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THE EFFECTS OF PSYCHOSTIMULANT DRUGS ON BRAIN DOPAMINERGIC AND SEROTONERGIC NEURONAL SYSTEMS: THE ROLE OF 5-HT_3 RECEPTORS

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ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Science of the University of Helsinki, for public criticism in Auditorium 1041 at Viikki Biocentre on August 16, 2002, at 12 o'clock noon.

Helsinki 2002

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Publications of the National Public Health Institute
KTL A12/2002

ISBN 951-740-287-2 (printed version)
ISSN 0359-3584

ISBN 951-740-288-0 (electronic version, pdf; http://ethesis.helsinki.fi)
ISSN 1458-6290

Yliopistopaino

To my family

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ABSTRACT

The psychostimulant drugs of abuse, amphetamine and cocaine, are believed to exert their stimulative and reinforcing effects by increasing the synaptic concentrations of dopamine and 5-hydroxytryptamine (5-HT, serotonin) as well as norepinephrine. In case of amphetamine, minor modifications in its molecular and/or spatial structure lead to a series of compounds whose subjective effects form a continuum which extends from stimulant to hallucinogenic.

To date, there is no consensus on the mechanism of 5-HT-dopamine interactions participating in the effects of psychostimulant drugs. One candidate for mediating such interactions is the 5-HT_3 receptor, which has been reported to modulate the central dopaminergic activity.

This study had two main aims: the first of which was to explore the effects of stimulative and hallucinogenic amphetamine derivatives on extracellular concentrations of dopamine and 5-HT in rat nucleus accumbens, and to assess the implication of spatial structure in the neurochemical and behavioral effects of 4-methylaminorex, an amphetamine-related compound which exists as four stereoisomers. The second main goal was to evaluate the role of 5-HT_3 receptors in the dopaminergic and reinforcement-related effects of cocaine, mazindol, amphetamine and methylphenidate, and to explore the role of released 5-HT in these interactions. The extracellular concentrations of the monoamine transmitters were monitored using *in vivo* microdialysis technique, and the reinforcement-related effects using conditioned place preference test.

The results showed that the hallucinogenic amphetamine derivative 4-methyl-2,5-dimethoxyamphetamine (DOM) failed to increase extracellular concentrations of either of the neurotransmitters studied. This indicates that extracellular levels of 5-HT do not reflect hallucinogenic activity, and that DOM lacks the dopaminergic effects of stimulative amphetamines. 3,4-Methyledioxyamphetamine (MDA) and its N-methylated analogue 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') strongly elevated extracellular levels of both 5-HT and dopamine, indicating that these compounds act via serotonergic mechanisms other than those of the hallucinogenic substances, but they may share dopaminergic mechanisms of stimulative amphetamines. The study of the four stereoisomers of 4-methylaminorex showed that the ability to elevate extracellular dopamine- and 5-HT-levels, and to induce behavioral changes, is associated with the S-configuration, which further confirms the established structure-activity relationships of amphetamine-related compounds. The behavioral effects of the three most potent isomers contained both dopaminergic and serotonergic components, which is in concert with their neurochemical effects. The changes in potency between the isomers are pharmacodynamic rather than pharmacokinetic, as evidenced by the observation that the dialysate concentrations of the least potent isomer, *trans*-4R,5R, were higher than those of the *cis*-isomers.

The experiments with the 5-HT_3 receptor antagonist showed that the 5-HT_3 receptors are involved in dopaminergic and the reinforcement-related effects of cocaine, mazindol and amphetamine. Instead, blockade of the 5-HT_3 receptors failed to modulate the effects of methylphenidate. Because cocaine, mazindol and amphetamine, but not methylphenidate, elevate extracellular levels of 5-HT in addition to dopamine, it is suggested that released 5-HT plays an essential role in 5-HT_3 receptor mediated regulation of dopamine release.

ABBREVIATIONS

2C-B	2-bromo-2,5-dimethoxyphenylethylamine
4-MTA	4-methylthioamphetamine
5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine, serotonin
6-OHDA	6-hydroxydopamine
7-OH-DPAT	7-hydroxydipropylaminotetralin
ADHD	Attention deficit hyperactivity disorder
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
COMT	Catechol-O-methyltransferase
DAT	Dopamine transporter
DAT-KO	Dopamine transporter knock-out
DOB	4-bromo-2,5-dimethoxyamphetamine
DOI	4-iodo-2,5-dimethoxyamphetamine
DOM	4-methyl-2,5-dimethoxyamphetamine
DOPAC	3,4-dihydroxyphenylacetic acid
ED_{50}	Median effective dose
EDTA	Ethylenediamine tetraacetic acid
GABA	γ-Aminobutyric acid
GBR 12909	1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-[3-phenylpropyl]piperazine
GC/MS	Gas chromatograph/mass spectrometer
HPLC	High-performance liquid chromatography
HVA	Homovanillic acid
i.p.	Intraperitoneal
L-DOPA	L-3,4-dihydroxyphenylalanine
LSD	Lysergic acid diethylamide
MAO	Monoamine oxidase
MDA	3,4-methylenedioxyamphetamine
MDEA	3,4-methylenedioxyethylamphetamine
MDL 72222	3-tropanyl-3,5-dichlorobenzoate
MDMA	3,4-methylenedioxymethamphetamine
PCP	Phencyclidine
s.c.	Subcutaneous
SCH 23390	7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine
U99194A	5,6-dimethoxy-2-(di-n-propylamino)indan
VMAT	Vesicular monoamine transporter

LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following publications, herein referred to by their Roman numerals (I-IV).

I Kankaanpää A, Meririnne E, Lillsunde P & Seppälä T (1998) The acute effects of amphetamine derivatives on extracellular serotonin and dopamine levels in rat nucleus accumbens. Pharmacol Biochem Behav 59: 1003-1009.[a]

II Kankaanpää A, Ellermaa S, Meririnne E, Hirsjärvi P & Seppälä T (2002) Acute neurochemical and behavioral effects of the stereoisomers of 4-methylaminorex in relation to brain drug concentrations. J Pharmacol Exp Ther 300: 450-459.[b]

III Kankaanpää A, Lillsunde P, Ruotsalainen M, Ahtee L & Seppälä T (1996) 5-HT_3 receptor antagonist MDL 72222 dose-dependently attenuates cocaine- and amphetamine-induced elevations of extracellular dopamine in the nucleus accumbens and the dorsal striatum. Pharmacol Toxicol 78: 317-321.[c]

IV Kankaanpää A, Meririnne E & Seppälä T (2002) 5-HT_3 receptor antagonist MDL 72222 attenuates cocaine- and mazindol-, but not methylphenidate-induced neurochemical and behavioral effects in the rat. Psychopharmacology 159: 341-350.[d]

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1. INTRODUCTION

Psychostimulants are compounds that strongly impact on behavior. Symptoms of psychostimulant intoxication in humans include: euphoria, agitation and raised self-respect, and with higher doses also restlessness, paranoia, panic reactions, confusion and even paranoid psychosis (DSM-IV, 1997). The rush following intake of the most renowned psychostimulant drugs, cocaine and amphetamine, is described by addicts as an euphoric state which resembles sexual orgasm (Fischman, 1989). In addition to traditional drugs of abuse which are classified in Schedule I of Controlled Substances or its equivalent, there is an increasing variety of so-called designer drugs in the illicit market. Designer drugs are compounds that often resemble traditional drugs in terms of efficacy and molecular structure. However, their distribution, possession, or use is outside of existing legal controls. Designer drugs are made by modifying the molecular structure of amphetamines as well as narcotic substances like fentanyl.

Drug addiction is a disease that is characterized by compulsive drug use, augmentation of the drug dose due to tolerance, difficulties in withdrawal, and weakening of physical and mental health. Typically psychostimulants produce strong psychological dependence, however cessation of their abuse does not induce physical withdrawal signs and symptoms characteristic to opiate withdrawal. The state of psychostimulant withdrawal in humans is characterized by symptoms such as fearfulness, irritability, sleeplessness or hypersomnia and depression, which last from several hours to four days (DSM-IV, 1997).

Experimental animals learn to self-administer intracranially, intravenously and orally drugs that are abused by humans. Drug self-administration behavior follows many similar rules with behavior reinforced by naturally rewarding functions such as eating, drinking, and sex (cf. Koob, 1992). Acute administration of almost all of the reinforcing drugs elevate extracellular dopamine levels in nucleus accumbens, leading to the suggestion that addictive properties of these substances result from the activation of brain mesocorticolimbic dopaminergic network, an integral part of which is the nucleus accumbens (cf. Fibiger & Phillips, 1979). Psychostimulants cocaine and amphetamine, which acutely affect primarily brain dopamine, also have the strongest potential for abuse.

In addition to drug dependence, repeated exposure to amphetamine derivatives is shown to cause long-lasting changes indicative of neurotoxicity in brain function and morphology of the neurons. An example is 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy'), a popular recreational drug at rave parties. These changes have been evidenced in the brains of humans with a long history of MDMA abuse (McCann et al., 1998), in addition to animal brain experiments (cf. Green et al., 1995).

2. REVIEW OF THE LITERATURE

2.1. Brain dopaminergic systems

In the late 1950's Arvid Carlsson (1959, cf. 1987) showed that dopamine acts as an independent neurotransmitter in the brain, and is concentrated in basal ganglia, which are involved in the control of motor functions. In addition, it was found that reserpine-induced depletion of monoamine transmitters from neuronal storage vesicles causes animals to lose their ability to perform spontaneous movements, resembling symptoms of Parkinson's disease in humans. Treatment with the dopamine precursor L-3,4-dihydroxyphenylalanine (L-DOPA) reversed these effects. Carlsson was awarded the 2000 Nobel Prize in Physiology or Medicine for his dopamine studies (Nobelförsamlingen, 2000).

2.1.1. Synthesis, storage, release and metabolism of dopamine

Catecholamine neurotransmitters are synthesized from the dietary amino acid L-tyrosine (cf. Cooper et al., 1996). The biosynthesis of dopamine is presented in Figure 2.1. The first reaction, catalyzed by tyrosine hydroxylase, is the rate-limiting step. Factors that acutely regulate the activity of tyrosine hydroxylase include negative feedback by the end-products dopamine, norepinephrine and epinephrine, as well as activity of the neuron and the phosphorylation rate of the protein itself (cf. Zigmond et al., 1989).

Synthesized dopamine is packed into storage vesicles (Figure 2.2) by the proton-pump powered vesicular monoamine transporter (VMAT). After being pumped into the vesicles, dopamine molecules become protonated in an acidic environment, and are trapped inside the vesicles. Action potential or other depolarizing stimulus induces exocytotic release of vesicular dopamine by a Ca^{2+} ion dependent mechanism into the synaptic cleft (Raiteri et al., 1979; Arbuthnott et al., 1990). Under certain circumstances dopamine transporter, which normally transports released neurotransmitter molecules back to the nerve ending, can function in reverse direction and release cytoplasmic dopamine in a Ca^{2+} independent mechanism (Raiteri et al., 1979; Arbuthnott et al., 1990). Catecholamine-releasing drugs

typically act by such a mechanism.

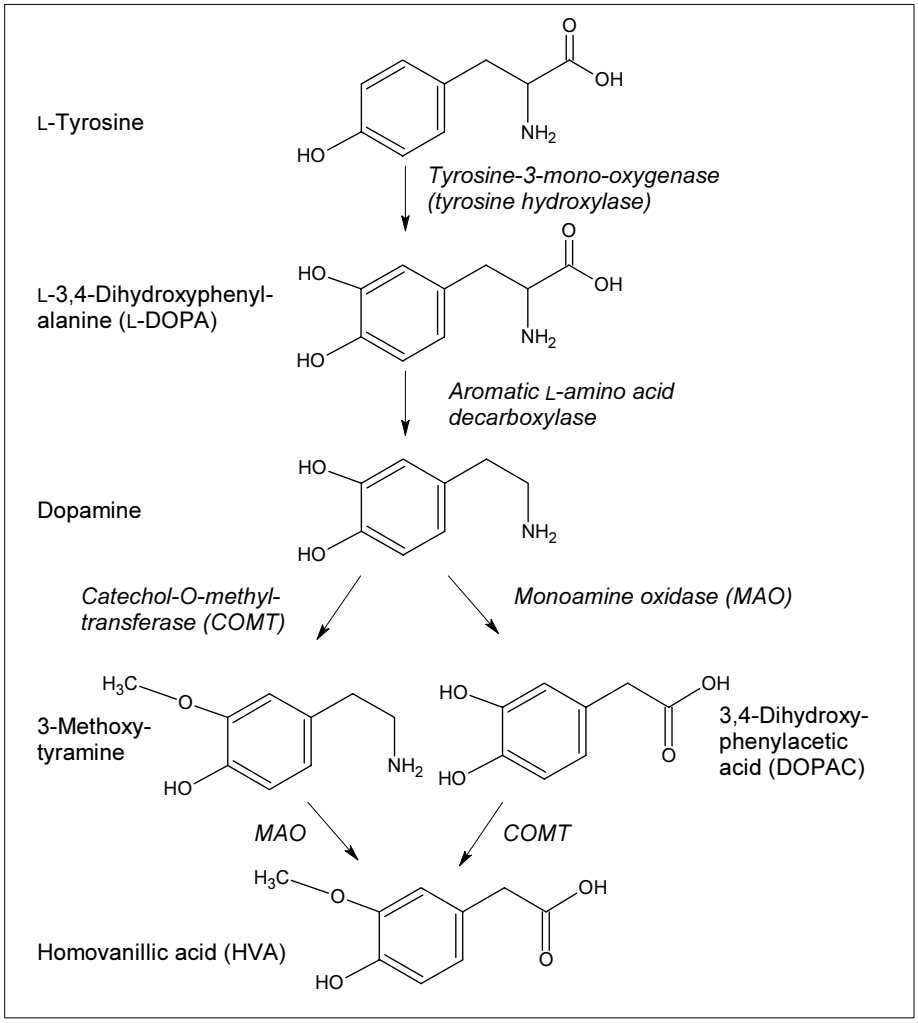

Figure 2.1. Simplified presentation of dopamine synthesis and metabolism.

Synaptic action of catecholamines is primarily terminated by efficient Na^+ and K^+ dependent transporter-mediated reuptake into nerve-endings (uptake 1). There is, however, also some uptake into surrounding tissue (uptake 2). Although reuptake terminates the synaptic action of the transmitters, there is a degrading machinery to prevent their accumulation in the nerve cells. The most important of the catecholamine metabolizing enzymes are catechol-*O*-methyl transferase (COMT; cf. Männistö & Kaakkola, 1999) and monoamine oxidase (MAO; Figures 2.1 and 2.2; cf. Kopin, 1985). MAO exists as two isoforms, MAO-A and MAO-B, of which the former deaminates norepinephrine and 5-HT, and the latter specifically phenylethylamine. Both isoenzymes metabolize dopamine. MAO is located in mitochondria of nerve endings and glial cells. COMT resides intracellularly in glial cells and postsynaptic neurons (cf. Männistö & Kaakkola, 1999).

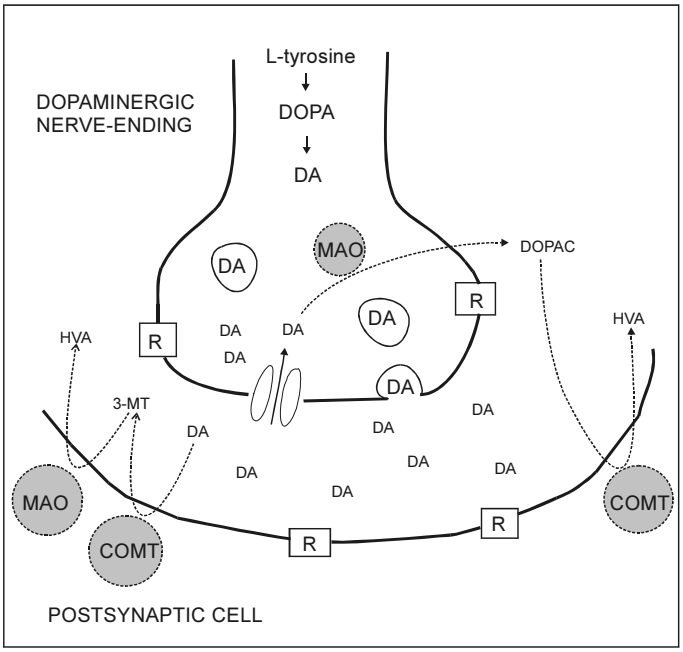

Figure 2.2. Dopaminergic nerve terminal and synapse. (DA) dopamine; (COMT) catechol-O-methyltransferase; (DOPAC) 3,4-dihydroxyphenylacetic acid; (HVA) homovanillic acid; (MAO) monoamine oxidase; (R) dopamine receptor; (3-MT) 3-methoxytyramine.

Dopamine metabolism may follow either of two routes, which is determined by whether the first step is catalyzed by MAO or COMT (Figures 2.1 and 2.2). Westerink (1985) has estimated that in rats MAO deaminates approximately 90% of dopamine in the striatum. This reaction produces 3,4-dihydroxyphenylacetaldehyde, which is immediately oxidized to 3,4-dihydroxyphenylacetic acid (DOPAC). Roughly 40% of DOPAC is removed from the brain as such, and the remaining 60% is methylated to homovanillic acid (HVA). Approximately 10% of total dopamine metabolism follows an extraneuronal route, in which COMT methylates dopamine to 3-methoxytyramine that is further converted to HVA via reactions catalyzed by MAO and aldehyde dehydrogenase.

2.1.2. Ascending dopaminergic pathways

The cell bodies of dopaminergic neurons projecting to forebrain are located in the brain stem, more specifically in substantia nigra pars compacta (A9), ventral tegmental area (A10), and retrorubral area (A8). Axons of A9- and A8- nigrostriatal dopamine neurons ascend along the medial forebrain bundle to innervate the nucleus caudatus, putamen and globus pallidus (Figure 2.3; Dahlström & Fuxe, 1964; cf. Lindvall, 1979; cf. Björklund & Lindvall, 1984; cf. Fuxe et al., 1985). In the striatum these nerve cells make synaptic contacts with medium spiny

neurons, from which striatum's efferents begin. Dopamine also inhibits large spineless cholinergic interneurons in the same area (Stoof et al., 1992). The nigrostriatal dopaminergic pathway participates in control of motor behavior.

