

REWARDING PROPERTIES OF PSYCHOMOTOR STIMULANTS AND MORPHINE:  
PHARMACOLOGICAL MODULATION OF THEIR CONDITIONING OR  
SENSITIZATION IN RATS

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## CONTENTS

ABSTRACT.....	5
LIST OF ORIGINAL PUBLICATIONS.....	6
ABBREVIATIONS.....	7
DEFINITIONS RELATED TO FREQUENTLY USED TERMS.....	8
1. INTRODUCTION.....	9
2. REVIEW OF THE LITERATURE.....	10
2.1 Behavioral processes underlying addiction.....	10
2.2 Experimental animal methods used in studying addiction.....	12
2.2.1 Models for the assessment of reward.....	12
2.2.2 Other methods.....	15
2.3 Brain neurotransmitter systems known as reward pathways.....	16
2.3.1 Dopaminergic system: the ascending mesolimbic and mesocortical pathways.....	17
2.3.2 Mesolimbic system as part of the reward-related neuronal circuits.....	20
2.4 Reward mechanisms of psychomotor stimulants with references to conditioning; cocaine, amphetamine, methylphenidate and mazindol.....	21
2.4.1 Dopaminergic system in the reward mechanisms of psychomotor stimulants.....	22
2.4.2 Modulation of the reward mechanisms of psychomotor stimulants by the $\gamma$ -aminobutyric acid-ergic system.....	24
2.4.3 Modulation of the reward mechanisms of psychomotor stimulants by the serotonergic system.....	26
2.5 Reward mechanisms of opiates with references to conditioning; morphine.....	30
2.5.1 Involvement of potassium channels in opioid receptor function.....	32
2.6 Sensitization phenomenon with emphasis on psychomotor stimulants.....	33
2.6.1 Involvement of the dopaminergic system.....	35
3. AIMS OF THE STUDY.....	38
4. MATERIALS AND METHODS.....	39
4.1 Animals.....	39
4.2 Conditioned place preference (studies I-IV).....	39
4.3 Motor activity measurements in acute experiments (studies II and IV).....	40
4.4 Microdialysis (study II).....	41
4.5 Psychomotor stimulant-induced withdrawal (study III).....	41
4.6 Conditioned taste aversion (study III).....	42
4.7 Drugs.....	42
4.8 Treatments.....	42
4.8.1 Effects of $\gamma$ -aminobutyric acid A receptor agonists on cocaine- and amphetamine-induced place preference (study I).....	42
4.8.2 Effects of a serotonin <sub>3</sub> receptor antagonist on place preference, dopamine increase and motor activity induced by cocaine, mazindol and methylphenidate (study II).....	43
4.8.3 Rewarding properties of methylphenidate: sensitization by prior exposure to the drug and the effects of dopamine <sub>1</sub> and dopamine <sub>2</sub> receptor antagonists (study III).....	44

4.8.4	Effects of unselective potassium channel blockers on place preference and changes in motor activity induced by morphine (study IV).....	45
4.9	Statistics.....	46
5.	RESULTS.....	48
5.1	Effects of $\gamma$ -aminobutyric acid A receptor agonists on cocaine- and amphetamine-induced place preference (study I).....	50
5.2	Effects of a serotonin <sub>3</sub> receptor antagonist on place preference, dopamine increase and motor activity induced by cocaine, mazindol and methylphenidate (study II).....	50
5.3	Rewarding properties of methylphenidate: sensitization by prior exposure to the drug and the effects of dopamine <sub>1</sub> and dopamine <sub>2</sub> receptor antagonists (study III).....	51
5.4	Effects of unselective potassium channel blockers on place preference and changes in motor activity induced by morphine (study IV).....	52
6.	DISCUSSION.....	53
6.1	Assessing the rewarding properties of the drugs and neurochemical mechanisms involved: the conditioned place preference method, dopamine levels in the nucleus accumbens and motor activity.....	53
6.1.1	Rewarding properties of mazindol.....	54
6.1.2	Rewarding properties of benzodiazepines.....	55
6.1.3	Rewarding properties of raclopride.....	56
6.2	Reward mechanisms of psychomotor stimulants and morphine with references to conditioning.....	57
6.2.1	Psychomotor stimulants and the involvement of the $\gamma$ -aminobutyric acid A receptors.....	57
6.2.2	Psychomotor stimulants and the involvement of serotonin <sub>3</sub> receptors.....	58
6.2.3	Psychomotor stimulants and the involvement of dopamine <sub>1</sub> and dopamine <sub>2</sub> receptors.....	60
6.2.4	Morphine and the involvement of potassium channels.....	61
6.3	Sensitization to the rewarding properties of psychomotor stimulants.....	62
6.3.1	Involvement of dopamine <sub>1</sub> and dopamine <sub>2</sub> receptors.....	62
6.4	Clinical implications.....	63
7.	CONCLUSIONS.....	66
8.	ACKNOWLEDGMENTS.....	67
9.	REFERENCES.....	68

## ABSTRACT

Psychomotor stimulants such as cocaine or amphetamine, and opiates such as morphine are notorious for their abuse potential. In the brain, the dopaminergic system in the nucleus accumbens and related areas is considered pivotal for mediating the rewarding properties of drugs, i.e. the properties of drugs that would reflect their abuse potential. The conditioning and sensitization phenomena are powerful modulators of addictive behavior, yet their neurochemical mechanisms are only partly understood. The present experiments were designed to evaluate the involvement of selected neuronal mediators in conditioning or sensitization of psychomotor stimulant- and morphine-reward. In the experiments the conditioned place preference method in rats was employed, since it provides a suitable means for assessing conditioning or sensitization of the reward. When appropriate, this was supplemented by accumbal dopamine (DA) and motor stimulation measurements.

The results show that conditioned reward of psychomotor stimulants, which activate the DA-ergic system directly, can be modulated by altering the  $\gamma$ -aminobutyric acid (GABA) A or serotonin(5-HT)<sub>3</sub> receptor mediated neurotransmission, although in a somewhat restricted manner. Conditioning of cocaine- and amphetamine-reward was prevented by a GABA A receptor agonist, diazepam, but not by another GABA A agonist, zolpidem, that has a distinguishable binding profile from that of diazepam. Thus, only a distinct subgroup of GABA A receptors appears to be responsible for the effect. Conditioning of cocaine- and mazindol-reward was likewise attenuated by a 5-HT<sub>3</sub> receptor antagonist, but only in animals that had not yet established the conditioned effect. Also psychomotor stimulant-induced increase in accumbal DA levels or motor stimulation were attenuated by the 5-HT<sub>3</sub> antagonism. Instead, the 5-HT<sub>3</sub> antagonism failed to attenuate the effects of methylphenidate, which unlike cocaine or mazindol does not enhance 5-HT-ergic neurotransmission, thus suggesting that the primary mechanisms of action psychomotor stimulants can significantly determine the roles of various regulatory processes.

When administered more chronically, methylphenidate's rewarding properties were sensitized. This sensitization was prevented by DA receptor antagonists, indicating the involvement of the DA-ergic system. Although methylphenidate is generally considered to possess relatively low abuse potential, our results suggest that repeated methylphenidate intake may enhance the vulnerability for abuse of methylphenidate and possibly other related drugs.

Unlike psychomotor stimulants, morphine and other opiates act via opioid receptors that are known to be linked to potassium (K<sup>+</sup>) channels. Quinine but not 4-aminopyridine, both unselective K<sup>+</sup> channel blockers, attenuated conditioned morphine-reward. The reason for the disparate result remains to be elucidated, but it might be attributable to differences in binding profiles of the two blockers. This is the first report to implicate the involvement of K<sup>+</sup> channels in morphine-reward, warranting further studies with more selective drugs.

In conclusion, the results show that, besides the brain DA-ergic system, also other neuronal systems can participate in regulatory control over the conditioning of psychomotor stimulant- or morphine-reward. Furthermore, the DA-ergic system also appears to be implicated in the sensitization to rewarding properties. These findings provide information on neuronal mechanisms involved in conditioning and sensitization of drug-reward, that may eventually contribute to the development of novel pharmacological approaches for the treatment of addiction.

## LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following publications, herein referred to by Roman numerals:

- I Meririnne E, Kankaanpää A, Lillsunde P, Seppälä T: The effects of diazepam and zolpidem on cocaine- and amphetamine-induced place preference. *Pharmacology, Biochemistry and Behavior* 62: 159-164, 1999.
- II Kankaanpää A, Meririnne E, Seppälä T: 5-HT<sub>3</sub> receptor antagonist MDL 72222 attenuates cocaine- and mazindol, but not methylphenidate-induced neurochemical and behavioral effects in the rat. *Psychopharmacology* 159: 341-350, 2002.
- III Meririnne E, Kankaanpää A, Seppälä T: Rewarding properties of methylphenidate: sensitization by prior exposure to the drug and effects of dopamine D<sub>1</sub>- and D<sub>2</sub>-receptor antagonists. *Journal of Pharmacology and Experimental Therapeutics* 298: 539-550, 2001.
- IV Meririnne E, Kankaanpää A, Vanakoski J, Lillsunde P, Seppälä T: The effects of quinine and 4-aminopyridine on conditioned place preference and changes in motor activity induced by morphine in rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 23: 713-730, 1999.

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## ABBREVIATIONS

ANOVA	analysis of variance
ANCOVA	analysis of covariance
AUC	area under the curve
CNS	central nervous system
COMT	catechol- <i>O</i> -methyltransferase
DA	dopamine
DAT	dopamine uptake transporter protein
DOPAC	3,4-dihydroxyphenylacetic acid
GABA	$\gamma$ -aminobutyric acid
5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine, serotonin
HPA	hypothalamus-pituitary-adrenal
HVA	homovanillic acid
i.p.	intraperitoneally
L-DOPA	L-dihydroxyphenylalanine
LH	lateral hypothalamus
MAO	monoamine oxidase
NAC	nucleus accumbens
PFC	prefrontal cortex
PTN	pedunculopontine tegmental nucleus
s.c.	subcutaneously
SNC	substantia nigra pars compacta
VP	ventral pallidum
VTA	ventral tegmental area

## DEFINITIONS RELATED TO FREQUENTLY USED TERMS

The scientific field of studying addiction is often complicated by using related terminology in an inconsistent manner. To avoid this, herein is provided a short glossary for the most vital terms used in the present study:

**Abuse** - drug intake for non-medical purposes in humans in a way that the drug interferes with the person's life, but not to the extent of addiction (dependence) (Kaplan et al. 1994);

**Addiction** - a disorder in humans that can be defined as a compulsive use with a loss of control over drug intake and narrowing of the behavioral repertoire toward excessive drug intake (Koob and Le Moal 1997). Somewhat related is the clinically used term substance dependence that may include also symptoms of tolerance and withdrawal (Kaplan et al. 1994);

**Conditioning** - a process, in which a primary property of a drug is associated with originally neutral stimuli (Cunningham 1993). Acquisition describes the process while the primary property is being associated with the stimuli, and expression describes the process when the stimuli alone can induce a response related to the primary property. For the sake of clarity, the term conditioning or conditioned reward etc. when used in the context of the place preference method (see section 2.2.1) describes the acquisition of conditioning, unless otherwise stated;

**Psychomotor stimulant** - The term describes a class of drugs that activate the central nervous system with simultaneous increase in motor activity, with amphetamine and cocaine being two prototypes (Wise and Bozarth 1987);

**Reinforcement** - a term often used in the context of the self-administration method in laboratory animals (see section 2.2.1). Refers to the ability of certain events to strengthen preceding stimulus-response associations (Feldman 1997). Positive or negative reinforcers are stimuli that increase the frequency of behavior that leads to or prevents, respectively, its presentation (Stolerman 1992).

**Reward** - a term that is used in the general literature in many different ways, sometimes even only as a synonym for pleasure. Throughout the present text, however, the term reward is used in a wider sense to describe any appetitive or motivational nature of a drug that can elicit approach behavior (Carr et al. 1989, Bardo and Bevins 2000), thus possibly reflecting addictive properties of the drug;

**Sensitization** - a process, in which repeated drug administration enhances a response to subsequent drug administration (Robinson and Berridge 1993).

## 1. INTRODUCTION

Human beings have been known to be susceptible to the intake of various intoxicants throughout the course of history. Nowadays, among such substances, psychomotor stimulants such as cocaine and amphetamine, or opiates such as heroin and morphine are particularly notorious for their abuse potential. Their repeated intake can result in a severe form of addiction that is disappointingly resistant to current treatment options. In order to understand the nature of addiction and to provide novel treatment strategies it is essential to comprehend behavioral elements and neurochemical mechanisms contributing to the development and maintenance of addictive behavior. In the past, addiction was interpreted simply as a desire avoiding physical withdrawal symptoms, but as knowledge has been gradually accumulated it has become clear that the addiction results from a more complex web of various behavioral elements. Two of the important elements appear to be the phenomena of conditioning and sensitization. They are particularly suggested to play a role in the development and subsequent relapse for addictive behavior (Robinson and Berridge 1993, Berridge and Robinson 1998, Di Chiara 1998, Di Chiara 1999, Robinson and Berridge 2000).

In the brain, the mesocorticolimbic DA-ergic system is considered to be particularly important in mediating the rewarding properties of psychomotor stimulants and morphine. This brain system, which projects from the ventral tegmental area (VTA) to the nucleus accumbens (NAC) and prefrontal cortex (PFC), intricately interplays with other regulatory neurotransmitters such as the GABA-ergic and 5-HT-ergic systems. Psychomotor stimulants can activate this system directly by releasing DA or inhibiting its re-uptake, whereas morphine acts via opioid-receptors that are linked to K<sup>+</sup> channels. The DA-ergic system is also important for the conditioning of reward, but data regarding roles of other neuronal mediators are more scarce or inconsistent. Similarly neurochemical mechanisms mediating sensitization to reward are only incompletely understood. Thus, the present series of experiments was addressed to evaluating roles of selected neuronal mediators, that are only partly understood at present, in conditioning or sensitization of the rewarding properties of psychomotor stimulants or morphine, by means of the conditioned place preference method in rats. As the name implies, the conditioning phenomenon is a key determinant for establishing place preference (Tzschentke 1998, Bardo 2000), and thus the method may be particularly suitable for studying the neuropharmacology of conditioned drug-reward. The method is also readily applicable to studying sensitization to drug-reward.

## 2. REVIEW OF THE LITERATURE

### 2.1 Behavioral processes underlying addiction

**Negative reinforcement.** By referring to opiate addiction, major emphasis was earlier put on physiological (physical) dependence as a main factor in drug dependence (Himmelsbach 1943). In this context, physiological dependence is understood as a need to use drugs repeatedly in order to avoid or alleviate physical withdrawal symptoms resulting from abrupt cessation of chronic high-dose drug-intake. Such withdrawal symptoms may include cramps, sweating, nausea, convulsions etc. This negative reinforcement, i.e. repeated drug use resulting from a desire to suppress physiological withdrawal symptoms, most probably plays a role in drug addiction. Yet the negative reinforcement model may not exclusively account for the addiction phenomenon. This assumption is based on several facts: an addictive drug can be self-administered in the absence of withdrawal symptoms (Ternes et al. 1985), it is generally known there is high tendency for relapse even after an extended period of abstinence when withdrawal symptoms have subsided, and the relief of withdrawal symptoms is only marginally effective in the treatment of addiction (see Wise and Bozarth 1987 p. 470 for references). Moreover, many therapeutic drugs such as antidepressants may produce withdrawal symptoms upon cessation of treatment, although they are not typically abused for non-medical purposes (Haddad 1999). More recently, the hypothesis has moved from physiological dependence toward a psychological one, in which emphasis is placed on a negative affective state and dysregulation of hedonic homeostasis resulting from abstinence after chronic drug intake as the motivational factors in addiction (Koob and Le Moal 1997, Koob et al. 1997).

**Positive reinforcement.** The term positive reinforcement is originally used to define a behavioral phenomenon in which the presentation of a stimulus increases the probability of behavior leading to the subsequent presentation of the stimulus. This hypothesis postulates that abused drugs are self-administered because of a state of mind they induce, which is often assumed to be euphoria and positive affect, but not because they alleviate an unpleasant state (Stewart et al. 1984, Wise and Bozarth 1987 p. 474, Wise 1988). Thus, addiction has been seen as a compulsive pleasure-seeking state (Bejerot 1980). Drug-induced euphoria is apparently involved in the development of addiction, but perhaps may not alone adequately account for addictive behavior. The fact that abused drugs often produce pleasurable effects, explains little about what is the force driving an addict to compulsively seek pleasure (Robinson and Berridge 1993).

**Role of conditioning.** By definition, classical or Pavlovian conditioning refers to a learning process, in which unconditioned stimuli (e.g drug-induced hypothermia) is associated with a conditioned stimuli (e.g specific environment) leading subsequently to a situation, in which the conditioned stimuli alone without the unconditioned stimuli can evoke conditioned response (e.g. hypothermia induced by the specific environment without the drug; Cunningham 1993). Accordingly, the acquisition and expression phases can be separated; in the acquisition phase a unconditioned stimulus is presented along with a conditioned stimulus allowing the associative learning to take place, and in the expression phase the conditioned response is assessed without the unconditioned stimulus. Furthermore, a separate type of conditioning, operant conditioning, can be distinguished according to the type of conditioned

response. Typically in operant conditioning the response is actively directed to modifying the accessibility of the unconditioned stimulus, while in classical conditioning it is not (e.g. conditioned hypothermia) (Stolerman 1992).

The conditioning phenomenon is considered to play an important role in the development and maintenance of drug addiction, particularly in drug-craving and reinstatement of drug-seeking behavior (Stewart et al. 1984, Wise and Bozarth 1987, Robinson and Berridge 1993, Berridge and Robinson 1998, Di Chiara 1998, Di Chiara 1999). The precise characteristics of the role of conditioning, however, are a subject of some debate. Some authors have suggested withdrawal-like symptoms evoked by conditioned environmental stimuli to provide a motivational source for drug-taking behavior (Siegel 1988). Other researchers have considered conditioned drug-like effects, supposedly euphoric and pleasurable, to be more important (Stewart et al. 1984). It is important to note that subjective and the motivational effects of drugs might be dissociable. In the study of Lamb et al. (1991) the authors found that opiate 'postaddicts' would work for an injection of low dose morphine, despite the fact that four of five subjects could not distinguish the subjective effects of morphine from those of placebo. Therefore, more recent theories have placed emphasis on motivational conditioning (Di Chiara 1998, Di Chiara 1999), or incentive salience attribution (a term referring to a psychological process in which perceived, originally neutral, stimuli are attributed with emphasis and importance; Robinson and Berridge 1993). That is, through associative learning activated by drugs and their euphoric effects, also the motivational aspects involved are conditioned, and hence the conditioned response might only include pathological 'wanting' without necessarily 'liking'.

**Role of sensitization and tolerance.** The term sensitization defines a phenomenon, in which repeated use of a drug results in an augmented response to the subsequent administration of the drug. This sensitization is contrasted with tolerance, in which repeated drug use leads to an attenuation of drug-induced response. For instance, in animals the repeated intermittent injections of a drug may induce sensitization, whereas continuous infusion of the drug typically results in tolerance (Robinson and Becker 1986). In humans tolerance is a well recognized phenomenon, but less data are available on the occurrence of the sensitization phenomenon. Most evidence for sensitization in humans has come from studies concerning with increased sensitivity for psychotic symptoms after repeated exposure to psychomotor stimulants (Sato et al. 1983). More recently, however, it has been shown that sensitization to the psychomotor stimulant effects of amphetamine can be induced in humans under laboratory conditions: motor activity/energy ratings, mood, rate and amount of speech, and eye-blink rates were shown to be significantly enhanced by repeated amphetamine administration (Strakowski et al. 1996, Strakowski and Sax 1998). While in humans tolerance might be reflected as a need to increase dose to achieve the same effect during repeated drug-intake, sensitization phenomenon is suggested to be of particular significance in predisposing an individual to relapse and reinstatement of drug-seeking behavior. Due to sensitization the motivational factors, linked to drug-related stimuli by associative learning, could be abnormally enhanced in an incremental fashion by every drug-intake, thus resulting in uncontrollable craving for drugs (Robinson and Berridge 1993, Di Chiara 1998, Robinson and Berridge 2000).

