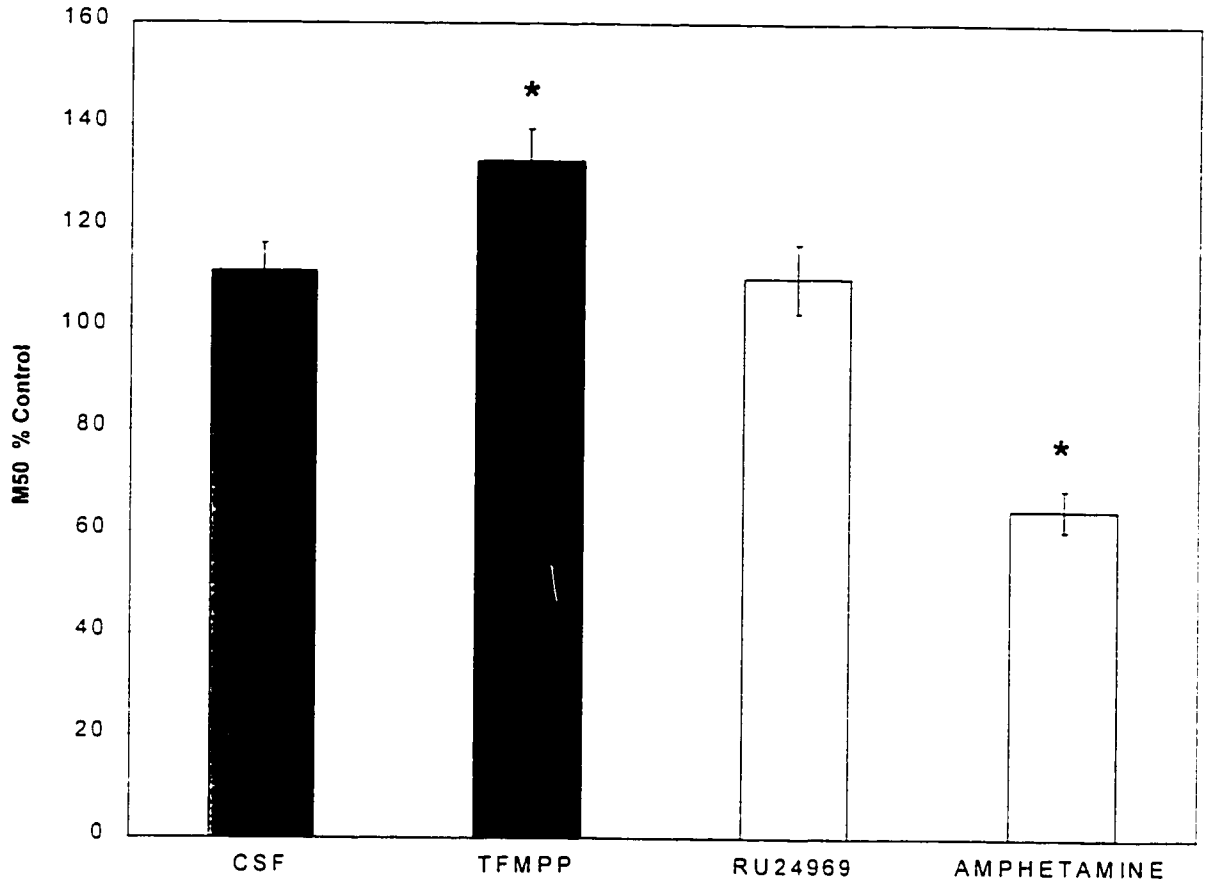


Figure 32. Effects of TFMPP (5 μg) and amphetamine (5 μg) injected into the shell of the nucleus accumbens on M50 of rats (n = 10) trained to self-stimulate on a continuous reinforcement schedule of electrical stimulation of the VTA at different stimulation frequencies. Significant differences ($p < 0.05$, ANOVA) between groups were denoted by • (compared with CSF-treated group).