Mesolimbic and mesocorticolimbic dopaminergic pathways originate mainly in the ventral tegmental area (A10), from where they project to: nucleus accumbens, olfactory tubercle, medial caudate-putamen, septum, amygdala, hippocampus, limbic cortex and other brain areas (Dahlström & Fuxe, 1964; cf. Lindvall, 1979; cf. Björklund & Lindvall, 1984; cf. Fuxe et al., 1985). In addition to controlling motor behavior, they participate in regulation of motivation, emotions, learning and approach behavior as well as higher cognitive functions (cf. Le Moal & Simon, 1991; cf. Schultz, 1998). Mesolimbic pathways are often considered a part of the mesocorticolimbic pathway.

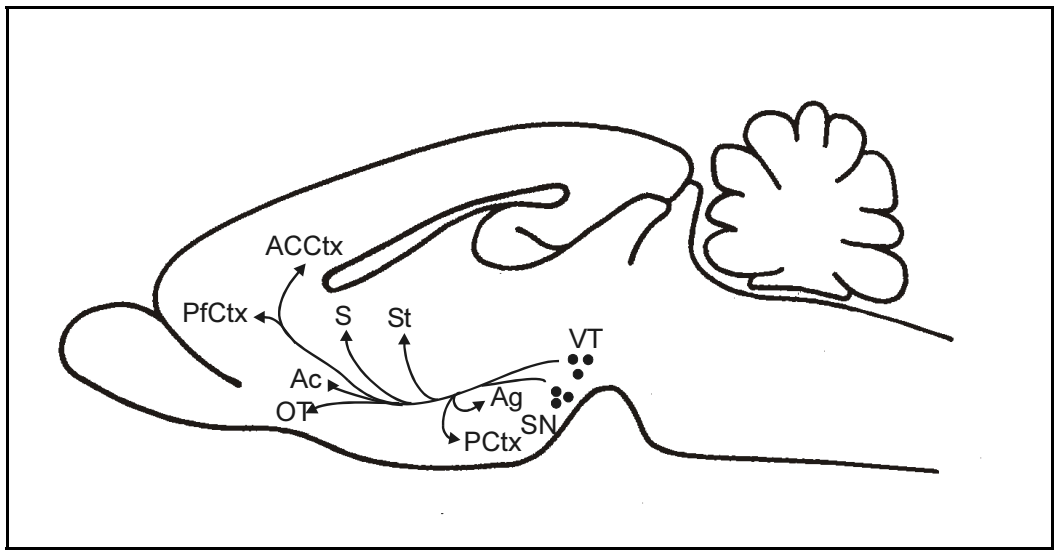

Figure 2.3. Ascending dopaminergic pathways in the rat brain. (Ac) nucleus accumbens; (ACCtx) anterior cingulate cortex; (Ag) amygdala; (OT) olfactory tubercle; (PfCtx) prefrontal cortex; (PCtx) piriform cortex; (S) septum; (SN) substantia nigra, A9; (St) striatum; (VT) ventral tegmental area, A10.

2.1.3. Dopamine receptors

Dopamine released into the synaptic cleft binds to receptors that belong in two families: D_1 and D_2 dopamine receptor families. The D_1 family consists of D_1 and D_5 receptors, D_2, D_3,

and D_4 receptors belong to the D_2 family (cf. Jackson & Westlund-Danielsson, 1994; cf. Sokoloff & Schwartz, 1995). The D_2 receptor exists in two isoforms, namely long and short, therefore there are a total of six different dopamine receptors. They are all G-protein coupled, seven times cell membrane spanning receptors. The receptors of the D_1 and D_2 families have opposite effects at the cellular level: activation of the former results in stimulation of the enzyme adenylate cyclase, while activation of the latter inhibits adenylate cyclase.

Dopamine receptors can be located either post- or presynaptically. In striatum, for example, postsynaptic dopamine receptors regulate neuronal feedback pathways, which enable striatal neurons to communicate with dopaminergic cell bodies in substantia nigra. In general, increasing stimulation of postsynaptic receptors decreases nigrostriatal dopaminergic activity. Stimulation of presynaptic somatodendritic receptors (autoreceptors) decreases firing rate of dopaminergic neurons, while stimulation of autoreceptors located in nerve endings inhibits dopamine synthesis and release (cf. Cooper et al., 1996). Autoreceptors belong to the D_2 family.

2.2. Brain serotonergic systems

In the 1930's Vittorio Erspamer and his co-workers isolated a gut stimulating substance they named enteramine, and in 1948 Irvine Page and his associates found a vasoconstrictive substance in blood serum they named serotonin. Later, they were found to be the same substance, 5-hydroxytryptamine (5-HT; cf. Whitaker-Azmitia, 1999).

Although only 1-2% of 5-HT in the human body is found in the central nervous system, it acts there as an important neurotransmitter. For example 5-HT reduces food intake apparently by affecting mechanisms of satiety, which enables serotonergic agonists such as fenfluramine to be used as anorectics. In addition, 5-HT probably controls impulsivity or similar psychological traits, since abnormally low serotonergic activity seems to be associated with suicidal or pathologically aggressive behavior. 5-HT has also been suggested to play a role in states such as obsessive-compulsive disorder (cf. Jacobs & Azmitia, 1992).

2.2.1. Synthesis, storage, release and metabolism of 5-HT

The biosynthesis of 5-HT is presented in Figure 2.4. Hydroxylation of dietary L-tryptophan is the rate-limiting step of 5-HT synthesis (cf. Boadle-Biber, 1993).

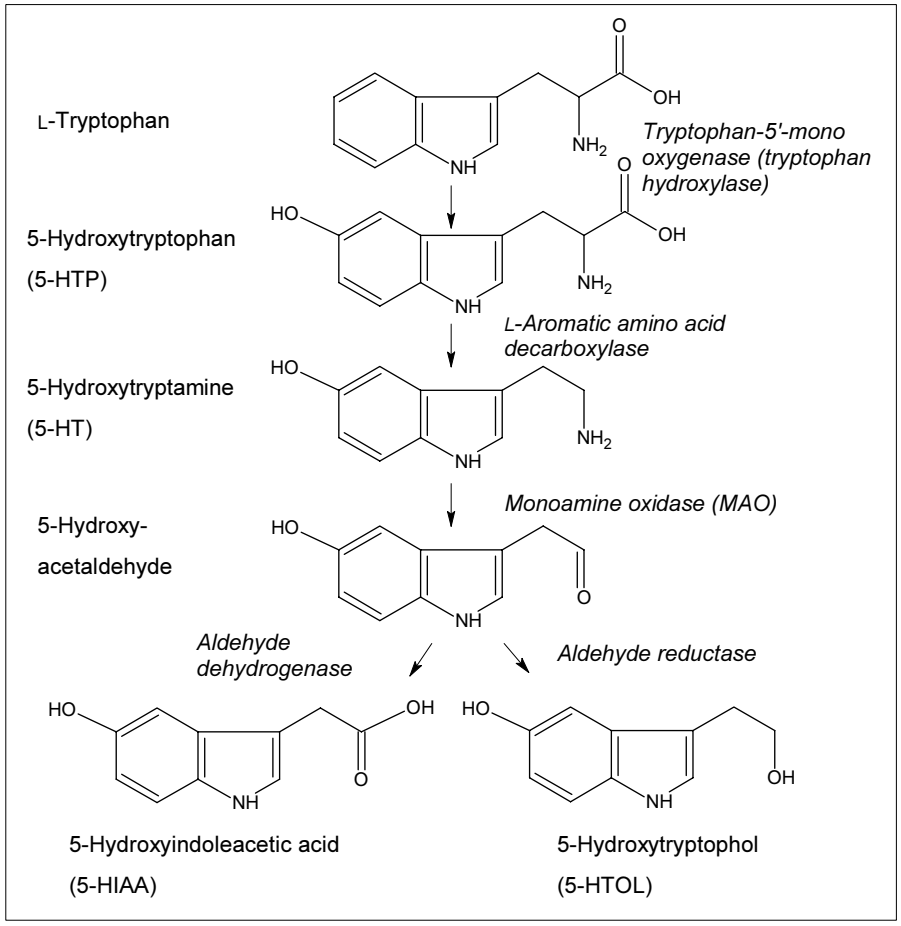

Figure 2.4.
5-HT synthesis and metabolism.

In nerve-endings 5-HT is stored in storage vesicles and released by means of exocytosis into the synaptic cleft, similarly with dopamine (cf. Sanders-Bush & Martin, 1982). Synaptic action of 5-HT is terminated by reuptake into the nerve-ending, in a process analogous to that of dopamine (Graham & Langer, 1992). The transporter proteins which carry out the reuptake processes, however, are not identical, but have different affinities to various monoamines, and are named correspondingly. After reuptake to a nerve-ending 5-HT is recycled or metabolized (Figure 2.4).

2.2.2. Brain serotonergic pathways

Dahlström and Fuxe (1964) described nine 5-HT-containing cell groups (B1-B9), which exist in midbrain and brainstem areas of raphe nuclei and reticular area (Figure 2.5). They can be divided into caudal and rostral systems (cf. Törk, 1990). The axons of caudal system brainstem neurons (cell groups B1-B4 in nucleus raphe pallidus, n. raphe obscurus, and n. raphe magnus) descend down to spinal cord via multiple routes, and mediate various roles of 5-HT in sensory, motor and autonomic functions.

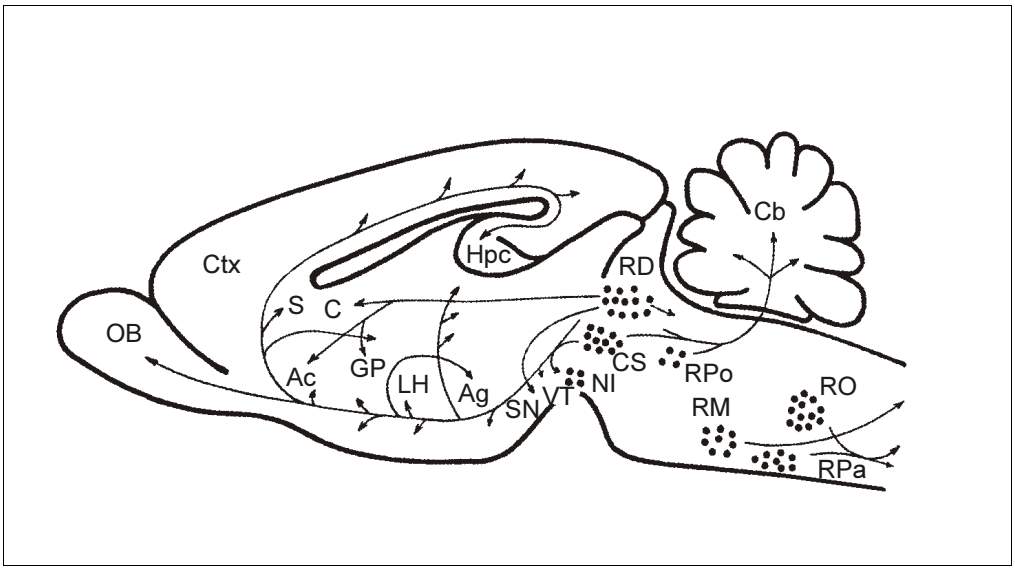

Figure 2.5. Serotonergic pathways in the rat brain. (Ac) nucleus accumbens; (Ag) amygdala; (C) nucleus caudatus; (Cb) cerebellum; (CS) nucleus centralis superior, B8; (Ctx) cortex; (GP) globus pallidus; (Hpc) hippocampus; (LH) lateral hypothalamus; (NI) nucleus interpeduncularis; (OB) olfactory bulb; (RD) nucleus raphe dorsalis, B7; (RM) n. raphe magnus, B3; (RO) n. raphe obscurus, B2; (RPa) nucleus raphe pallidus, B1; (Rpo) nucleus raphe pontis; (S) septum; (SN) substantia nigra; (VT) ventral tegmental area.

Rostral 5-HT system consists of midbrain neurons (cell groups B5-B9 in n. raphe dorsalis and n. raphe medianus), from which arises two ascending projections, the ventral and dorsal pathways (cf. Törk, 1990). The ventral ascending pathways originate mainly from cell groups B6-B8 and projects to many structures in the diencephalon, basal ganglia, limbic system and cerebral cortex. These nerve fibers traverse the midbrain where they innervate substantia nigra, ventral tegmental area and nucleus interpeduncularis. The dorsal and ventral striatum (caudate-putamen and nucleus accumbens), amygdala, hippocampus, septum,

olfactory tubercle, olfactory bulb and neocortex are also innervated by other serotonergic fibers. The dorsal ascending 5-HT pathways mainly originate in cell groups B7 and B8, which project, for example, to mesencephalic gray. Most of the axons of the dorsal pathways ultimately merge with the medial forebrain bundle, where they join the ventral pathways to form a combined ascending system.

2.2.3. 5-HT receptors

5-HT mediates neuron function via 5-HT receptors located pre- and postsynaptically. First clues of the existence of multiple 5-HT receptor subtypes came from a study of Gaddum and Picarelli (1957), which showed that morphine and dibenzyline (phenoxybenzamine) given separately decreased the contractile effect of 5-HT on guinea pig ileum, while given together they completely prevented it. This led the researchers to a conclusion that 5-HT receptors exist in two subtypes, which they named after morphine and dibenzyline as M and D receptors.

To date, at least fourteen different 5-HT receptors have been identified in seven receptor families (5-HT_1 - 5-HT_7; cf. Cooper et al., 1996; cf. Hoyer et al., 1994; cf. 2001 Receptor & Ion Channel Nomenclature Supplement). The 5-HT_1 family consists of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D},5-HT_{1E} and 5-HT_{1F} receptors. The members of 5-HT_2 receptor family are 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}, and the 5-HT_5 family consists of 5-HT_{5A} and 5-HT_{5B} receptors. At present there are no known subtypes of 5-HT_3, 5-HT_4 and 5-HT_6 receptors. All 5-HT receptors, except the ion-channel coupled 5-HT_3 receptor, are G-protein coupled receptors and mediate their effects via adenylate cyclase or phospholipase C. The receptors of the 5-HT_1 family mediate inhibitory effects of 5-HT by being negatively coupled to adenylate cyclase, whereas, the receptors of the 5-HT_2 family mediate excitatory effects of 5-HT via activation of phospholipase C. 5-HT_4, 5-HT_6 and 5-HT_7 receptors are positively coupled to adenylate cyclase. The second messenger system utilized by 5-HT_5 receptors is not known. At least 5-HT_{1A} and 5-HT_{1B} receptors can be located presynaptically where they function as autoreceptors to take part in regulation of serotonergic nerve cell firing and release of 5-HT. There is evidence that 5-HT receptors of different families can interact in mediating the numerous behavioral effects of 5-HT (Glennon et al., 1991).

5-HT_{1A} receptor is abundant in hippocampus, lateral septum, medial amygdala as well as frontal and entorhinal cortex, where they are thought to be located postsynaptically. These receptors have also been found in dorsal and medial raphe-nuclei, which suits their presumed role as somatodendritic autoreceptors (Hamon et al., 1990).

In the 5-HT_2 receptor family the 5-HT_{2A} receptor represents the classical 5-HT_2 receptor, which corresponds to the D receptor of Gaddum and Picarelli. The 5-HT_{2C} receptor was formerly classified as the 5-HT_{1C} receptor, while the 5-HT_{2B} receptor is a newcomer to this family (Baxter et al., 1995). Non-specific agonists of this receptor family are, for example, the 5-HT analog α-methyl-5-HT and the hallucinogenic phenylalkylamines DOM, DOI and DOB (4-methyl-2,5-dimethoxyamphetamine and its iodinated and brominated analogs; Glennon et al., 1984; cf. Glennon, 1990). In the rat brain 5-HT_2 receptors are abundant especially in the claustrum, nucleus accumbens, olfactory tubercle, piriform cortex, and in layers one and two of the neocortex (cf. Hoyer et al., 1994).

The only ion-channel coupled 5-HT receptor, the 5-HT_3 receptor, resembles both structurally and functionally the cholinergic nicotine receptor (Peters et al., 1992). 5-HT_3 receptor activation opens a cation channel after which the nerve cell is rapidly depolarized. Although the 5-HT_3 receptor, the M receptor of Gaddum and Picarelli, was identified at an early stage, knowledge about its functions has increased only since the development of selective agonists and antagonists in the 1980's (cf. Middlemiss & Tricklebank, 1992). 5-HT_3 receptors are widely distributed both in the periphery and the central nervous system, where the highest receptor densities have been found in the area postrema, entorhinal cortex, amygdala and in certain brainstem nuclei (Kilpatrick et al., 1987; cf. Hoyer et al., 1994). 5-HT_3 receptors have been found active in a variety of test systems including hyperlocomotion induced by dopamine agonists, many anxiety models, nociception, food intake, effects of drugs and withdrawal symptoms induced by cessation of their use, as well as nausea and vomiting induced by drugs or radiation therapy (cf. Barnes et al., 1992; cf. Grant, 1995).

2.3. Psychostimulants, dopamine and 5-HT

2.3.1. Cocaine

The history of cocaine use is thoroughly described in the literature (cf. Angrist & Sudilowsky, 1978). For thousands of years primitive peoples have been familiar with the stimulative qualities of coca leaves. Chewing coca leaves was a widely distributed habit in western and north-western areas of South America even before the bloom of the Inca culture. In 1860 Albert Niemann isolated an alkaloid from coca leaves, and named it cocaine. Chemically, cocaine is a benzoylecgonine methylester, which is an optically active [l(-)] crystalline base (Figure 2.6). After experimenting with the substance on himself, Sigmund Freud published a series of writings praising its effects in the 1880's, whilst his friend and colleague Karl Koller discovered its usefulness as a local anaesthetic. In 1888 a coca extract containing soft drink named Coca-Cola® appeared on the market, and soon after, it became one of the most popular soft drinks. In the 1890's the disadvantages of cocaine use - development of dependence and toxic effects - were noticed. In the year 1906 cocaine use became restricted in the United States, and since 1914 possession, selling and delivery of coca-products has been illegal. Today cocaine is the most used psychostimulant drug of abuse.

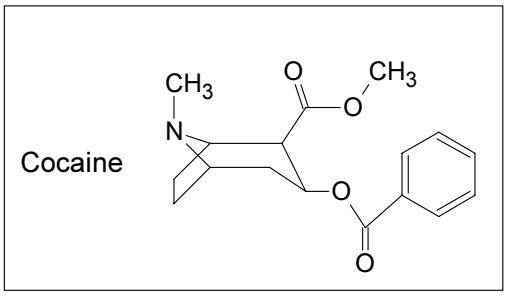

Figure 2.6. Molecular structure of cocaine.