**Summary.** The different processes mentioned above are by no means mutually exclusive. That is, the avoidance of distress, pleasure seeking, conditioning phenomenon, as well as sensitization and tolerance, most probably each play some role in drug addiction. Which of them is emphasized may vary depending on drug of abuse, state of addiction, personal mental characteristics etc. In addition, other factors that are not usually considered in biological approaches such as the availability of drugs, peer pressure, an individual's values related to drug abuse derived from friends and parents, genetic susceptibility etc. also influence drug addiction. Taken together, it appears that there are several factors with varying emphasis affecting the development of addiction with the most common factor being the addictive properties of abused drugs.

## 2.2 Experimental animal methods used in studying addiction

There are number of animal models using different approaches for assessing the addictive properties of drugs, but due to diversity in methodology they appear to measure different aspects of drug-reward instead of a single common factor.

### 2.2.1 Models for the assessment of reward

**Conditioned place preference.** In the conditioned place preference method the rewarding properties of drugs, as well as other stimuli, are evaluated by means of conditioning (Carr et al. 1989, Hoffman 1989, Tzschentke 1998). Virtually all drugs known to possess addictive properties in humans can induce place preference in this method, thereby reflecting their rewarding properties. The effect is most prominent with readily abused drugs such as cocaine, amphetamine and heroin, but also other psychomotor stimulants and opiates, as well as nicotine, ethanol, tetra-9-hydrocannabinol, LSD, benzodiazepines, gamma-hydroxybutyrate, phencyclidine, testosterone are able to induce place preference (Schechter and Calcagnetti 1993, Tzschentke 1998). In addition, also naturally rewarding stimuli such as food, water, saccharin or sucrose solutions, sexual attraction and ejaculation, social behavior, and even in some cases aggressive behavior, have been reported to induce place preference (Schechter and Calcagnetti 1993, Tzschentke 1998).

In the conditioned place preference method rats are conditioned to associate a distinct environment with the effects of a drug; if they show a preference for that drug-associated environment after conditioning, it is interpreted as the drug having rewarding properties. The test is conducted in an apparatus, which is usually rectangular in shape, divided into two compartments by a separating wall with a doorway, although the number of compartments or the shape of the apparatus can vary. The compartments can be distinguished from each other on the basis of different lighting, color, texture, odor etc. Typical apparatus and conditioning procedure are presented in Figure 2.1 and Table 2.1, respectively. There are two main alternative methods for conducting place preference experiments. In the biased method, animals show an initial preference for one compartment before conditioning; during conditioning drug-effects can be then associated with initially either a preferred or less-preferred compartment. In the unbiased method this initial preference is absent, and consequently drug-effects are associated in a balanced fashion with both preferred and less-preferred compartments. Furthermore, the procedure differentiates the acquisition and expression phases of conditioning (Table 2.1; see section 2.1). For the sake of clarity, in the

context of the place preference method ‘conditioning’ refers to the acquisition phase unless otherwise stated.

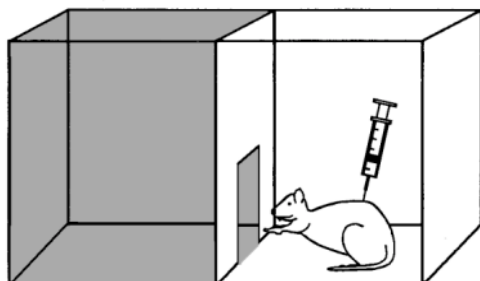


FIGURE 2.1. Schematic drawing illustrating a test apparatus for conditioned place preference experiments. Modified from Watson et al. (1989).

There are certain advantages in the conditioned place preference method (Carr et al. 1989, Schechter and Calcagnetti 1993). It is a relatively simple and inexpensive method, and no surgical operations are necessary. In addition to drugs, also the rewarding properties of non-pharmacological stimuli can be assessed. After conditioning the animals are tested in a drug-free state, thus minimizing the interference of acute motor effects as well as any other effects of the drug. Importantly, this applies also to experiments that assess the effects of antagonists on drug-induced place preference. Further, besides rewarding properties, the same method can also be used to measure the aversive properties of drugs and non-pharmacological stimuli. As with most behavioral methods, it also has some disadvantages (Carr et al. 1989, Schechter and Calcagnetti 1993, Tzschentke 1998). Disruption in memory processes by drugs may affect the results. In particular, drug-induced disturbance in habituation for the drug-paired compartment can be speculated to result falsely in novelty-induced place

TABLE 2.1. Schematic presentation of a typical conditioning procedure in the conditioned place preference method.

	Days	Doorway	Procedural events of the phase
Preconditioning phase	1 to 3	open	Rats are daily allowed to move freely between compartments for a period of time. On the last day times spent in the compartments are measured (basal value; preconditioning time).
Conditioning phase (Acquisition of conditioning)	3 to 8	closed	After a drug-injection(s) animals are restricted to a predetermined compartment (drug-paired compartment) for a period of time.  After a vehicle-injection(s) animals are restricted to the opposite compartment (vehicle-paired compartment) for a period of time.  These drug/vehicle-compartment pairings can be conducted once or twice a day.
Postconditioning phase (Expression of conditioning)	1	open	Rats are allowed to move freely between the compartments for a period of time. Time spent in the drug-paired compartment is measured (postconditioning time).

preference. Also anxiolytic effects of drugs may lead to errors in the biased method. Finally, the role of state dependent learning can not be totally excluded, although the evidence suggest that it does not significantly contribute to place preference (Carr et al. 1989).

Although the detailed mechanisms are not fully understood, it is generally assumed that in the method the primary rewarding properties of a drug (unconditioned stimulus) are attached to originally neutral environmental stimuli (conditioned stimulus) through associative learning. As a result, these environmental stimuli acquire secondary rewarding properties, and subsequently they will be able to elicit approach behavior (conditioned response) when the animal is exposed to them without the effect of the drug (Carr et al. 1989, Hoffman 1989, Tzschentke 1998, Bardo and Bevins 2000). Thus, for the development of place preference, the conditioning phenomenon is a key determinant, which appears to follow mainly (but not exclusively) the principles of classical conditioning (Tzschentke 1998, Bardo and Bevins 2000). The resulting approach behavior evoked by reward-associated stimuli may be relevant in terms of the mechanisms by which drug-associated cues come to maintain drug-taking and elicit craving. Besides assessment of the conditioned rewarding properties of drugs as such, the conditioned place preference method has been suggested to be a suitable model to study the neuropharmacology of drug-reward (Hoffman 1989), and to test non-addictive drugs for anti-craving activity (Tzschentke 1998).

**Self-administration.** Self-administration is probably the most direct method for the assessment of the positively reinforcing properties of drugs. It is based on operant responding; that is, in this method animals are trained to perform a task, such as lever pressing, in order to get a drug that is usually administered intravenously, although other routes of administration have also been successfully employed (Stolerman 1992, Meisch and Lemaire 1993). Thus, in the context of self-administration a drug can be considered positively reinforcing when its administration activates the behavior resulting in a subsequent administration of the drug. The self-administration method is particularly versatile for assessments of various reward-related behaviors (Markou et al. 1993, Shalev et al. 2002); some of them such as the progressive-ratio paradigm can be understood to provide a measure that reflects both unconditioned and conditioned reinforcing properties of a drug, whereas others such as the conditioned reinforcement paradigm, are more specifically concerned with the conditioning aspect of drug-reinforcement.

Practically all drugs abused by humans are self-administered by animals in this method, and its validity is regarded to be high (Markou et al. 1993). Notably, there appears to be a general correlation between the results of self-administration and conditioned place preference methods; most drugs that are self-administered are also able to induce place preference and *vice versa* (Carr et al. 1989), yet the neural mechanisms mediating the two behavioral phenomena may not be identical (Bardo and Bevins 2000). The self-administration method, however, also possesses some disadvantages: the execution of an experiment is technically demanding, and sometimes interpretation of obtained data can be problematic (Meisch and Lemaire 1993).

**Intracranial self-stimulation.** Intracranial self-stimulation can be used as an indirect method for the assessment of the rewarding properties of abused drugs. In this method electrodes are placed in a brain area during surgery, and after recovery the animals are trained to stimulate

these electrodes by pressing a lever. In certain brain areas, such as the medial forebrain bundle including the lateral hypothalamus (LH), the stimulation of electrodes is apparently reinforcing; animals can repeatedly stimulate themselves up to exhaustion while ignoring food or drink. Several drugs abused by humans, when administered under such conditions, can enhance or facilitate the response of this self-stimulation, which is typically interpreted to reflect the rewarding effects of drugs (Lewis 1993).

### 2.2.2 Other methods

**Drug discrimination.** In the drug discrimination method animals are trained to determine whether the intrinsic stimuli induced by a drug is present or absent. Typically, the response of the animal is to press a certain level when the training drug is injected, and to press another level when a placebo is injected. It is believed that studying the discriminative properties of abused drugs may be of value in understanding drug abuse in humans. This is based on the assumptions that the effects of an abused drug apparent as discriminative stimulus are closely related to their euphoriant and ‘subjective’ effects in humans, and that these effects might be important determinants of the extent to which the drug will be abused. Particularly, the abuse potential of new compounds can be compared to that of known abused drugs by means of discriminative stimulus. The lack of a discriminative stimulus similar to that of a commonly abused drug may indicate that the compound is unlikely to be abused to the extent of the drug (Goudie and Leathley 1993). The discriminative stimulus method can also be used for *in vivo* assessment of the pharmacological mechanisms involved in drug-induced effects (Goudie and Leathley 1993).

**Locomotor activity.** The term ‘locomotor’ means movement from one location to another, and there is a diversity of methods to the measure locomotor activity of animals. The rationale for measuring locomotor activity while assessing drug-reward lies in the putative relationship between locomotor stimulating effects and reinforcing properties of abused drugs as suggested by Wise and Bozarth (1987). The psychomotor stimulant theory of addiction holds that all addictive drugs have locomotor stimulating effects, the stimulating effects of these drugs have a shared biological mechanism, and the biological mechanism of these stimulating effects is homologous to the biological mechanism of positive reinforcement (Wise and Bozarth 1987, Wise 1988). It should be not surprising that although there are parallelisms between drug-induced locomotor stimulation and reinforcement, changes in locomotor activity does not necessarily fully reflect changes in drug-reinforcement, as they are two separate behavioral phenomena (Watson et al. 1989). Despite this, however, measuring drug-induced locomotor activity may provide a useful additional means of studying drug-reward that is inexpensive and simple to conduct. In particular, measuring locomotor activity has been widely used in studies assessing the sensitization phenomenon induced by the repeated administration of abused drugs.

**Microdialysis.** The *in vivo* microdialysis technique allows the measurement of extracellular neurotransmitter concentrations in the brain of freely moving animals. In fact, the microdialysis technique can be performed locally for almost every organ and tissue of the body. There are several modifications of the method, but basically the procedure includes placing a microdialysis probe in a brain area such as the NAC or striatum. The microdialysis probe is then perfused with artificial cerebrospinal fluid or buffer solution; substances in

extracellular fluid diffuse according to a concentration gradient across the membrane of the probe into the perfusion fluid, which is then collected and analyzed for neurotransmitters of interest with, for instance, HPLC using an electrochemical detector. The technique is quite sensitive to certain changes in experimental conditions. For instance, changes in temperature, the flow rate or chemical properties of perfusion fluid can profoundly affect the results (Robinson and Justice 1991), and hence the experimental system used needs to be carefully tested prior to running experiments. Concentrations measured in the perfusion fluid do not fully correspond to actual concentrations in the extracellular fluid, and quite delicate methods have been developed to correct these discrepancies (Robinson and Justice 1991). Fortunately, usually there is no need to know the actual concentrations in the extracellular fluid; in most of studies an assessment of relative change in measured concentrations induced by a treatment is adequate (Westerink 1995).

The microdialysis technique has been widely used to assess the effects of abused drugs alone and in combination with other drugs on neurotransmitter concentrations in selected brain areas. General interest has been focused particularly on the DA system of the NAC, as this brain area may play a substantial role in drug addiction. A great number of microdialysis studies have dealt with neurochemical correlates during self-stimulation and drug self-administration (Westerink 1995), and these have provided important information on the neurochemical basis of reward-related behavior.

### 2.3 Brain neurotransmitter systems known as reward pathways

Despite the fact that abused drugs have many distinct actions, their rewarding actions appear to have a common denominator; namely, similar effects in the brain mechanisms of reward. Our current understanding of the brain circuitry through which various rewards gain control over behavior has developed from the early intracranial brain stimulation studies of Olds and Milner (1954), whose initial finding was that rats would return to places where they received stimulation of the septal area. Subsequent studies indicated that the most sensitive sites for intracranial self-stimulation were along medial forebrain bundle, a complex neural system involving major ascending monoaminergic pathways, descending fibers from reward sites in various limbic structures, and a network of intrinsic neurons with dendrites radiating to the sensory and motor projection systems (Lewis 1993). Ever since, advancements in neurobiological research have identified specific subunits and neurochemical components of the brain reward system. Currently, it is believed that there are a number of receptor systems, neuronal pathways and brain regions involved to varying extents. An important part of this system are the mesolimbic and mesocortical DA-ergic pathways with their extensive connections to related brain regions and neurotransmitter systems (Koob 1992, Wise 1996, Bardo 1998, Wise 1998, McBride et al. 1999). This brain circuitry is considered to have originally evolved for mediating actions of natural rewards such as food, drink and sexual contact, and thus many drugs of abuse seem to pathologically mimic natural rewards in this respect (Di Chiara et al. 1993, Di Chiara 1998, Di Chiara 1999).

### 2.3.1 Dopaminergic system: the ascending mesolimbic and mesocortical pathways

**Neuroanatomy.** Major ascending DA-ergic pathways originate from cell bodies in the brainstem, mainly in the substantia nigra pars compacta (SNc, A9 area) and the VTA (A10 area).

*Nigrostriatal pathway.* The neurons from the SNc project through the internal capsule mainly to the striatum, thus forming the nigrostriatal DA-ergic system (Figure 2.2; Fuxe et al. 1985), which controls motor behavior and muscle tonus.

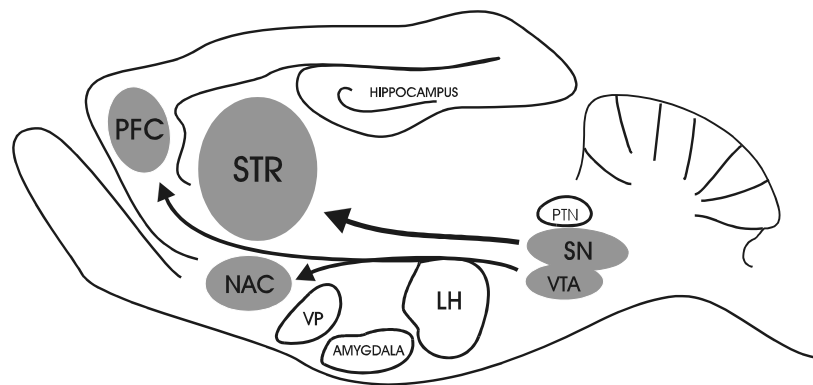


FIGURE 2.2. Simplified presentation of a sagittal rat brain section illustrating the nigrostriatal dopaminergic pathway (arrow from the substantia nigra to the striatum), and the mesolimbic and mesocortical dopaminergic pathways (arrows from the ventral tegmental area to the nucleus accumbens or prefrontal cortex, respectively). SN: substantia nigra, STR: striatum, NAC: nucleus accumbens, VTA: ventral tegmental area, PFC: prefrontal cortex, LH: lateral hypothalamus, VP: ventral pallidum, PTN: pedunculopontine tegmental nucleus.

*Mesolimbic pathway.* The mesolimbic DA-ergic system consists of neurons that originate from the VTA, travel through the medial forebrain bundle, and innervate the NAC, olfactory tubercle, and other limbic area such as the amygdala, hippocampus and septum (Figures 2.2 and 2.3; Fuxe et al. 1985). The mesolimbic system is considered to play a role in the control of motivation, emotion, and motor behavior. The system is also important in mediating the rewarding properties of abused drugs (Figure 2.3). The NAC receives the strongest projections of the neurons from the VTA, and a particular role for the NAC in reward-related behavior has been suggested (see below). Anatomically the NAC is comprised of subterritories, of these the ventromedial shell and dorsolateral core appear to be dominant. The shell, which receives afferents from subcortical and brainstem structures, exhibits greater neuroanatomical diversity than the core. The shell sends outputs to the core via the feed-forward striatopallido-thalamocortico-striatal pathway, but it also strongly innervates the lateral preoptico-lateral hypothalamic continuum and VTA (Zahm 1999). Both the shell and the core area appear to be implicated in reward-related behavior, yet they may subserve different roles (Zahm 1999, Parkinson et al. 1999).

*Mesocortical pathway.* The neurons forming the mesocortical DA-ergic system originates also from the VTA, and they travel to cortical areas including the PFC, and the entorhinal and cingulate cortices (Figures 2.2 and 2.3; Fuxe et al. 1985, Cooper 1996). This brain system is involved in control of higher cognitive functions. The PFC has also been implicated in drug-reward (Figure 2.3); chronic intake of an abused drug is suggested to produce DA-ergic hypofunction in the PFC that would underlie impulsivity and loss of control of drug-seeking behavior (Jentsch and Taylor 1999).

*Other pathways.* In addition to these major ascending systems, there are also other DA-ergic systems that are shorter in length, such as the tuberoinfundibular DA-ergic system connecting the pituitary gland and the median eminence of the hypothalamus (Cooper et al. 1996), but they are probably not so intensively involved in mediating the rewarding properties of abused drugs.

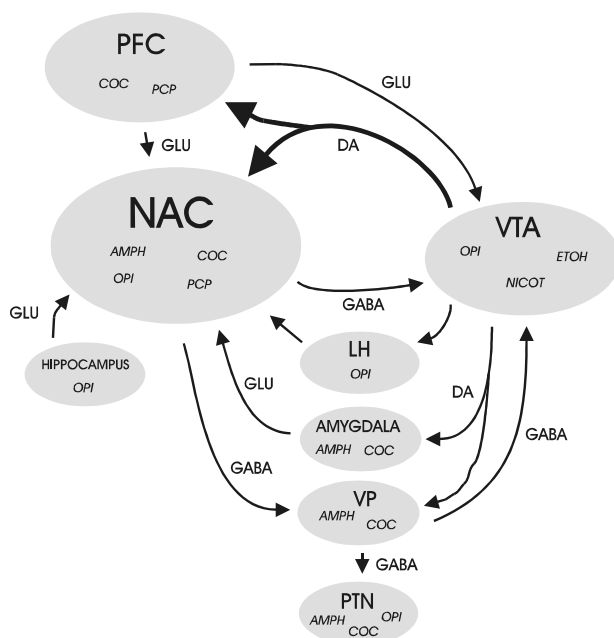


FIGURE 2.3. Simplified schematic presentation of reward-related brain areas, and their interconnections of different neurotransmitters. Abbreviation of a drug of abuse within a brain area indicates the involvement of the brain area in mediating the drug's rewarding properties. NAC: nucleus accumbens, VTA: ventral tegmental area, PFC: prefrontal cortex, LH: lateral hypothalamus, VP: ventral pallidum, PTN: pedunculo-pontine tegmental nucleus. DA: dopamine, GABA:  $\gamma$ -aminobutyric acid, GLU: glutamate. COC: cocaine, AMPH: amphetamine, OPI: opiates, ETOH: ethanol, NICOT: nicotine, PCP: phencyclidine. Modified from Bardo (1998) and McBride et al. (1999).