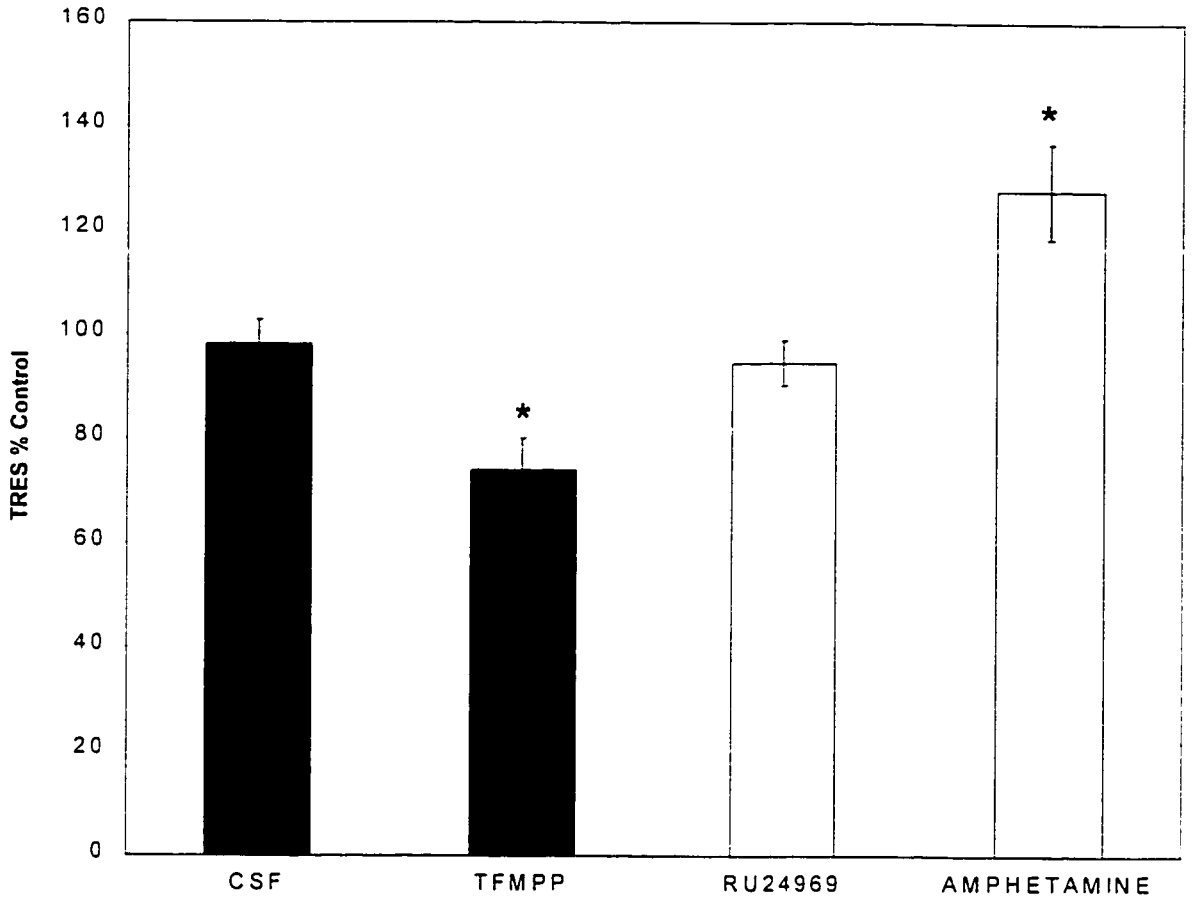


Figure 33. Effects of TFMPP (5 μg) and amphetamine (5 μg) injected into the shell of the nucleus accumbens on TRES of rats (n = 10) trained to self-stimulate on a continuous reinforcement schedule of electrical stimulation of the VTA at different stimulation frequencies. Significant differences ($p < 0.05$, ANOVA) between groups were denoted by * (compared with CSF-treated group).

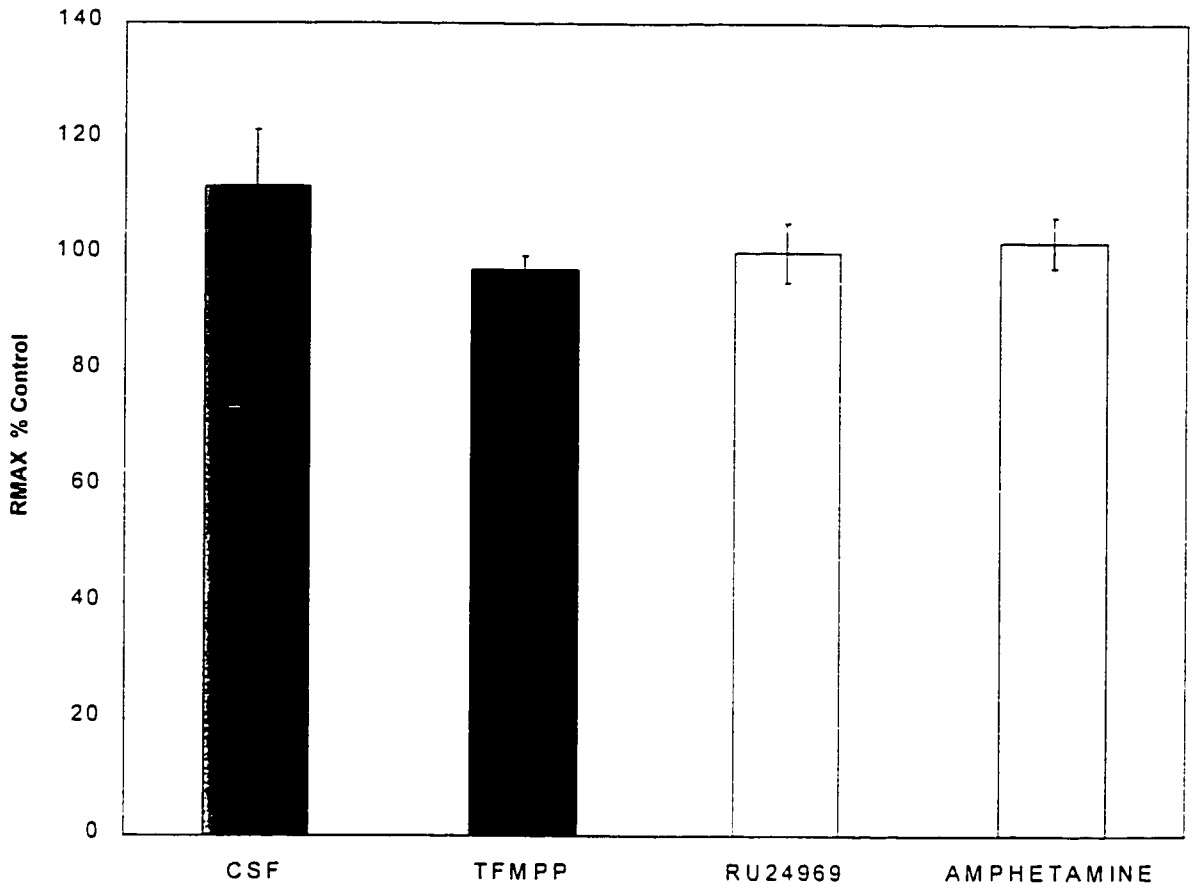


Figure 34. Lack effects of TFMPP (5 μg), RU 24969 (5 μg) and amphetamine (5 μg) injected into the shell of the nucleus accumbens on RMAX of rats (n = 10) trained to self-stimulate on a continuous reinforcement schedule of electrical stimulation of the VTA at different stimulation frequencies. Significant level, $p < 0.05$, ANOVA