In rats the behaviors induced by cocaine include those typical to psychostimulants: increased locomotor activity, and with higher doses of the drug stereotyped behavior, that both have been shown to be dependent on the mesocorticolimbic dopaminergic network (Kelly & Iversen, 1976). Cocaine increases synaptic concentrations of dopamine, 5-HT and norepinephrine by blocking their reuptake into nerve endings, without inducing transmitter

release (Heikkila et al., 1975; Koe, 1976). As measured by the *in vivo* microdialysis technique, cocaine strongly elevates extracellular levels of dopamine, norepinephrine and 5-HT at least in the nucleus accumbens, striatum, prefrontal cortex and ventral tegmental area, without any marked effect on neurotransmitter metabolites (Carboni et al., 1989a; Hurd & Ungerstedt, 1989a, 1989b; Moghaddam & Bunney, 1989; Nomikos et al., 1990; Kuczenski et al., 1991; Reith et al., 1997; Andrews & Lucki, 2001). Consistent with cocaine's mechanism of action based on neurotransmitter reuptake inhibition, the effect of cocaine on extracellular dopamine levels is prevented by treatment with γ-butyrolactone and tetrodotoxin, which indicates that action potential is necessary for elevation of dopamine concentration (Carboni et al., 1989a; Nomikos et al.,1990).

The electrophysiological effects of cocaine include inhibition of: dopaminergic nerve cell firing in substantia nigra and ventral tegmental area (Einhorn et al., 1988; Lacey et al., 1990), noradrenergic in locus caeruleus (Pitts & Marwah, 1987) and serotonergic in dorsal raphe (Cunningham & Lakoski, 1990). These effects are mediated, at least partly, via inhibitory autoreceptors which are activated by increased synaptic transmitter concentrations caused by inhibition of transmitter reuptake. Negative feedback from target neurons (for example in the nucleus accumbens) is another mechanism by which cocaine can inhibit monoaminergic nerve cell firing (Einhorn et al., 1988).

2.3.2. Amphetamine and its derivatives

Amphetamine is a synthetic compound that was developed in the late 1880's. In the 1930's its central nervous system stimulating effect was discovered, after which it became a popular stimulant drug among university students in the United States. The possible medical applications of amphetamine were intensively studied from the late 1930's - by 1946 it had been tested as a treatment for almost forty different diseases. After reports describing toxic effects and amphetamine psychosis, amphetamine preparations became available only on prescription in 1939. For military purposes amphetamine was first used in the Spanish Civil War and after that more widely in the Second World War (cf. Angrist & Sudilowsky, 1978).

In the 1960's, derivatives of amphetamine, such as 3,4-methylenedioxyamphetamine

(MDA, 'love'; Figure 2.7) and 4-methyl-2,5-dimethoxyamphetamine (DOM, 'STP'), appeared in the clandestine market. Their effects were markedly different from those of the parent compound (cf. Haislip, 1989). Many of these compounds were initially developed by the pharmaceutical industry, but have never become available on the licit market. The N-methyl- and N-ethyl-derivatives of MDA, MDMA and 3,4-methylenedioxyethylamphetamine (MDEA, 'eve'), appeared in the clandestine market since the early 1980's, during the second wave of illicit drug manufacturing (cf. Haislip, 1989). These are usually classified designer drugs; however in this study such a distinction is not made. The similarities of these drugs with their parent compound MDA were the basis for this alignment.

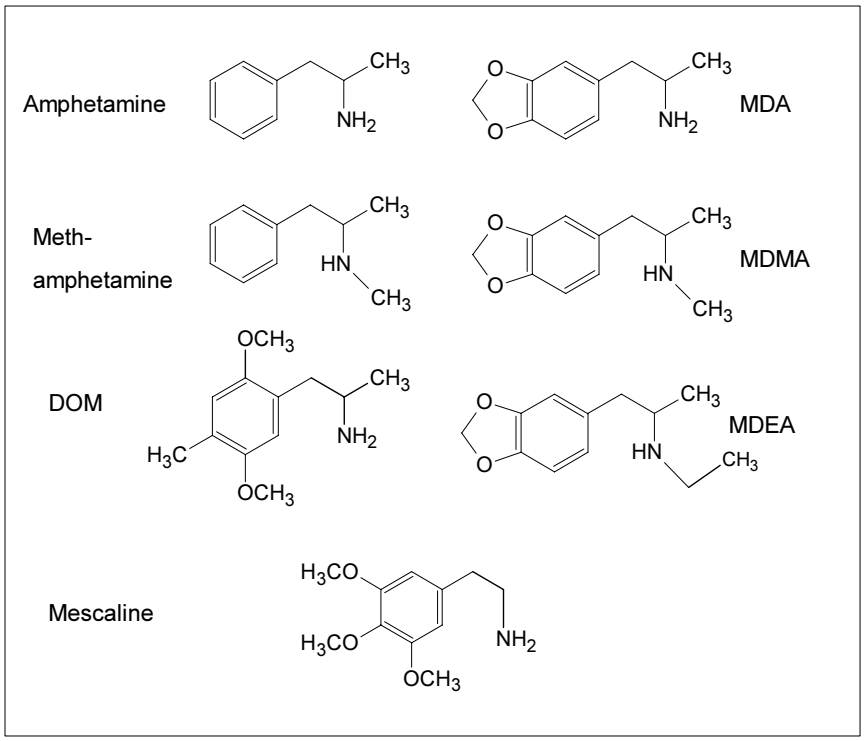

Figure 2.7. Molecular structures of selected amphetamine derivatives and mescaline.

The spectrum of amphetamine derivatives' subjective effects extends from the stimulant amphetamine to the hallucinogenic DOM. The chemical structure of DOM resembles that of mescaline (Figure 2.7), and it has been shown to produce hallucinations similar to those induced by LSD and mescaline at total doses of 5-10 mg (Snyder et al., 1967). In rats the discriminative stimulus produced by DOM resembles that of mescaline, but not that of amphetamine (Silverman & Ho, 1980), while subjective effects of MDA in humans, and its discriminative effects in experimental animals, resemble both those of LSD and psychostimulants (Naranjo et al., 1967; Glennon et al., 1982; Glennon & Young, 1984a,

1984b). N-methylation of racemic MDA to MDMA decreases or abolishes its DOM-like effect, but increases its amphetamine-like effect (Glennon et al., 1982; Glennon & Young, 1984a, 1984b), whereas N-ethylation to MDEA decreases both of these effects (Glennon, 1989; Glennon & Misenheimer, 1989a; cf. Glennon, 1991). MDMA also has unique subjective effects, due to which it has been proposed to form together with some other amphetamines a new group of drugs, named 'entactogens' (Nichols, 1986; Nichols & Oberlender, 1990). Methamphetamine is a stimulant drug, the effects of which resemble those of amphetamine.

The structure-activity relationships of amphetamines are further complicated by optical isomerism, a characteristic of chiral molecules. The central effects of the amphetamine enantiomers differ at least quantitatively: S(+)-amphetamine (*d*-amphetamine, dextroamphetamine) is on average three times more efficacious than R(-)-amphetamine (*l*-amphetamine, levoamphetamine) (Taylor & Snyder, 1971; Moore, 1978). Similarly with amphetamine itself, its derivatives also exist as optical isomers. The psychotomimetic effects of DOM are associated with the R(-)-, or *l*-isomer (Shulgin, 1978). In the case of MDA, the effects of the enantiomers differ qualitatively: the effects of the S(+)-isomer resemble those of amphetamine (Shulgin, 1978), while the effects of R(-)-isomer are associated with LSD (Glennon & Young, 1984a, 1984b). Drugs of abuse found in the clandestine market are almost without exception a mixture of the enantiomers, or a racemic mixture, whereas pure enantiomers are also used in scientific experiments. In this review of literature prefixes are used to indicate enantiomers, while the name of the substance without prefix indicates racemic mixture.

In rats acute behavioral effects of amphetamine include strong locomotor activation, and with higher dose of the drug, stereotyped activity characterized by intense sniffing and oral stereotypies such as gnawing and licking, which have been shown to be of dopaminergic origin (Randrup & Munkvad, 1970; Taylor & Snyder, 1971; Creese & Iversen, 1973, 1974). Amphetamine increases synaptic concentrations of dopamine by multiple mechanisms; by drifting into the nerve endings and displacing dopamine (and norepinephrine) from storage vesicles into the cytoplasmic pool from where it is released via reversal of the transporter, by inhibiting dopamine (and norepinephrine) reuptake and by inhibiting MAO (Blaschko, 1952;

Ferris et al., 1972; Fuller, 1972; Heikkila et al., 1975; Taylor & Ho, 1978; Raiteri et al., 1979; cf. Garattini & Samanin, 1981; Liang & Rutledge, 1982). Amphetamine releases dopamine from reserpinized tissue efficiently (cf. Garattini & Samanin, 1981). Its dopamine-releasing effect is not prevented by treatment with tetrodotoxin, which indicates it is independent of neuronal activity (Moore, 1978; Nomikos, 1990). As measured using the *in vivo* microdialysis technique, amphetamine strongly elevates extracellular dopamine levels in, for example, the nucleus accumbens and the striatum (Zetterström et al., 1983, 1986; Hernandez et al., 1987; Carboni et al., 1989a; Kuczenski & Segal, 1989), which are implicated in locomotor and stereotyped activity, respectively. In addition, amphetamine elevates extracellular dopamine levels in the prefrontal cortex (Moghaddam & Bunney, 1989).

Similarly with amphetamine, MDA, MDMA and MDEA increase extracellular dopamine levels in the striatum and the nucleus accumbens (Yamamoto & Spanos, 1988; Hiramatsu & Cho, 1990; Nash, 1990; Gough et al., 1991; Nash & Brodkin, 1991; Nash & Nichols, 1991) via dopamine (and norepinephrine) transporters both by release and inhibition of their reuptake (Johnson et al., 1986; Schmidt, 1987a; Steele et al., 1987; Fitzgerald & Reid, 1990; Hekmatpanah & Peroutka, 1990; Nash & Brodkin, 1991). Increasing the size of the N-substituent decreases the ability of methylenedioxyamphetamines to release dopamine and to elevate its extracellular levels (MDA > MDMA > MDEA; Johnson et al., 1986, 1987; McKenna et al., 1991; Nash & Nichols, 1991). MDA, as a metabolite of MDMA, does not have a significant role in elevation of dopamine levels following treatment with MDMA (Hiramatsu et al., 1991). The dopaminergic effects of methylenedioxyamphetamines are stereoselective: the S(+)-isomers of MDA and MDMA are markedly more efficacious than the R(-)-isomers, which have only negligible effects (Johnson et al., 1986; Steele et al., 1987; Hiramatsu & Cho, 1990; McKenna et al., 1991).

Extracellular 5-HT levels are elevated by higher doses of amphetamine, however less efficiently than dopamine (Hernandez et al., 1987; Kuczenski & Segal, 1989). Methylenedioxyamphetamines increase extracellular concentrations of 5-HT comparable to those of dopamine (Hiramatsu & Cho, 1990; Gough et al., 1991). Indeed, they have been shown to affect 5-HT release and reuptake more than those of dopamine (Nichols et al., 1982;

Johnson et al., 1986; Schmidt et al., 1987; Steele et al., 1987; Fitzgerald & Reid, 1990, 1993; McKenna et al., 1991; Berger et al., 1992). MDMA releases 5-HT by the mechanism of reverse-transport (Rudnick & Wall, 1992). As a result of massive 5-HT release markedly decreased concentrations of 5-HT and 5-HIAA have been measured in many forebrain regions following treatment with MDA, MDMA and MDEA (Ricaurte et al., 1985; Stone et al., 1986, 1987; Schmidt, 1987a). Regarding the effects of methylenedioxyamphetamines on 5-HT release and reuptake, there is no such stereoselectivity as in their dopaminergic effects (Johnson et al., 1986; Steele et al., 1987). Consistent with its neurochemical effects, acute injection of MDMA induces behavioral effects indicative of the so-called 5-HT-syndrome, which in rats includes Straub tail and splaying of hind limbs (Slikker et al., 1989; Spanos & Yamamoto, 1989). The MDMA-induced increase in locomotor activity is also 5-HT-dependent (Callaway et al., 1991).

Electrophysiologically, amphetamines depress firing activity of dopaminergic, noradrenergic and with larger doses serotonergic nerve cells (Graham & Aghajanian, 1971; Bunney et al., 1973; Engberg & Svensson, 1979; Rebec et al., 1982). These effects are likely to result from activation of both somatodendritic autoreceptors and the negative feed-back loop (Bunney & Aghajanian, 1978).

As measured using the *in vivo* microdialysis technique, amphetamine, unlike cocaine, decreases extracellular concentrations of dopamine metabolites DOPAC and HVA (Zetterström et al., 1983, 1986; Hernandez et al., 1987; Carboni et al., 1989a; Kuczenski & Segal, 1989). This is because the majority of DOPAC is presumably derived from metabolism of intraneuronal dopamine and the cytoplasmic dopamine is partially depleted as a result of dopamine release (Kuczenski, 1980; Zetterström et al., 1986). HVA is a secondary metabolite of DOPAC, and thus changes in its concentrations should presumably parallel the those of DOPAC (Westerink, 1985). The effects of amphetamine on HVA indeed parallel the effects on DOPAC with small to moderate doses, but diverge at higher doses. The increase in extracellular HVA concentration observed after administration of a high dose of amphetamine is suggested to result from dopamine metabolism shifting to an extraneuronal pathway, which is linked to dopamine uptake inhibition (Kuczenski, 1980).

Elevation of extracellular 5-HT levels induced by amphetamines is accompanied by a

decrease in 5-HIAA levels (Kuczenski & Segal, 1989; Hiramatsu & Cho, 1990; Gough et al., 1991). This can be assumed to result from depletion of cytoplasmic 5-HT, because 5-HIAA mirrors the metabolism of intraneuronal 5-HT (Grahame-Smith, 1971), and amphetamines are known to interact with cytoplasmic 5-HT (Kuczenski & Segal, 1989; Johnson et al., 1991; Fitzgerald & Reid, 1993). In addition, both enantiomers of MDMA inhibit MAO-A (Leonardi & Azmitia, 1994), which may also result in decreased extracellular 5-HIAA concentrations.

In vitro, DOM does not induce 5-HT release (McKenna et al., 1991) or inhibit its reuptake (Steele et al., 1987). Instead, DOM and its iodinated and brominated analogues, DOI and DOB respectively, act directly as agonists or partial agonists on receptors of the 5-HT_2 family (Glennon et al., 1983, 1984; Rasmussen & Aghajanian, 1986; Titeler et al., 1988; Glennon, 1990), which has lead to a suggestion that hallucinogenic effects are mediated via these receptors (Glennon et al., 1984; Titeler et al., 1988; Glennon, 1990, Wing et al., 1990). Concerning the 5-HT_2 receptor subtypes, the most probable candidate is the 5-HT_{2A} receptor, although hallucinogenic substances also have affinity for the 5-HT_{2C} (former 5-HT_{1C}) and the 5-HT_{2B} receptors (Sanders-Bush et al., 1988; Sanders-Bush & Breeding, 1991; Nelson et al., 1999). The R(-) isomer of MDA, which, based on discriminative stimulus experiments has been associated with hallucinogenic-like effects, has moderate affinity for 5-HT_2 receptors (Lyon et al., 1986).

Many amphetamine derivatives, in addition to those more profoundly discussed here, for example, methamphetamine and p-chloroamphetamine, induce long lasting changes indicative of neurotoxicity in brain dopaminergic and/or serotonergic neuronal systems. Analogous to their acute effects, amphetamine degenerates dopaminergic, methamphetamine both dopaminergic and serotonergic, and methylenedioxyamphetamines exclusively serotonergic nerve cells (Koda & Gibb, 1973; Hotchkiss & Gibb, 1980; Ricaurte et al., 1980, 1984, 1985, 1987; Wagner et al., 1980; Stone et al., 1986, 1987, 1988; Commins et al., 1987; Schmidt, 1987b; Series & Molliver, 1994). A comparison of the methylenedioxyamphetamine potencies indicates that MDEA is the least potent (Stone et al., 1987; Johnson et al., 1987; Barrionuevo et al., 2000).

The toxic effects of MDA, MDMA and methamphetamine seem to localize in serotonergic nerve endings, but not in the cell bodies or *en passant* axons (Battaglia et al.,

1987, 1991; O'Hearn et al., 1988; Axt & Molliver, 1991; Mamounas et al., 1991). To date, mechanisms of MDMA-induced degeneration of 5-HT neurons remain obscure. However it seems that the 5-HT transporter plays a central role, because it has been shown that exposure to methamphetamine or 4-chloroamphetamine only degenerates 5-HT transporter containing axons in the nucleus accumbens, while the *en passant* axons lacking the transporter, are spared (Brown & Molliver, 2000).

2.3.2.1. Designer drugs

Designer drugs are potent and toxic analogues of traditional drugs which are outside existing legal controls. Some of them are compounds that initially have been developed by the pharmaceutical industry, but more recently have appeared on the market as products of clandestine laboratories. Since the advent of the Internet, designer drugs have been promoted world-wide in drug-culture related web sites. These commonly include presentations of both synthesis and use of these substances, together with users' descriptions of their subjective effects. For example, 'PIHKAL (Phenethylamines I Have Known and Loved): A Chemical Love Story, Part 2: The Chemical Story' by Alexander and Ann Shulgin contains descriptions of nearly 200 amphetamine-related substances.

As authorities become aware of their abuse potential, these substances are placed on the Schedule of Controlled Substances. However their abuse is, almost without exception, not detected by immunogical drug-tests commonly used in drug-screening procedures (Cody, 1990a, 1990b; Cody & Schwarzhoff, 1993; Kankaanpää et al., 2001a). Examples of recent designer drugs include 4-methylaminorex, 4-methylthioamphetamine (4-MTA), and 4-bromo-2,5-dimethoxyphenylethylamine (2C-B, 'Nexus').

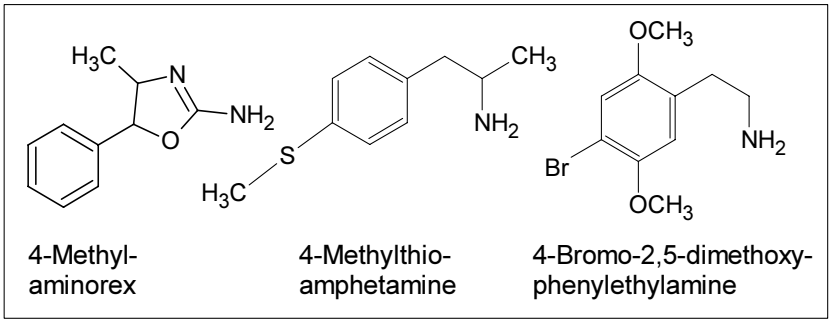

Figure 2.8. Molecular structures of selected designer drugs.