**DA synthesis and metabolism.** DA synthesis (Cooper et al. 1996) starts from the amino acid tyrosine, which is transported across the blood-brain barrier, and then taken up into the DA neuron. The rate-limiting step in DA synthesis once tyrosine has gained entry into the neuron is the conversion of L-tyrosine to L-dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine hydroxylase. Subsequently, L-DOPA is then converted to DA by L-aromatic amino acid decarboxylase so rapidly that endogenous L-DOPA levels in the brain are negligible. DA is released from storage vesicles following an action potential. Once released the amount of DA in the synaptic cleft is regulated primarily by transporting it back to the nerve terminal by the DA uptake transporter protein (DAT). Dopamine metabolism (Cooper et al. 1996) can occur both outside and inside the DA-ergic neurons. DA that is not taken up is metabolized outside the DA-ergic cells by glial catechol-*O*-methyltransferase (COMT) to 3-methoxytyramine, which is subsequently converted to homovanillic acid (HVA) by

monoamine oxidase (MAO; Westerink 1985). Synthesized or taken up intracellular DA that is not stored in vesicles is metabolised by MAO to 3,4-dihydroxyphenylacetic acid (DOPAC), which then diffuses out of the cell and can be conjugated to glucuronides or further metabolized to HVA by COMT (Westerink 1985). In rat the brain, DOPAC is the major end-product of DA metabolism, while in the human brain the major end-product is HVA.

**DA receptors.** DA receptors are divided between the DA1- and DA2-like receptor families. DA1-like receptors are comprised of DA1 and DA5, and DA2-like receptors DA2, DA3 and DA4 receptor subtypes. The receptors can be differentiated on the basis of biochemical functioning, cellular location, and distribution profile over the brain (Cooper et al. 1996). DA1-like receptors, but not DA2-like receptors, activate the adenylate cyclase of the second messenger system. Both DA1 and DA5 receptors are located postsynaptically, where they regulate the activity of non-DA-ergic neurons. However, DA2 receptors can be found postsynaptically, or as autoreceptors on the soma, somatodendrite or terminal area of an axon regulating the activity of the DA neuron. The two other subtypes of DA2-like receptors, DA3 and DA4 receptors, are also present postsynaptically, but they are expressed to a lesser extent as autoreceptors. Along the mesolimbic and mesocortical pathways and their related brain areas in the central nervous system (CNS), DA1 receptors are enriched in the NAC, olfactory tubercle, and amygdala, but they can be found also in the cortex, hippocampus and hypothalamus. In comparison, DA5 receptors are rather discretely distributed: they are found only in the hippocampus, thalamus and hypothalamus. Postsynaptic DA2 receptors are primarily located in the NAC and olfactory tubercle, and to a certain degree in the amygdala, cortex, hippocampus and hypothalamus. DA2 autoreceptors, on the other hand, are most abundant in the VTA, in which virtually no DA1 receptors can be detected. The pattern of distribution of DA3 and DA4 receptors, both postsynaptic and autoreceptors, somewhat follows that of DA2 receptors. In particular, DA3 receptors are as abundant as DA2 receptors in the NAC.

**Role of mesolimbic DA in reward-related behavior.** Although the mesolimbic DA-ergic system is thought to participate in mediating the rewarding properties of abused drugs, there is disagreement to some degree about the exact nature of this brain system in the process. Historically, mesolimbic DA transmission particularly in the NAC with outputs to pallidal motor pathways has been linked to regulating behavioral activation evoked by emotions and motivational stimuli (Watson et al. 1989). One of the first proposals of a unique relationship between DA and reward was provided by Wise, who suggested in the anhedonia hypothesis that brain DA systems mediate the pleasure produced by rewarding stimuli; a suggestion based on the assumption that observed neuroleptic-induced blockade of the rewarding properties of drugs or food would result from a decrease in the pleasure, i.e. anhedonia (Wise et al. 1978, Wise 1982). Accordingly, more recently some authors have proposed that the suppression of mesolimbic DA may be involved in mediating anhedonia resulting from exposure to uncontrollable aversive experiences (Cabib and Puglisi-Allegra 1996), and that a decrease in food-induced DA transmission in the NAC could actually serve as a biochemical marker for anhedonia (Di Chiara and Tanda 1997). In line with this view, the suppression of the mesolimbic DA-ergic system during withdrawal from abused drugs is suggested to contribute to anhedonia and a negative affective state during withdrawal (Weiss et al. 1992, Koob et al. 1997).

However, also other suggestions for the role of DA have been put forward. According to the incentive-sensitization theory, the mesolimbic DA mediates the attribution of incentive salience to the neural presentations of reward-related stimuli (Robinson and Berridge 1993, Berridge and Robinson 1998). This means that during the DA-mediated process the reward-related stimuli are attributed with emphasis and importance in order to make them attractive and 'wanted'. This is an unconscious process; only the product of this process, the perception of the object as 'wanted', would be experienced and interpreted consciously. Notably, incentive salience would be a distinct component of motivation and reward, and hence the DA systems are necessary for 'wanting' but not 'liking' (Robinson and Berridge 1993, Berridge and Robinson 1998). Apparently somewhat compatible with the role of DA in mediating incentive salience is the view that naturally DA (not restricted solely to the mesolimbic system) codes errors in reward predictability (Hollerman and Schultz 1998, Schultz 1998). An unexpected reward elicits a strong positive DA signal, this declines with repeated presentation and learning, in such a manner that the presentation of predicted reward eventually no longer elicits a DA response. By contrast, the omission of a predicted reward leads to suppression of the DA signal. Thus, the DA signal appears to label a stimuli with appetitive value, predict and detect rewards and signal alerting and motivational events (Schultz 1998). A hypothesis formulated by Di Chiara also attributes the DA-ergic system in the NAC to reward-related associative learning processes, both for the acquisition of classical conditioning and the expression of conditioned response (Di Chiara 1998, Di Chiara 1999). This view distinguishes associative learning due to DA-ergic activation induced by naturally rewarding stimuli from abnormal associative learning following pathological DA activation induced by abused drugs. An activation of the DA-ergic system in the NAC shell area by naturally rewarding stimuli is characterized by habituation; in contrast, drugs of abuse have non-habituating effects resulting in nonadaptive or even sensitized DA release after repeated drug intake. These neurochemical consequences of abused drugs are thought to strengthen reward-related associative learning processes, thus constituting the basis of addictive behavior (Di Chiara 1998, Di Chiara 1999).

### 2.3.2 Mesolimbic system as part of the reward-related neuronal circuits

Despite the relative importance of the mesolimbic DA-ergic system, other brain areas are also known to provide extensive interconnections with the mesolimbic system, thus forming a complex interacting neural net which has been implicated in modulating the rewarding properties of abused drugs.

The ventral pallidum (VP; ventral part of the globus pallidus) serves as a major anatomical target for GABA-ergic neural information coursing from the NAC, but there is also a direct DA projection from the VTA to the VP (Figure 2.3; Watson et al. 1989, McBride et al. 1999). From the VP some information is relayed back to the VTA, as well as diffusively to other midbrain, thalamic, and limbic structures (Bardo 1998). Although the GABA-ergic NAC-VP projection is proposed to exclusively process locomotor stimulating actions (Gong et al. 1997a), the involvement of the VP may be of particular significance in mediating the rewarding properties of the psychomotor stimulants (Figure 2.3; Bardo 1998, McBride et al. 1999).

Another brain area implicated in psychomotor stimulant-reward is the amygdala, which receives DA-ergic inputs from the VTA and sends glutamatergic outputs to the NAC (Figure 2.3; Watson et al. 1989, Bardo 1998). The exact role of the amygdala remains to be elucidated, but it has been suggested that the amygdala could exert particular, yet complex, control over associative learning processes including classical conditioning, in conjunction with the related brain area NAC (Everitt et al. 1999), and that DA-ergic elements within the amygdala might modulate (amphetamine) reward, while non-DA-ergic elements might be more generally involved in learning processes (Bardo 1998).

Along with the amygdala, primarily glutamatergic projections from the hippocampus provide important input for the NAC (Watson et al. 1989, Bardo 1998). The hippocampus appears to be particularly implicated in opiate-reward (Figure 2.3), the most robust area being the CA3 region that is rich in opiate receptors and endogenous opiate peptides (Wise 1996, Bardo 1998, McBride 1999).

The pedunculopontine tegmental nucleus (PTN) is hypothesized to serve as an important interface between the NAC-VP system and the basal ganglia, recruiting reward-relevant information from the NAC-VP system in order to activate behavioral response in motor-related areas (Bardo 1998). Both psychomotor stimulant- and opiate-reward appears to involve the PTN (Figure 2.3; Wise 1996, Bardo 1998), and it has been proposed to function more in the acquisition than in the maintenance of drug-rewarded behavior (Bardo 1998).

A brain area known to be involved in a number of motivated behaviors, including feeding, drinking and sexual behavior, is the hypothalamus. Particularly well-known is the reinforcing properties of electrical stimulation in the LH (Lewis 1993), which receives inputs from the NAC and send outputs to the VTA (Figure 2.3; Watson et al. 1989, Bardo 1998). DA within the LH is supposed to provide tonic inhibition of excitatory output to the mesolimbic system (McBride et al. 1999). However as far as drugs of abuse are concerned, the role of the LH may be primarily restricted to opiate-reward (Figure 2.3; Bardo 1998, McBride 1999).

In addition, not surprisingly also the involvement of other brain structures beyond the ones cited above has been proposed. These areas include the central gray, the dorsal reticular formation and the area postrema, but how exactly these structures contribute to or parallel the brain reward mechanisms remains to be elucidated.

#### 2.4 Reward mechanisms of psychomotor stimulants with references to conditioning; cocaine, amphetamine, methylphenidate and mazindol

Cocaine and amphetamine are the prototypes of psychomotor stimulant drugs; they are particularly well recognized for their notorious addiction potential, and therefore the use of cocaine and amphetamine for therapeutic purposes as a local anaesthetic or for the treatment of hyperactivity disorder, respectively, is restricted to selected cases. The chemical structure of methylphenidate is related to that of amphetamine, and likewise also methylphenidate is used therapeutically for the treatment of hyperactivity disorder and narcolepsy. Mazindol also possesses properties characteristic for a psychomotor stimulant, and it is most often used as an anorectic agent.

Despite belonging to the same class of psychomotor stimulants, there are distinctive features in their mechanism of action between cocaine, amphetamine, methylphenidate and mazindol. Cocaine binds to a specific site at the DAT; this prevents the DAT from functioning and thus DA is not taken up from the synaptic cleft resulting in increased extracellular levels of DA (White and Wolfe 1991). In addition, cocaine blocks the uptake of 5-HT and noradrenaline (Koe 1976). Amphetamine also binds to the DAT, although to a site separate from that of cocaine. Amphetamine can prevent DA uptake, but it also allows intracellular DA to diffuse out through the DAT, and usually it is considered as a DA releaser (White and Wolfe 1991). Furthermore, amphetamine is also able to prevent DA metabolism by inhibiting MAO (White and Wolfe 1991). Similar to cocaine, amphetamine can also increase the extracellular levels of 5-HT and noradrenaline (Kuczenski and Segal 1997, Kankaanpää et al. 1998). Although the chemical structure of methylphenidate bears some resemblance to that of amphetamine, its mechanism of action includes blocking the DAT similarly to cocaine (White and Wolfe 1991). Methylphenidate also increases the extracellular levels of noradrenaline, but unlike cocaine or amphetamine it does not affect the 5-HT-ergic system (Koe 1976, Kuczenski and Segal 1997, Segal and Kuczenski 1999). Mazindol has a distinct chemical structure, but its neurochemical profile is nevertheless quite similar to that of cocaine: mazindol blocks the uptake of DA, 5-HT and noradrenaline (Koe 1976). Regardless of the differences in the neurochemical profiles of these psychomotor stimulants, they appear to exert comparable behavioral profiles in animals; at lower doses locomotor activity prevails, but as the dose increase stereotyped behaviors begin to emerge, turning into more exclusive stereotyped behavior with increasing doses (Babbini et al. 1977, Rang et al. 1999).

#### 2.4.1 Dopaminergic system in the reward mechanisms of psychomotor stimulants

**DA receptors and the rewarding properties of cocaine and amphetamine.** An early study of Yokel and Wise (1975) showed that an unselective DA receptor antagonist pimozide administered systemically altered level pressing for amphetamine in a manner consistent with reward reduction. Ever since a substantial amount of evidence for the role of DA receptors in psychomotor stimulant-reward has been accumulating. For instance, DA1 and DA2 receptor antagonists have attenuated the reinforcing properties of cocaine or amphetamine in the self-administration method (Caine and Koob 1994, Phillips et al. 1994b, Fletcher 1998), consistent with the findings that DA1-like and DA2-like agonists can be self-administered (Self and Stein 1992, Caine et al. 1999). In particular, the conditioning of reward appears to be modifiable by DA1 and DA2 receptor ligands (Beninger and Miller 1998). Both DA1 and DA2 receptor antagonists have attenuated cocaine-induced operant responding for a cocaine-associated stimulus (Weissenborn et al. 1996), or amphetamine-induced enhancement of responding for conditioned food-reward (Ranaldi and Beninger 1993). Also conditioned cue-induced relapse to cocaine self-administration has been attenuated by DA1 and DA2 receptor antagonists (Shalev et al. 2002). Furthermore, in the place preference method, DA1 receptor antagonist have repeatedly attenuated the rewarding properties of amphetamine, cocaine, and a number of other psychomotor stimulants (Tzschentke 1998), although this is contrasted by the finding that cocaine can induce place preference in DA1 receptor knockout mice (Miner et al. 1995). Also DA2 receptor antagonists attenuated place preference induced by amphetamine, but for reasons still to be elucidated they have been generally ineffective in modifying cocaine-induced place preference (Tzschentke 1998).

Taken together, the evidence indicates that DA1 receptors are involved in conditioning of psychomotor stimulant-reward, although this view is somewhat compromised by the finding with knockout mice. Instead, DA2 receptors appear to mediate only amphetamine-, but not cocaine-induced place preference, which suggests that some differences may exist between the two psychomotor stimulants in the neurochemical processes related to the conditioning of their reward.

**NAC and the rewarding properties of cocaine and amphetamine.** One of the major brain areas involved in the rewarding properties of cocaine and particularly of amphetamine appears to be the NAC (Bardo 1998, McBride et al. 1999). Amphetamine is readily self-administered to the NAC (Hoebel et al. 1983), and DA-ergic lesions in the NAC or intra-accumbal infusions of DA receptor antagonists attenuate the rewarding properties of amphetamine in the self-administration model (Lyness et al. 1979, Phillips et al. 1994a, Phillips et al. 1994b). For cocaine, however, the data is somewhat less parallel. Originally, in an early study of Goeders and Smith (1983) cocaine was not found to be self-administered into the NAC, which led to the suggestion that the NAC may not initiate cocaine-reward. More recent studies, however, have shown that cocaine can be self-administered to the shell area of the NAC (McKinzie et al. 1999). Furthermore, DA-ergic lesions in the NAC, as well as intra-accumbal infusions of DA receptor antagonists, attenuate the rewarding properties of self-administered cocaine (Roberts et al. 1977, Phillips et al. 1994a). Considering reward-conditioning in particular, intra-accumbal amphetamine has repeatedly shown to induce place preference (Carr and White 1986, Schiltein et al. 1998), and intra-accumbal DA-ergic lesions or DA receptor antagonists have attenuated amphetamine-induced place preference (Spyraki et al. 1982a, Hiroi and White 1991a). Furthermore, recent studies have shown that intra-accumbal amphetamine can increase responding for conditioned food-reward (Fletcher et al. 1998), and a excitotoxic lesion in the NAC core impairs expression of the appetitive Pavlovian approach behavior (Parkinson et al. 1999). While accumbal DA appear to be significant for conditioned amphetamine-reward, this may not be extendable to cocaine. Neither cocaine infusions into the NAC induced place preference nor DA-ergic lesions in the NAC attenuated place preference induced by systemic cocaine (Spyraki et al. 1982b, Hemby et al. 1992). Also DA2 receptor antagonists in the NAC have failed to affect cocaine-induced place preference (Kaddis et al. 1995, Baker et al. 1996).

Taken together, it appears that the DA-ergic system in the NAC mediates various reward-related behaviors of amphetamine, as well as primary cocaine-reward as measured by the self-administration method, but this may not be extendable to the conditioning of cocaine-reward as measured by the conditioned place preference method. It needs to be noted, however, that the DA1 receptor antagonist SCH 23390 injected into the NAC prevented cocaine-induced place preference (Baker et al. 1998), and hence the involvement of the accumbal DA-ergic system can not be entirely ignored in mediating conditioned cocaine-reward.

**DA-ergic system in other brain nuclei and the rewarding properties of cocaine and amphetamine.** Besides the NAC, also other related brain areas appear to contribute to the rewarding properties of psychomotor stimulants. For example, cocaine is self-administered into the PFC (Goeders and Smith 1983, Goeders and Smith 1986, Goeders et al. 1986). The involvement of the DA-ergic system is emphasized by the findings that DA-ergic

manipulations in the PFC can modulate cocaine self-administration (Goeders and Smith 1983, Goeders and Smith 1986, McGregor and Roberts 1995). On the other hand, amphetamine injected into the PFC does not support self-administration or conditioned place preference, and DA-ergic lesions in the brain area do not affect amphetamine self-administration (Carr and White 1986, Goeders et al. 1986, Leccese and Lyness 1987).

Considering the secondary conditioned reward of psychomotor stimulants as measured by the place preference method, the VP which receives a major input from the NAC appears to be of particular interest. Unlike the NAC, both cocaine and amphetamine can induce place preference when injected directly into the VP (Gong et al. 1996), and DA-ergic lesions in the VP attenuates cocaine-induced place preference (Gong et al. 1997b). Furthermore, responding for conditioned food-reward can be enhanced by injecting amphetamine into the VP (Fletcher et al. 1998).

Finally, given the importance of the amygdala in the associative learning processes of appetitive stimuli (Everitt et al. 1999), not unexpectedly, evidence exist for its involvement in mediating conditioned psychomotor stimulant-reward. Both cocaine- and amphetamine-induced place preferences can be attenuated by amygdaloid lesions (Hiroi and White 1991b, Brown and Fibiger 1993), and intra-amygdaloid amphetamine can induce place preference (O'Dell et al. 1999) or enhance stimulus-reward association (Hitchcott et al. 1997). Furthermore, the amygdala has been suggested to be involved in conditioned cue-induced relapse to cocaine seeking, and a recent study indicates that it is the amygdaloid DA1 receptor not the DA2 receptor that mediates responding for cocaine-paired stimuli (See et al. 2001, Shalev et al. 2002).

**DA in the rewarding properties of methylphenidate and mazindol.** In contrast to the two aforementioned prototypes of psychomotor stimulants, considerably less is known about the neurobiology of the rewarding properties of other psychomotor stimulants such as methylphenidate and mazindol, which are known to possess rewarding properties (Martin-Iverson et al. 1985, Gevaerd and Takahashi 1996, Kaminski et al. 1996). Although the DA-ergic system is apparently involved in several behavioral responses to methylphenidate, somewhat unexpectedly various DA-ergic manipulations, such as 6-hydroxyDA lesions, DA2 antagonists or gene-mediated disruption of DAT, have failed to affect methylphenidate-induced place preference (Martin-Iverson et al. 1985, Mithani et al. 1986, Sora et al. 1998). Furthermore, there are virtually no studies which are concerned with the reward-related mechanisms of mazindol. Thus, the clarification of the neuronal reward-related mechanisms of these psychomotor stimulants awaits further studies, particularly since neuronal mechanisms contributing to rewarding properties of various psychomotor stimulants are not necessarily identical, as exemplified by the differences between cocaine and amphetamine.

#### 2.4.2 Modulation of the reward mechanisms of psychomotor stimulants by the $\gamma$ -aminobutyric acid-ergic system

GABA is the major inhibitory neurotransmitter ubiquitously present in the brain (Cooper et al. 1996, Rang et al. 1999), but in other tissues GABA occurs only in trace amounts. It has been estimated that GABA serves as a transmitter at about 30 % of all the synapses in the brain. GABA is mainly found in short local interneurons that form synapses with neurons

containing other mediators such as classic transmitters DA, 5-HT, acetylcholine. In the rat, the corpora quadrigemina and diencephalic regions are particularly rich in GABA. The precursor for GABA synthesis is glutamate, which is turned into GABA by the enzyme glutamic acid decarboxylase; synthesized GABA is metabolized mainly by the enzyme GABA transaminase into succinic semialdehyde.