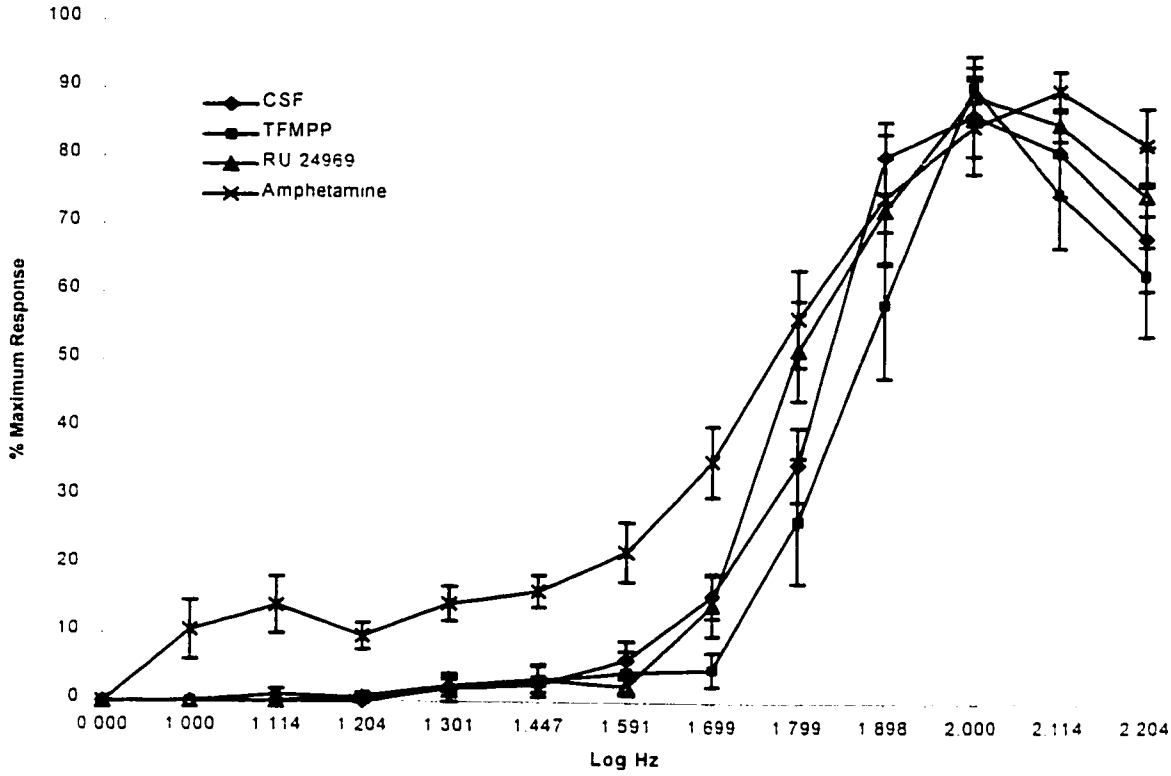


Figure 35. Effects of microinjections of amphetamine, TFMPP and RU 24969 into the nucleus accumbens on rate-frequency responses for VTA self-stimulation (n = 10).

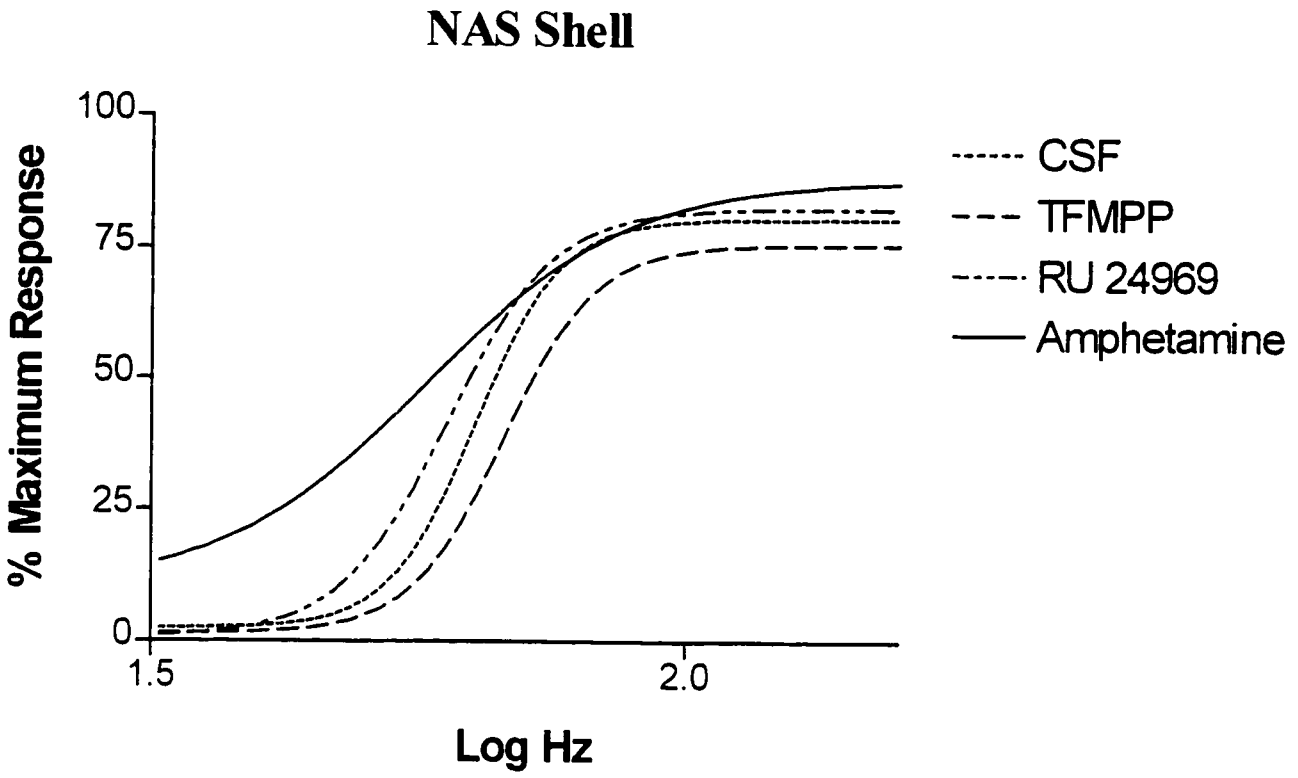


Figure 36. Sigmoidal curves prepared from the rate-frequency curves for the NAS by multiple regression.