The phenylisopropylamine derivative 4-methylaminorex (Figure 2.8) is a sympathomimetic amine, which exists as four stereoisomers, *cis*-4R,5S-, *cis*-4S,5R-, *trans*-4R,5R-, and *trans*-4S,5S-4-methylaminorex (Poos et al., 1963; Roszkowski & Kelly, 1963; Yelnosky & Katz, 1963). This compound was originally developed by the pharmaceutical industry, and later mixtures of *cis*-isomers appeared on the illegal market with street names of 'U4Euh' (euphoria) and 'ice' (Davis & Brewster, 1987; Klein et al., 1989). The high abuse potential of *cis*-(±)-4-methylaminorex has been demonstrated in animal models (Glennon & Misenheimer, 1989b; Mansbach et al., 1990; Young & Glennon, 1993), and it has been classified as a Schedule I substance in the US (Schedule I; Federal Register, Schedules of Controlled Substances, 1989).

4-MTA (Figure 2.8) is an amphetamine derivative, which has been sold and used as 'ecstasy' since 1997 in many European countries including Finland. There are already reports of 4-MTA related deaths (Europol-EMCDDA, 1999). 4-MTA induces a strong release of 5-HT (Scorza et al., 1999), but in contrast to MDMA, it does not seem to have a marked effect on the dopaminergic system (Huang et al., 1992).

2C-B (Figure 2.8) is marketed actively on the Internet, and is sold, similarly with 4-MTA, as 'ecstasy' (Giroud et al., 1998; De Boer, 1999). Structurally it resembles hallucinogenic amphetamines (Figure 2.7), however both human and animal studies suggest that it induces both stimulatory and hallucinogenic effects (Shulgin & Carter, 1975; Glennon et al., 1988, Bronson et al., 1995). The mechanisms mediating the central effects of 2C-B have not yet been studied.

2.3.3. Methylphenidate

Methylphenidate is a clinically used psychostimulant drug, that is widely used in the treatment of attention deficit hyperactivity disorder (ADHD) and narcolepsy (cf. Chiarello & Cole, 1987). In controlled treatment, abuse of methylphenidate is rare, but there is evidence of illegal misuse (Rappley, 1997). Structurally methylphenidate is a ritalinic acid ester, which includes a piperidin ring, and may thus be considered a cyclic amphetamine derivative (cf. Biel & Bopp, 1978; Figure 2.9). However, the molecule has also certain features common

with cocaine. As a molecule with two asymmetric centers, methylphenidate exists in four stereoisomers, of which *threo* forms are active, while *erythro* forms are not (Ferris et al., 1972; cf. Biel & Bopp, 1978; Schweri et al. 1985).

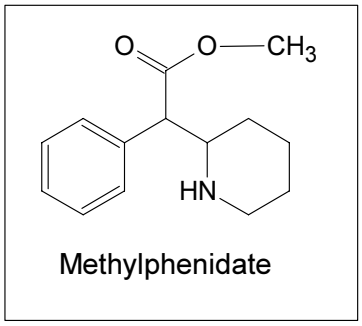

Figure 2.9.
Molecular structure of methylphenidate.

In rats, behavioral effects of methylphenidate include augmented locomotor activity and stereotyped behavior, resembling the effects of amphetamine and cocaine. As measured by *in vivo* microdialysis, methylphenidate increases extracellular concentration of dopamine (and norepinephrine), but not 5-HT in the brain (Kuczenski & Segal, 1997; Segal & Kuczenski, 1999). Methylphenidate acts predominantly by inhibiting dopamine (and norepinephrine) reuptake, without causing an effect on 5-HT (Ferris et al., 1972; Koe, 1976; Taylor & Ho, 1978; Schweri et al., 1985; Nomikos et al., 1990). Characteristically to drugs that act by inhibiting dopamine reuptake, methylphenidate is not able to release dopamine from tissues of reserpinized animals (cf. Garattini & Samanin, 1981). Its ability to increase extracellular dopamine concentrations is inhibited by tetrodotoxin treatment, this indicates that action potential is a prerequisite for elevation of synaptic concentrations of dopamine (Nomikos et al.,1990).

Reports concerning the effects of methylphenidate on extracellular levels of DOPAC and HVA seem to be contradictory; some researchers have reported a decrease (Aoyama et al., 1996), while others have reported no change or even an increase (Kuczenski & Segal, 1997).

Methylphenidate binds to substrate and non-substrate sites in the dopamine transporter, suggesting that it shares binding sites with both cocaine and amphetamine (Schweri et al., 1985; Wayment et al., 1999). Transporter binding correlates with its stimulant effects (Schweri et al., 1985).

2.4. Psychostimulants and addiction

The most important components of drug addiction are drug craving and the compulsive abuse of the drug (psychological dependence). In addition, some drugs of abuse, although not psychostimulants, cause physical dependence, in which physiological changes induced by repeated exposure to the drug lead to withdrawal symptoms when ceasing drug intake.

In order to maintain drug-seeking behavior, a drug must be able to act as a positive reinforcer. A reinforcer can be functionally defined as any incidence which increases probability of a response. Reward can be similarly defined, but it also includes a positive affective state, a pleasure (cf. Koob, 1992). In experimental animals reinforcement and potentially drug-reward can be assessed with behavioral tests such as drug-discrimination, self-administration, intra-cranial self-stimulation and conditioned place preference.

All psychostimulants studied so far, including cocaine, amphetamine, MDMA and methylphenidate, have reinforcing effects as assessed using self-administration and/or place preference tests (Roberts et al., 1977, 1980; Spyraki et al., 1982a,1982b; Pettit et al., 1984; Roberts & Vickers, 1984; Martin-Iverson et al., 1985; Mithani et al., 1986; Morency & Beninger, 1986; Woolverton, 1986; Bilsky et al., 1990, 1991; Meririnne et al., 2001).

2.4.1. Animal models of drug addiction

2.4.1.1. Drug discrimination

The ability to produce a discriminative stimulus effect is considered a key feature in dependence and abuse-promoting effects of a drug. Reinforcing stimuli, which may or may not follow emission of an operant response, are considered major determinants in the behavior of animals. In addition, stimuli that provide information about whether reward is available for a particular response at a particular time are fundamental in control of behavior. These are called discriminative stimuli, because animals have to use the stimuli to discriminate whether to respond or not. Discriminative stimuli may be either exteroceptive or interoceptive such as drug stimuli (cf. Goudie & Leathley, 1993).

Almost all of the centrally active drugs, including psychostimulants, anxiolytics, sedative-hypnotics, opioids, hallucinogenic substances, tetrahydrocannabinol, ethanol and nicotine can act as discriminative stimuli. Thus, experimental animals can recognize the effects of a particular drug and differentiate it from other drugs. It is assumed that the actions of drugs as discriminative stimuli are closely related to their subjective effects in humans. It is believed that analysis of the discriminative properties of a drug in experimental animals may increase understanding of human drug abuse, and predict whether any drug is prone to abuse as the subjective effects in humans are considered to largely determine the extent of abuse (cf. Stolerman, 1992, cf. Goudie & Leathley, 1993). Discriminative stimulus properties of drugs are typically expressed with lower doses than the effects on motor activity or on reinforcement.

2.4.1.2. Self-administration

Drug self-administration is an operant procedure, in which experimental animals perform certain tasks, for example lever presses to receive drug infusions, which serve as positive reinforcers. Typically drugs are delivered by chronic intravenous or intracranial catheters. Oral delivery is less commonly used. Experimental animals learn to self-administer most of drugs abused by humans, for example, psychostimulants, opioids, dissociative anesthetics such as phencyclidine, barbiturates, benzodiazepines, ethanol, nicotine, and some solvents, but not constituents of cannabis or hallucinogenic substances such as LSD and DOM (cf. Stolerman, 1992; cf. Caine et al., 1993). Drug self-administration behavior follows many of the same rules as behavior reinforced by natural rewards, food, drink and sex. Psychostimulants are very powerful reinforcers even in non-dependent states (restricted availability of the drug). In a dependent state motivation is further increased by negative reinforcement, in which the drug prevents or alleviates the presumably aversive state of withdrawal (cf. Koob, 1992).

2.4.1.3. Intracranial self-stimulation

Intracranial self-stimulation is an operant procedure, in which experimental animals, for example, by pressing a lever receive an electric shock to a certain brain area through an intracranial electrode. Since animals learn the tasks required to receive the shock very rapidly, and perform it to the exclusion of eating and drinking, intracranial self-stimulation is assumed to be a very strong positive, possibly rewarding reinforcer. According to this view, intracranial self-administration provides an opportunity to study the biology of nervous pathways involved in motivation and positive reinforcement without disturbing input from other systems (cf. Markou & Koob, 1993).

2.4.1.4. Conditioned place preference

In conditioned place preference experiments the drug is repeatedly administered in the same distinctive environment, therefore the animal learns to associate the drug effect (pleasure, aversion, neutral) with the environment. Correspondingly the drug-free state (vehicle injection) is paired with a different environment. Typically the environments are designed in such a way that they differ by means of visual, sensory and olfactory cues (cf. Carr et al., 1989; cf. Hoffman, 1989; cf. Stolerman, 1992). After an adequate number of these pairings, or conditioning sessions (different drugs require different number of sessions) the animals are allowed to choose between the drug-paired and vehicle-paired environments. If there is then an increase in the time spent in the drug-paired compartment relative to the vehicle-paired compartment, this shift is called place preference and is considered the measure of the drugs' reinforcing effects.

Conditioned place preference experiments can be conducted using either biased or unbiased methods. In the biased (unbalanced) model the animals initially (before drug-treatments) prefer either compartment, whereas in the unbiased (balanced) model no such preference exists (cf. Carr et al., 1989).

2.4.2. Brain areas implicated in psychostimulant reinforcement

The mesocorticolimbic dopaminergic pathway plays an important role in the reinforcement circuitry of the brain. Many vital, highly satisfying functions such as eating, drinking and sex have been shown to activate this system and to release dopamine in the nucleus accumbens. The effects of addictive drugs (psychostimulants, alcohol, nicotine, opioids) seem to parallel these effects of natural rewards. For example, cocaine and amphetamine, both of which increase the concentration of free dopamine very efficiently, also belong to those drugs that induce the strongest euphoria and drug dependence. In experimental animals activation of mesocorticolimbic dopaminergic pathway is expressed, in addition to addiction, as increase in motor activity (Wise & Bozarth, 1987). Regarding subtypes of motor activity, locomotor activity in particular is mediated via the nucleus accumbens, while stereotyped behaviors result from activation of the nigrostriatal dopaminergic pathway (cf. Garattini & Samanin, 1981).

The nucleus accumbens consists of many anatomically and pharmacologically distinct subterritories, that have different afferent and efferent connections. The core division of nucleus accumbens (Figure 2.10), which surrounds the anterior commissure, projects predominantly to the motor areas of the basal ganglia, whereas the shell, located ventromedially of the core and partly surrounding it, projects mainly to limbic structures (Heimer et al., 1991). Therefore, the shell is considered part of the limbic system and the core belongs to the sensorimotor striatal complex (cf. Bardo, 1998). Dopamine in the shell division of nucleus accumbens in particular, is considered essential in the actions of psychostimulants (cf. Di Chiara, 1999; cf. Koob & Le Moal, 2001). In many studies, however, nucleus accumbens is treated as one entity, as is the case with many microdialysis experiments, because both the size of the dialysis probe and diffusion area of the transmitters makes it difficult to differentiate between the subdivisions reliably in a repeatable manner (Figure 2.10).

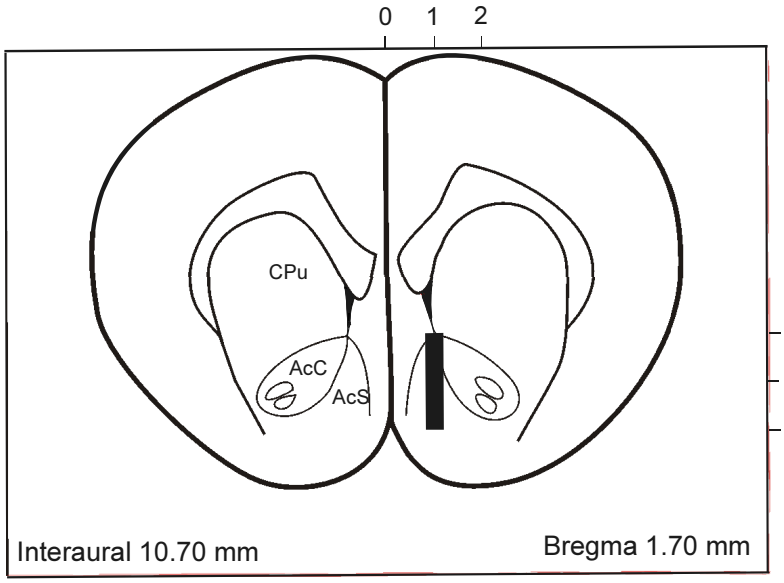

Figure 2.10. Schematic presentation showing subdivisions of nucleus accumbens in rat brain, and the size of a standard microdialysis probe (2 mm membrane length, 0.5 mm membrane diameter). (AcC) nucleus accumbens core; (AcS) nucleus accumbens shell; (CPu) caudate-putamen. Modified from Paxinos & Watson, 1986.

Amphetamine injected directly into the nucleus accumbens is reinforcing, as measured with both self-administration and conditioned place preference tests (Hoebel et al., 1983; Carr & White, 1986). This effect has been blocked by 6-hydroxydopamine lesions of same nucleus (Spyraki et al., 1982a), and the blockade of accumbal D_1 and D_2 receptors (Hiroi & White, 1991a; Phillips et al., 1994a), thus showing that accumbal dopamine plays a key role in the reinforcing properties of amphetamine (Figure 2.11).

Dopamine in the nucleus accumbens seems to be important also in the reinforcing effects of cocaine, as evidenced by the effects of 6-hydroxydopamine lesions of this structure and of locally applied D_1 and D_2 receptor antagonists on cocaine self-administration (Roberts et al., 1977, 1980; Pettit et al., 1984; Maldonado et al., 1993; Phillips et al., 1994b). Direct injections of cocaine into the nucleus accumbens, however, do not induce conditioned place preference (Hemby et al., 1992). Locally applied dopamine receptor antagonists (Kaddis, et al., 1995; Baker et al., 1996) or the elimination of presynaptic dopaminergic input with 6-hydroxydopamine (Spyraki et al., 1982b) does not change conditioned place preference induced by systemic injection of cocaine. The reports concerning the ability of cocaine infused into the nucleus accumbens to maintain self-administration behavior are inconsistent; in the study of Goeders and Smith (1983) cocaine was unable to maintain self-administration, but more recently cocaine infused into the shell division of the nucleus accumbens has been shown to maintain self-administration (Carlezon et al., 1995; McKinzie

et al., 1999). In addition, taken the ability of locally applied selective D_1 receptor antagonist SCH 23390 to decrease reinforcing effects of cocaine as measured by both conditioned place preference (Baker et al., 1998) and self-administration (Maldonado et al., 1993; Caine et al., 1995), it seems safe to conclude that nucleus accumbens has a central role in the reinforcing effects of cocaine (Figure 2.11).

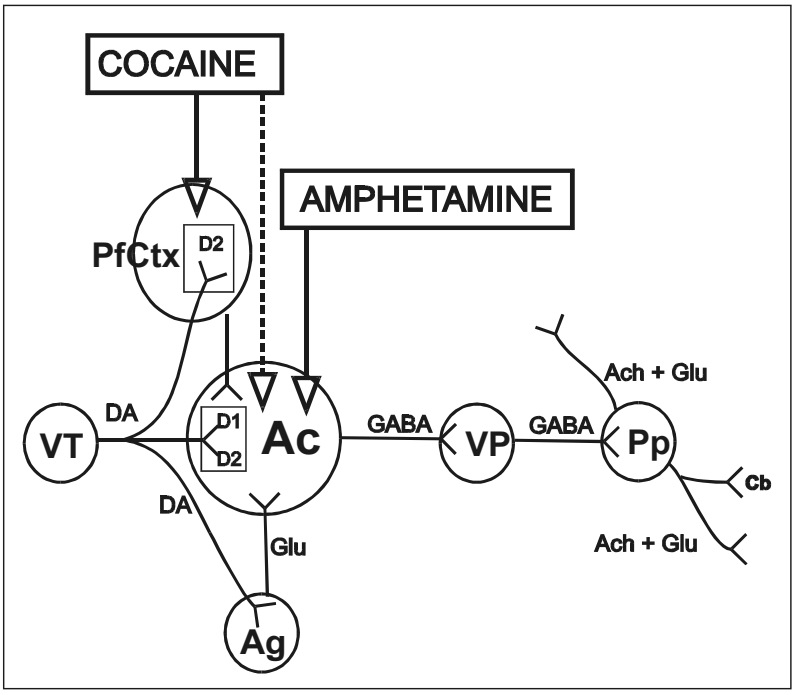

Figure 2.11. Brain areas implicated in psychostimulant reinforcement. Infusion of drugs into nuclei indicated by arrows maintain self-administration (dotted line indicates inconsistent results). (Ac) nucleus accumbens; (Ach) acetylcholine; (Ag) amygdala; (Cb) cerebellum; (D1, D2) dopamine D_1 and D_2 receptors; (DA) dopamine; (GABA) γ-aminobutyric acid; (Glu) glutamate; (PfCtx) prefrontal cortex; (Pp) pedunculopontine nucleus; (VP) ventral pallidum; (VT) ventral tegmentum. Modified from Bardo, 1998.

The prefrontal cortex receives input from dopaminergic cell bodies in the ventral midbrain, similar to the nucleus accumbens. Prefrontal cortex plays an important role in the reinforcing effects of cocaine (Figure 2.11). This is evidenced by the fact that cocaine infusion into this brain area maintains self-administration (Goeders & Smith, 1983, 1986; Goeders et al., 1986), as well as other study designs (Isaac et al., 1989; McGregor et al., 1996). Unlike the effects of cocaine, the effects of amphetamine do not seem to depend on the prefrontal cortex (Goeders et al., 1986; Carr & White, 1986; Leccese & Lyness, 1987).