**GABA receptors.** GABA acts primarily by binding to GABA receptors, although physiologically relevant binding occurs also at the GABA transporter protein and enzymes involved in GABA metabolism (Cooper et al. 1996). Some debate have existed over the nomenclature of GABA receptors, but they can be simplistically divided into three classes: GABA A, B and C receptors (Barnard et al. 1998, Bormann 2000), of which GABA A receptors are the most prevalent in the CNS.

The activation of GABA A receptors results in the opening of chloride channels, thereby causing hyperpolarization and inhibiting the firing of the neuron. GABA A receptors possess modulatory binding sites for drugs of different classes such as benzodiazepines, barbiturates, neurosteroids, ethanol (Bormann 2000). For example, benzodiazepines as diazepam and flunitrazepam do not usually have intrinsic activity for GABA A receptors, but they enhance the action of GABA by increasing the frequency of chloride channel opening and their bursting (Barnard et al. 1998). Structurally GABA A receptors are pentameric molecules that in the CNS are most often formed by  $\alpha$ ,  $\beta$  and  $\gamma$  subunits, yet other combinations exist which accommodate in special ways the other subunits types  $\delta$ ,  $\epsilon$ ,  $\pi$  and  $\rho$ . As these various subunits additionally have different forms (for instance,  $\alpha 1$ - $\alpha 6$ ,  $\beta 1$ - $\beta 4$ ,  $\gamma 1$ - $\gamma 3$ ), theoretically there are several combinations of GABA A receptors which can be assembled. Although only a more limited number of these has been actually identified, still the GABA A receptor can form several subgroups (Barnard et al. 1998). The diversity of GABA A receptors is illustrated by the findings that the binding profile of benzodiazepines can be differentiated from that of certain non-benzodiazepine drugs such as zolpidem, despite their using the same binding site at the GABA A receptor (Niddam et al. 1987, Sanger et al. 1994, Barnard et al. 1998). Receptors showing only low or intermediate affinity for zolpidem are mostly located in limbic structures such as the NAC and certain parts of the hippocampus and hypothalamus, as well as in the striatum, spinal cord and entorhinal cortex (Niddam et al. 1987, Benavides et al. 1993, Sanger et al. 1994). More recently it has been shown that particularly receptors made up of  $\alpha 1$   $\beta 1, 2$  or  $3$   $\gamma 3$  and  $\alpha 5$   $\beta 1$  or  $3$   $\gamma 2$  subunits are insensitive to zolpidem (Barnard et al. 1998). Thus, GABA A receptors appear not to form a single entity, but instead represent a form of structural diverseness. Currently, however, the functional roles and pharmacological implications of these multiple subtypes are incompletely understood.

Another important GABA receptor type is the GABA B receptor, which seems to be coupled to  $Ca^{2+}$  and  $K^{+}$  channels via the second messenger system; GABA B receptors are located both pre- and postsynaptically, and they can be activated by a specific agonist, baclofen, that results presynaptically in attenuating the release of neurotransmitters and postsynaptically in an inhibitory potential (IPSP; Cooper et al. 1996). Finally there are GABA C receptors, which are distinguishable from two other GABA receptors by their insensitivity to baclofen and to a competitive GABA A receptor antagonist, bicuculline. GABA C receptors are enriched in the vertebrate retina, and they are also insensitive to GABA A modulatory drugs such as benzodiazepines, imidazopyridines, barbiturates and neurosteroids (Bormann 2000).

**GABA receptors and reward.** Widely distributed GABA A receptors can also be found in reward-related brain areas (Churchill et al. 1990, Zhang et al. 1991, Churchill et al. 1992, Gruen et al. 1999). Co-localization of inhibitory GABA A receptors with DA neurons is suggested by the findings that DA-ergic neurotransmission can be modified by drugs binding to GABA A receptors when administered locally into psychomotor stimulant reward-related brain areas such as the NAC, VTA, PFC, VP and the amygdala (Zetterström and Fillenz 1990, Finlay et al. 1995, Chu and Lin 1996, Westerink et al. 1996, Giorgetti et al. 1998, Gong et al. 1998, Westerink et al. 1998, Xi and Stein 1998). The role of GABA A receptors in the regulation of DA neurons is usually of an inhibitory nature. In some cases, however, also an excitatory effect can be observed possibly due to an indirect disinhibition, i.e. inhibition of inhibitory GABA-ergic interneurons (Xi and Stein 1998). Nevertheless, both systemic and local administration of benzodiazepines that enhance GABA A receptor activity, reduce the extracellular DA levels in the NAC and PFC (Zetterström and Fillenz 1990, Invernizzi et al. 1991, Finlay et al. 1992, Finlay et al. 1995). In particular, cocaine-induced elevations in accumbal DA levels can be attenuated by benzodiazepine-enhanced GABA-ergic activation (Dewey et al. 1997, Giorgetti et al. 1998). Thus, these instances of neurochemical evidence imply that GABA A receptor manipulation might modulate the rewarding properties of psychomotor stimulants.

Some behavioral evidence supporting for the regulatory role of the GABA-ergic system in psychomotor stimulant-reward have already been obtained; the benzodiazepines, alprazolam and chlordiazepoxide, have been shown to attenuate cocaine self-administration (Goeders et al. 1989, Goeders et al. 1993). The self-administration model is susceptible to biases caused by motor disturbances, and therefore benzodiazepine-induced motor deficits may have complicated the interpretation of these results. These motor deficits, however, probably can not solely account for the attenuating effect of benzodiazepines, since after a few days of administration (presumably after the development of tolerance to the motor deficient effects) at least alprazolam left food-induced responding unaffected (Goeders et al. 1993). Furthermore, another benzodiazepine triazolam inhibited amphetamine-induced place preference (Pettit et al. 1989). In the same study, morphine-induced place preference was unaffected by triazolam treatment, thereby excluding the possibility that the inhibition of amphetamine-induced place preference results merely from the amnesic effects of triazolam. These inhibitory effects appear not to be restricted to GABA A receptors; also the GABA B agonist, baclofen, has attenuated cocaine self-administration (Roberts et al. 1996). Furthermore, vigabatrin ( $\gamma$ -vinyl GABA) that increase GABA levels by inhibiting GABA transaminase has been shown to attenuate both elevations in accumbal DA levels and the lowering of brain stimulation reward thresholds induced by cocaine (Kushner et al. 1997, Morgan and Dewey 1998). Not all the evidence, however, tally with the aforementioned findings: a GABA mimetic, progabide, was shown to be inefficient in attenuating amphetamine-induced place preference (Di Scala et al. 1985).

#### 2.4.3 Modulation of the reward mechanisms of psychomotor stimulants by the serotonergic system

Despite its importance as a neurotransmitter in the brain, most 5-HT is found outside the CNS. 5-HT can not cross the blood-brain barrier, and therefore it is obvious that brain cells synthesise their own 5-HT (Cooper et al. 1996). The precursor for 5-HT synthesis is the

amino acid tryptophan that is able to cross the blood-brain barrier actively by means of a carrier protein; once taken up in a brain cell tryptophan is converted to 5-hydroxytryptophan by the enzyme tryptophan hydroxylase; 5-hydroxytryptophan is in turn converted to 5-HT by the enzyme L-aromatic amino acid decarboxylase (the same enzyme that synthesizes DA). In 5-HT synthesis important regulating factors are thought to be the availability of food-derived tryptophan, plasma concentrations of other neutral amino acids as they compete with tryptophan for the same carrier protein, and the activity of the tryptophan hydroxylase enzyme. The degradation of 5-HT involves the enzyme MAO (the same enzyme found in DA metabolism) that converts 5-HT into 5-hydroxyindoleacetaldehyde, which can be further oxidized to 5-hydroxyindoleacetic acid (5-HIAA) or reduced to 5-hydroxytryptophol (Cooper et al. 1996).

The distribution of 5-HT in the brain is very wide-spread, but there is known to be clusters of 5-HT containing cells lying in or near the midline (raphe in Latin) of the pons and upper brain stem referred to as the raphe nuclei; of these clusters more rostrally located ones (raphe dorsalis, medianus and centralis superior) provide extensive 5-HT innervation to the forebrain, while more caudal groups project largely to medulla and brain stem (Cooper et al. 1996). In general, most raphe neurons appear to innervate quite diffusively overlapping terminal fields thus they lack any obvious topography, with the exceptions of the neurons originating from the raphe medianus which furnish a very large component to the limbic system including the amygdala, NAC and the hippocampus, and the neurons originating from the raphe dorsalis which project with greater density to the neostriatum, cerebral and cerebellar cortices, and the thalamus.

**5-HT and reward.** In psychomotor stimulant-reward, the role of the 5-HT-ergic system appears not to be unambiguous. In neurochemical studies, often an enhancement of DA-ergic neurotransmission in reward-related brain areas, such as the VTA, NAC and PFC, by the activation of the 5-HT-ergic system can be observed, although some opposite results have also been reported (Guan and McBride 1989, Parsons and Justice 1993, Iyer and Bradberry 1996, De Deurwaerdère et al. 1998, Lorrain et al. 1999). Furthermore, the administration of fluoxetine, a 5-HT uptake blocker, appears to augment both cocaine- and amphetamine-induced DA increase in the NAC (Clark et al. 1996, Sills et al. 1999), although the latter finding may result from a pharmacokinetic interaction (Sills et al. 1999).

In the self-administration model, an increase in brain 5-HT levels with fluoxetine or L-tryptophan appears to reduce, whereas a decrease in 5-HT levels with 5,7-dihydrotryptamine lesions appears to augment psychomotor stimulant self-administration (Porrino et al. 1989, Carroll et al. 1990, Kleven and Woolverton 1993, McGregor et al. 1993, Roberts et al. 1994, Munzar et al. 1999). In several studies, however, the 5-HT-ergic manipulations have also affected self-administration of non-drug reinforcers such as food or glucose/saccharin solutions that are used as control groups (Carroll et al. 1990, Kleven and Woolverton 1993, Roberts et al. 1994). Furthermore, not all the data are parallel: both 5-HT-ergic lesions by 5,7-dihydrotryptamine or fluoxetine at relevant doses have also failed to affect psychomotor stimulant self-administration (Fletcher et al. 1999, Porrino et al. 1989). Despite some shortcomings, nevertheless, a seemingly simplistic interpretation of the self-administration data is that the contribution of 5-HT to psychomotor stimulant-reward is inhibitory, but caution in interpreting these findings has been recommended as changes in self-

administration can reflect either an increase or decrease in reinforcing efficacy (Walsh and Cunningham 1997, Sills et al. 1999). In fact, in the extinction paradigm of the self-administration method, in which an operant responding after removing a reinforcer such as cocaine is taken as a measure of the motivational properties of the reinforcer, 5-HT depletion by para-chlorophenylalanine lesions decreases rather than increases responding for absent cocaine; that is, 5-HT depletion attenuates incentive motivation for cocaine (Tran-Nguyen et al. 1999). Only a limited number of studies have evaluated the involvement of the 5-HT-ergic system in conditioned psychomotor stimulant-reward using the place preference method. For example, amphetamine-induced place preference can be prevented by the 5-HT uptake blocker zimelidine (Kruszewska et al. 1986), whereas accumbal 5-HT lesions by 5,7-dihydrotryptamine were ineffective (Spyraki et al. 1988). Furthermore, a recent study has shown that combined 5-HT uptake transporter protein and DAT knockout in mice eliminates cocaine-induced place preference, although both knockouts were separately ineffective, which indicates compensatory interplay between the 5-HT- and DA-ergic systems (Sora et al. 2001). Taken together, it seems that the 5-HT-ergic system is intricately involved in regulating psychomotor stimulant-reward, yet its exact role remains incompletely understood for the time being.

**5-HT receptors.** One factor that may complicate the interpretation of the results obtained with general manipulations on the 5-HT-ergic system is the existence of multiple 5-HT receptor subtypes. Depending on the criteria applied, at least 7 subtypes can be classified, e.g. 5-HT1- and 5-HT2-like receptor families, as well as 5-HT3, 5-HT4, 5-HT5, 5-HT6 and 5-HT7 receptors (Barnes and Sharp 1999). Specific ligands are not available for all of them, which has hampered the progress of evaluation of their characteristics in brain function. However, based on the studies conducted with relatively specific agonists or antagonists, thus far at least 5-HT1, 5-HT2 and 5-HT3 receptors appear to be implicated in mediating the rewarding properties of psychomotor stimulants.

**5-HT3 receptors and reward.** During the past decade, particular attention has been focussed on 5-HT3 receptors. The primary distinction from other 5-HT receptors is that 5-HT3 receptors are not coupled to a G protein, but are functionally linked directly to a cation channel instead (Kilpatrick and Tyers 1992). Although in the CNS 5-HT3 receptors are not as abundant as other ligand gated ion-channels, the highest densities are routinely found in the cortex and limbic areas such as the hippocampus and amygdala; they are suggested to be located presynaptically, such as in terminal areas of the mesolimbic system including the NAC, where they apparently mediate some excitatory effect of 5-HT (Kilpatrick and Tyers 1992, Hagan et al. 1993, Kilpatrick et al. 1996). The immediate consequence of 5-HT3 receptor activation is the release of stored neurotransmitters (Kilpatrick and Tyers 1992). For instance, studies have found that the activation of 5-HT3 receptors by the agonists 2-methyl-5-HT and 1-phenylguanide, applied locally or intracerebroventricularly, results in increased DA release in the NAC; this effect can be blocked by 5-HT3 antagonists (Jiang et al. 1990, Chen et al. 1991). Accordingly, 5-HT3 blockade has attenuated the increased DA neurotransmission in the NAC induced by injecting a neurokinin agonist in the VTA (a model for stimulating the mesolimbic DA-ergic system; Hagan et al. 1987), local application of 5-HT in the NAC (Parsons and Justice 1993), electrical stimulation of the raphe dorsalis (De Deurwaerdère et al. 1998), stress (Imperato et al. 1990) or DA-ergic system indirectly activating drugs of abuse such as ethanol, morphine and nicotine (Carboni et al. 1989a).

Further studies indicated that also in other terminal areas of the mesolimbic and mesocortical systems, e.g. in the amygdala and PFC, the DA-ergic neurotransmission can be modulated by 5-HT<sub>3</sub> receptors (Costall et al. 1987, Chen et al. 1992, Tanda et al. 1995, Ge et al. 1997). Consequently, based on findings like these it might be suggested that 5-HT<sub>3</sub> receptors could modify drug-reward.

When considering psychomotor stimulants, however, that activate the DA-ergic system directly the picture appears to be less straightforward. A number of studies have found that psychomotor stimulant-induced changes in the mesolimbic system are not modified by 5-HT<sub>3</sub> receptor antagonists: 5-HT<sub>3</sub> antagonists have neither altered elevation of accumbal DA concentrations (Carboni et al. 1989a, Cervo et al. 1996), changes in accumbal DA metabolism (Koulu et al. 1990), nor suppression of A10 DA neurons (Batsche et al. 1992) induced by amphetamine or cocaine. These studies contrast with a few more recent findings, one from our laboratory, showing that both cocaine- and amphetamine-induced increase in accumbal DA levels can be attenuated by the 5-HT<sub>3</sub>-antagonists (McNeish et al. 1993, Kankaanpää et al. 1996). In concert with these latter results, acute locomotor stimulation by cocaine and amphetamine has been repeatedly shown to be blocked by 5-HT<sub>3</sub>-antagonists (Costall et al. 1987, Svingos and Hitzemann 1992, McNeish et al. 1993), although the blockade of the DA-ergic system in the NAC may not fully account for this effect (McNeish et al. 1993). Furthermore, chronic administration of cocaine can result in an attenuation of 5-HT<sub>3</sub> receptor mediated DA-release, which may represent a partial mechanism for behavioral tolerance to cocaine (King et al. 1999). Thus, although the data are quite inconclusive, some evidence exist to suggest that DA-ergic mechanisms of psychomotor stimulants could be partially regulated by 5-HT<sub>3</sub> receptors.

Despite some supporting neurochemical data, however, studies have shown that self-administration of cocaine or the facilitating effect of amphetamine on intracranial self-stimulation, are not modified by 5-HT<sub>3</sub>-antagonists, thereby indicating that 5-HT<sub>3</sub> receptors could not be directly involved in the reinforcing properties of psychomotor stimulants (Peltier and Schenk 1991, Lane et al. 1992, Montgomery et al. 1993). This view is further strengthened by the findings that 5-HT<sub>3</sub>-antagonists do not affect the discriminative stimulus of cocaine or amphetamine (Lane et al. 1992, Moser 1992). In the place preference paradigm, which relies more on conditioning aspect of reward, studies conducted thus far have yielded quite inconsistent results. In two studies cocaine- and amphetamine-induced place preference has been reported to be unaffected by 5-HT<sub>3</sub> antagonists (Carboni et al. 1989b, Cervo et al. 1996). On the other hand, in two other studies cocaine-, as well as methamphetamine- and methylenedioxymethamphetamine-induced place preference has been shown to be attenuated by 5-HT<sub>3</sub> antagonism (Bilsky and Reid 1991, Suzuki et al. 1992), which implies that at least under certain circumstances 5-HT<sub>3</sub> receptors are involved in conditioning of the rewarding properties of psychomotor stimulants. This may be further emphasized by the finding that in the self-administration paradigm a 5-HT<sub>3</sub> antagonist ondansetron can attenuate amphetamine-induced enhancement of conditioned responding for food-reward (Fletcher and Higgins 1997).

Taken together, although 5-HT<sub>3</sub> receptors may modify the activated DA-ergic neurotransmission in reward-related brain areas, they do not appear to mediate the rewarding

properties of psychomotor stimulants. However, it can still be speculated whether they have some modulatory role in conditioning processes of psychomotor stimulant-reward.

**Other 5-HT receptors.** There is accumulating evidence that also 5-HT1 and 5-HT2 receptors are implicated in modifying the rewarding properties of psychomotor stimulants. For instance, in some previous studies various drugs binding to 5-HT1 or 5-HT2 receptor subtypes with different affinities have been reported to be able to modify psychomotor stimulant-reward (Nomikos and Spyraiki 1988, Porrino et al. 1989, Nader and Barrett 1990, Peltier and Schenk 1993), although the general view is complicated by the dissimilar outcomes in different studies, and questions about the selectivity of some used drugs (Walsh and Cunningham 1997). In recent studies using more specific agonists and antagonists, at least the involvement of 5-HT1B and 5-HT2C receptors are further confirmed, yet not necessarily clarified. 5-HT1B receptor agonists have enhanced cocaine self-administration (Parsons et al. 1998), but somewhat inconsistently antagonized the threshold-reducing effects of cocaine on intracranial self-stimulation behavior (Harrison et al. 1999), or amphetamine-induced enhancement of responding for conditioned reward (Fletcher and Korth 1999). On the other hand, 5-HT2C receptor antagonist, when measured in various self-administration models such as fixed ratio, progressive ratio or extinction paradigms, has decreased reinforcing properties of cocaine, which is in accordance with previous neurochemical evidence (Grottick et al. 2000).

## 2.5 Reward mechanisms of opiates with references to conditioning; morphine

The term opiates refers to compounds that can be extracted or synthesized from opium, a product of the poppy, *Papaver somniferum*. The term opioids, instead, is used in a wider sense to include all the opiates, semisynthetic and fully synthetic compounds, and endogenous peptides that are linked to the opioidergic receptor system in the brain. The first opiate to be identified and characterized was morphine, which is still the most important drug for the treatment of pain, and it remains a standard for any new analgesic. Other opiates, for instance, which have related chemical structure with morphine include heroin, ethylmorphine, codeine and oxycodone. As a general rule, these opiates cause analgesia, respiratory depression, suppress cough, inhibit gastrointestinal transit etc. Unfortunately, morphine and most of the other opiates possess abuse potential, which restricts their clinical use. Particularly notorious is the semisynthetic morphine derivative, heroin (diacetylmorphine), but its effects are believed to be due to its metabolic conversion to morphine in the brain (Feldman et al. 1997).