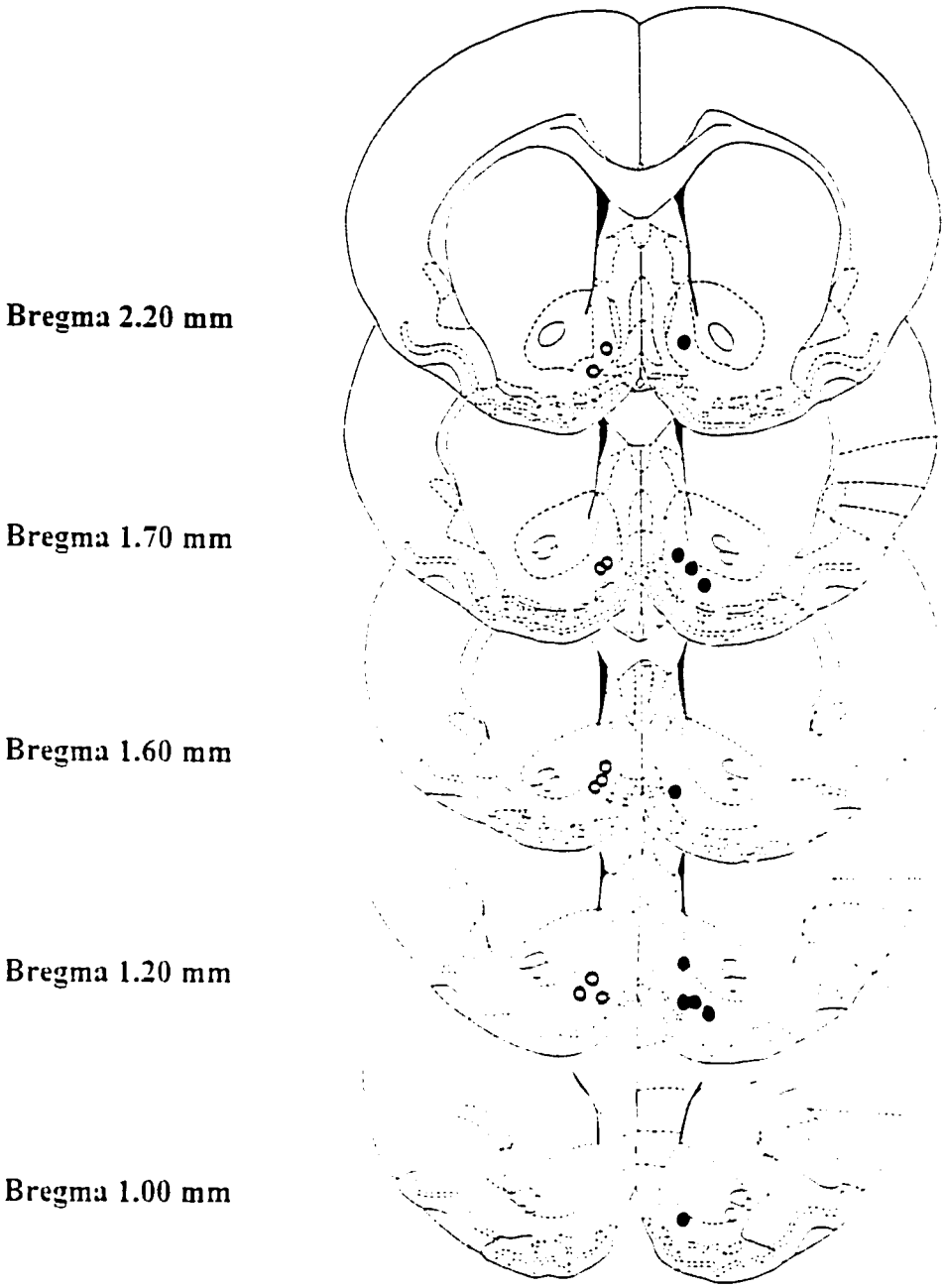


Figure 37. Schematic reconstructions showing the approximate placements of bilateral injection sites in the nucleus accumbens shell (● for ipsilateral to the VTA electrode and O for contralateral to the VTA electrode, n = 10). The sections were redrawn from the atlas of Paxinos and Watson (1986).

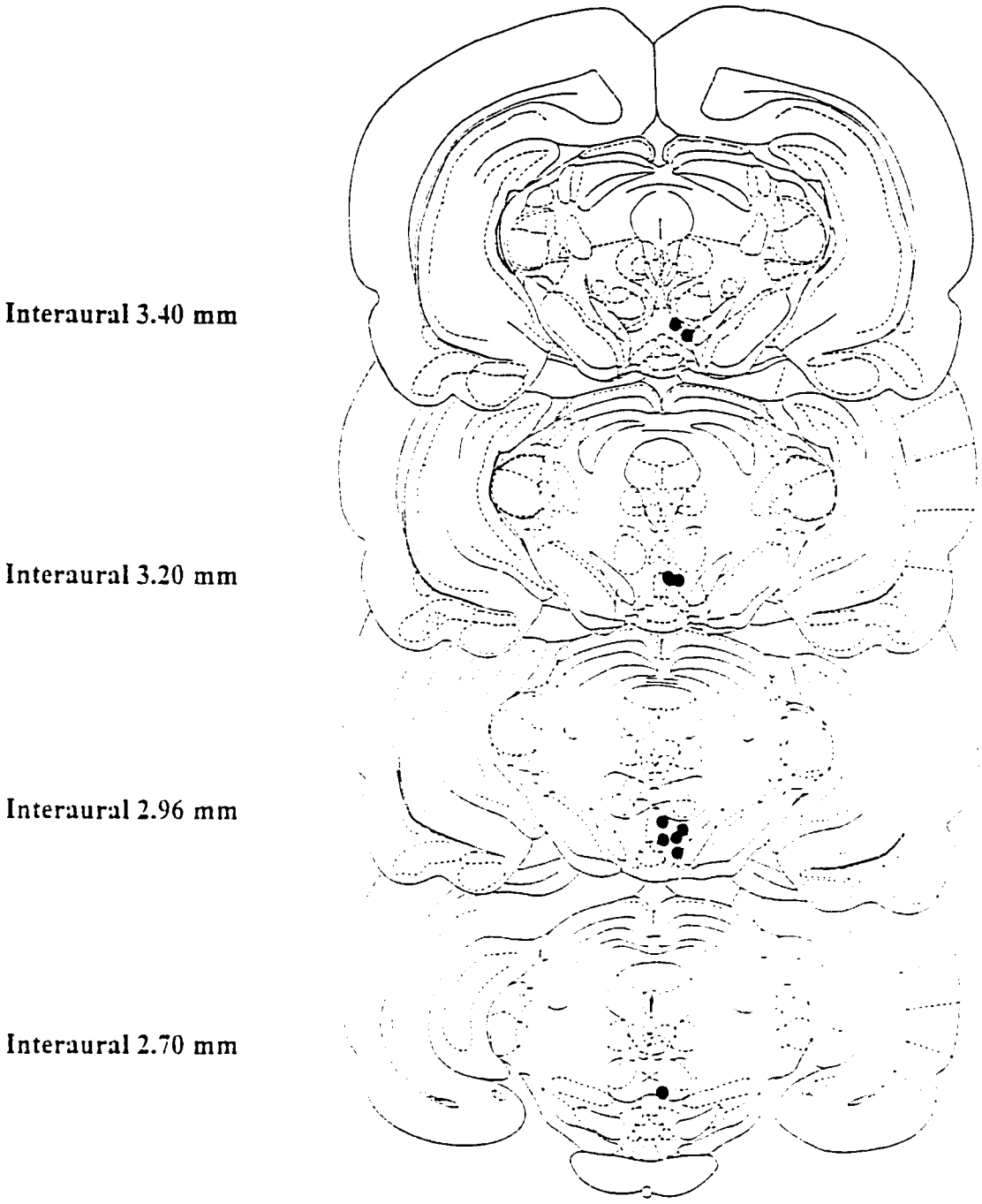


Figure 38. Schematic reconstructions showing the approximate placements of electrode-stimulating sites in the ventral tegmental area (●, n = 10) with the cannulation of the NAS shell. The sections were redrawn from the atlas of Paxinos and Watson (1986).