Amygdala is also implicated in psychostimulant reinforcement, although in a modulatory rather than initiative manner (Hiroi & White, 1991b; Carr & White, 1986; Deminiere et al., 1988; Brown & Fibiger, 1993; McGregor et al., 1994; Robledo & Koob, 1993). It has also been suggested that neurotransmitters other than dopamine participate in these processes (Loh & Roberts, 1990). The role of amygdala in the reinforcing properties of

psychostimulants is likely to result from its glutamatergic output into the spiny neurons inside the nucleus accumbens.

Nucleus accumbens efferents follow four anatomic routes, many nuclei of which have been shown to be involved in the reinforcing effects of psychostimulant drugs. Efferents that use GABA as their primary transmitter descend from nucleus accumbens shell into the ventral pallidum, from which the information is sent back to the ventral tegmental area, as well as diffusely to other structures in the midbrain, thalamus and limbic areas (cf. Pennartz et al., 1994). The second accumbal-pallidal route connects with midbrain nucleus pedunculopontinus which consists mainly of cholinergic cell bodies, and the third route innervates dorsomedial thalamus, which forms reciprocal connections with cortical areas such as the prefrontal and cingulate cortex. The fourth route connects nucleus accumbens with preoptic area and lateral hypothalamus.

The ventral pallidum is situated in a strategic position to affect midbrain dopaminergic nuclei involved in drug reinforcement and to provide output to neural systems that serve drug-taking behavior (Figure 2.11). Drug-induced hyperactivity is at least partially dependent on ventral pallidum (Austin & Kalivas, 1990; Mogenson & Nielsen, 1983), and because locomotor behavior and drug reinforcement share neural substrates, it can be assumed that it has a role in mediating drug reinforcement as well. Ventral pallidum is also known to participate in reinforcing effects of non-drug stimuli (Johnson et al., 1993; McAlonan et al., 1993). Accordingly, the ventral pallidum is implicated in the reinforcing properties of cocaine (Hubner & Koob, 1990; Gong et al., 1996,1997; Sizemore et al., 2000).

Nucleus pedunculopontinus, which is considered the anatomical interface between motivational output from accumbal-pallidal system and motor output of the basal ganglia (Inglis & Winn, 1995), seems to have a role as mediator of reinforcing effects of drugs (Bechara & van der Kooy, 1989; Olmstead & Franklin, 1994). Since nerve fibers projecting from accumbal-pallidal systems and basal ganglia into the nucleus pedunculopontinus use GABA, their effect is likely to be inhibitory.

Thus it seems that the neuropharmacological mechanisms mediating the reinforcing effects of different psychostimulants may differ, at least to some extent, and that in addition to nucleus accumbens (shell), at least prefrontal cortex, ventral pallidum, amygdala and

nucleus pedunculopontinus are involved. Studies with selective agonists and antagonists have revealed that, in addition to dopamine and 5-HT, at least acetylcholine, glutamate and many peptides play key roles in these processes (cf. Bardo, 1998).

2.4.3. Dopamine and psychostimulant reinforcement

As evidenced by numerous studies in which dopamine receptor blockade has prevented or attenuated self-administration or place preference induced by psychostimulant drugs, dopamine has a central role in reinforcing properties of these drugs. Dopamine receptor antagonists such as haloperidol and pimozide increase intravenous amphetamine and cocaine self-administration (Roberts & Vickers, 1984; Woolverton, 1986), which is interpreted as a decrease in reinforcement, which the animal tries to compensate by increasing drug intake. The same antagonists also prevent amphetamine- and cocaine-induced conditioned place preference (Spyraki et al., 1982a,1982b; Morency & Beninger, 1986). The predominant affinity of these antagonists to D_2 receptors (although they are not specific) indicates that these receptors are involved in the reinforcing effects of psychostimulants.

D_3 receptors, which resemble D_2 receptors, have also shown to participate in drug reinforcement. In contrast to D_2 receptors, the effect of the D_3 receptor seems inhibitory. For example, experimental animals do not self-administer the D_3 receptor agonist 7-OH-DPAT (Caine & Koob, 1993), neither does it induce conditioned place preference (Rodriguez De Fonseca et al., 1995), whereas the selective D_3 antagonist U99194A does induce conditioned place preference (Kling-Petersen et al., 1995).

Reports concerning the D_4 receptors seem contradictory; D_4 antagonist clozapine has shown to increase both cocaine self-administration (Roberts & Vickers, 1984; Vanover et al., 1993), and breaking points on progressive schedule of self-administration (Loh et al., 1992), the former of which is interpreted as decrease and the latter as increase in reinforcement.

In addition to aforementioned receptors of the D_2 family, recent evidence suggests that D_1 receptors also have a role in the reinforcing properties of drugs. New D_1 receptor agonists, which are able to traverse the blood-brain-barrier, are self-administered by experimental animals (Self & Stein, 1992; Weed et al., 1993; Weed & Woolverton, 1995), and D_1

antagonists such as SCH 23390 decrease the reinforcing effects of psychostimulant drugs, as measured by both self-administration (Caine & Koob, 1994; Hubner & Moreton, 1991) and conditioned place preference tests (Hoffman & Beninger, 1989; Cervo & Samanin, 1995; Bardo et al., 1999; Meririnne et al., 2001).

Taken together, there is a considerable number of findings supporting the role of dopamine in reinforcing effects of psychostimulant drugs. During the past few years this role, however, has been questioned by studies using mice that lack the dopamine transporter (DAT) gene (DAT knock-out, DAT-KO). In these studies DAT-KO -mice have self-administered cocaine (Rocha et al., 1998a), and both cocaine and methylphenidate have induced conditioned place preference in them, although dopamine levels in caudate-putamen have remained unchanged (Sora et al., 1998). There is however, evidence that in similar DAT-KO mice cocaine and amphetamine (which have an effect on norepinephrine and 5-HT transporters in addition to dopamine transporter) but not GBR 12909 which is specific to dopamine transporter, increase extracellular levels of dopamine in nucleus accumbens while those of caudate-putamen remain unchanged (Carboni et al., 2001). The latter study provides an explanation for the persistence of drugs' reinforcing effects in DAT-KO mice that is compatible with the dopamine hypothesis. Carboni and coworkers (2001) suggest that in DAT-KO mice the norepinephrine transporter which is able to clear dopamine from synaptic cleft at least as effectively as the dopamine transporter, would in absence of dopamine transporter perform the dopamine reuptake. In this case blockade of norepinephrine transporter is reinforcing in DAT-KO mice.

2.4.4. Sensitization

Repeated exposure to a drug typically leads to tolerance, or a decrease in the effect of the drug due to habituation. To date it is believed that tolerance is a physiological phenomenon, by means of which an organism tries to neutralize the effect of the drug and to maintain its homeostasis. While repeated exposure decreases some effects of a drug, the magnitude of other effects may increase, or became sensitized.

Prior treatment with drugs of abuse often increases the effect of subsequent treatments

on locomotor and stereotyped activity (Ahtee, 1974; Segal & Mandell, 1974; Browne & Segal, 1977; Ahtee & Attila, 1987). There is a large body of evidence suggesting that dopamine, which has a vital role in control of motor behavior, is also a key factor in sensitization phenomenon (Ahtee, 1974; Robinson & Becker, 1982, 1986; Ahtee & Attila, 1987; Kalivas & Stewart, 1991). This is also supported by findings indicating that repeated exposure to amphetamine can trigger a psychotic state resembling schizophrenia in humans, which can be reversed by dopamine receptor antagonists (Bell, 1965; Snyder, 1972, 1973). In fact amphetamine-induced sensitization is used as an animal model of schizophrenia (Randrup & Munkvad, 1970; Robinson & Becker, 1986; Lillrank et al., 1991). Although the mechanisms underlying sensitization are not yet known in detail, it has been suggested that D_1 receptors in the ventral tegmental area activated by drug-induced somatodendritical release of dopamine, and down-regulation of inhibitory D_2 autoreceptor function by repeated exposure of the drug, would participate in the development of sensitization (Kalivas & Stewart, 1991; Henry et al., 1998).

In addition to motor activation, repeated exposure to amphetamine, cocaine and methylphenidate have been shown to sensitize their reinforcing effects (Lett, 1989; Shippenberg & Heidbreder, 1995; Le Pen et al., 1998; Pierre & Vezina, 1998; Meririnne et al., 2001). According to certain theories of drug addiction, sensitization of drugs' reinforcing effects is thought to play a central role in the development of drug dependence (Robinson & Berridge, 1993).

2.4.5. Neurobiological theories of drug addiction

The early theories of drug addiction, which utilized opiate dependence as their model, emphasized the impact of physical dependence (Himmelsbach, 1943). According to this view drugs are abused to prevent symptoms of withdrawal, i.e., the physical withdrawal symptoms would act as negative reinforcers, and maintain recurrent drug use. It is nevertheless clear that physical dependence is not a factor sufficient to explain drug addiction, since for example psychostimulants, which are highly addictive, do not induce physical dependence at all.

According to theories based on positive reinforcement, the key feature in development

of drug dependence is the subjective feeling of pleasure induced by drugs of abuse. Wise (1982) discovered the association between dopamine and pleasure in his studies, in which primary operant reinforcement maintained by natural reinforcers, intracranial self-stimulation, and drugs of abuse was weakened by neuroleptic drugs. From these observations he interpreted that neuroleptics prevent the primary reinforcement based on hedonic effects of positive reinforcers by causing anhedonia, an incapability to feel pleasure. In this case dopamine would specifically mediate the primary hedonic effects of positive reinforcers. After having noticed that neuroleptics diminish response to conditioned stimuli (secondary reinforcement) as well as primary reinforcement, Wise himself replaced the 'anhedonia hypothesis' with 'incentive motivation -hypothesis' (Wise, 1985).

Theories that combine positive and negative reinforcement describe addiction as a cycle of alternating positive and negative reinforcement. In such a cycle, drug craving and relapse based on sensitization, alternates with a psychological and physical withdrawal state caused by cessation of drug-intake, which can also motivate to continue drug use. As the cycle is repeated, disorder of controlling brain reward system strengthens, and eventually leads to compulsive drug use and incapability to control drug use. At the neurobiological level the mesolimbic dopaminergic pathway, opioid peptide systems, as well as brain and hormonal stress systems, which have to adapt alternately to drugged and un-drugged states, are known to participate in the mechanisms of this disorder of homeostatic control (Koob & Le Moal, 1997).

Reward learning theories emphasize the importance of reward prediction in drug addiction. Most of the dopamine neurons become activated as a result of both primary reward, for example, food or drink, and conditioned reward, a visual or auditory stimulus predicting availability of a reward. In reward learning theories it is assumed that dopamine neurons label environmental stimuli as desirable, predict and detect reward, and signal important and motivating events. All responses to rewards or stimuli predicting them would depend on the predictability of the event in such a way that the dopamine neurons would become activated from events that are better than predicted, remain inactive if events are such as predicted, and become depressed from events that are worse than predicted. When mediating reward according to error in predictability, dopamine response formally fulfills the requirements of

learning signals postulated by theories on reinforcing learning. During the learning process dopamine response changes from detecting primary rewards to prediction of reward (Schultz, 1998).

According to theories emphasizing the incentive sensitization, or sensitization of 'wanting', the characteristics of drug dependence, drug craving and relapse, result directly from changes in functions whose neuronal regulation has been adapted to a sensitized state due to repeated exposure to a drug. Dependence-producing drugs activate the mesocorticolimbic dopaminergic system, psychologic function of which is to label stimuli that activate it as desirable and important. In some individuals, repeated exposure to drugs induce activation-increasing adaptations in this system, which is expressed as hypersensitivity (sensitization) to the drugs and stimuli associated with them. By means of associative learning (conditioning) the drug and stimuli associated with it, are labeled excessively desirable and the normal 'wanting' turns into drug-craving. Sensitization of neural systems mediating motivation may occur independently of changes in systems mediating subjective feelings of pleasure induced by the drug, and neural systems mediating withdrawal-related emotions. Thus the sensitization of 'wanting' may induce drug-dependence even if expectations concerning the pleasurable effects of the drug, or adverse effects of withdrawal, were diminished (Robinson & Berridge, 1993; Berridge & Robinson, 1998).

Di Chiara (1999) emphasizes the importance of adaptive changes in nucleus accumbens shell dopamine response, or absence of them, as the neurobiological basis for development of drug-dependence. Both addictive drugs and natural reinforcers stimulate dopamine transmission primarily in the nucleus accumbens shell. In the case of drugs, but not natural rewards, there is no one-trial habituation of this response. As a result of this resistance to habituation, repeated exposure to drugs is capable of activating dopamine transmission in nucleus accumbens shell recurrently, without decrease in activation. In this theory it is assumed that this process abnormally strengthens association between stimulus and drug, and leads to excessive motivational value of a drug or a stimulus predicting its availability. That is, in an addicted state repeated activation of nucleus accumbens dopamine transmission leads to abnormal associative learning, which makes drug-related stimuli govern behavior. According to this theory dopamine is necessary to associate a stimulus to the reward, and to

transform it as desirable, which is then expressed in later operant tests as capability of functioning as positively reinforcing stimulus. Dopamine release in nucleus accumbens shell by unknown and unpredictable attractive primary rewards might function as the link between the discriminative properties of primary rewards and their biological effects. Instead, the features of dopamine transmission in the nucleus accumbens core and the prefrontal cortex are congruent with those involved in expression of motivation, which is consistent with the presumed role of nucleus accumbens as an interface between motivation and action (Mogenson et al., 1980).

2.5. 5-HT-dopamine interactions

Anatomically, brain dopaminergic and serotonergic pathways intersect both in the areas of dopaminergic cell bodies in the ventral tegmental area and the substantia nigra, as well as their projection areas in nucleus accumbens, prefrontal cortex and striatum (see sections 2.1.2 and 2.2.2). Although investigations carried out with various experimental techniques have resulted in vast evidence of functional interactions of these neuronal systems, the true nature of the interactions still remains obscure. Traditionally the net effect of 5-HT on the dopaminergic system has been considered inhibitory, but many of the more recent studies suggest a facilitating role, because various serotonergic treatments have been shown to increase dopamine release in many brain areas, as well as motor activity of experimental animals (Blandina et al., 1988; Guan & McBride, 1989; Benloucif & Galloway, 1991; Jacocks & Cox, 1992; Parsons & Justice, 1993; De Deurwaerdere et al., 1996, 1998; De Deurwaerdere & Spampinato, 1999; Sasaki-Adams & Kelley, 2001). The dopaminergic activity stimulating effect of 5-HT has been revealed, for example, by stimulating dorsal raphe nucleus electrically and by infusing 5-HT directly to the area of mesocorticolimbic cell bodies, as well as their projection areas. In addition to, or instead of, direct effects, this regulation can naturally be mediated indirectly via another transmitter system(s).

Dopamine also increases 5-HT release (Matsumoto et al., 1996). For example dopamine release in nucleus accumbens and striatum strongly increases 5-HT release in the same areas (Broderick & Phelix, 1997). It was also noticed that open field motor activity was increased

concomitantly with 5-HT release.

The picture is further complicated by the different 5-HT receptor subtypes; for example 5-HT_{1B}, 5-HT_{2A} and 5-HT_3 receptors facilitate dopaminergic effects (Carboni et al., 1989c; Jiang et al., 1990; Chen et al., 1991; Parsons et al., 1996; De Deurwaerdere et al., 1998, De Deurwaerdere & Spampinato, 1999; Lucas et al., 2000a), whereas 5-HT_{2C} receptors are reported to inhibit them (Walsh & Cunningham, 1997; Lucas et al., 2000a). In addition, these effects seem to be specific to brain area and other conditions. For example, electrical stimulation of the dorsal raphe nucleus releases dopamine via 5-HT_3 and 5-HT_{2A} receptor activation in nucleus accumbens but not in the striatum (De Deurwaerdere et al., 1998, De Deurwaerdere & Spampinato, 1999). During nigrostriatal activation, however, 5-HT seems to be able to release dopamine also in the striatum (Lucas et al., 2000b). Activation of the 5-$HT_{2B/2C}$ receptors seems to inhibit dopamine activity more consistently in both the nucleus accumbens and the striatum (De Deurwaerdere & Spampinato, 1999; Di Giovanni et al., 1999; Lucas et al., 2000a).

Psychostimulant drugs increase synaptic concentrations of dopamine and 5-HT to a varied extent depending on the substance, without a direct receptor effect. Numerous studies have tried to reveal the role and mechanisms of 5-HT-dopamine interactions in the effects of psychostimulants, but results remain contradictory. The most compelling evidence of such interactions has been obtained with psychostimulants that have strong impact on synaptic concentrations of both neurotransmitters. The ability of MDMA, for example, to release dopamine does not entirely rely on its effect on the dopamine transporter, but also on 5-HT released by the drug, since the blockade of 5-HT release by various means decreases dopamine release markedly (Schmidt et al., 1991, 1992a, 1992b, 1994; Yamamoto et al., 1995; Gudelsky & Nash, 1996). Thus, a portion of synaptic dopamine seems to be released secondarily as a result of 5-HT release.

Regarding the role of 5-HT in the reinforcing effects of psychostimulants, increasing rat brain 5-HT content has been shown to decrease amphetamine and cocaine self-administration (Leccese & Lynes, 1984; Smith et al., 1986; Carroll et al., 1989, 1990; Porrino et al., 1989), and decrease the breaking-points on a progressive-ratio schedule of cocaine self-administration (McGregor et al., 1993). Decrease in drug self-administration is typically

interpreted as an increase in reinforcing effects. The study of McGregor and coworkers (1993) using a model which is thought to reflect reinforcing effects of drugs in a more unequivocal manner, indicates that the reinforcing effects of cocaine are actually decreased as brain 5-HT content increases. An inhibitory role is also supported by studies, in which depleting nerve cells of 5-HT by the neurotoxin 5,7-dihydroxytryptamine increases breaking-points on a progressive-ratio schedule of cocaine reinforcement (Roberts et al., 1994), and response to a conditioned reward (Fletcher et al., 1999). However, treatment with the tryptophan hydroxylase-inhibitor para-chlorophenylalanine decreases cocaine-seeking in rats, which indicates that a decrease in 5-HT transmission decreases the motivation for cocaine (Tran-Nguyen et al., 1999).