**Opioid receptors.** Even though opiates were long anticipated to act via a specific receptor system, it was only in 1973 that the existence of opioid receptors was demonstrated (Pert and Snyder 1973). Three main opioid receptor types have been classically distinguished, these are  $\mu$ -,  $\delta$ - and  $\kappa$ -receptors, each of which has specific endogenous ligand:  $\beta$ -endorphine, enkephalins and dynorphins, respectively (Reisine 1995). An overview of selected ligands, distribution and effects after pharmacological stimulation of the three main opioid receptors is presented in Table 2.2. Morphine has preferential affinity for  $\mu$ -receptors (Reisine 1995), and it appears that most of rewarding effects of opiates are mediated by  $\mu$ -receptors, although the involvement of the two other receptors should not be totally ignored (Matthes et al. 1996, Bardo 1998, Van Ree et al. 1999). More recently, opioid receptors have been further

classified into subtypes ( $\mu_1$ ,  $\mu_2$ ,  $\delta_1$ ,  $\delta_2$ ,  $\kappa_1$ ,  $\kappa_2$ ,  $\kappa_3$ ) (Van Ree et al. 1999); there is some evidence that at least  $\mu_1$ -receptors may mediate the rewarding properties of morphine and heroin (Negus et al. 1993, Piepponen et al. 1997).

TABLE 2.2. An overview of three main classes of opioid receptors, their ligands, distribution, and responses to pharmacological stimulation. The data is assembled from Mansour et al. (1995), Feldman et al. (1997), Gutstein and Akil (2001).

	$\mu$ -Receptor	$\delta$ -Receptor	$\kappa$ -Receptor
Receptor agonist	endorphins, morphine	enkephalins	dynorphins
Receptor antagonist	naloxonazine, $\beta$ -funaltrexamine	naltrindole	nor-binaltorphimine
Distribution in the CNS	widely over the CNS with particularly high densities in the thalamus, interpeduncular nucleus, caudate-putamen, limbic structures (nucleus accumbens, olfactory nucleus, amygdala)	predominantly found in forebrain structures such as the neocortex, striatum, olfactory areas, substantia nigra, nucleus accumbens  a subgroup may co-localize and interact with $\mu$ -receptors.	the nucleus accumbens, preoptic area of the hypothalamus, caudate-putamen, olfactory area, interpeduncular nucleus, paraventricular nucleus, paraventricular thalamus, amygdala, neural lobe of pituitary, supraoptic nucleus
Pharmacological effects of receptor agonists	analgesia, reward, sedation, changes in food intake, inhibition of gastrointestinal transit, respiratory depression, cardiovascular depression, neuroendocrine effects	analgesia, reward, sedation, changes in food intake, inhibition of gastrointestinal transit	analgesia, dysphoria, sedation, changes in food intake, neuroendocrine effects

**Opioid reward: brain areas involved.** A major brain site involved in mediating the opiate reward appears to be the VTA (Figure 2.3; Bardo 1998, McBride et al. 1999, Van Ree et al. 1999). For instance, morphine injected into the VTA at relatively low doses can maintain self-administration and induce place preference (Phillips and LePiane 1980, Bozarth and Wise 1981). In line with this, microinjections of the  $\mu$ -receptor agonist DAMGO into the VTA can maintain self-administration or induce place preference (Bals-Kubik et al. 1993, Devine and Wise 1994). While the  $\delta$ -agonist DPDPE is also self-administered into the VTA, a 100-fold greater dose of DPDPE is required relative to DAMGO, which led to the suggestion that the major contribution comes from the  $\mu$ -receptors (Devine and Wise 1994).

The main effect of  $\mu$ -receptor stimulation in the VTA seems to be inhibition of GABA-ergic interneurons; this in turn leads to disinhibition of the DA containing output neurons, activation of mesolimbic neural transmission and increased release of DA in the terminal area NAC (Di Chiara and North 1992). Also the NAC may play a role in opiate reward (Figure 2.3). Morphine can be self-administered into the NAC, and intra-accumbal injections of morphine can induce place preference (Olds 1982, Van der Kooy et al. 1982), but also contrasting results have been obtained (Olmstead and Franklin 1997, Schiltein et al. 1998). Despite the fact that morphine is preferential a  $\mu$ -receptor agonist, a selective  $\mu$ -agonist DAMGO injected into the NAC did not induced place preference (Bals-Kubik et al. 1993).

Instead, met-enkephalin that has an affinity for both  $\mu$ - and  $\delta$ -receptors, can be self-administered into the NAC (Goeders et al. 1984). Thus, it appears that  $\delta$ -receptors, and perhaps  $\mu$ -receptors, could mediate opiate reward in the NAC. However, although stimulation of the VTA results in activation of accumbal DA neurons, on which local opioid receptors can be found presynaptically, the mesolimbic DA-ergic system may not exclusively form a critical component of opiate reward. The available current data favors the view that place preference induced by opiates is susceptible to DA-ergic manipulations, whereas self-administration of opiates is not (Van Ree et al. 1999). Thus, given the putative differences between the two models, it has been suggested that some conditional, incentive or motivational aspects, but not consummatory or reinforcing aspects, of opiate reward could be DA dependent (Di Chiara and North 1992, Van Ree et al. 1999).

There are also a number of other brain sites that have been proposed to support the rewarding properties of morphine. Particularly the hippocampus and LH appear to be involved (Figure 2.3; Bardo 1998, McBride et al. 1999, Van Ree et al. 1999). For instance, morphine can be self-administered when injected into the hippocampus or LH (Olds 1979, Self and Stein 1993). Intrahippocampal morphine can also induce place preference (Corrigal and Linseman 1988). In addition, there is some evidence that PTN, VP, and periaqueductal gray could play a role in opiate reward, but their involvements have not been studied thoroughly yet (Van Ree et al. 1999).

Taken together, it appears that morphine and other opiates have a particular profile of reward-related brain areas, which are distinguishable from those for psychomotor stimulants, although in the mesolimbic system some overlapping can be observed.

#### 2.5.1 Involvement of potassium channels in opioid receptor function

Opioid receptors belong to the family of G protein coupled receptors with seven transmembrane domains, and activation of these receptors results in a combination of multiple components of cellular processes acting in concert. The G-proteins, that consist of a single  $\alpha$ -subunit combined with a dimer of  $\beta\gamma$ -subunits, couple the receptors to the intracellular effectors (Taylor and Fleming 2001). These G proteins mediate a number of downstream actions, which include inhibition of adenylyl cyclase, activation of phospholipase A2, alterations in ion channel conductance, as well as phosphorylation of proteins leading to desensitization of the receptor and alterations in transcription factors (Taylor and Fleming 2001). One major consequence is apparently an increase in K<sup>+</sup> conductance in different parts of the CNS (Taylor and Fleming 2001), which may be ultimately responsible for most of the acute effects of systemically administered morphine (Di Chiara and North 1992).

**K<sup>+</sup> channels.** There is a diversity of K<sup>+</sup> channels, currently more than 200 genes encoding a variety of K<sup>+</sup> channels have been identified, all containing a homologous pore segment for K<sup>+</sup> ions (Shieh et al. 2000). A recent classification is based on a number of transmembrane segments: three groups of six, four, or two putative transmembrane segments with one or two pores are recognized (Shieh et al. 2000). 1) Six transmembrane one-pore channels: voltage-gated K<sup>+</sup> channels including *Shaker*-related or calcium activated channels. These channels are activated by depolarization, typically allowing to permeant ions to flow outward. 2) Two

transmembrane one-pore channels: inward rectifier channels that conduct ion current inward unlike other K<sup>+</sup> channel, are important in setting resting membrane potential. ATP sensitive K<sup>+</sup> channels belong to this group. 3) Four transmembrane two-pore channels. These more recently discovered channels are weak inward rectifiers, and they represent perhaps most abundant class of K<sup>+</sup> channels with more than 50 members.

**Morphine and K<sup>+</sup> channels.** Biochemical evidence links all three opioid receptors with G protein mediated opening of inward rectifier K<sup>+</sup> channels (Wimpey and Chavkin 1991, Grudt and Williams 1993, Shi et al. 2000, Taylor and Fleming 2001), which would ultimately result in hyperpolarization, inhibition of action potential discharge, and contributing to a decrease in neurotransmitter release (Christie 1991, North 1992, Feldman 1997). Some evidence also exist for coupling opioid receptors with voltage- or calcium-activated K<sup>+</sup> channels (Wimpey and Chavkin 1991, Twitchell and Rane 1994).

As for the biochemical data, most behavioral data relates the effects of morphine to the ATP sensitive K<sup>+</sup> channel, a subgroup of inward rectifier K<sup>+</sup> channels. For instance, a number of studies have been concerned with attenuation of morphine-induced analgesia by ATP sensitive K<sup>+</sup> channel blockers (Ocaña et al. 1990, Narita et al. 1992b, Ocaña et al. 1995, Raffa and Martinez 1995). Furthermore, *weaver* mutant mice that have a point mutation in the inward rectifying K<sup>+</sup> channel gene, displayed attenuated analgesia for morphine (Ikeda et al. 2000). Accordingly, ATP sensitive K<sup>+</sup> openers can potentiate morphine analgesia, and they appear to have analgesic properties of their own (Vergoni et al. 1992, Lohmann and Welch 1999). In addition, ATP sensitive K<sup>+</sup> channel blockers are effective in attenuation morphine-induced hyperthermia or locomotor stimulation (Narita et al. 1992a, Ocaña et al. 1993). Some studies indicate that also *Shaker*-related voltage-gated K<sup>+</sup> channels might be involved: morphine-induced analgesia is blunted in mice lacking the *Shaker*-like K<sup>+</sup> channel Kv1.1 gene, or when this gene is inactivated with antisense oligonucleotides (Galeotti et al. 1997, Clark and Tempel 1998).

However, while evidence for the role of K<sup>+</sup> channels in morphine analgesia is accumulating, only a few studies have been concerned with a putative role of K<sup>+</sup> channels in morphine's abuse potential. In one study ATP sensitive K<sup>+</sup> channels openers were shown to be able to attenuate withdrawal symptoms after chronic morphine administration (Robles et al. 1994), but no data are available on the role of K<sup>+</sup> channels in actual rewarding properties. Only indirect evidence exists: two unselective K<sup>+</sup> channel blockers quinine and 4-aminopyridine blocked morphine-induced elevation in accumbal DA or locomotor stimulation (Pei et al. 1993), which imply that at least some K<sup>+</sup> channels might be involved in morphine reward. Thus, the involvement of various K<sup>+</sup> channels in the rewarding properties of morphine awaits further clarification. One ultimate goal of such studies could be evaluating whether the uncoupling of the analgesic and rewarding properties of morphine-like drugs is possible.

## 2.6 Sensitization phenomenon with emphasis on psychomotor stimulants

As mentioned previously (see section 2.1), the sensitization term describes a phenomenon, in which repeated use of drugs results in an augmented response upon re-administration of the drug. Interest in behavioral sensitization is vitalized by the notion that the chronic abuse of psychomotor stimulants, which increase DA-ergic neurotransmission, can exacerbate

sensitivity to paranoid psychosis in humans (Sato et al. 1983). Consequently, amphetamine-induced behavioral sensitization in laboratory animals has been used as an experimental model for schizophrenia (Robinson and Becker 1986). More recently, the sensitization phenomenon has also been suggested to be implicated in drug addiction; sensitization as a result of chronic drug administration is suggested to lead to an increased sensitivity of motivational and conditional factors involved, thereby strengthening the development of drug-seeking behavior on recurrent drug intake (see section 2.1). For the most part this idea is based on the results of animal studies (and indirect evidence from sensitization to psychotic effects in humans), since more direct evidence for the sensitization to the psychomotor stimulant effects in humans has been lacking until quite recently (Strakowski et al. 1996, Strakowski and Sax 1998).

In laboratory animals sensitization was first described to occur with repeated cocaine administration as early as in the 1930's (Downs and Eddy 1932). Ever since, several compounds have been shown to be able to induce sensitization, including the psychomotor stimulants cocaine, amphetamine, methylphenidate and mazindol, and the opiate morphine (Table 2.3). The sensitization phenomenon has been particularly well established with cocaine and amphetamine under various circumstances, but this robustness may not be extendable to all psychomotor stimulants. For instance, with methylphenidate somewhat dissimilar results have been obtained; in some studies locomotor activity have been reported to be sensitized (Shuster et al. 1982, Gaytan et al. 1997, McDougall et al. 1999), whereas in other studies no such effect was observed (McNamara et al. 1993, Izenwasser et al. 1999).

TABLE 2.3. Selected drugs of abuse that can induce sensitization to locomotor-stimulating effects or rewarding properties. The species of choice has been the rat, except with ethanol since it induces sensitization more reliably in the mouse. In the References only one example of the appropriate studies is given.

Drug of abuse	Species	Method	Reference
Cocaine	rat	locomotor activity	White et al. 1998
	rat	self-administration	Schenk and Partridge 2000
	rat	conditioned place preference	Lett 1989
Amphetamine	rat	locomotor activity	Vanderschuren et al. 1999
	rat	self-administration	Pierre and Vezina 1998
	rat	conditioned place preference	Lett 1989
Methamphetamine	rat	locomotor activity	Kuribara and Uchihashi 1993
Methylphenidate	rat	locomotor activity	Gaytan et al. 1997
Mazindol	rat	locomotor activity	Zanin and Takahashi 1994
Morphine	rat	locomotor activity	Babbini and Davis 1972
	rat	conditioned place preference	Lett 1989
Heroin	rat	locomotor activity	Marinelli et al. 1998
Nicotine	rat	locomotor activity	Shoaib et al. 1997
Ethanol	mouse	locomotor activity	Lessov and Phillips 1998
Phencyclidine	rat	locomotor activity	Phillips et al. 2001

**Characteristics of sensitization.** Two main phases of sensitization can be distinguished: during the initiation (also referred to as induction or development) phase a transient sequence of cellular and molecular event precipitated by a drug leads to enduring changes in neural function, while during the expression phase these enduring neural alterations mediate the augmented behavioral response (Pierce and Kalivas 1997). The development of sensitization appears to be a complex and delicate phenomenon. Typically sensitization occurs most reliably with intermittent drug administration, while constant drug infusion may result in tolerance (Robinson and Becker 1986). The dosage used appears to be also important, relatively high doses being more efficient (Browman et al. 1998). Furthermore, expression of sensitization seems to be time-dependent. That is, sensitization is not necessarily most evident immediately after the discontinuation of a drug treatment, and some intensification in sensitization might occur during withdrawal (Paulson et al. 1991, Shippenberg and Heidbreder 1995). One remarkable feature of sensitization is its persistence; once developed it has been described to last even as long as a year (Paulson et al. 1991). In most studies, alterations in locomotor activity, stereotypes or rotational behavior have been used as a measure of sensitization. A more limited number of studies have employed specific reward-models such as conditioned place preference or self-administration for the assessment of sensitization. In these studies, morphine-, cocaine-, amphetamine-induced place preference, or cocaine- and amphetamine-induced self-administration have been shown to be sensitized by prior exposure to the drug (Table 2.3), which is in obvious accordance with the results of the motor activity studies.

#### 2.6.1 Involvement of the dopaminergic system

**Brain areas involved.** In initiation and expression of behavioral sensitization to cocaine and amphetamine, nuclei in the DA-ergic mesocorticolimbic pathways are thought to form key substrate, yet there appear to be a few important distinctions between the two psychomotor stimulants (Table 2.4). In the initiation of behavioral sensitization the VTA, the origin for the DA-ergic pathways, is considered to be crucially involved (Table 2.4; Kalivas and Stewart 1991). Repeated amphetamine-injections into the VTA can induce behavioral sensitization (Cador et al. 1999). Also the development of sensitization to systemic cocaine can be modified by intra-VTA manipulation (Sorg and Ulibarri 1995), although the data is somewhat inconclusive concerning whether intra-VTA injections of cocaine can induce sensitization (see Vanderschuren and Kalivas 2000 p. 105). The neuronal events associated with the expression of sensitization are distributed over the terminal areas of the mesocorticolimbic pathways and their interconnected nuclei, with the NAC being one of the most often implicated (Table 2.4; Pierce and Kalivas 1997). For instance, enhanced behavioral response in sensitized animals can be elicited by intra-accumbal administration of cocaine or amphetamine (Cador et al. 1995).

**DA-ergic system.** Although other neurotransmitters are certainly involved, DA is one important neurotransmitter in mediating sensitization (Kalivas and Stewart 1991, Pierce and Kalivas 1997, Vanderschuren and Kalivas 2000). DA1 or DA2 receptor antagonists can inhibit the development of sensitization to amphetamines (Kuribara and Uchihashi 1993, Meng et al. 1998), and enhanced response in amphetamine-sensitized animals, i.e. expression of sensitization, can be induced by the selective DA uptake blocker GBR12909 (Vanderschuren et al. 1999). Furthermore, in cocaine-sensitized animals, the enhanced

TABLE 2.4. Involvement of the dopaminergic system, and the mesolimbic and mesocortical pathways in initiation and expression of behavioural sensitization to psychostimulants. 'Yes/no' indicates roughly equivalent evidence for and against. The data are modified from Vanderschuren and Kalivas (2000).

Phase of sensitization	Neurochemistry	Cocaine	Amphetamine
Initiation	Dopamine transmission	no	yes
	Ventral tegmental area	yes	yes
	Nucleus accumbens	yes	no
	Prefrontal cortex	yes	yes/no
Expression	Dopamine transmission	yes	yes
	Ventral tegmental area	no	no
	Nucleus accumbens	yes	yes
	Prefrontal cortex	yes	no

response can be modified by DA1 and DA2 antagonists or the DA-ergic neurotoxin MPTP in a manner that indicates DA-ergic involvement during the expression phase (Tella 1994, Itzhak et al. 1999). Induction of cocaine-sensitization, however, has generally been insensitive to DA-ergic lesions or DA1 and DA2 receptor antagonists (Kuribara and Uchihashi 1993, Mattingly et al. 1994, Itzhak et al. 1999, White et al. 1998), which is quite perplexing given the crucial role of DA in both locomotor activity and cocaine's mode of operation.

Within the VTA, the involvement of both the DA1 receptors on GABA-ergic interneurons and DA2 autoreceptors on the soma of the DA-ergic neurons, both activated by drug-induced somatodendritic DA release, in the initiation of sensitization has been suggested (Kalivas and Stewart 1991). The critical step could be the activation of DA1 receptors, that would trigger alterations in local GABA-ergic regulation of the DA neuron. Another important mediator could be the desensitization of impulse-regulating DA2 autoreceptors, thereby attenuating inhibitory control of the DA neuron leading to enhanced activity. This desensitization, however, is only a transient phenomenon lasting typically less than a week, which contrasts with the often more persistent behavioral sensitization, and thus the desensitization DA2 autoreceptors may initialize the cascade rather than *per se* mediate it (Wolf et al. 1993). Within the NAC, enhanced presynaptic DA release is likely to participate in expression of sensitization (Pierce and Kalivas 1997). This may, at least partly, involve alterations in the density of the DA uptake protein, sensitivity of DA uptake presynaptic DA2 autoreceptors (again, which lasts typically less than a week) or in the rate of DA synthesis (Pierce and Kalivas 1997). The enhanced DA release, however, does not temporally fully coincide with the behavioral sensitized response; the enhancement is not typically evident until a week or more after drug discontinuation, while the sensitization is apparent already within a day or two, and therefore the enhanced DA release is considered to be responsible mainly for the long-term expression of sensitization (Wolf et al. 1993). In addition, although changes in DA receptor densities appear not to be of particular significance, enhanced sensitivity of postsynaptic DA1 in the NAC receptors may further contribute to the expression, and at the very least their intact functioning is required (Pierce and Kalivas 1997). Finally, alterations also within other DA-ergic terminal areas and their interconnected nuclei appear to play a role in the expression of sensitization (Pierce and Kalivas 1997): for instance, a decrease in

DA neurotransmission in the PFC of cocaine-sensitized animals is suggested to increase the activity of glutamatergic neurons projecting to the VTA and NAC, and subsequently stimulate the DA neurons in these areas.