DISCUSSION

Differential Effects of Microinjections of 8-OH-DPAT into the Dorsal and Median Raphé Nucleus: Locomotor Activity

In the present study, locomotor activity was differentially affected by microinjections of 8-OH-DPAT (a 5-HT_{1A} receptor agonist) into the DRN (hypoactivity) and the MRN (hyperactivity). The effects were prominent 10 minutes after microinjections into the respective raphé nuclei. These results support and extend the results of previous studies published by Hillegaart and Hjorth (1989), Hillegaart (1990) and Higgins and Elliott (1991). The present results are consistent with these authors' observations that 5 µg of 8-OH-DPAT decreased locomotor activity and rearing after administration into the DRN whereas this dose of 8-OH-DPAT into the MRN significantly increased locomotor activity. By contrast, one study reported similar effects of microinjections of 5-HT into the DRN and the MRN (Hillegaart, 1991). The present data are consistent with the proposal for distinct roles of the respective ascending DRN and MRN serotonergic projections (Hillegaart, 1990; Higgins and Elliott, 1991; Hogg *et al.*, 1994; File and Gonzalez, 1996).

The differential behavioural effects in the present study may be supported by the suggestion that the effect of 5-HT in the VTA is likely to be mediated by different types of 5-HT fibres originating from the various raphé nuclei (Halliday and Törk, 1989). The VTA consists of important DA cell bodies which project to the NAS. The NAS is a dopaminergic terminal area that is associated with locomotor activity. Moreover, some

anterograde and retrograde tracer labeling studies (Parent *et al.*, 1981; Vertes and Martin, 1988; Vertes, 1988) have also suggested the possibility of differential roles for the DRN and the MRN, having identified differential projections from those raphé nuclei to forebrain bundle area.

It has been proposed that the contrasting behavioural profiles of a 5-HT_{1A} agonist such as 8-OH-DPAT observed after microinjections into the DRN or MRN are related to the differential innervation of forebrain structures by these respective nuclei (Higgins and Elliott, 1991). Activation of 5-HT_{1A} receptors in these raphé nuclei produces different effects on 5-HT synthesis in different brain regions. Following injections of 8-OH-DPAT into the DRN there is a reduction of 5-HT synthesis in the striatum (STR), the NAS and the prefrontal cortex (PFC). By contrast, following injection of 8-OH-DPAT into the MRN there was a decrease in 5-HT synthesis in the NAS and the PFC (Invernizzi *et al.*, 1991; Casanovas and Artigas, 1996). These findings support the view that most serotonergic innervation of the STR originates in the DRN (Steinbusch *et al.*, 1981). Moreover, it is apparent that 5-HT release in the STR and the hippocampus may be controlled respectively by 5-HT_{1A} autoreceptors of the DRN and the MRN. Kreiss and Lucki (1994) reported that local administration of 8-OH-DPAT into the DRN reduced 5-HT release in the striatum, but not in the hippocampus. Conversely, administration of 8-OH-DPAT into the MRN reduced 5-HT release in the hippocampus, but not in the striatum. Casanovas and Artigas (1996) reported that the differential reduction of 5-HT release in the MRN and hippocampus may be related to the presence of complex mechanisms of control of 5-HT release in these neuron. Therefore, the significant reduction in the hippocampal 5-HT following the MRN, but not the DRN (Kreiss and

Lucki, 1994; López *et al.*, 1996) may be considered a critical difference accounting for the present differential locomotor effects of 8-OH-DPAT applied to the DRN and MRN respectively.

A possible specific role for 5-HT_{1A} receptors in the present study is supported by a previous study (Hillegaart, 1990) using (-) pindolol, a 5-HT antagonist, to antagonize 8-OH-DPAT-induced hyperactivity after application into the MRN. However, 8-OH-DPAT has been shown to activate somatodendritic 5-HT receptors at low doses and to stimulate postsynaptic 5-HT receptors at high doses (Tricklebank *et al.*, 1984; Dourish *et al.*, 1988; Montgomery *et al.*, 1991). Therefore, the observation of Hillegaart *et al.* (1989) that systemic injection of 8-OH-DPAT suppressed motor activity and rearing was attributable to predominant effects of 8-OH-DPAT in the DRN and perhaps also to postsynaptic effects in the forebrain. The predominent effects in the DRN after systemic injection of 8-OH-DPAT may be explained by an electrophysiological study (Sinton and Fallon, 1988) that reported that inhibition of neuronal firing rates in the DRN is more pronounced than the comparative effects of this compound in the MRN or the SN (Sinton and Fallon, 1988), because the DRN appears to be richer in 5-HT_{1A} sites than the MRN (Pazos and Palacios, 1985). Therefore, differential localization of 5-HT_{1A} receptors could possibly lead to opposing effects of serotonergic agents between the DRN and the MRN. Moreover, systemically administered 8-OH-DPAT seems to bind easily to postsynaptic 5-HT_{1A} receptors in forebrain, because a relatively high dose is often administered to induce a behavioural effect. Thus, the suppression of locomotor activity followed systemic administration of 8-OH-DPAT may be at least partially attributable to an inhibitory effect via postsynaptic 5-HT_{1A} receptors. On the other hand, 8-OH-DPAT used

in the present study presumably decreased total activity via somatodendritic 5-HT_{1A} receptors, reducing 5-HT release in the DRN-innervated structures such as the NAS and the STR whereas 8-OH-DPAT might increase total activity via MRN somatodendritic 5-HT_{1A} receptors, reducing 5-HT release in MRN-innervated structures such as the NAS and the hippocampus. Because both mesocorticolimbic and nigrostriatal DA pathways have been implicated in locomotor activity (Beninger, 1983) and DA activity may be modulated by changes to firing rates of 5-HT cells (Sinton and Fallon, 1988), the interactions between 5-HT and DA in these systems are highly relevant for interpreting the 5-HT-related changes in locomotor activity.