In studies aimed to clarify the role of different 5-HT receptors in psychostimulant reinforcement it has been shown that the unselective 5-HT receptor blocker methysergide, and the 5-HT_2 receptor blocker cinanserin decrease amphetamine self-administration (Leccese & Lyness, 1984; Porrino et al., 1989). Cocaine self-administration remains unchanged despite the 5-HT_1, 5-HT_2, and 5-HT_3 receptor blockade (Porrino et al.,1989; Peltier & Schenk, 1991; Lacosta & Roberts, 1993; Peltier et al., 1994). Concerning the role of 5-HT_{1B} receptors, results are contradictory: Stimulation of this receptor has been shown to increase the reinforcing effects of cocaine (Parsons et al., 1998), but 5-HT_{1B} receptor gene knock-out mice are more motivated to self-administer cocaine than control mice (Rocha et al., 1998b). Although blockade of the 5-HT_2 receptors had no effect on cocaine self-administration, blockade of the 5-HT_{2A} receptors seems to decrease both the ability of cocaine to produce a discriminative stimulus effect and its other behavioral effects (McMahon & Cunningham, 2001). Similarly, 5-HT_3 receptor antagonists decrease conditioned place preference induced by cocaine and MDMA (Bilsky & Reid, 1991; Suzuki et al., 1992), although they do not prevent cocaine self-administration.

For research reports concerning implication of 5-HT-dopamine interactions in the effects of psychostimulant drugs, it is rather typical that the results obtained by different groups using similar study protocols oppose each other. For example, blockade of 5-HT_3 receptors decreases the elevation of extracellular dopamine levels, conditioned place preference and locomotor activation induced by cocaine in majority of studies (Costall et al.,

1987; Reith, 1990; Van der Hoek & Cooper, 1990; Suzuki et al., 1992; Svingos & Hitzemann, 1992; McNeish et al., 1993), while there are also similar studies, in which no such effect is found (King et al., 1994; Cervo et al., 1996; Lê et al., 1997).

Taken together, it seems that to date, it has not been possible to create an unambiguous picture of 5-HT-dopamine interactions either in drug-free conditions or under the influence of psychostimulant drugs. According to the theory of Bankson and Cunningham (2001) the brain serotonergic system, including the 5-HT receptors, is plastic, thus it can function in many different ways depending on the neurochemical environment, which makes it very complicated. In such a state, changes in brain neurochemical environment, for example, drug-induced dopamine release, would contribute to the net effect to which activation of a certain type of 5-HT receptor leads at levels of both neurochemistry and behavior. On the other hand, it is also presumable that many 5-HT receptor types mediate, for example, the reinforcing and rewarding effects of psychostimulant drugs simultaneously (cf. Bardo, 1998), when the activation of one type of receptor would be sufficient to mediate the effects of 5-HT. Similarly, the blockade of one receptor type would not be enough to prevent the effect, since it would be mediated via a receptor of another type.

3. AIMS OF THE STUDY

The psychostimulant drugs of abuse, amphetamine and cocaine, are known to exert their central effects by increasing the synaptic concentrations of dopamine and 5-HT, as well as norepinephrine. A large body of evidence suggests that their ability to induce a strong elevation of extracellular dopamine levels in the nucleus accumbens, especially, plays a crucial role in their stimulative and reinforcing effects. In case of amphetamine, however, minor modifications in its molecular and/or spatial structure results in compounds whose subjective effects extend from stimulatory to hallucinogenic. Although these compounds are abused by humans, their neurochemical effects remain largely unknown.

Despite extensive research, the picture of 5-HT-dopamine interactions either in drug-free conditions or under the influence of psychostimulant drugs, has not yet been clarified. One candidate for mediating such interactions is the 5-HT_3 receptor, which has been reported to modulate the central dopaminergic activity.

The aim of this series of studies was firstly to evaluate the dopaminergic and serotonergic effects of designer derivatives of amphetamine, and secondly to explore the role of 5-HT_3 receptors in mediating the neurochemical and behavioral effects of psychostimulant drugs which differ in their mechanism of action.

Specific goals of this work were:

1) To evaluate the neurochemical effects of hallucinogenic and stimulative amphetamine derivatives.
2) To assess the implication of spatial structure in neurochemical and behavioral effects of psychostimulant drugs.
3) To elucidate the role of 5-HT_3 receptors in the dopaminergic effects of cocaine, mazindol, amphetamine and methylphenidate which differ both in their dopaminergic mechanism of action, and the magnitude of their effect on 5-HT.

4. MATERIALS AND METHODS

4.1. Animals

Adult male Wistar rats were housed two per cage in a temperature-controlled room (20 ± 2°C) with a 12-h light cycle (all the experiments were conducted during the light phase). The animals had free access to standard laboratory chow and tap water. The animal experiments were approved by the local institutional Animal Care and Use Committee and the Chief Veterinarian of the County Administrative Board, and they were conducted according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

4.2. Drugs

Amphetamine sulphate (Sigma Chemical Co., MO, USA), MDA hydrochloride (donated by NIDA, USA), MDMA hydrochloride (NIDA), DOM hydrochloride (NIDA), cocaine hydrochloride (Sigma Chemical Co.) and methylphenidate hydrochloride (RBI, MA, USA) were dissolved in saline (0.9% NaCl). The four optical isomers (*trans*-4R,5R, *trans*-4S,5S, *cis*-4R,5S, and *cis*-4S,5R) of 4-methylaminorex were prepared in the Laboratory of Organic Chemistry, University of Helsinki, Finland, using synthesis methods described by Poos et al. (1963) and Klein et al. (1989). For animal experiments, the isomers were dissolved in a small volume of saline acidified with a drop of glacial acetic acid. The pH was then adjusted to physiological level with 2 M NaOH and the solution was made up to volume with saline. MDL 72222 and mazindol (RBI) were dissolved in acidified saline as described above, the exception being that mazindol was suspended in saline and 0.1% (v/v) Tween 80 for the place preference test.

Amphetamine, MDA and MDMA were administered at doses of 1.0, 3.0 and 9.0 mg/kg, while DOM was injected at doses of 0.5 and 1.0 mg/kg. The doses of the 4-methylaminorex-isomers were 2.5, 5.0 and 10 mg/kg. Cocaine and methylphenidate were injected at doses of 10 and 20 mg/kg, and mazindol at a dose of 10 mg/kg. MDL 72222 was administered at

dosage rates of 0.025, 0.050, 0.100 and 1.000 mg/kg. The concentrations of the drugs in the solutions, all expressed as free base, were adjusted so that they could be injected in a volume of 1.0 ml/kg. The drugs and their corresponding vehicles were injected intraperitoneally (i.p.), with the exception of MDL 72222 which was administered subcutaneously (s.c.).

4.3. Neurochemical experiments

Neurochemical effects of the drugs were evaluated using *in vivo* microdialysis technique, which enables the experimenter to monitor the extracellular fluid of alive animals in discrete brain areas. In addition to endogenous substances such as neurotransmitters and their metabolites, the brain extracellular concentrations of exogenous substances (for example drugs) can be estimated from dialysate samples.

4.3.1. Surgery and brain dialysis

When performing *in vivo* microdialysis in anesthetized animals (paper III) microdialysis probes (CMA/12 Microdialysis probe, Carnegie Medicin Ab, Sweden) were implanted directly into the nucleus accumbens (A +2.0, L -1.2, V -8.0; Paxinos & Watson, 1986) and the dorsal striatum (A +1.0, L +2.7, V -6.5; Paxinos & Watson, 1986) under halothane anesthesia. The probes were then connected to a microinjection pump and perfused with modified Ringer-solution (147 mM NaCl, 1.2 mM $CaCl_2$, 2.7 mM KCl, 1.0 mM $MgCl_2$, 0.02 mM ascorbic acid, pH 6) at a flow rate of 2 µl/min under constant flow of halothane. After a stabilization period of 30 min, sampling time was 30 minutes. Samples were frozen immediately and stored at -70°C until assayed.

In experiments that were conducted using freely-moving animals (papers I, II, and IV), a guide cannula (CMA/12, CMA Microdialysis, Sweden) was implanted 2 mm above the nucleus accumbens (A: +2.0, L: -1.2, V: -6.0; Paxinos & Watson, 1986) of a halothane-anesthetized animal and secured with dental cement and two small screws. The rats were allowed to recover from the surgery for 2 days (paper I) or 6-7 days (papers II and IV), and then on the day of the experiment (paper I) or 1 day prior to the experiment (papers II and IV)

a microdialysis probe (CMA/12, membrane length 2 mm) was inserted through the guide cannula into the nucleus accumbens. The rat was then placed in a test cage, the probe was connected to a microinjection pump and perfused with modified Ringer's solution (as above, but without ascorbic acid) at a flow rate of 2 µl/min. After a 60-min period of stabilization, the sampling interval was 30 (papers I and IV) or 20 min (paper II). In papers II and IV, aliquots of antioxidant solution (1.0 mM oxalic acid, 3.0 mM L-cysteine, 0.1 M acetic acid) were added to the vials prior to collection of the samples (Kankaanpää et al., 2001b). After collection of basal samples the drug(s) or their corresponding vehicles were administered as described in the original publications. Aliquots of the samples were immediately assayed for neurotransmitters and their metabolites using HPLC. In paper II part of each dialysate sample was placed in a deep freezer (-70°C) to be assayed for *cis*- and *trans*-4-methylaminorex. At the end of the experiment the animal was exposed to halothane and decapitated, after which the brain was dissected and immersed in 10% buffered formalin solution. Correct placement of the microdialysis probe was verified, and the data was included only from animals with accurate placements.

4.3.2. Chemical analyses

In paper I the dialysate samples were analyzed for dopamine, 5-HT and their metabolites by two separate HPLC systems, both using Brownlee ODS 5 μ (100 x 2.1 mm) reverse-phase columns (Applied Biosystems, Inc., USA) and electrochemical amperometric detectors (ANTEC, The Netherlands). One of the detectors was set at +780 mV for dopamine, DOPAC, HVA and 5-HIAA assay, and the other at +600 mV for 5-HT assay. The mobile phases were similar in the two systems (0.1 M NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid, 7% acetonitrile and 0.1 M EDTA), with the exception that the concentration of octanesulphonic acid was 1 mM for the dopamine, DOPAC, HVA and 5-HIAA assay, and 0.01 mM for the 5-HT assay. The flow rate was 0.60 ml/min in both systems.

In order to determine 5-HT and dopamine simultaneously, a modification of this method was developed (Kankaanpää et al., 2001b), and then employed in papers II and IV. Briefly, the changes made to the dopamine assay described above included a new column, Inertsil ODS-

3V 5 µm (250 mm x 4.6 mm I.D.; GL-Sciences Inc., Tokyo, Japan), as well as some modifications in the consistence of the mobile phase, which was a mixture of buffer containing 50 mM NaH_2PO_4, 0.1 mM EDTA, 2.3 mM octanesulfonic acid and acetonitrile (21% v/v in the final solution), with the pH adjusted to 3.0 with phosphoric acid. The flow rate was 1.2 ml/min.

In paper III the dialysate samples were analyzed for dopamine and its metabolites at the Department of Pharmacy, Division of Pharmacology and Toxicology, University of Helsinki, Finland using a method that is essentially similar to that described above, except that a Spherisorb ODS 5 µ (250 x 4.6 mm) column was used and the mobile phase consisted of a mixture of 0.1 M NaH_2PO_4 (adjusted to pH 4.1 with 0.1 M citric acid), methanol (10-12%), 1 mM octanesulphonic acid and 0.2 mM EDTA. The flow rate was 1.0 ml/min.

The *cis*- and *trans*-4-methylaminorex were quantitated with GC/MS as *tert*-butyldimethylsilyl derivatives using a previously described method (Kankaanpää et al., 2001a) adapted for dialysate samples. The procedure began with the extraction of 4-methylaminorex from the dialysate samples, mixed with 1 ml of 0.5 M NaOH and 5 ml of toluene containing carbamazepine (10 µg/100 ml) as internal standard. After centrifugation, the toluene layer was transferred into a clean test tube and evaporated to dryness. The derivatization reagent, 120 µl of *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide/acetonitrile (1:6), was added to the dry residue. After incubation for 2 h at 55°C, the mixture was injected in a volume of 1 µl into GC/MS apparatus (Hewlett-Packard G1800A GCD System, Hewlett-Packard Company, Palo Alto, CA, USA). The system was operated in the splitless injector mode. The GC column was an HP-5 of 30 m length, 0.25 mm I.D., and 0.25 µm film thickness. Helium was used as a carrier gas. The inlet and detector temperatures were maintained at 250 and 280°C, respectively. The column temperature was initially 150°C with a hold time of 2.0 min and was increased 15°C/min to 320°C with a final hold time of 3.0 min.

4.4. Behavioral experiments

4.4.1. Characterization of drug-induced behavioral changes

Behavior of the rats treated with 4-methylaminorex isomers was characterized by an observer blind to drug conditions from video tapes recorded during the microdialysis experiments. Locomotor activity of the animals was estimated from the number of complete passes (all four legs) across the midline of the test-cage during 20 min intervals (corresponding to the sampling interval in the microdialysis experiments).

A more detailed behavioral analysis was carried out with the rats receiving 5.0- and 10-mg/kg doses of the 4-methylaminorex isomers: the onset, frequency, and/or duration of different behaviors were monitored visually for 1 min every 5th min by an observer blind to the drug conditions. This behavioral analysis began 20 min before drug injection, after which monitoring was discontinued for 20 min to exclude the effect of injection on the behavior, and then continued for 200 min at 20-min intervals.

The behavioral patterns characteristic for each isomer were then scored according to a rating scale modified from scales based on observations of rats exposed to increasing doses of amphetamine (Creese & Iversen, 1973) and phencyclidine (PCP; Sturgeon et al., 1979). The rats were given a single behavioral score per each 20-min sampling interval as follows: 0, passive or long-lasting (≥ 10 min) motionlessness; 1, active motionlessness; 2, active motionlessness with occasional rearings; 3, locomotor activity with bursts of rearings; 4, stereotyped behavior; 5, intense stereotyped behavior; 6, ataxia; 7, catatonia.

When rated passive motionless the animal was stationary, lying or sleeping, or sniffing peacefully, while active motionlessness included alertness, movements of the head, and active sniffing in various directions. Locomotor activity was defined as movement of the animal over the surface of the observation cage floor. Stereotyped behavior was defined as compulsive-like, rapid, and repetitive purposeless behavior, that occurred at an abnormally high frequency. Intensive sniffing, head bobbing, or head weaving performed at a moderate rate, were rated milder forms of stereotyped behavior, in comparison to vigorous backward walking, circling, and body weaving, which were considered intense stereotyped behavior.

Ataxia was defined as inability of the animal to execute coordinated motor responses. When rated catatonic, rats were lying flat and were unresponsive to touch.

4.4.2. Motor activity measurements with an Animex activity meter

Motor activity was recorded by an Animex activity meter (LKB Farad, Sweden), the general sensitivity of which was set at 40 μA and the measuring channel was set at 39 μA. The sensitivity was chosen in such a way that the overall motor activity was measured, and the possible ceiling effect of activity counts approaching zero was avoided.

After a 60-min period of habituation to the test box, three basal activity values, 15 min each, were recorded prior to any drug treatments. After drug treatments activity was then recorded at 15-min intervals for 3 h.

4.4.3. Conditioned place preference

The conditioned place preference experiments were conducted as described earlier in more detail (Meririnne et al., 2001). Six identical rectangular boxes (60 x 30 x 45 cm) were used. Each box was divided into two compartments of equal size by a separating wall with a guillotine door: one compartment was black with a smooth floor and small drops of acetic acid added in the back corners, and the other was white with wire mesh on the floor and no acetic acid added.

The place preference procedure consisted of three phases: 1) Preconditioning phase (days 1-3), during which the rats were allowed to explore both compartments freely for 15 min (900 sec). On the third day the times spent in both compartments were measured, and the rats were assigned to treatment groups with a less-preferred compartment serving as a drug-paired compartment. 2) Conditioning phase (days 4-6) consisted of two conditioning sessions each day. In the first session of the day the rats first received vehicle, or no injection, and 30 min later a vehicle injection after which they were immediately confined to the saline-paired compartment for 40 min. After an interval of at least 90 min the second session of the day was commenced: the rats received first vehicle, MDL 72222 (pretreatment) or no injection,

and 30 min later they received cocaine, mazindol, methylphenidate or vehicle. The rats were then immediately confined to the drug-paired compartment for 40 min. During this phase the guillotine door was closed. 3) Postconditioning phase (day 7): The guillotine door was opened and the time the rats spent in the drug-paired compartment was measured for 15 min. When appropriate (see below), this measurement was preceded by a vehicle or MDL 72222 injection given 30 min earlier.

Three kinds of experiments were carried out: first, effects of MDL 72222 alone were evaluated, then effects of MDL 72222 on acquisition of place preference induced by cocaine, mazindol or methylphenidate was assessed, and finally effects of MDL 72222 on the expression of cocaine-induced place preference were tested.

4.5. Statistical analyses

In the microdialysis experiments the mean of the samples before the drug treatments (two to four samples) was considered to represent basal release (100%) of neurotransmitters according to which relative changes after the injections were calculated. For statistical evaluations of both neurochemical and motor activity data in papers II and IV, as well as concentrations of the isomers in paper II, areas under the curves (AUC) during the indicated intervals were calculated with the trapezoidal method, and peak effect or minimum value where appropriate were taken directly from the data (cf. Matthews et al., 1990), after which the data was then subjected to one-way ANOVA followed by Tukey's or Bonferroni's test, or to two-sample t-test where appropriate. In papers I and III, two-way ANOVA followed by Tukey's test were used.

In paper II the behavioral scores were analyzed with Kruskall-Wallis non-parametric ANOVA, and multiple comparisons were made using the Mann-Whitney U test with Bonferroni protection.

In the place preference test (paper IV) the postconditioning time served as a measure of place preference. The one-way ANCOVA (preconditioning time as covariant) was used for statistical evaluation of the effects of the drugs alone and combined with MDL 72222. Since there is evidence that the effect of another 5-HT_3 receptor antagonist, ondansetron, at high

doses, is dependent on whether the drug-paired compartment is white or black (Higgins et al., 1992), a two-way ANCOVA (treatment doses x drug-paired compartments) was used to evaluate the effects of MDL 72222 alone. Bonferroni's test was used for paired comparisons.

5. RESULTS

5.1. Effects of amphetamine derivatives and 4-methylaminorex isomers on dopamine and 5-HT

Table 5.1. summarizes the effects of amphetamine derivatives and 4-methylaminorex isomers on extracellular concentrations of dopamine, 5-HT and their metabolites.

Table 5.1. Summary of the acute effects of psychostimulant drugs on the extracellular concentrations of dopamine, 5-HT and their metabolites in the nucleus accumbens.