Since the data discussed above are largely based on results obtained from studies assessing sensitization in terms of motor stimulating effects, employing some caution is advisable when generalizing to reward-related behavior. The importance of this can be highlighted with the finding that a DA1 receptor antagonist can block the development of sensitization to cocaine-induced place preference (Shippenberg and Heidbreder 1995), despite the well documented inefficacy of DA1 antagonism in the development of sensitization to cocaine-induced motor stimulation, as mentioned above. Unfortunately, not much data are available on neurochemical mechanisms mediating sensitization to the rewarding properties of drugs. In addition to cocaine-induced place preference, DA1 inhibition can also block the development of sensitization to amphetamine self-administration, further emphasizing the importance of DA1 receptors (Pierre and Vezina 1998). However, DA2 inhibition has been reported to be ineffective in inhibiting the enhancement of cocaine place preference by prior exposure, although some modulation could be observed (Shippenberg and Heidbreder 1995).

### 3. AIMS OF THE STUDY

In order to understand the characteristics of addictive behavior and providing novel pharmacological treatment strategies for addiction diseases, it is essential to understand neurochemical mechanisms mediating the rewarding properties of drugs of abuse. Conditioning and sensitization are powerful modulators of addictive behavior. The involvement of the brain DA-ergic system in the conditioning of the rewarding properties of drugs of abuse is generally recognized. Roles of other neuronal systems, however, are more incompletely understood, and even less is known about the mechanisms mediating sensitization of the rewarding properties of drugs of abuse. In the present series of studies we examined the involvement of selected neuronal systems in conditioning or sensitization of the rewarding properties of psychomotor stimulants or morphine mainly using the conditioned place preference method.

The specific aims of the study were as following:

1. To study the involvement of the GABA A and 5-HT<sub>3</sub> receptor-mediated neurotransmission in conditioning of the rewarding properties of the psychomotor stimulants cocaine, amphetamine, mazindol or methylphenidate
2. To evaluate whether the rewarding properties of methylphenidate are sensitized by prior exposure to the drug, and to study the involvement of DA-ergic neurotransmission both in conditioning and sensitization of the rewarding properties of methylphenidate
3. To study the involvement of K<sup>+</sup> channel-related mechanisms of morphine's action in the conditioning of its rewarding properties

## 4. MATERIALS AND METHODS

### 4.1 Animals

Adult male Wistar rats weighing 180-350 g were used throughout the study. The rats were delivered from Helsinki University Laboratory Animal Center, Finland (studies I, II and IV), or from Harlan Nederland B.V., the Netherlands (study III) at least 1 week prior to the experiments. Two rats were housed per cage in a temperature-controlled room ( $20-23 \pm 2^\circ\text{C}$ ) with a 12-h light cycle. The experiments were conducted during the lights-on phase of the cycle, unless otherwise stated. The animals had free access to standard laboratory chow and tap water, except in the conditioned taste aversion test (see section 4.6). The animal experiments were approved by the local institutional animal care and use committee, and if necessary by the chief veterinarian of the county administrative board. All the experiments were conducted according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

### 4.2 Conditioned place preference (studies I-IV)

**Apparatus.** The conditioned place preference test was conducted in rectangular polyvinylchloride boxes (60x30x45 cm), divided into two compartments of equal size by a separating wall (Figure 2.1) with a guillotine door (8x6 cm). Both compartments were covered with loose-fitting transparent plastic lids. One compartment was black with a smooth floor, and the other was white with wire mesh on the floor. Small drops of acetic acid were added in both back corners of either the black (studies I, III and IV) or white (study II) compartment. Thus, the compartments differed in three modalities: visual, tactile and olfactory. The boxes were placed in a quiet dim room with masking noise present provided by an air ventilation system or a CD player.

#### **Place conditioning procedure**

*Unbiased method* (see section 2.2.1; studies I and IV):

Preconditioning phase (day 1): During the preconditioning phase the guillotine door was open and the rats were allowed to freely explore the compartments for 15 min (900 sec). The time the rats spent in both compartments was measured using a stopwatch; if this preconditioning time for a rat was less than 300 sec (study I) or 340 sec (study IV) in either of the compartments, the rat was excluded from further testing. The number of rats in each treatment group was counterbalanced, i.e. half of the rats were assigned to their less-preferred compartment, and other half were assigned to their preferred compartment as the drug-paired compartment. Furthermore, the rats were also counterbalanced between the black and white compartments. Care was taken that in each treatment group the average preconditioning time was between 435 and 465 sec.

Conditioning phase (days 2-9): The guillotine door was closed during this phase. The rats experienced one conditioning session per day. On even-numbered days the rats were injected twice (pretreatment + treatment) at an appropriate interval (10 - 50 min) followed by immediate confinement in the drug-paired compartment for 50 min. On odd-numbered days the rats were injected correspondingly twice (pretreatment or its vehicle + treatment vehicle)

at an appropriate interval followed by immediate confinement in the vehicle-paired compartment for 50 min.

Postconditioning phase (day 10): The guillotine door was opened, and the time the rats spent in the drug-paired compartment was measured for 15 min. This postconditioning time served as a measure of place preference.

*Biased method* (see section 2.2.1; studies II and III):

Preconditioning phase (days 1-3): The guillotine door was open and the rats were allowed daily to freely explore the compartments for 15 min (900 sec). On the last day the time the rats spent in both compartments was measured with a stopwatch; if the preconditioning time for a rat was less than 200 sec in either of the compartments, the rat was excluded from further testing. According to this preconditioning time the less-preferred compartment usually served as drug-paired compartment. The number of rats in each treatment group was counterbalanced between the black and white compartments.

Conditioning phase (4-6): During this phase the guillotine door was closed. The rats experienced two conditioning sessions each day. In the first session they were always administered with vehicle to avoid a carry-over effect in the second session: two injections (pretreatment vehicle + treatment vehicle) were given at an appropriate interval (10 - 40 min), after which the rats were immediately confined to the vehicle-paired compartment for 40 min. After an interval of at least 90 min the second session of the day was started. The rats were again injected twice (pretreatment + treatment) at an appropriate interval followed by immediate confinement in the drug-paired compartment for 40 min.

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. The actual postconditioning time or shift in preference for the drug-paired compartment (postconditioning time - preconditioning time) served as a measure of place preference.

#### 4.3 Motor activity measurements in acute experiments (studies II and IV)

The motor activity was recorded by an Animex-activity meter (LKB Farad, Sweden), on which a modified transparent Macrolon III-type cage (18 x 33 x 30 cm) was placed. The activity meter induces a magnetic field at the bottom of the cage, the magnitude of which changes every time a rat moves in it resulting in an increase in counting. The general sensitivity of the meter was set at 40  $\mu$ A, and the measuring channel was set at 39  $\mu$ A (study II) or 10  $\mu$ A (study IV). The former sensitivity measures general activity, while the latter sensitivity is more focused on locomotor activity.

After a 60-min period of habituation to the cage, three basal activity values were recorded prior to any drug treatments. Then the rats were injected twice (pretreatment + treatment) at an appropriate interval, after which there was 1-min break before the activity recording was begun. The motor activity was recorded either at 15-min intervals for 3 h (study II) or at 30-min intervals for 5 h (study IV).

#### 4.4 Microdialysis (study II)

The rats were anaesthetized using 4% halothane gas (Trothane, I.S.C. Chemicals Ltd, UK). A guide cannula was implanted 2 mm above the NAC (A: +2.0, L: -1.2, V: -6.0 as calculated relative to the bregma according to Paxinos and Watson (1986)) and secured with dental cement (Aqualox, VOCO, Germany) and two small screws. During surgery halothane was administered at a concentration of 2% and the animal's body temperature was maintained at 38°C using a temperature control system.

On the day 6 or 7 after surgery experiments were run on conscious animals. Microdialysis probes (membrane length 2 mm, CMA/12, CMA Microdialysis, Sweden) were inserted into the NAC, connected to a microinjection pump and perfused with modified Ringer's solution (147 mM NaCl, 1.2 mM CaCl<sub>2</sub>, 2.7 mM KCl, 1.0 mM MgCl<sub>2</sub>, pH 6) at a flow rate of 2 µL/min. A 10-µL aliquot of an antioxidant solution was added to the vials prior to collection of the dialysate samples (Kankaanpää et al. 2001). The perfusate was discarded during the first 60 min, after which samples were collected at 30-min intervals. The rats were injected twice at 2 h 30 min and at 3 h after beginning the perfusion.

Aliquots (20 µL) of the samples were assayed for DA, 5-HT, and their metabolites DOPAC, HVA, and 5-HIAA (Kankaanpää et al. 2001) using an HPLC system (Varian, USA) with an electrochemical detector (ANTEC Analytical Technology Leyden, the Netherlands) and an Inertsil ODS-3 V 5 µm (250 mm x 4.6 mm ID) reverse-phase column (GL-Sciences, Japan). At the end of the experiment the animals were decapitated, and their brains were dissected out and immersed in 10% buffered formalin solution. The correct placement of the microdialysis probe was verified, and data were included only from animals with accurate placements.

#### 4.5 Psychomotor stimulant-induced withdrawal (study III)

The rats were injected once daily for seven consecutive days. For the three following days the animals were weighed and their food consumption was measured daily, and on the last day anxiety-like behavior in the black-white box test and changes in motor activity were measured.

**Black-white box test.** The boxes used in the place preference experiments were also used in the black-white box test. Unlike in the place preference experiments, however, no wire mesh or acetic acid were used. Instead, the black compartment was illuminated by a 20 W red bulb, and the white compartment was illuminated by a 10 W white bulb. The compartments differed in the magnitude of luminousness, but otherwise they were similar. Each rat was tested separately once by placing it gently in the middle of the black compartment facing away from the doorway. The following parameters were measured for 8 min: time to enter the white compartment for the first time, total time spent in the white compartment, activity both in the black and white compartments (number of crosses over a midline) and number of crosses between the black and white compartments.

**Motor activity.** After the black-white box test, the motor activity of rats was measured for 40 min in a test cage (transparent Macrolon III-type cage) by means of computer controlled

photobeam activity system (San Diego Instruments, USA). Ambulatory and rearing activities were recorded as interruptions of photobeams at the heights of 5 and 12.5 cm from the cage floor, respectively.

#### 4.6 Conditioned taste aversion (study III)

The rats were housed in pairs with laboratory chow freely available. Throughout the experiment daily drinking was restricted as follows: 1) from 08.15 to 08.30 (habituation/pairing/test session) the rats were placed singly in separate test cages with a drinking bottle filled with water or 0.2 % saccharin; 2) from 12.00 to 18.00 (freely drinking session) the rats were allowed to drink water freely in their home cages. During the preconditioning phase (days 1-3), the bottle in the habituation session was filled with water. In the subsequent conditioning phase (days 4-6), the bottle in the pairing session was filled with saccharin. Eight to 16 min after the session the rats were injected once. Finally, in the postconditioning phase (day 7), two bottles were used in the test session: one filled with water and the other with saccharin. Fluid consumption from both bottles was measured by weighing the bottles before and after the session. The saccharin preference ratio, calculated as saccharin fluid consumption / (saccharin fluid + water consumption), was taken as a measure of taste aversion.

#### 4.7 Drugs

4-Aminopyridine (Sigma, A 0152, USA), ( $\pm$ )amphetamine sulphate (Sigma, A 1263), cocaine hydrochloride (Sigma, C 5776), quinine hydrochloride (Sigma, Q 1125), methylphenidate hydrochloride (RBI, M 147, USA (study II) or donated by Novartis Pharma AG, Switzerland (study III)), morphine sulphate (RBI, M 122), raclopride L-tartrate (RBI, R 121), SCH 23390 hydrochloride (RBI, D 054) and zolpidem hemitartrate (donated by Leiras-Synthelabo, Finland) were dissolved in saline. Diazepam (donated by Orion, Finland) was suspended in a solution of saline and 0.1% (v/v) Tween 80. MDL 72222 (RBI, T 102) was dissolved in acidified saline, the final pH of which was adjusted to 5-7. Mazindol (RBI, M 130) was dissolved in 0.1% Tween 80 (place preference studies) or in acidified saline (microdialysis studies).

#### 4.8 Treatments

All the drugs were injected i.p. at a volume of 1 ml/kg, with the exceptions that MDL and raclopride were administered s.c. and in the conditioned taste aversion test methylphenidate was given at a volume of 8 ml/kg. All the drug doses were calculated as free base.

##### 4.8.1 Effects of $\gamma$ -aminobutyric acid A receptor agonists on cocaine- and amphetamine-induced place preference (study I)

In this unbiased place preference study (see section 4.2) diazepam (0.2-5 mg/kg) and zolpidem (2.5-10 mg/kg), two GABA A receptor agonists with a distinct binding profile, were used (Barnard et al. 1998). For the schedule of the experimental procedure and drug treatments see Table 4.1.

TABLE 4.1. Schedule for experimental procedure and drug treatments in study I. The number after the abbreviation refer to dose in mg/kg. The intervals between two injections for a compartment were 50 min for diazepam- and 10 min for zolpidem-pretreated animals. V: vehicle, COC: cocaine, AMP: amphetamine, DZP: diazepam, ZOL: zolpidem.

Day	1	2 - 9		10
	Pre-conditioning phase No injection	Conditioning phase Injections:		Post-conditioning phase No injection
		Drug-paired compartment	Vehicle-paired compartment	
		V	V	
		V + COC 15	V + V	
		V + AMP 9	V + V	
		DZP 0.2 - 5	V	
		ZOL 2.5 - 10	V	
		DZP 0.2 - 5 + COC 15	V + V	
		ZOL 2.5 - 10 + COC 15	V + V	
		DZP 0.2 - 5 + AMP 9	V + V	
		ZOL 2.5 - 10 + AMP 9	V + V	

#### 4.8.2 Effects of a serotonin3 receptor antagonist on place preference, dopamine increase and motor activity induced by cocaine, mazindol and methylphenidate (study II)

In this study MDL 72222 (0.1 and 1 mg/kg) was used as 5-HT<sub>3</sub> receptor antagonists (Kilpatrick and Tyers 1992). The biased place preference method (see section 4.2) was used to study conditioning of psychomotor stimulant-reward, supplemented by a microdialysis test for monitoring accumbal DA levels (see section 4.4) and motor activity measurements (see section 4.3). For the schedules of the experimental procedures and drug treatments see Table 4.2. Note that in the place preference test the effect of MDL 72222 was tested, apart from the acquisition of conditioned psychomotor stimulant-reward as usual, also on expression of conditioned cocaine-reward. Furthermore, since the experiments indicated that methylphenidate at the initial dose of 20 mg/kg could be more resistant to MDL 72222 than

TABLE 4.2. Schedule for experimental procedure and drug treatments of the conditioned place preference test in study II. The number after the abbreviation refer to dose in mg/kg. The interval between two injections for a compartment was 30 min. Drug treatments of the microdialysis or motor activity tests correspond to injections in the drug-paired compartment, with the exception that also the methylphenidate dose of 10 mg/kg was tested when appropriate. V: vehicle, COC: cocaine, MAZ: mazindol, MET: methylphenidate, MDL: MDL 72222.

Day	1 - 3	4 - 6		7
	Pre-conditioning phase No injection	Conditioning phase Injections:		Post-conditioning phase Injections:
		Vehicle-paired compartment	Drug-paired compartment	
		V	V	-
		V + V	MDL 0.1 and 1 + V	-
		V + V	V + COC 20	-
		V + V	MDL 0.1 and 1 + COC 20	-
		V + V	V + MAZ 10	-
		V + V	MDL 0.1 and 1 + MAZ 10	-
		V + V	V + MET 20 or 5	-
		V + V	MDL 0.1 and 1 + MET 20 or 5	-
		V	COC 20	V
		V	COC 20	MDL 0.1 and 1

cocaine and mazindol, lower methylphenidate doses (5 or 10 mg/kg) were included in the experimental procedures to confirm that the ineffectiveness of MDL 72222 would not be attributable to an inappropriately high dose of methylphenidate.

#### 4.8.3 Rewarding properties of methylphenidate: sensitization by prior exposure to the drug and the effects of dopamine1 and dopamine2 receptor antagonists (study III)

In this study, four separate tests were conducted. For the schedules of the experimental procedures and drug treatments in the tests see Table 4.3.

First, the dose-response profile for place preference induced by methylphenidate (0.31-20 mg/kg) was assessed employing the biased method (see section 4.2), and the involvement of DA1 and DA2 receptors in this conditioned reward was evaluated by using the respective antagonists SCH 23390 (0.05-0.2 mg/kg) and raclopride (0.2-0.8 mg/kg) (Cooper et al. 1996). Also the effects of both antagonists alone in the place preference method was tested.

Second, tests were made to determine whether prior exposure sensitizes the rewarding properties of methylphenidate. On the basis of the dose-response profile the highest dose that was clearly ineffective was chosen as the reference dose (0.31 mg/kg). The rats were first subjected to the 7-day sensitization treatment with methylphenidate, followed by the place preference test conducted with the reference dose to find out whether it would now induce place preference, i.e. whether the prior administration has sensitized the rewarding properties of methylphenidate. The following sensitization treatment was applied: once daily the rats received vehicle or methylphenidate (0.31-20 mg/kg) for seven days in their home cages. On the same day as the the last injection the biased place conditioning procedure with methylphenidate at the reference dose was initiated. The first 15-min habituation period of the preconditioning phase was conducted at least 3 h before the last sensitization treatment injection. In this way there was a 3-day interval between the sensitization treatment and the conditioning phase of the place preference test. The involvement of DA1 and DA2 receptors in the development of this sensitization was tested by administering SCH 23390 (0.1 and 0.2 mg/kg) or raclopride (0.2 and 0.4 mg/kg) with methylphenidate (5 mg/kg) during the sensitization treatment phase.

Third, it is possible that the enhancement of place preference induced by the prior administration would reflect the alleviation of sensitization treatment-induced withdrawal by methylphenidate re-administered during the conditioning phase, rather than a true sensitization phenomenon. Therefore, to assess the magnitude of sensitization treatment-induced withdrawal the rats were treated daily with vehicle or methylphenidate (5 and 20 mg/kg) for 7 days (similarly to the sensitization treatment) followed by measurements of withdrawal-parameters (see section 4.5).

Fourth, another possibility is that after the sensitization treatment methylphenidate would induce place preference because tolerance would have developed to its aversive properties at the reference dose (0.31 mg/kg), instead of the true sensitization phenomenon. Therefore, to assess the aversive properties of methylphenidate at the reference dose the conditioned taste aversion test was conducted with vehicle or methylphenidate (0.31 mg/kg) as described in section 4.6.

TABLE 4.3. Schedule for experimental procedures and drug treatments in study III. The number after the abbreviation refer to dose in mg/kg. The intervals between the injections of the dopamine antagonist and methylphenidate were 40 min for SCH 23390- and 10 min for raclopride-pretreated animals. V: vehicle, MET methylphenidate, SCH: SCH 23390, RAC: raclopride, LMA: locomotor activity, ALB: anxiety-like behavior.