In the present study, 1 μg of 8-OH-DPAT injected into the MRN was not effective at all, although 1 μg of 8-OH-DPAT was reported to be more effective than 5 μg in Hillegaart's earlier study (1990). Different rat strains may show different behavioural effects such as feeding or locomotion responses to 5-HT agonists (Aulakh *et al.*, 1989). Similar strain differences have been observed with mice in relation to ICSS (Garrigues and Cazala, 1983). Interestingly, 2.5 μg of 8-OH-DPAT was effective on total activity following microinjection into the DRN, but not when microinjected into the MRN, in the present study. These results are in agreement with a reported higher sensitivity of the DRN than the MRN to the systemic administration of 8-OH-DPAT (Sinton and Fallon, 1988; Casanovas and Artigas, 1996).

A prominent decrease in rearing was observed 10 minutes after microinjections of 5 μg of 8-OH-DPAT into the DRN, in contrast to a significant decrease in rearing 5 minutes after microinjections of 5 μg of 8-OH-DPAT into the MRN. It is interesting that the MRN injections decreased rearing although locomotor activity was increased. Higgins

and Elliott (1991) previously described a reduction in rearing observed after microinjections of 8-OH-DPAT into the both raphé nuclei. The present analysis of interaction between dose and time suggests that the two different target sites (the DRN and MRN) may have a differential latency to effect at the same dose.

There were no significant effects of microinjections of 8-OH-DPAT on consecutive behaviour in the present study. Consecutive behaviour such as grooming may be less sensitive to drug administration than locomotor activity or rearing (Jacobs, 1976). When rats are administered compounds which either dramatically increase synaptic 5-HT or directly stimulate postsynaptic 5-HT receptors, an increase in stereotyped behaviour such as the 5-HT stereotyped syndrome is expected (Jacobs, 1976). 8-OH-DPAT in the DRN, but not in the MRN, induces flattened body posture without inducing any other behaviour characteristic for the 5-HT syndrome such as forepaw treading, head weaving and hindlimb abduction (Hillegaart and Hjorth, 1989; Higgins and Elliott, 1991). The flattened body posture seen after DRN microinjection is distinct from that of the 5-HT syndrome and may be due to the hypotensive properties of 8-OH-DPAT such as marked falls in blood pressure and heart rate (Connor and Higgins, 1989; Higgins and Elliott, 1991). Therefore, a relatively high dose of systemically injected 5-HT agonists may be required to induce an increase in the present consecutive behaviour measure. The lack of effects on consecutive behaviour in the present study is consistent with the proposal that the behavioural effects observed here were caused by somatodendritic stimulation, but not by postsynaptic receptor activation, through microinjections of 8-OH-DPAT.

Effects of Microinjections of 8-OH-DPAT into the Dorsal and Median Raphé Nucleus on Electrical Self-Stimulation Responses of the Ventral Tegmental Area

Microinjection of 8-OH-DPAT directly into either the DRN or MRN induced a decrease in VTA self-stimulation frequency thresholds (M50). These results are consistent with those of Fletcher *et al.* (1995) who found that microinjections of 8-OH-DPAT into the MRN lowered an equivalent threshold measure for lateral hypothalamic self-stimulation. Both of these self-stimulation sites may receive a similar type of projection from the MRN (Vertes and Martin, 1988). Thus, self-stimulation of both the VTA and hypothalamus may have similar behavioural responses to 8-OH-DPAT, although Jenkins *et al.* (1983) have suggested that behavioural effects of brain self-stimulation may be stimulation site-dependent.

Injections of the 5-HT_{1A} receptor agonist 8-OH-DPAT (Arvidsson *et al.*, 1981) into both the DRN and MRN reduce 5-HT synthesis and/or release in terminals (Invernizzi *et al.*, 1991). Fletcher *et al.* (1995) first suggested that lowering brain 5-HT activity facilitates responding for rewarding brain stimulation, at least when the stimulating electrode is located within the medial forebrain bundle. The present study confirms this observation and extends these effects to the DRN. These results lend further support to the proposal that brain 5-HT neurons may play an important role in modulating the activity of the brain's reward circuitry (Fletcher et al., 1995). The present study did not address directly the involvement of DA in mediating the effects of 8-OH-DPAT, but the activity of DA neurons may be influenced by alterations in 5-HT activity. Several reports indicate that reductions in 5-HT activity may facilitate DA function (Dray *et al.*, 1978;

Drescher and Hetey, 1988). Thus, the present facilitatory effect of 8-OH-DPAT on brain stimulation reward may be due to reduction of an inhibitory influence of 5-HT on DA activity (Dray *et al.*, 1978).

Another rat lateral hypothalamic self-stimulation study (Montgomery *et al.*, 1991) illustrated that the systemic effects of 8-OH-DPAT were biphasic depending on the drug dose. According to that study, lower doses increased reward responding through an agonist action at 5-HT_{1A} somatodendritic autoreceptors while higher doses decreased reward responding through stimulation of 5-HT_{1A} postsynaptic receptors. However, it is difficult to compare these systemic biphasic effects with the results of microinjections of 8-OH-DPAT in the present study. In a previous study in this laboratory with systemic 8-OH-DPAT injections (Ahn *et al.* 1997), a dose-dependent increase (0, 0.003, 0.01, 0.03, 0.1, 0.3 mg kg^{-1} SC) in frequency threshold (M50) was observed while dose-dependent decreases were observed in total response (TRES) and maximal response (RMAX). These results would be consistent with postsynaptic effects of 8-OH-DPAT, because this drug did not facilitate ICSS of the VTA through stimulation of somatodendritic 5-HT receptors at low doses but inhibited ICSS of the VTA at high doses.

Of the behavioural measures in the present study, 8-OH-DPAT-induced frequency change (M50) is associated with a change in reward strength or reinforcement (Edmonds and Gallistel, 1974; Wise, 1996). Therefore, the pattern of the present results may be interpreted as an effect of 8-OH-DPAT on reward via somatodendritic 5-HT autoreceptors, rather than a non-specific performance deficit. The hypothesis that 5-HT may inhibit DA activity in the mesolimbic pathway is supported by the present behavioural results. Removal of inhibitory 5-HT inputs as a result of local 8-OH-DPAT

injections into the DRN and the MRN may increase dopaminergic activity, resulting in decrease of self- stimulation thresholds. In the present study, the measure of motor performance, RMAX, was unaffected after microinjections of 8-OH-DPAT into either the DRN or MRN. As discussed before, these results are different from the data obtained with systemic 8-OH-DPAT in the prior study in this laboratory. This difference underscores the need for caution in interpreting effects of systemically administered 8-OH-DPAT in terms of actions at somatodendritic receptors.