Treatment	Dopamine	DOPAC	HVA	5-HT	5-HIAA
Amphetamine	↑↑↑	↓↓↓	↓↓	↑	-
MDA	↑↑	↓↓	↓	↑↑↑	↓↓
MDMA	↑↑	↓↓	↓	↑↑	↓↓
DOM	-	-	-	-	-
4-Methylaminorex					
Trans-4R,5R	-	↓↓	↓↓	-	↓
Cis-4R,5S	↑↑↑	↓↓	↓↓	↑↑↑	↓↓
Cis-4S,5R	↑↑↑	↓↓↓	↓	↑↑↑	↓
Trans-4S,5S	↑↑↑	↓↓↓	↓↓	↑↑↑	↓↓

The arrows show the changes induced by drug treatments: ↑ maximal increase to more than 200% of the basal output, ↑↑ maximal increase to more than 400%, ↑↑↑ maximal increase to more than 600%, ↓ maximal decrease to less than 80% of the basal output, ↓↓ maximal decrease to less than 60%, ↓↓↓ maximal decrease to less than 40%. All results presented here with arrows have been found statistically significant ($p < 0.05$) as evaluated by statistical tests in the original papers; a dash (-) indicates no significant change.

Concerning the isomers of 4-methylaminorex, it should be noted that although the isomers *cis*-4R,5S, *cis*-4S,5R, and *trans*-4S,5S all increased extracellular levels of dopamine and 5-HT with similar efficacy, they were not equipotent. As evident from Figure 5.1., the *cis*-4S,5R isomer had the strongest effect on 5-HT levels while the *trans*-4S,5S isomer elevated dopamine levels most potently. Thus the rankings for elevation of 5-HT were *cis*-4S,5R > *trans*-4S,5S ≈ *cis*-4R,5S > *trans*-4R,5R, while the corresponding order for elevating extracellular dopamine was *trans*-4S,5S > *cis*-4S,5R ≈ *cis*-4R,5S > *trans*-4R,5R.

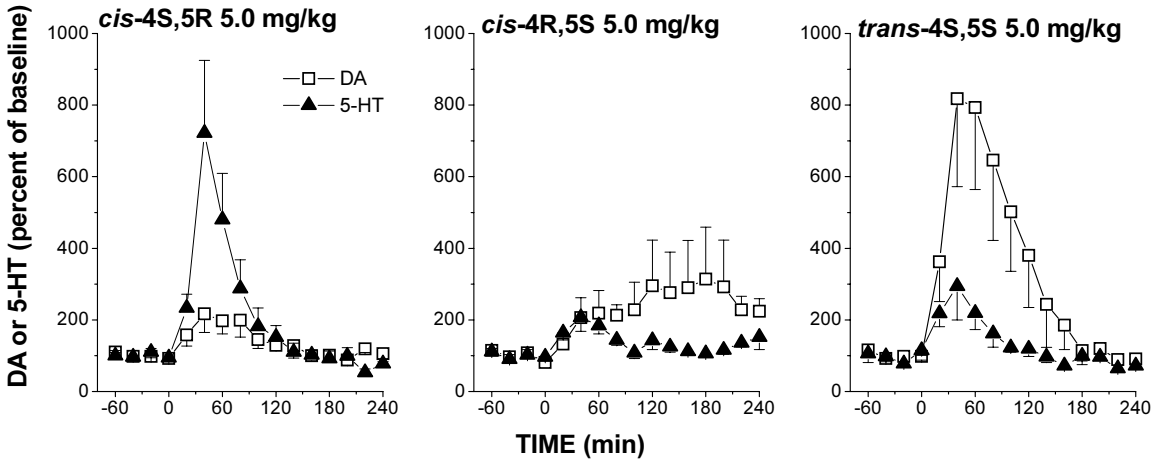

Figure 5.1. Effects of the 5.0-mg/kg dose of the three most potent 4-methylaminorex isomers on extracellular levels of 5-HT and dopamine (DA) in the nucleus accumbens. *Cis*-4S,5R-, *cis*-4R,5S, and *trans*-4S,5S-4-methylaminorex were administered at 0 min.Data are given as means ± SEM (n = 6). At this intermediate dose, only *cis*-4S,5R-induced elevation of 5-HT-level and *trans*-4S,5S-induced elevation of dopamine level were found statistically significant ($p < 0.05$) as evaluated by Tukey's *post-hoc* test in the original paper.

5.2. Behavioral changes induced by the neurochemical effects of 4-methylaminorex

The behavioral effects of the 4-methylaminorex isomers are plotted in Figure 5.2. together with the neurochemical data and brain concentrations of the isomers. The behavioral data is scored according to the rating scale used. The rats were given a single behavioral score per each 20-min sampling interval as follows: 0, passive or long-lasting (>10 min) motionlessness; 1, active motionlessness; 2, active motionlessness with occasional rearing; 3, locomotor activity with bursts of rearing; 4, stereotyped behavior; 5, intense stereotyped behavior; 6, ataxia; 7, catatonia.

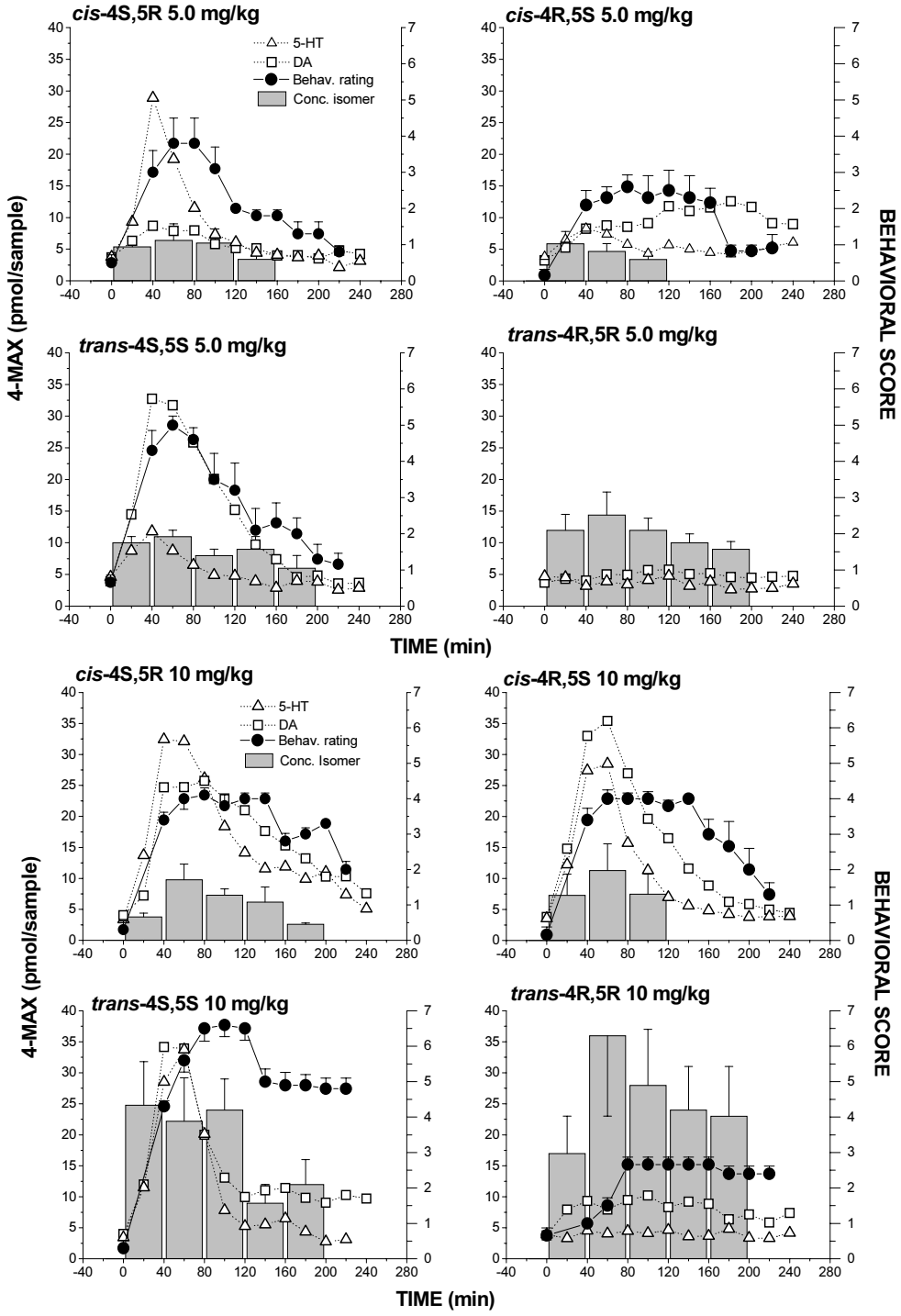

Figure 5.2. The behavioral effects of the 4-methylaminorex (4-MAX) isomers at doses of 5.0 and 10 mg/kg, together with neurochemical data and brain concentrations of the isomers. *Cis*-4S,5R-, *cis*-4R,5S-, *trans*-4S,5S-, and *trans*-4R,5R-4-methylaminorex were administered at 0 min. Data are given as means ± SEM (n = 6). The scaling of the neurochemical data corresponds to that of Figure 5.1 (0-1000% of the basal level). Behavioral scores following administration of each isomer at both doses differed statistically significantly ($p < 0.05$, Mann-Whitney U test) from those of control animals as evaluated in the original paper, with a following rank order of potency between isomers: *trans*-4S,5S > *cis*-4S,5R ≈ *cis*-4R,5S > *trans*-4R,5R. Although the isomer *trans*-4R,5R was the least potent, its concentrations were statistically significantly ($p < 0.05$, Tukey's test) higher than those of the cis-isomers in the dialysate samples.

In general, the means of the scores plotted in Figure 5.2 are self-explanatory, and they illustrate the behaviors induced by the isomers satisfactorily, but there are few points that need further consideration. Firstly, the behavior induced by both *cis*-isomers at a dose of 5.0 mg/kg can be described as a compulsive-like pattern of locomotor activity, rearing and stops. However, more detailed analysis revealed that administration of the isomer *cis*-4S,5R, but not *cis*-4R,5S, produces apractic movements and falling, indicative of ataxia, as well as circling and coprophagy, in addition to the common behavior described above. Due to these behaviors, *cis*-4S,5R-induced changes were scored higher than those induced by the isomer *cis*-4R,5S.

Secondly, all the isomers except *trans*-4R,5R induced a strong but transient peak in locomotor activity shortly after injection of the drug, which does not become evident from Figure 5.2. because locomotor activity data is not included, and the detailed analysis of rats' behavior began only 20 min after injection.

5.3. Effects of 5-HT_3 receptor blockade on psychostimulant-induced neurochemical and behavioral changes

S.c. administration of the 5-HT_3 receptor antagonist MDL 72222 did not affect the spontaneous release of dopamine, 5-HT or their metabolites at the doses used. Neither did it induce conditioned place preference or motor activity when administered alone.

Instead, all the psychostimulants tested, i.e. cocaine, mazindol, amphetamine and methylphenidate elevated extracellular dopamine levels significantly ($p < 0.05$) as evaluated by statistical tests in the original papers. The 5-HT levels were significantly elevated after administration of cocaine, mazindol and amphetamine whereas methylphenidate had no effect on 5-HT when compared with vehicle-treated rats. In addition, administration of amphetamine decreased extracellular levels of both DOPAC and HVA, and methylphenidate decreased the levels of DOPAC only, otherwise there were no statistically significant changes in the levels of the metabolites.

Similarly with their effects on dopamine, cocaine, mazindol and methylphenidate induced conditioned place preference and locomotor activity ($p < 0.05$) as revealed by statistical tests in original papers.

The effects of the 5-HT_3 receptor antagonist MDL 72222 on neurochemical and behavioral changes induced by the psychostimulant drugs are summarized in Table 5.2.

Table 5.2. Summary of the effects of 5-HT_3 receptor blockade on neurochemical and behavioral changes induced by psychostimulant drugs.

Treatment	Dopamine ⇈ NACC	Dopamine ⇈ Striatum	LMA ⇈	aCPP ⇈	eCPP ⇈
MDL + Amph	↓[a]	↓[a]	N.A.	N.A.	N.A.
MDL + Coc	↓[a,f]	↓[a]	↓	↓	-
MDL + Maz	↓[f]	N.A.	↓	↓	N.A
MDL + MP	-[f]	N.A.	-	-	N.A

The arrows indicate the changes induced by psychostimulant drugs alone (column headings) and effect of the 5-HT_3 receptor antagonist MDL 72222 (MDL) on these changes semiquantitatively, i.e. MDL attenuates, not prevents, for example the elevation of dopamine levels induced by amphetamine. The changes presented here with arrows have been found statistically significant ($p < 0.05$) as evaluated by statistical tests in the original papers; a dash (-) indicates no significant change. (Amph) amphetamine; (Coc) cocaine; (Maz) mazindol; (MP) methylphenidate; (NACC) nucleus accumbens; (LMA) motor activity; (aCPP) acquisition of conditioned place preference; (eCPP) expression of conditioned place preference; ([a]) microdialysis with anesthetized animals; ([f]) microdialysis with freely moving animals; (N.A) not assessed.

Although the main findings of the studies with the 5-HT_3 receptor antagonist are summarized in Table 5.2, it should be noted that although MDL 72222 attenuated the effects of both amphetamine and cocaine in paper III, a larger dose of MDL 72222 was needed to reduce amphetamine-induced elevation of extacellular dopamine levels in both nuclei studied.

6. DISCUSSION

6.1. Effects of amphetamine derivatives and 4-methylaminorex isomers on dopamine and 5-HT

As evidenced by this study, the hallucinogenic amphetamine derivative DOM did not induce any significant changes in the extracellular concentrations of either 5-HT or dopamine in the nucleus accumbens, whereas MDA and MDMA elevated the concentrations of both transmitters, and amphetamine elevated predominantly dopamine levels. These results are consistent with the *in vitro* comparison studies which show that DOM has no effect on 5-HT and dopamine release or uptake inhibition, while MDA and MDMA induce the release, or inhibit the uptake, of both 5-HT and dopamine (Nichols et al., 1982; Johnson et al., 1986; Steele et al., 1987; McKenna et al., 1991). Our results with amphetamine and methylenedioxyamphetamines are also in concert with earlier *in vivo* studies (Zetterström et al., 1983, 1986; Yamamoto & Spanos, 1988; Carboni et al., 1989a; Hiramatsu & Cho, 1990; Gough et al., 1991; Nash & Nichols, 1991). Since the results concerning the dopaminergic effects of the stimulative amphetamine derivatives are largely confirmatory, they are not further discussed here.

The doses of DOM used in this study were chosen on the basis of the potency of the drug: the reported ED_{50} value for DOM in drug discrimination studies is 0.44 mg/kg in the rat (Glennon et al., 1984), whereas that of MDA, for example, is 0.97 mg/kg (Nichols, 1986). Furthermore, DOM has been shown to produce hallucinogenic effects in humans at total doses of 5-10 mg (Snyder et al., 1967), while the recreational total doses of MDMA range from 60 to 250 mg (Peroutka, 1990). Thus, the ineffectiveness of DOM observed in this study is unlikely to result from inadequate dosing.

As stated in the Review of the Literature (Section 2.3.2) it is well established that hallucinogenic activity involves serotonergic, in particular $5\text{-}HT_2$ receptor, mediated mechanisms (Glennon et al., 1984; Titeler et al., 1988). Nevertheless, in our study DOM failed to affect the extracellular levels of 5-HT in the nucleus accumbens. Thus it seems that acute hallucinogenic activity is not reflected in increases or decreases of extracellular 5-HT

concentrations, but rather in some other mechanisms mediated by postsynaptic 5-HT receptors. In contrast MDA, which is also considered to possess hallucinogenic properties (Glennon, 1989, 1991), increased the extracellular 5-HT concentrations, similarly with MDMA that is presumably devoid of appreciable hallucinogenic effects (Glennon et al., 1982; Glennon & Young, 1984a, 1984b). The strong effects of MDA and MDMA on 5-HT concentrations indicates that their serotonergic mechanisms of action involve other components, in addition to, or instead of, those mediating hallucinogenic effects.

Similarly with MDA and MDMA, acute administration of the 4-methylaminorex isomers *trans*-4S,5S, *cis*-4S,5R and *cis*-4R,5S elevated extracellular levels of both 5-HT and dopamine in rat nucleus accumbens. In contrast, the effects of the isomer *trans*-4R,5R on both transmitters were negligible. The rank order for elevating 5-HT was *cis*-4S,5R > *trans*-4S,5S ≈ *cis*-4R,5S > *trans*-4R,5R, while for elevating extracellular dopamine *trans*-4S,5S > *cis*-4S,5R ≈ *cis*-4R,5S > *trans*-4R,5R. The latter is consistent with the preliminary microdialysis report of Eberle et al. (1992) and also with observations concerning the ability of the isomers to substitute S-amphetamine in a drug-discrimination paradigm (Glennon & Misenheimer, 1989b), to induce locomotor activation and stereotypies (Batsche et al., 1994), and to suppress firing of A10 dopamine cells (Ashby et al., 1995). Such a rank order can also be predicted from the established structure-activity relationships of amphetamine-related drugs (Glennon & Misenheimer, 1989b). Given the role of accumbal dopamine in the reinforcing and rewarding properties of drugs of abuse (cf. Wise & Bozarth, 1987), the isomer *trans*-4S,5S also seems to possess the strongest potential for abuse, and should thus be classified as a Schedule I substance.

The dopaminergic effects of the three most potent isomers of 4-methylaminorex - strong elevation of dopamine and decrease in DOPAC and HVA - resemble those of amphetamine (Zetterström et al., 1983, 1986). As is the case with amphetamine (see Review of the Literature, section 2.3.2), effects of these isomers on HVA paralleled their effects on DOPAC at low doses, but diverged at high doses, presumably as a result from dopamine metabolism shifting to the extraneuronal pathway, which can be interpreted to reflect dopamine uptake inhibition. Such dual mechanism is supported by observations of Ashby et al. (1995) who have shown that the suppressant effects of *trans*-4S,5S-4-methylaminorex on

A10 dopamine cells are attenuated by pretreating animals with α-methyl-p-tyrosine or reserpine, which deplete newly synthesized cytoplasmic dopamine and vesicular stores of monoamines, respectively (cf. Garattini & Samanin, 1981). Taken together, it appears that both cytoplasmic and vesicular dopamine mediate the effects of *trans*-4S,5S-, *cis*-4S,5R- and *cis*-4R,5S-4-methylaminorex, which implies an action mechanism involving both dopamine release and uptake inhibition.