Day	1-6	7	8	9	10	11	12	13	
Place preference test assessing conditioning of reward		Pre-conditioning phase No injection			Conditioning phase Injections:				Post-conditioning phase No injection
					Vehicle-paired compartment	Drug-paired compartment			
					V	V			
					V	MET 0.31 - 20			
					V + V	V + MET 5			
					V + V	SCH 0.05 - 0.2 + MET 5			
					V + V	RAC 0.2 - 0.8 + MET 5			
					V	SCH 0.05 - 0.2			
					V	RAC 0.2 - 0.8			
Place preference test assessing sensitization to reward	Sensitization treatment phase Injections:		Pre-conditioning phase No injection		Conditioning phase Injections:				Post-conditioning phase No injection
					Vehicle-paired compartment	Drug-paired compartment			
	V				V	MET 0.31			
	MET 0.31 - 20				V	MET 0.31			
	MET 20				V	VEH			
	V + MET 5				V	MET 0.31			
	SCH 0.1 and 0.2 + MET 5				V	MET 0.31			
	RAC 0.2 and 0.4 + MET 5				V	MET 0.31			
	SCH 0.2				V	V or MET 0.31			
	RAC 0.4				V	V or MET 0.31			
Tests assessing withdrawal	Sensitization treatment phase Injections: V MET 5 and 20		Weight change and food consumption measurements						
									LMA and ALB measurements
Taste aversion test assessing aversive properties			Pre-conditioning phase No injection		Conditioning phase Injections: V MET 0.31			Post-conditioning phase No injection	

#### 4.8.4 Effects of unselective potassium channel blockers on place preference and changes in motor activity induced by morphine (study IV)

In the place preference study, the effects of (12.5, 25 and 50 mg/kg) and 4-aminopyridine (1 and 2 mg/kg), two unselective K<sup>+</sup> channel blockers (Fatherazi and Cook 1991, Shieh et al. 2000), on place preference induced by morphine (10 mg/kg) were assessed using the unbiased

method (see section 4.2). When the effects of quinine or 4-aminopyridine alone were first assessed by conditioning them alone in the drug-paired compartments, both drugs showed some tendency to induce place aversion. Therefore, to avoid the bias when their interaction with morphine was evaluated, in subsequent experiments quinine and 4-aminopyridine were conditioned in both compartments.

In the motor activity test (see section 4.3) the effects of quinine (12.5, 25 and 50 mg/kg) or 4-aminopyridine (1 and 2 mg/kg) on alterations in motor activity induced by morphine (10 mg/kg) was assessed. When testing the blockers alone, a separate experiment was run also during the lights-off phase. As then the rats are more active, and thus any motor suppressant effect of the blockers alone would be more reliably observed. For the schedules of the experimental procedures and drug treatments in both tests see Table 4.4.

TABLE 4.4. Schedule for experimental procedure and drug treatments of the conditioned place preference test in study IV. The number after the abbreviation refer to dose in mg/kg. The interval between two injections for a compartment was 20 min. Drug treatments of the motor activity test correspond to injections in the drug-paired compartment. V: vehicle, MOR: morphine, Q: quinine, AP: 4-aminopyridine.

Day	1	2 - 9		10
	Pre-conditioning phase No injection	Conditioning phase Injections:		Post-conditioning phase No injection
		Drug-paired compartment	Vehicle-paired compartment	
		V	V	
		V + MOR 10	V + V	
		Q 50	V or Q 50	
		AP 2	V or AP 2	
		Q 12.5 - 50 + MOR 10	V + V	
		AP 1 and 2 + MOR 10	V + V	

#### 4.9 Statistics

In the place preference experiments the postconditioning time or shift in preference (postconditioning time - preconditioning time) served as a measure of reward. Statistical evaluation was conducted with one-way ANOVA (study I) or with one-way and two-way ANCOVAs (preconditioning time as covariant; studies II, III and IV). *Post hoc* comparisons were tested with Dunnet's t-test or Bonferroni's test, respectively. In study III Bonferroni's test was adjusted for appropriate number of comparisons.

The results of motor activity measurements in acute experiments were evaluated as absolute counts (study II) or as relative changes to the mean of three basal values (study IV). In study II, peak effects taken directly from the data and AUCs calculated by the trapezoidal method served as measures of motor activity. The obtained data were then subjected to one-way ANOVA followed by Dunnet's t-test. In study IV, the hypoactivity and hyperactivity phases were assessed separately using two-way ANOVA (pretreatment x treatment) for repeated measures. When needed this was followed by one-way ANOVA for repeated measures with Bonferroni's protection (the normal level of acceptance 0.05 divided by the number of comparisons made) to evaluate separately the effects of different doses.

In the microdialysis experiment the results were calculated as relative changes to the mean of two basal values. Peak effects and AUCs obtained as described above were subjected to one-way ANOVA followed by Dunnet's t-test.

In the assessment of withdrawal symptoms weight change, food consumption, and motor activity were analyzed using one-way ANOVA for repeated measures, while the parameters of anxiety-like behavior in the black-white box test were assessed with one-way ANOVA followed by Bonferroni's test.

In addition, paired and two-sample t-tests were used throughout the study whenever appropriate.

## 5. RESULTS

The main findings of all experiments are summarized in Table 5.1, while the key results of the conditioned place preference and microdialysis tests are further illustrated in Figures 5.1 and 5.2. In the following sections the results are described in more detail.

In the place preference experiments the postconditioning time was measured for 900 s and thus the average time between the compartments is 450 s. Using the biased method the preconditioning times approximated 360-380 s. Increased preference for the drug-paired compartment may not therefore necessarily reflect a true place preference; that is, the postconditioning time may not have exceeded 450 s. For this reason, postconditioning times of selected groups are given in parentheses to show that true place preference was obtained.

TABLE 5.1. A summary of the effects of selected pretreatments on the acquisition of conditioned place preference (CPP), motor stimulation and increase in accumbal DA levels induced by the psychostimulants and morphine. '↓↓↓' refers to prevention, '↓↓' to attenuation, and '-' to no effect. Empty cells indicate that no corresponding test was conducted.

Drug of Abuse	Pre-treatment	Mechanism of action	CPP	Motor Stimulation	Accumbal DA	Other
Cocaine	Diazepam	GABA A agonist	↓↓↓			
	Zolpidem	GABA A agonist	-			
	MDL 72222	5-HT3 antagonist	↓↓	↓↓	↓↓	MDL 72222 had no effect on expression of cocaine-induced CPP
Amphetamine	Diazepam	GABA A agonist	↓↓↓			
	Zolpidem	GABA A agonist	-			
Methylphenidate	MDL 72222	5-HT3 antagonist	-	-	-	
	SCH 23390	DA1 antagonist	↓↓↓			SCH 23390 prevented sensitization to methylphenidate-induced CPP
	Raclopride	DA2 antagonist	-			Raclopride prevented sensitization to methylphenidate-induced CPP
Mazindol	MDL 72222	5-HT3 antagonist	↓↓	↓↓	↓↓	
Morphine	Quinine	K+ blocker	↓↓	↓↓↓		
	4-Aminopyridine	K+ blocker	-	↓↓↓		

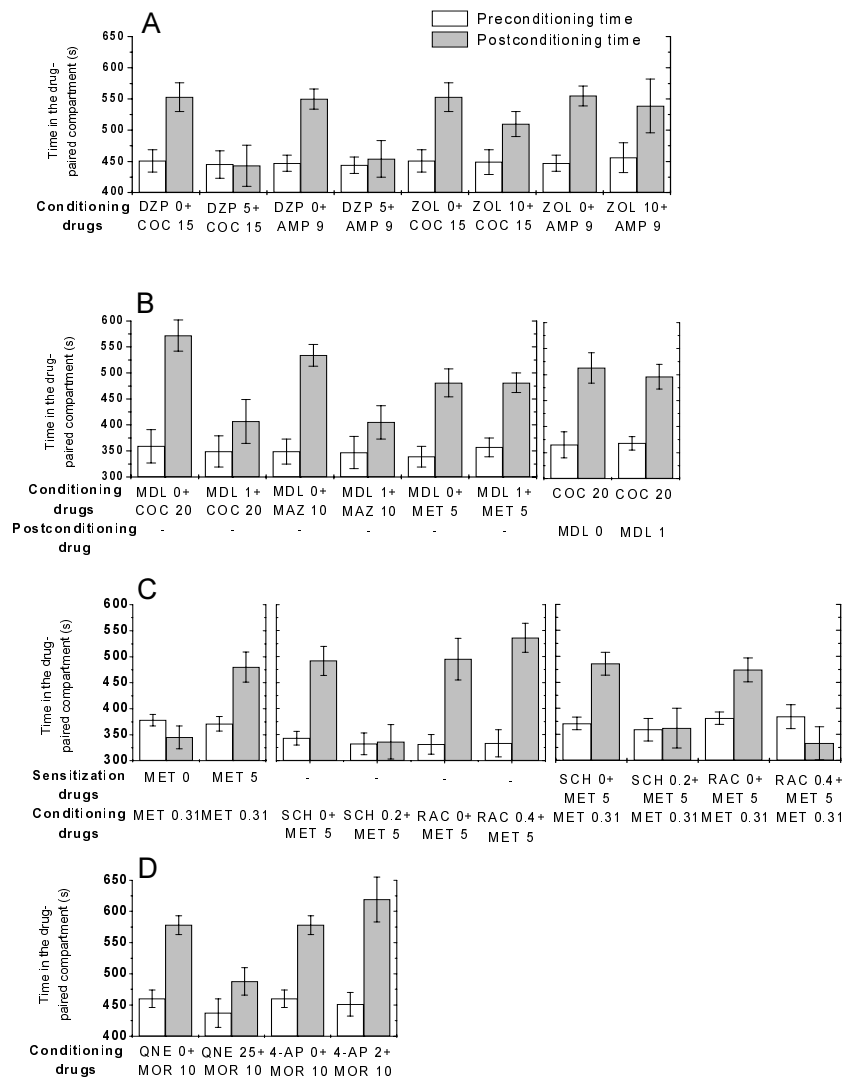


FIGURE 5.1. Main findings in the conditioned place preference experiments. A) Effects of GABA A receptor agonists on psychomotor stimulant-induced place preference. During the conditioning phase the rats were administered diazepam or zolpidem with a psychomotor stimulant (Conditioning drugs). B) Effects of 5-HT3 antagonist on acquisition and expression of psychomotor stimulant-induced place preference. MDL 72222 was administered either during the conditioning phase with a psychomotor stimulant (Conditioning drugs) or during the postconditioning phase alone (Postconditioning drug). C) Sensitization to methylphenidate-induced place preference, and the effects of DA1 and DA2 receptor antagonists. During the conditioning phase the rats received methylphenidate alone, with SCH 23390 or with raclopride (Conditioning drugs). When appropriate, this was preceded by the 7-day sensitization treatment, during which once a day the rats received methylphenidate alone, with SCH 23390 or with raclopride (Sensitization drugs). D) Effects of unselective K<sup>+</sup> channel blockers on morphine-induced place preference. During the conditioning phase the rats were administered quinine or 4-aminopyridine with morphine (Conditioning drugs). DZP: diazepam, ZOL: zolpidem, COC: cocaine, AMP: amphetamine, MDL: MDL 72222, MET: methylphenidate, SCH: SCH 23390, RAC: raclopride, QNE: quinine, 4-AP: 4-aminopyridine. The number after the abbreviation refers to dose in mg/kg.

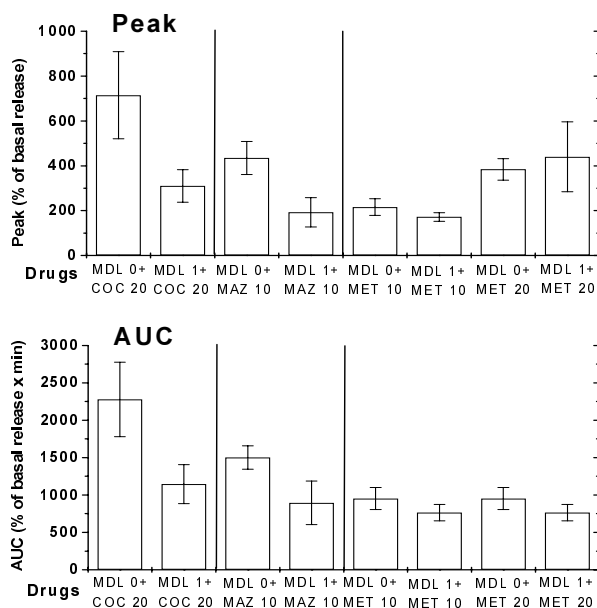


FIGURE 5.2. Main findings in the microdialysis experiment: the effects of the 5-HT<sub>3</sub> receptor antagonist MDL 72222 on psychomotor stimulant-induced DA increase in the NAC. The results are presented as a peak effect or AUC of relative changes, which were calculated with the mean of two samples before drug treatments as basal value (100%). MDL: MDL 72222, COC: cocaine, MAZ: mazindol, MET: methylphenidate. The number after the abbreviation refers to dose in mg/kg.

### 5.1 Effects of $\gamma$ -aminobutyric acid A receptor agonists on cocaine- and amphetamine-induced place preference (study I)

Neither diazepam nor zolpidem alone caused place preference or aversion. Instead, cocaine and amphetamine alone induced place preference, which both were abolished by pretreatment with diazepam ( $p=0.039$  and  $p=0.005$ , respectively), but not with zolpidem.

### 5.2 Effects of a serotonin<sub>3</sub> receptor antagonist on place preference, dopamine increase and motor activity induced by cocaine, mazindol and methylphenidate (study II)

MDL 72222 given alone did not induce place preference or aversion, affect the extracellular levels of DA, 5-HT or their metabolites in the NAC, or cause changes in spontaneous motor activity. Instead, both cocaine and mazindol induced place preference ( $572\pm30$  s and  $534\pm21$  s, respectively), increased the levels of DA and 5-HT without effects on their metabolite levels, and stimulated motor activity. Methylphenidate induced place preference (5 mg/kg and 20 mg/kg ( $516\pm31$  s)), increased accumbal DA levels (only 20 mg/kg) and stimulated motor activity (only 10 and 20 mg/kg). A notable exception from the two other psychomotor stimulants was that methylphenidate did not affect the 5-HT levels. Furthermore, it was the only drug that decreased the levels of DOPAC; the levels of other metabolites remained unaffected.

MDL 72222 attenuated approximately by 60 to 70% the acquisition of both cocaine- and mazindol-induced place preference ( $p=0.007$  and  $p=0.016$ , respectively). Instead, expression of cocaine-induced place preference remained unaffected. MDL 72222 attenuated the increased DA levels and motor stimulation induced by cocaine (AUC:  $p=0.01$  and  $p=0.09$ ,

respectively) and mazindol (AUC:  $p=0.012$  and  $p=0.012$ , respectively). Acquisition of place preference induced by methylphenidate at either dose was not attenuated by MDL 72222. MDL 72222 also generally failed to affect methylphenidate-induced increase in accumbal DA or motor stimulation, irrespective of the methylphenidate dose used. Other psychomotor stimulant-induced neurochemical changes, i.e. cocaine- and mazindol-induced increase in 5-HT levels or methylphenidate-induced decrease in DOPAC levels, were not affected by MDL 72222.

### 5.3 Rewarding properties of methylphenidate: sensitization by prior exposure to the drug and the effects of dopamine1 and dopamine2 receptor antagonists (study III)

Methylphenidate induced place preference dose-dependently ( $p<0.001$ ). The dose of 0.31 mg/kg was chosen as the reference dose for the sensitization experiments since it was clearly ineffective. When tested after the 7-day sensitization treatment with methylphenidate, conditioning with the reference dose now resulted in an increased preference for the drug-paired compartment ( $p=0.002$ ), i.e. the prior administration seemed to sensitize the rewarding properties of methylphenidate. A separate analysis with rats having only mild initial aversion for the drug-paired compartment (preconditioning time  $> 375$  s) revealed that, besides increased preference, a true place preference ( $515\pm 14$  s) can be obtained by conditioning after the sensitization treatment.

SCH 23390 ( $p=0.006$ ), but not raclopride, co-administered with methylphenidate during the conditioning phase prevented the place preference, i.e. conditioning of methylphenidate-reward was prevented by the DA1 but not DA2 antagonism. Conditioning with SCH 23390 caused no place preference or aversion, whereas the highest dose of raclopride (0.8 mg/kg) unexpectedly appeared to increase preference for the drug-paired compartment ( $p=0.05$ ), although only when conditioned with initially less-preferred side.

Both SCH 23390 and raclopride (at a dose lower than that increasing preference) co-administered with methylphenidate during the sensitization treatment phase prevented the enhancement of place preference ( $p=0.031$  and  $p=0.003$ , respectively), i.e. sensitization to the rewarding properties of methylphenidate was prevented by both DA1 and DA2 antagonism. Administration of SCH 23390 or raclopride alone during the sensitization treatment phase followed by conditioning with vehicle or methylphenidate had no effect.

No indication of withdrawal symptoms, as assessed with changes in body weight, food consumption, anxiety-like behavior or motor activity, was observed after the 7-day treatment with methylphenidate at doses sufficient to induce enhancement of place preference. It was only after treatment with the highest dose (20 mg/kg) when the rats showed some anxiety-like behavior in the black-white box test ( $p=0.032$  for the time to enter the white compartment for the first time), although otherwise no withdrawal symptoms were observed.

Finally, methylphenidate at the reference dose had no aversive properties when tested in the conditioned taste aversion test.

#### 5.4 Effects of unselective potassium channel blockers on place preference and changes in motor activity induced by morphine (study IV)

In the place preference experiments, when associated only with the drug-paired compartment, quinine was ineffective but 4-aminopyridine showed a tendency for place aversion ( $p=0.082$ ). Therefore, to avoid this bias when their interaction with morphine was studied, in subsequent experiments quinine and 4-aminopyridine were conditioned in both drug- and vehicle-paired compartments. As expected, then neither quinine nor 4-aminopyridine showed tendency a for place aversion anymore. Instead, morphine induced place preference, and this morphine-induced place preference was attenuated by approximately 70% by pretreatment with quinine ( $p=0.008$ ), whereas 4-aminopyridine was ineffective.

In the motor activity experiments, two phases of morphine-induced motor activity were separated on the basis of the preliminary experiments: 1) A hypoactivity phase: for 0-120 min when morphine attenuated motor activity as compared to the vehicle treated rats; 2) A hyperactivity phase: for 120-240 min when morphine increased motor activity as compared to the vehicle treated rats. Neither quinine nor 4-aminopyridine alone significantly affected the spontaneous motor activity of the rats. When administered together with morphine, quinine or 4-aminopyridine did not affect the hypoactivity, while both drugs attenuated the hyperactivity ( $p=0.032$  and  $p=0.004$ , respectively) induced by morphine.

## 6. DISCUSSION

### 6.1 Assessing the rewarding properties of the drugs and neurochemical mechanisms involved: the conditioned place preference method, dopamine levels in the nucleus accumbens and motor activity.

In the present study, the conditioned place preference method was employed for assessing the rewarding properties of drugs and neurochemical mechanisms mediating their conditioning and sensitization. In two studies this was complemented by measuring alterations in accumbal DA levels and/or motor activity induced by drugs of abuse.

**Conditioned place preference method.** A conditioning phenomenon is considered to play an important role in the development and maintenance of drug addiction, particularly in drug-craving and reinstatement of drug-seeking behavior (Stewart et al. 1984, Wise and Bozarth 1987, Robinson and Berridge 1993, Berridge and Robinson 1998, Di Chiara 1998, Di Chiara 1999). Therefore, the conditioned place preference was chosen as a method of choice for assessing neurochemical mechanisms mediating conditioning of drug-reward; in particular since the method possesses several advantages (see section 2.2.1). It is relatively simple and inexpensive to conduct, no surgical procedures are needed, and the result can be measured while the animal is in a drug-free state. In addition to conditioning, another important factor in the drug addiction is the sensitization phenomenon (Robinson and Berridge 1993, Robinson and Berridge 2000). The conditioned place preference method is also readily applicable to studies concerning with sensitization to drug-reward. For instance, place preference induced by cocaine, amphetamine or morphine has been shown to be enhanced in animals exposed to the drug prior to the test (Lett 1989). Of course, studying conditioning and sensitization of the drug-reward is not restricted to the conditioned place preference method; also other methods, such as certain models of the self-administration method, are very suitable for the purposes, although they may be methodologically more demanding.

Both the biased and unbiased models of the conditioned place preference method were employed in the present work (see section 2.2.1). In the unbiased model the drug-effect was associated in a counterbalanced fashion between both less-preferred and preferred compartments. Consequently, even a vehicle-treated animal could show substantial preference for the drug-paired compartment without any treatment-induced increase in preference. Therefore, the unbiased model, in which the drug-effect was associated with the less-preferred compartment (preconditioning time < 450 s), was considered somewhat more sensitive. However, a concern with the biased model is that increased preference for the drug-paired compartment may reflect the anti-aversive rather than rewarding properties of the drug, particularly with drugs possessing anxiolytic properties such as benzodiazepines (Tzschentke 1998). That is, drugs may only reduce initial aversiveness of the drug-paired compartment, instead of inducing a true preference for it (postconditioning time > 450 s).