The effects of 8-OH-DPAT on VTA self-stimulation contrast markedly with the differential effects on locomotor activity observed in the previous experiment and other studies (Hillegaart, 1990 & 1991; Higgins and Elliott, 1991). The threshold reducing effects following microinjections of 8-OH-DPAT into both DRN and MRN may be attributed to specificity of the ICSS paradigm for reward in contrast to locomotor activity measures. It may be argued that ICSS of the VTA is limited to stimulation of the mesocorticolimbic but not the nigrostriatal pathway (Nakahara *et al.*, 1989 & 1992). In fact, rewarding stimulation of the ventral tegmentum is associated with DA release in mesolimbic terminal regions, including the NAS (Phillips and Fibiger, 1989; Blaha and Phillips, 1990; Fiorino *et al.*, 1993) while locomotor activity may be changed by a drug manipulation of nigrostriatal and / or mesocorticolimbic pathways (Beninger, 1983).

WAY 100635, a selective 5-HT_{1A} antagonist (Fletcher *et al.*, 1994), has no intrinsic influence on raphé cell firing *in vivo*, whilst antagonising the inhibition of firing induced by 8-OH-DPAT (Forster *et al.*, 1995). The data of Critchley *et al.* (1994) support a role for WAY 100635 as a specific 5-HT_{1A} antagonist. These authors demonstrated that WAY 100635 may inhibit the stimulatory effect of 8-OH-DPAT on plasma

adrenocorticotrophin (ACTH) levels in the rat. In the present study, systemic administration of WAY 100635 blocked the change in reward responses (M50) induced by intracranial injection of 8-OH-DPAT. The present results with WAY 100635 are consistent with 5-HT_{1A} receptor mediation of these effects of 8-OH-DPAT. A wide range of doses of WAY 100635 (0.0125, 0.0250, 0.0500 or 0.1000 mg kg^{-1} SC) had no effects on VTA self-stimulation in a prior study in this laboratory (Ahn *et al.*, 1997). A similar lack of effects was observed with systemic administration of the less selective 5-HT_{1A} receptor antagonist, pindolol (Ahn *et al.*, 1997).

Decreased Ventral Tegmental ICSS Responses Induced by Microinjections of TFMPP, but not RU 24969, into the Shell of Nucleus Accumbens

In the present study TFMPP microinjected into the shell of the NAS increased frequency thresholds (M50) for VTA self-stimulation, corresponding to a decrease in reward responses. It is very interesting that intra-NAS application of RU 24969 had no significant effect on VTA reward in the present study. It has recently been reported that subcutaneous administration of RU 24969 elevated lateral hypothalamic ICSS thresholds without affecting response latency, a measure of general motoric activity (Harrison *et al.*, 1999). In that study, the blockade of RU 24969 effects by GR 127935, a 5-$HT_{1B/D}$ receptor antagonist, led the authors to argue for an involvement of 5-HT_{1B} receptors in the elevation of the ICSS threshold. The elevation of the ICSS threshold by RU 24969 might be caused by 5-HT_{1B} receptors on any specific pathway in the brain but not on the NAS. It is difficult to contrast the findings of Harrison *et al.* (1999) directly with the present results in view of the difference in route of administration and the different stimulation site. Nevertheless, recent data from this laboratory have revealed threshold-elevating effects of systemic RU 24969 on VTA ICSS (Ahn *et al.*, 1997). In the present study, the dose (5 μg bilateral) of microinjected RU 24969 may be too low to get a behavioural effect, because there may be non-specific binding to various types of receptors in the NAS. Actually, Higgins *et al.* (1991) applied 25 μg of RU 24969 unilaterally into the SN to induce significant contralateral rotation, and the potency for RU 24969 in inducing this behaviour is lower than that of other 5-HT agonists such as 5-carboxamidotryptamine (5-CT) and sumatriptan. Due to problems related to solubility

and non-specific actions of high concentrations of microinjection solutions, 5 μg was the highest intracranial dose used in the present study.

On the basis of electrolytic lesion studies, Hervé and colleagues (1979 & 1981) proposed that there were direct projections from the DRN and the MRN to the NAS to regulate the activity of the terminals of the mesolimbic dopaminergic neuron projections. Thus, the regulation by these direct 5-HT projections is mediated through 5-HT receptors in the NAS. TFMPP and RU 24969 are non-selective 5-HT_{1B} receptor agonists. RU 24969 has a higher affinity for 5-HT_{1A} and 5-HT_{1B} receptors than does TFMPP (RU 24969 > TFMPP for 5-HT_{1A} and 5-HT_{1B} receptors) (Middlemiss and Tricklebank, 1992; Boess and Martin, 1994; Chopin *et al.*, 1994). Both TFMPP and RU 24969 have approximately the same affinity for 5-HT_{2C} receptors (RU 24969 = TFMPP for 5-HT_{2C} receptor), while TFMPP has slightly higher affinity for 5-HT_{2A} (RU 24969 < TFMPP for 5-HT_{2A} receptor) and lower affinity for 5-HT_{2B} receptors (RU 24969 > TFMPP for 5-HT_{2B} receptor) than does RU 24969 (Middlemiss and Huston, 1990; Peroutka *et al.*, 1990; Middlemiss and Tricklebank, 1992; Boess and Martin, 1994; Chopin *et al.*, 1994; Saudou and Hen, 1994; Sexena, 1995). The rank order of potency for 5-HT receptor subtypes is 5-HT_{2C} > 5-HT_{1B} > 5-HT_{1A} > 5-HT_{2A} for TFMPP and 5-HT_{1B} > 5-HT_{1A} > 5-HT_{2C} > 5-HT_{2A} for RU 24969 (Middlemiss and Tricklebank, 1992; Chopin *et al.*, 1994).