The nearly equal elevation of extracellular levels of dopamine and 5-HT induced by the three most potent 4-methylaminorex isomers resembles the effects of MDMA. Since drug-induced release of endogenous dopamine is implicated in the development of MDMA-induced degeneration of serotonergic neurons (cf. Green et al., 1995), it can be speculated whether administration of the 4-methylaminorex isomers would similarly induce neurotoxic effects. In fact, there is evidence that administration of multiple doses of racemic *cis*-(±)-4-methylaminorex causes long-term (7-day) declines in striatal tryptophan hydroxylase activity, which may be indicative of neurotoxicity (Hanson et al., 1992).

The negligible effects of the isomer *trans*-4R,5R on extracellular levels of 5-HT and dopamine resemble the inefficacy of DOM. In addition, as hallucinogenic properties of DOM and some other amphetamine derivatives have been associated with R-configuration, it seems possible that the *trans*-4R,5R would have hallucinogenic activity. This aspect, however, remains to be elucidated.

6.2. Behavioral changes induced by the neurochemical effects of 4-methylaminorex

Acute administration of each 4-methylaminorex isomers induced locomotor activation, which, in the case of higher doses of the *cis*-isomers and the isomer *trans*-4S,5S, rapidly turned into stereotypic behavior maintained at one location, and often into ataxia or even catatonia following the highest dose of *trans*-4S,5S. When scored according to the rating scale used, the rank order of potency was *trans*-4S,5S > *cis*-4S,5R ≈ *cis*-4R,5S > *trans*-4R,5R, which matches that observed in the behavioral study by Batsche et al. (1994), as well as that observed when measuring dopaminergic parameters (present results; Eberle et al., 1992; Ashby et al., 1995). Furthermore, dopaminergic manipulations attenuate locomotor

activity induced by *trans*-4S,5S-4-methylaminorex (Batsche et al., 1994) and the ability of the same isomer to suppress basal firing rates of A10 dopamine cells (Ashby et al., 1995). Thus, it seems that dopaminergic mechanisms may mediate the behavioral effects of 4-methylaminorex.

Oral stereotypies such as gnawing or licking characteristic of high dose amphetamine-treatment (Creese & Iversen, 1973), however, were rarely seen after administration of the three most potent isomers at a dose of 10 mg/kg. Instead, behaviors observed included both lateral and vertical repetitive head movements, vigorous sniffing, forepaw treading, head-weaving, ataxia and/or catatonia, many of which resemble behaviors characteristic of the so-called 5-HT syndrome in the rat (Grahame-Smith, 1971; Green & Heal, 1985). Since the same isomers also had a marked effect on 5-HT in addition to dopamine, it seems that there may be serotonergic components in their behavioral effects, similarly with MDMA (Slikker et al., 1989; Spanos & Yamamoto, 1989). In the behavioral study of Batsche et al. (1994), however, serotonergic manipulations had no effect on locomotor activation induced by the isomer *trans*-4S,5S at a dose of 3 mg/kg. This results does not necessarily contradict ours, because in the present study we found the effect of a 5.0-mg/kg dose of the same isomer on 5-HT significantly smaller than its effect on dopamine, and the serotonergic-like behavioral effects were absent as well. Thus it seems possible that the 5-HT effect is masked by the strong dopaminergic effect induced by the isomer *trans*-4S,5S at this dose, or alternatively the serotonergic effect is not strong enough to induce perceivable behavioral changes. Instead, at the same 5.0-mg/kg dose the isomer *cis*-4S,5R, which produced apractic movements and falling indicative of ataxia, also affects 5-HT more profoundly than dopamine. Taken together, it can be suggested that the serotonergic-like behaviors appear only after very strong elevation of synaptic 5-HT-levels, or under conditions when serotonergic stimulation is more pronounced than dopaminergic stimulation. Furthermore, regarding the study of Batsche et al. (1994), serotonergic mechanisms may mediate behavioral effects other than locomotor activation, which appears to be of dopaminergic origin.

Although the behavioral, as well as neurochemical, effects of the isomer *trans*-4R,5R were not as pronounced as those of all the other isomers, the concentrations of both *trans*-isomers, especially the isomer *trans*-4R,5R, were higher than those of the *cis*-isomers in the

dialysate (Figure 5.2). The same phenomenon was also seen in brain tissue and plasma samples obtained from rats treated with i.p. injections of the 4-methylaminorex isomers in our earlier study (Kankaanpää et al., 2001a). These differences may be due to the rapid conversion of one isomer to another, but because there were no signs of *cis*-isomers after administration of *trans*-isomers or vice versa, this appears rather unlikely. In addition, because racemic 4-methylaminorex has been shown to be excreted predominantly as the parent compound (Henderson et al., 1995), massive conversion of some isomers to unknown metabolites is not a very plausible explanation either. Nevertheless, determination of the concentration of *cis*- and *trans*-4-methylaminorex revealed that the relative ineffectiveness of *trans*-4R,5R was not due to pharmacokinetic factors, since its concentrations in the samples were at least as high as those of any other isomers.

6.3. Effects of 5-HT_3 receptor blockade on psychostimulant-induced neurochemical and behavioral changes

The main finding of our series of experiments aimed at clarifying the role of 5-HT_3 receptors in mediating the effects of psychostimulant drugs is that pretreatment with the specific 5-HT_3 receptor antagonist MDL 72222 robustly attenuates the elevation of extracellular dopamine, acquisition of place preference, and motor activation induced by cocaine and mazindol, while the effects of methylphenidate remain unchanged. Our results concerning the attenuation of cocaine's effects by 5-HT_3 receptor blockade are consistent with earlier studies of Reith (1990), Suzuki et al. (1992), Svingos and Hitzemann (1992) and McNeish et al. (1993). They are further supported by our findings with mazindol, which has a neurochemical profile similar to that of cocaine. Thus together these results confirm that the 5-HT_3 receptor antagonist MDL 72222 is able to modulate the effects of psychostimulant drugs that nonselectively elevate extracellular concentrations of dopamine, 5-HT and norepinephrine.

5-HT_3 receptor blockade also attenuated amphetamine-induced elevation of extracellular dopamine levels, but only with a relatively high dose of MDL 72222. It must be noted, however, that amphetamine's interactions with 5-HT_3 receptor antagonist was assessed only

with microdialysis technique in anesthetized animals, and thus they cannot be discussed as extensively as those with other psychostimulants. Nevertheless, our results are consistent with findings that 5-HT_3 receptor antagonists reduce behavioral activation following intra-accumbens amphetamine or dopamine (Costall et al., 1987), but apparently not with those of Carboni and coworkers (1989c), who have reported that another 5-HT_3 receptor antagonist ICS 205-930 failed to inhibit the amphetamine-induced elevation of extracellular dopamine in the nucleus accumbens. The latter study, however, does not necessarily conflict with our findings, because the highest dose of ICS 205-930 was 30 μg/kg. The same dose of 30 μg/kg was also used as the highest dose in studies in which ICS 205-930 and MDL 72222 failed to attenuate conditioned place preference induced by amphetamine (Carboni et al., 1989b). Taken together, it seems that the action of amphetamine is more resistant to 5-HT_3 receptor antagonism than that of cocaine and mazindol.

In contrast, MDL 72222 failed to antagonize the methylphenidate-induced neurochemical and behavioral changes. In addition, methylphenidate was the only drug showing no effect on 5-HT levels, consistently with earlier studies (Ferris et al., 1972; Koe, 1976; Schweri et al., 1985; Kuczenski & Segal, 1997). Given the finding of Svingos and Hitzemann (1992) that 5-HT_3 receptor mediated antagonism of cocaine-induced locomotor activity is dependent on 5-HT, together with our results, it seems likely that the ability of a drug to elevate extracellular levels of 5-HT makes it susceptible to modulation by 5-HT_3 receptor antagonists. In addition, the elevation of dopamine in the medial prefrontal cortex induced by the selective 5-HT uptake inhibitor fluoxetine is attenuated by the 5-HT_3 receptor blockade (Tanda et al., 1995). Furthermore, several drugs of abuse such as MDMA, morphine, ethanol and nicotine, which are known to be sensitive to the 5-HT_3 receptor blockade (Carboni et al., 1989b,c; Bilsky and Reid, 1991), have also been reported to increase 5-HT levels (Ribeiro et al., 1993; Tao & Auerbach, 1995; Selim & Bradberry, 1996).

Dopamine release in the nucleus accumbens is shown to be enhanced by 5-HT_3 receptor agonists applied directly into the same brain structure (Jiang et al., 1990; Chen et al., 1991). This has led to a postulation that 5-HT_3 receptors in the nucleus accumbens are located presynaptically on dopaminergic nerve terminals. The effects of 5-HT_3 receptor antagonists given to drug-free animals, however, are negligible, as shown in our studies, as well as

numerous earlier studies (e.g. Costall et al., 1987; Koulu et al., 1990; Reith, 1990). This lack of effect may be related to the low endogenous tone within the serotonergic system coupled to these receptors, as suggested by Tricklebank (1993). Accordingly, the accumbal 5-HT_3 receptors would be activated due to increased synaptic 5-HT concentration following administration of cocaine, mazindol or amphetamine, which in turn would result in enhanced release of dopamine. Correspondingly, the blockade of 5-HT_3 receptors would attenuate but not completely abolish the drug-induced elevation of extracellular dopamine concentration, as seen in this study.

Unspecifically, MDL 72222 might interfere with the actions of psychostimulant drugs by binding to the dopamine transporter. Considering the close structural resemblance of cocaine and MDL 72222, which both carry the tropane-moiety, such an interaction might well occur. Nevertheless, because Svingos and Hitzemann (1992) have reported that neither ICS 205-903, another 5-HT_3 receptor antagonist with tropane-structure, nor zacopride interacts with cocaine at the dopamine transporter, this kind of interaction between cocaine and MDL 72222 does not seem very likely either.

As stated in the Review of the Literature (Section 2.4), activation of the dopaminergic neuronal system in mesocorticolimbic brain areas, including the nucleus accumbens, is thought to play a major role in both the reinforcing properties of abused drugs and the locomotor stimulation induced by them. Regarding the reinforcing properties of cocaine, however, there is evidence that mesolimbic brain areas other than the nucleus accumbens, may play even more important roles (see Section 2.4.2). Plenty of evidence exists, however, for the relevance of the nucleus accumbens in cocaine reinforcement; 6-OHDA lesions in the nucleus accumbens are shown to attenuate cocaine self-administration (Roberts et al., 1977; Pettit et al., 1984) and microinjections of cocaine directly in the shell region of the nucleus accumbens support self-administration (Carlezon et al., 1995; McKinzie et al., 1999; but see Goeders & Smith, 1983). Furthermore, local infusion of selective D_1 receptor antagonist SCH 23390 in the nucleus accumbens attenuated reinforcement induced by cocaine, as measured by place preference (Baker et al., 1998) and self-administration tests (Maldonado et al., 1993; Caine et al., 1995). Taken together, it seems most likely that extensive connections between the nucleus accumbens and other anatomically related brain nuclei may

be critically important for the reinforcing properties of cocaine.

Since, in the present study, MDL 72222 attenuated the cocaine- and mazindol-induced effects in accumbal microdialysis and in place preference and motor activity experiments, which together provide a fairly reliable means of assessment of drug reinforcement, it seems reasonable to assume that 5-HT_3 receptors are involved in the reinforcing properties of these drugs. In self-administration and drug discrimination tests, however, 5-HT_3 receptor antagonists have repeatedly failed to antagonize the effects of cocaine (Paris & Cunningham, 1991; Peltier & Schenk, 1991; Lane et al., 1992). These results do not, however, necessarily conflict with each other, because place preference tests and the self-administration tests quoted here measure somewhat different aspects of reinforcement. In the place preference test, the antagonist is typically administered together with the drug from the beginning of the experiment, i.e. the *acquisition* of conditioned response is subjected to receptor blockade, whereas in the self-administration test the rats have already learned to use the drug by the time the antagonist is administered. Temporally, the latter corresponds to the *expression* of the learned drug effect in the place preference paradigm. The importance of this difference is exemplified by our finding that acquisition, but not expression, of cocaine-induced place preference was attenuated by MDL 72222 -pretreatment. Thus, our results may in fact indicate that 5-HT_3 receptor blockade primarily affects the development of neurochemical changes leading to drug self-administration, rather than reverses any already established changes.

What is more difficult to explain, is that there are also studies in which 5-HT_3 receptor antagonists have no effect on elevation of extracellular dopamine, acquisition of place preference (Cervo et al., 1996), or locomotor activation (King et al., 1994; Lê et al., 1997) induced by cocaine. There is however, evidence that the effects of 5-HT_3 antagonist may be compound-specific, at least in memory- and learning-related tests (Bratt et al., 1994; Pitsikas & Borsini, 1997; Díez-Ariza et al., 1998). Thus it can be speculated whether the use of different compounds may have confused the results concerning the interactions of 5-HT_3 receptor antagonists and cocaine. Indeed, according to our knowledge MDL 72222 has attenuated the effects of cocaine in all studies (Suzuki et al., 1992; Svingos & Hitzemann, 1992) including ours, with the exception of the place preference experiment by Cervo et al.

(1996). In contrast, ondansetron (GR38032F) has generally failed to block the effects of cocaine (King et al., 1994; Cervo et al., 1996; Lê et al., 1997). Taken together, it appears that 5-HT_3 receptor antagonists may differ in their ability to antagonize the effects of cocaine, ondansetron being less effective than MDL 72222 at the doses used.

7. SUMMARY AND CONCLUSIONS

This study showed that the hallucinogenic amphetamine derivative DOM lacks any effect on 5-HT or dopamine in the nucleus accumbens, whereas MDA and MDMA strongly elevate extracellular levels of both transmitters. The 4-methylaminorex isomers *trans*-4S,5S, *cis*-4S,5R and *cis*-4R,5S elevated 5-HT and dopamine in the nucleus accumbens, and induced major behavioral changes, while the effects of the isomer *trans*-4R,5R were less profound. However, the dialysate concentrations of *trans*-4R,5R-4-methylaminorex were higher than those of the *cis*-isomers. Blockade of the 5-HT_3 receptors attenuated the dopaminergic and reinforcement-related effects of cocaine, mazindol and amphetamine while the effects induced by methylphenidate remained unchanged.

To conclude:

1) The inability of the hallucinogenic amphetamine derivative DOM to elevate 5-HT levels indicates that extracellular levels of 5-HT cannot be considered to reflect hallucinogenic activity. Consequently, the strong increase in synaptic 5-HT concentrations induced by MDA does not support its purported hallucinogenic activity, it rather resembles the effect of MDMA. The methylenedioxyamphetamines, but not DOM, may act via dopaminergic mechanisms similar to amphetamine.
2) The ability of the stereoisomers of 4-methylaminorex to elevate extracellular 5-HT- and dopamine-levels, as well as to induce motor activation, is associated with the S-configuration of the molecule. Behavioral effects of the three most potent 4-methylaminorex isomers, *trans*-4S,5S, *cis*-4S,5R, and *cis*-4R,5S, contain both dopaminergic- and serotonergic-like elements. The differences in potencies between the isomers are pharmacodynamic rather than pharmacokinetic.
3) 5-HT_3 receptors are involved in cocaine- and mazindol-induced neurochemical and behavioral effects, in particular the acquisition of conditioned reward. Instead, blockade of the 5-HT_3 receptors failed to modify the effects of methylphenidate, the only drug with no significant effect on the serotonergic system. Thus it is suggested that drug-induced serotonergic stimulation plays an important role in 5-HT_3 receptor mediated regulation of dopamine release.

8. ACKNOWLEDGMENTS

The present study was carried out in the Drug Research Unit, Department of Mental Health and Alcohol Research, National Public Health Institute. I am indebted to Professor Jussi Huttunen, the Director General of the Institute and Professor Jouko Lönnqvist, the Head of the Department of Mental Health and Alcohol Research, for the excellent facilities the institute has provided.

I express my sincerest gratitude to my supervisors, Docent Timo Seppälä and Professor Liisa Ahtee. Docent Seppälä gave me the privilege to concentrate on research work, and has been guiding, supporting and trusting me throughout the course of these studies. Professor Ahtee welcomed me to the Division of Pharmacology and Toxicology, Department of Pharmacy, University of Helsinki, to perform my postgraduate studies, and has been my teacher in the highly stimulating field of neuropharmacology. Furthermore, I wish to thank both of my supervisors for their valuable comments during preparation of this manuscript.

Professor Kalervo Kiianmaa and Docent Martti Attila offered constructive criticism on the manuscript and provided useful suggestions, which led to its improvement; to them I extend my sincere thanks. I am furthermore grateful to Ms Jennifer Kimpton (Moorebank, NSW, Australia) for the linguistic revision of the manuscript.

I am deeply grateful to my co-investigators who have all significantly contributed to this work. Dr Pirjo Lillsunde and Dr Minna Ruotsalainen introduced me to the world of microdialysis in the first place. More recently, M.Sc. Satu Ellermaa has been helping me a lot with her practical skills and knowledge on animal physiology. M.Sc. Paula Hirsjärvi has been our expert on animal behavior and welfare since the very beginning of these studies. Last but not least, I would like to especially acknowledge my fellow researcher Dr Esa Meririnne, who has always been there for a discussion, and has had both willingness and means to help, whether problem was statistical, scientific, or something else.

I owe my appreciation to Ms Riitta Tirkkonen-Vesikko and B.Sc. Miina Kajos for skillful technical assistance. Special thanks are due to Mr Kari Ariniemi, our chromatography specialist, who has shared his knowledge of the secrets of chromatography with me.

I also want to thank my friend Satu Ylä-Outinen (Tervonen) for giving me, more than ten

years ago, a hint of a nice summer job in the Laboratory of Pharmacology and Toxicology, National Public Health Institute. I got the job, which, as judged now, probably has had quite an impact on the course of my working career. Since those early days my responsibilities in the lab have changed, and the name of the lab as well, but most of the people, who taught me the very basics of laboratory work are still there, strengthened with new forces. Thus I want to extend my thanks for the entire staff at the Drug Research Unit and the Laboratory of Substance Abuse for helping me in so many ways, and for creating a unique down-to-earth and sociable work community, in which it is a pleasure to belong.

Finally, I owe my warmest thanks to my husband Harri, our daughter Hilla, and my parents Outi and Olli for their love, support, patience and encouragement. They have given me a Life.

Financial support from the Yrjö Jahnsson Foundation, Helsinki, and drug supplies from the National Institute on Drug Abuse (NIDA), US, are gratefully acknowledged.

Helsinki, June 2002

Aino Kankaanpää

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