Behaviours induced by 5-HT receptor-related compounds have been largely attributed to activation of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptors or interactions between these receptors (Berendsen, 1995). The NAS, targeted in the present study, expresses various subtypes of 5-HT receptors, including 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3 and 5-HT_4 receptors. As there is no evidence for the expression of 5-HT_{1A}

and 5-HT_7 receptors in the NAS (Pazos and Palacios, 1985; Saudou and Hen, 1994; Martin and Humphrey, 1994; Peroutka, 1994; Boess and Martin, 1994; Pandey *et al.*, 1994), these receptors may be ruled out in relation to the present effects of TFMPP in this region. 5-HT_{1B} terminal autoreceptors or heteroreceptors may possibly mediate the present effects of TFMPP, because a decrease of 5-HT release is observed after local administration of TFMPP into the diencephalon (Auerbach *et al.*, 1991) and a reduction of rat brain 5-HT synthesis after systemic TFMPP is mediated by 5-HT_{1B} autoreceptors located on the serotonergic axon terminals (Hjorth *et al.*, 1995). 5-HT_{2A} receptors may be considered for the present effects because TFMPP has a somewhat higher affinity for these receptors than does RU 24969, as described above. In addition, it has been suggested that postsynaptic 5-HT_{2C} receptors may mediate locomotor hypoactivity induced by systemic injection of TFMPP (Lucki and Frazer, 1982; Kennett and Curzon, 1988). Systemic injections of RU 24969 increase locomotor activity (Green *et al.*, 1984), and this effect has been proposed to result from the activation of 5-HT_{1B} receptors (Pranzatelli *et al.*, 1987; Chopin *et al.*, 1994). These results reveal a differential profile of the effect of TFMPP and RU 24969 that needs to be examined more thoroughly in further studies.

The potential interaction between effects of stimulating different 5-HT receptor subtypes should be considered when attempting to interpret the differential effects of TFMPP and RU 24969 on VTA self-stimulation thresholds. Berendsen (1990 & 1995) proposed that functional interactions between 5-HT_{2A} and 5-HT_{2C} receptors may take place and that care is needed in drawing conclusions from functional measurements when compounds have more or less equal affinities for more than one 5-HT receptor. A

synergistic effect of stimulation of both 5-HT_{1A} and 5-HT_{1B} receptors on locomotor activity was recently described (O'Neill and Parameswaran, 1997). In addition, 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors may play an interactive role in the regulation of sexual behaviour (Wolf *et al.*, 1998). Because TFMPP and RU 24969 act at more than one 5-HT receptor subtype, the behavioural effects may be the result of an interaction between effects at different receptor subtypes within the NAS.

Amphetamine was injected into the shell of the NAS at the end of the present experiment in order to compare with the effects of 5-HT-related compounds such as TFMPP and RU 24969. Amphetamine increases DA release and blocks its uptake (Westerink, 1979). Therefore, it was expected that amphetamine would enhance the rewarding effects. There were, in fact, marked effects: decreased M50 and increased TRES, but no effect in RMAX. In contrast to the effects of TFMPP, these effects of amphetamine may be mediated by DA receptors in the shell of the NAS. It is well established that amphetamine induces an increase in extracelluar DA release in the shell of the NAS (Seiden and Sabol, 1993; Pierce and Kalivas, 1995; Pontieri *et al.*, 1995). These studies suggest that amphetamine enhanced the rewarding effects of the VTA stimulation through dopaminergic mechanisms in the shell of the NAS. Local administration of amphetamine decreases ICSS thresholds, showing differential effects between rostral (core) and caudal (shell) compartments of the NAS (Ranaldi and Beninger, 1994). These latter workers demonstrated that the shell of the NAS is much more sensitive to amphetamine treatment than the core of the NAS.

CONCLUSION

Based on the present studies of locomotor activity, it may be stated that the ascending 5-HT projections from the DRN and the MRN are differentially involved in the control of motor functions. The present results suggest an excitatory role of the DRN and an inhibitory role of the MRN, possibly because of the differential effects in the nigrostriatal and the mesolimbic pathways on locomotor activity. This differential effect may be mediated by brain 5-HT_{1A} receptors. A specific role for brain 5-HT_{1A} receptors was investigated by means of a 5-HT_{1A} receptor agonist, 8-OH-DPAT. These receptors are known to exist as somatodendritic autoreceptors and postsynaptic receptors. Therefore, local administration of 8-OH-DPAT into the 5-HT cell bodies may result in behavioural effects through the somatodendritic autoreceptors that differ from those produced by systemic administration of 8-OH-DPAT. The concept of interactive regulation of the dopaminergic system by 5-HT is supported by the present results.

Self-stimulation of the VTA of the rat brain can increase DA release in the NAS, which may be modulated by the stimulatory effects of 8-OH-DPAT injected into the DRN and the MRN. Therefore, there may be direct or indirect 5-HT inhibitory projections from both of these raphé nuclei. Local administration of 8-OH-DPAT into the DRN and the MRN may stimulate 5-HT_{1A} somatodendritic autoreceptors and reduce 5-HT release from terminals to attenuate the inhibitory effects of 5-HT in the NAS. Because 8-OH-DPAT may selectively inhibit 5-HT neurotransmission, the present results are interpreted in terms of interactions between 5-HT and DA. This interpretation is

consistent with proposals for an important modulatory role for 5-HT neurons from the DRN and the MRN in the regulation of limbic system activity.

The differential effects of TFMPP and RU 24969 on the VTA ICSS responses are attributed to the pharmacological actions of these drugs at more than one 5-HT receptor subtype. Because of problems of drug selectivity at receptors, it is difficult to determine the specific receptors involved in the mediation of behavioural changes. Only by combining data from various related compounds can a tentative conclusion be made. Involvement of 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptors is a possible explanation for reduced ICSS reward response by TFMPP but not by RU 24969. These effects may be mediated by a single receptor class or by interactions between effects at multiple receptor subtypes. Further studies using more selective drugs such as MDL 100,907 for 5-HT_{2A} receptor antagonism and mesulergine for 5-HT_{2C} receptor antagonism are required to distinguish which subtypes of 5-HT receptor are associated with the reduced reinforcement by intra-NAS TFMPP.

The data presented in this thesis confirm and extend the analysis of effects of 5-HT receptor-related compounds on motor activity and brain stimulation reward. The differential effects of DRN- and MRN-mediated effects on locomotor activity and reward indicate a selective and neuronal path-dependent role for 5-HT in the regulation of mesolimbic DA activity. The present results indicate a need for caution in interpreting the actions of systemically administered 8-OH-DPAT in terms of actions at somatodendritic receptors. Further research with more selective compounds will be necessary to elucidate

the exact role of 5-HT in regulating the activity of the reward-relevant neurons in the NAS.

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