In the present work, studies II and III employed the biased model, in which conditioning of the psychomotor stimulants cocaine, methylphenidate and mazindol increased preference for the drug-paired compartment. As mentioned above, the nature of this increased preference can be questioned. However, there are several reasons to believe that these results truly reflect their rewarding properties. First, psychomotor stimulants appear to be anxiogenic rather than

anxiolytic (Yang et al. 1992). Second, besides increased preference, the psychomotor stimulants appeared to induce a true place preference for the drug-paired compartment. Thirdly, the biased model has been validated for measuring psychomotor stimulant-reward (Calcagnetti and Schechter 1993): extinction of the established cocaine-induced place preference can be induced by re-associating the originally cocaine-paired environment with saline, which indicates the involvement of a conditioning phenomenon. Taken together, it appears that despite the use of the biased model, the rewarding properties of cocaine, mazindol and methylphenidate were truly measured.

**Microdialysis and motor activity measurements.** The conditioned place preference method was complemented by measuring accumbal DA and 5-HT levels in study II. The exact role of the accumbal DA is a subject of some disagreement, but several researchers have attributed it with various processes related to associative learning (Berridge and Robinson 1998, Di Chiara 1998, Schultz 1998, Di Chiara 1999). Considered in this sense, measuring accumbal DA levels could provide a suitable means of elucidating the neurochemistry underlying the findings in the conditioned place preference experiment. However, this may not pertain to all psychomotor stimulants, since at least cocaine-induced place preference might be dissociable from accumbal DA (see section 2.4.1). Despite this, DA in the NAC is certainly involved in psychomotor stimulant-reward, and therefore accumbal DA measurements may further extend the implications of place preference experiments. Recent studies have dissociated subcompartments within the NAC, of which both the shell and core areas may be distinctively implicated in reward-related behavior (Zahm 1999, Parkinson et al. 1999). Unfortunately, due to a size of the microdialysis probe used for DA measurements, we were not able to dissociate the accumbal compartments of such small areas. Thus, the results obtained represent alterations of DA levels within the NAC as a whole. Also motor activity measurements were included in the experimental setups of studies II and IV. However, the acute motor stimulating effects of drugs most likely do not reflect fully their rewarding properties. Yet again, given the association between the DA-ergic system and motor stimulation, some further support for the results obtained in other models can be gained from motor activity measurements. In particular, when the three methods - the conditioned place preference, monitoring alterations in accumbal DA levels and measuring motor activity - are conducted together, as done in study II, they should jointly form a relatively reliable means of studying the drug-reward.

Throughout the present series of experiments, the effects of the drugs alone in the tests were quite similar to what was anticipated on the basis of their known abuse potential. That is, drugs recognized to possess abuse potential induced place preference, increased accumbal DA levels, or stimulated motor behavior as expected, whereas drugs generally considered to be non-abused were mainly ineffective. However, there were a few findings that deserve further attention, and they are discussed in the three following sections.

#### 6.1.1 Rewarding properties of mazindol

In our study with rats, mazindol increased dose-dependently preference for the drug-paired compartment. This finding extends that of Gevaerd and Takahashi (1996), who previously showed that mazindol can induce place preference in mice. Mazindol can also maintain self-administration in non-human primates (Kaminski et al. 1996), which is considered to be of excellent predictability for human abuse. Furthermore, the discriminative stimulus of

mazindol can be generalized to that of cocaine in rats (Witkin et al. 1991). Finally, in the present study mazindol increased extracellular DA levels in the NAC and stimulated motor activity, which both are typical for a drug of abuse. Taken together, these animal data uniformly indicate that mazindol possesses rewarding properties, which can be expected given its similar neurochemical profile with cocaine (Koe 1976). Thus it might be suggested that mazindol could have abuse potential in humans.

Primary human data, however, supporting abuse potential is scarce. While mazindol shared discriminative stimulus with amphetamine under laboratory conditions, it produced no amphetamine-like subjective effects (Chait et al. 1986). In another study with healthy volunteers mazindol had no reinforcing properties, it actually produced only dysphoric subjective effects and decreased drug-liking (Chait et al. 1987). In cocaine abusers, only some stimulant-like subjective effects were observed at similar doses of mazindol, otherwise it had no effect on subjective measures and it was not distinguished from a placebo (Preston et al. 1993). Furthermore, epidemiological data indicating abuse potential for mazindol is virtually absent. Thus, these human data appear to be in obvious contradiction to the animal data, but the reasons for this can only be speculated at present.

One potential interpretation for the discrepancy is related to the dose and the route of administration used in the humans studies. For instance, while oral doses up to 2 mg were used in the studies mentioned above, it has been estimated in a recent study that as high a dose as 30 mg of mazindol orally would be required to produce 50% occupancy of DAT in the human brain, the degree of blockade predicted to alter cocaine's euphorogenic effects (Malison et al. 1998). Furthermore, in the human studies mazindol was administered orally, which contrasts with the animal studies using parenteral routes. After oral ingestion, some effects of mazindol have not been evident until 3 h, with strengthening observable up to 6 h (Chait et al. 1986, Chait et al. 1987), and thus it may be that the long delay in the onset of effects after oral mazindol have attenuated its reinforcing properties. Nevertheless, taken together it seems that mazindol can be regarded as a low potency drug for abuse. Yet some caution is warranted with higher doses or parenteral routes of administration, although issues of tolerability may well limit these possibilities (Preston et al. 1993).

#### 6.1.2 Rewarding properties of benzodiazepines

The abuse potential of benzodiazepines has been long recognised in humans. Given their wide and extensive clinical use, however, it can be considered to be of trivial risk among the general population (Woods et al. 1992). In human laboratory experiments, benzodiazepines have been shown to possess reinforcing properties indicating their abuse potential, yet only in subjects with histories of drug abuse, and not in normal subjects without histories of moderate drinking, anxiety or insomnia (Griffiths and Weerts 1997).

In line with the human data above, animal experiments show that benzodiazepines possess some abuse potential, but less than other classic sedative or stimulant drugs of abuse (Griffiths and Weerts 1997). For instance, although negative results have also been reported, in the self-administration method benzodiazepines can be self-administered. Typically, however, this has required intravenous injections, along with several training sessions or first establishing stable responding to another more reinforcing drug (Griffiths and Weerts 1997). In place preference

studies benzodiazepines have yielded quite inconsistent results: sometimes they have and sometimes they do not have induced place preference. A closer inspection of the literature reveals that in the studies with successful establishment of place preference the biased model has been employed (Nomikos and Spyraiki 1988, Spyraiki et al. 1988, File 1986, Acquas et al. 1989, Gray et al. 1999), which raises speculation about the role of anxiolysis (Tzschentke 1998). However, when the unbiased method is employed, like in our study, no place preference has been observed (Di Scala et al. 1992, Suzuki et al. 1995, Parker et al. 1998, Leri and Franklin 2000a). However, on the basis of these latter results it should not be misconcluded that benzodiazepines or related drugs would not possess rewarding properties at all, since in the place preference method typically only two to four drug-injections (e.g. four in our study) is given to drug-naive animals. Although this number of drug-environment pairings is normally adequate for inducing place preference, considered in the light of the self-administration experiments, more drug-environment pairings might be required to establish place preference with benzodiazepines in the unbiased model.

In conclusion, the results of the present study agree well with the view that benzodiazepines possess only limited rewarding properties in drug-naive subjects. Furthermore, this appears to be extendable to the non-benzodiazepine GABA-ergic drug zolpidem, which like diazepam failed to induce place preference.

### 6.1.3 Rewarding properties of raclopride

In the present study the DA<sub>2</sub> receptor antagonist raclopride at the highest dose tested increased preference for the drug-paired compartment, although this was evident only when raclopride was associated with the less-preferred compartment. It appears that drug-induced anxiolysis may not account for this, because raclopride has been actually shown to cause anxiety-like behavior in the rat (yet only low doses of 0.05-0.1 mg/kg were tested; Timothy et al. 1999). Thus, our finding implies that raclopride could possess some rewarding properties, but this idea finds little support from the literature. Raclopride attenuated rather than enhanced the reinforcing properties of amphetamine in the self-administration method (Fletcher 1998), and in the conditioned place preference method raclopride administration alone has repeatedly been shown not to possess rewarding properties (although in the studies using rats somewhat lower doses, when compared to our study, appear to have been given; Ågmo et al. 1993, Hoffman and Donovan 1995, Meisel et al. 1996). In general, despite being tested in a number of studies, DA<sub>2</sub> receptor antagonists have induced no place preference apart from one study (Tzschentke 1998). In this study, metoclopramide was reported to increase preference when associated with a less-preferred compartment, and this was speculated to result from drug-induced disturbance of habituation in the paired environment leading to novelty-induced place preference (Hoffman and Beninger 1989). Whether this could also account for the present finding remains unclear. Another potential interpretation involves the binding profile of raclopride. Although generally regarded as a DA<sub>2</sub> antagonist, raclopride also possesses some affinity for DA<sub>3</sub> receptors (Cooper et al. 1996). Interestingly, a putative DA<sub>3</sub> receptor antagonist U99194 has been reported to induce place preference (Kling-Petersen et al. 1995). Thus, the participation of DA<sub>3</sub> antagonism in the effects of raclopride at higher doses can be speculated, although no direct evidence supporting this idea is available.

In conclusion, it appears that some DA2 antagonists can occasionally induce ‘drug of abuse’-like increased preference for the drug-paired compartment, but the reasons for this - as well as implications of it - are unclear for the time being.

## 6.2 Reward mechanisms of psychomotor stimulants and morphine with references to conditioning

The results of the present study show that also other neuronal systems than the DA-ergic one can importantly contribute to regulating the conditioned rewarding properties of psychomotor stimulants and morphine. It may be, however, that at least in the case of psychomotor stimulants their effects are mainly mediated via the DA-ergic system, given psychomotor stimulants’ primary mechanism of action. The results of the present study concerning the selected neuronal systems are discussed separately below.

### 6.2.1 Psychomotor stimulants and the involvement of the $\gamma$ -aminobutyric acid A receptors

A substantial amount of neurochemical evidence suggests that the GABA A receptors can control the brain DA-ergic system in the reward-related areas; usually activation of the GABA A receptor results in inhibition of the spontaneous or drug-induced DA-ergic neurotransmission. Previous behavioral data are mainly supportive of this: GABA A receptor activation with the benzodiazepines alprazolam and chlordiazepoxide have attenuated cocaine self-administration (Goeders et al. 1989, Goeders et al. 1993), and amphetamine-induced place preference was attenuated by the benzodiazepine triazolam (Pettit et al. 1989), although not by progabide, a GABA mimetic that presumably non-selectively activates various GABA receptors (Di Scala et al. 1985). In the present study, the benzodiazepine diazepam prevented both cocaine- and amphetamine-induced place preference, which suggests an inhibiting regulatory involvement of GABA A receptors in conditioning of the psychomotor stimulant-reward. More recently, it has been shown that not only acquisition but also expression of amphetamine-induced place preference can be attenuated by diazepam (Leri and Franklin 2000a).

Thus, the data quite consistently show that GABA A receptor activation can robustly attenuate various aspects, including conditioning, of psychomotor stimulant-reward, making no distinction between cocaine and amphetamine.

**Memory disruption.** Unlike diazepam, another GABA A receptor agonist zolpidem, that has a distinct binding profile from that of diazepam (Niddam et al. 1987, Sanger et al. 1994, Barnard et al. 1998), failed to affect cocaine- or amphetamine-induced place preference in the present study. This finding has an important implication: diazepam is well known for its amnesic properties, which may be argued to interfere with establishing the appropriate psychomotor stimulant-environment association resulting in the inhibition of place preference. Like diazepam, however, also zolpidem has been shown to disrupt learning and memory processes in various behavioral methods, even at the doses well below those used in the present study (Sanger et al. 1986, Chodera et al. 1994, Tang et al. 1995, Edgar et al. 1997), and still it failed to modify the psychomotor stimulant-induced place preference. Thus, it appears that the inhibitory effect of diazepam may not be solely attributed to a disruption of learning and memory functions.

**Brain areas involved.** The distinct binding profile in reward-related brain areas between the two GABA A receptor agonists may account for the relatively ineffectiveness of zolpidem, and it leads us to consider the neuroanatomy involved in mediating the inhibitory effect of diazepam. For instance, in amphetamine-induced place preference the DA-ergic system in the NAC is considered to play a crucial role, accumbal DA is under the inhibitory regulation of the local GABA-ergic system, and zolpidem binding in the NAC is considerably less than that of classical GABA A receptor ligands (Niddam et al. 1987, Benavides et al. 1993). Thus, these data convergently suggest the involvement of the NAC, and this has been subsequently confirmed by the recent finding that intra-accumbal diazepam can inhibit the expression of place preference induced by amphetamine (Leri and Franklin 2000b).

The picture is somewhat more obscure with cocaine. The NAC appears not to be critical for place preference induced by cocaine. Instead, the VP may play an essential role in cocaine-place preference, but zolpidem binding in that brain area is no different from that for the classical GABA A receptor ligands (Niddam et al. 1987, Benavides et al. 1993). Furthermore, two recent studies dissociate GABA-ergic modulation in the VP from both place preference inducing properties (Gong et al. 1997a) and cocaine-induced DA increase (Gong et al. 1998). In the VTA, zolpidem binding is relatively low (Duncan et al. 1995), but the involvement of this brain area in cocaine-induced place preference is unclear. In addition, GABA-ergic regulation in the VTA has been reported to be able to both inhibit and excite the downstream DA-ergic neurotransmission (Westerink et al. 1996, Xi and Stein 1998), which further complicates the interpretation. In the amygdala, 88% of benzodiazepine receptors showed high or intermediate affinity for zolpidem (Benavides 1993), thereby not supporting the involvement of the brain area, although in another study its binding was only 30-38% of that of the benzodiazepine, flunitrazepam (Niddam et al. 1987). Finally, the PFC probably contributes to cocaine-induced place preference, but the extent of zolpidem binding in the brain area is not known. In the frontal cortex, 42% of benzodiazepine receptors was reported to show high affinity for zolpidem (Benavides et al. 1993), but this brain area may not fully correspond to the PFC.

Considered together, it appears GABA A receptors can universally modulate the rewarding properties of the psychomotor stimulants cocaine and amphetamine, including both acquisition and expression of their conditioning, possibly via 'zolpidem-insensitive' GABA A receptors, i.e. receptors compiled of  $\alpha 1 \beta 1, 2$  or  $\gamma 3$ , or  $\alpha 5 \beta 1$  or  $\gamma 2$  subunits (Barnard et al. 1998). Furthermore, while the NAC may play a crucial role in inhibition of amphetamine-induced place preference by diazepam, some other brain area thus far unidentified may be responsible for diazepam's inhibitory effect on cocaine-induced place preference.

#### 6.2.2 Psychomotor stimulants and the involvement of serotonin<sub>3</sub> receptors.

In our study cocaine-induced place preference, increases in accumbal DA levels and stimulation of motor activity were attenuated by the 5-HT<sub>3</sub> receptor antagonist MDL 72222. Also mazindol-induced effects were similarly attenuated by the 5-HT<sub>3</sub> antagonism, which further confirms the results obtained with cocaine given the analogous neurochemical profile of the two psychomotor stimulants. However, in previous studies, which employed similar methods, quite inconsistent results have been obtained. In some studies 5-HT<sub>3</sub> antagonists have attenuated the reward-related effects of psychomotor stimulants, whereas in others no

such effect was observed (see section 2.4.3). There is no obvious explanation for this lack of consistency. In general, 5-HT<sub>3</sub> antagonists including MDL 72222 are considered to be highly selective (Kilpatrick and Tyers 1992). However, since some antagonists may bind to separate sites of the 5-HT<sub>3</sub> receptor (Barnes and Barnes 1993), of which different subtypes are now known to exist (Barnes and Sharp 1999), it can be speculated whether the use of different antagonists may have confused the results. Nevertheless, a very recent study shows that in transgenic mice over-expressing 5-HT<sub>3</sub> receptors cocaine-induced place preference is attenuated (Allan et al. 2001). Although this may seemingly be contradictory to our results, these animals were in fact hypersensitive to some effects of cocaine, and the authors speculated whether this lack of preference reflected aversive properties of cocaine ‘overdose’ rather than a true decrease in reward.

Taken together, despite some inconsistencies in previous studies, our results nevertheless quite uniformly support the findings which show that the 5-HT<sub>3</sub> antagonism can modulate the rewarding properties of cocaine, at least under certain circumstances.

**Limited efficacy of the 5-HT<sub>3</sub> antagonism.** Our findings may appear to be in contradiction with the results obtained previously in the self-administration and drug discrimination experiments, which have repeatedly shown that reinforcing and discriminative properties of cocaine are insensitive to 5-HT<sub>3</sub> antagonism. There are, however, a few noteworthy aspects that may account for the dissimilarities. First, unlike the self-administration and drug discrimination methods, the place preference method relies predominantly on classical-like conditioning (Tzschentke 1998, Bardo and Bevins 2000). Thus, it can be speculated whether the 5-HT<sub>3</sub> antagonism selectively interferes with the conditioning processes rather than attenuates directly any rewarding or reinforcing properties. This suggestion would be in harmony with the previous findings that also drug-induced place aversions or amphetamine-induced enhancement of conditioned responding for food can be modulated by the 5-HT<sub>3</sub> antagonism (Acquas et al. 1990, Fletcher and Higgins 1997). Second, in the present study only acquisition but not expression of cocaine-induced place preference was attenuated by MDL 72222. In the self-administration and drug discrimination experiments an antagonist is typically administered when the animal has already learned the effects of a drug. This would temporarily correspond to the expression phase in the conditioned place preference method: the antagonist is administered when the animals have already learned the drug-environment association. Since in our study only the acquisition but not expression phase was attenuated, this finding may in fact indicate that the 5-HT<sub>3</sub> antagonism prevents neurochemical mechanisms involved in initiating events leading to drug-seeking behavior rather than reverses any already established changes.

Taken together, the results support the view that 5-HT<sub>3</sub> receptors can modulate psychomotor stimulant-reward, but only in a very limited manner by interfering selectively with the acquisition of reward-related conditioning. This may provide some interpretation for the dissimilar results obtained in various reward-assessing methods.

**Significance of the 5-HT-ergic system.** Unlike the situation for cocaine and mazindol, the 5-HT<sub>3</sub> antagonism did not interfere with the effects of methylphenidate. Importantly, this failure occurred irrespective of the methylphenidate doses used, thereby indicating that it might not be solely attributable to the use of an inappropriately high dose of methylphenidate.

Compared with cocaine and mazindol that are known to block the uptake of DA and 5-HT, a primary distinct feature of methylphenidate is its ineffectiveness in altering 5-HT-ergic neurotransmission, as found also in the present study. Thus, it may be suggested that the ability of a drug to increase 5-HT-ergic neurotransmission is a prerequisite for the drug to be susceptible to the 5-HT<sub>3</sub> antagonism. This assumption is in line with the finding that the attenuation of cocaine-induced motor stimulation by the 5-HT<sub>3</sub> antagonism is dependent on intact 5-HT neurotransmission (Svingos and Hitzemann 1992). In addition, further support can be gained from neurochemical studies showing that an enhancement of the DA-ergic neurotransmission in reward-related brain areas induced by increasing 5-HT neurotransmission is mediated at least partly via 5-HT<sub>3</sub> receptors (see section 2.4.3).

The results considered together with these data suggest the importance of 5-HT via 5-HT<sub>3</sub> receptors in certain neurochemical and behavioral effects of cocaine and mazindol but not methylphenidate. Consequently, it appears that differences in the psychomotor stimulants' primary mechanism of actions can determine significantly roles of various regulatory processes in neuronal mechanisms mediating reward.

### 6.2.3 Psychomotor stimulants and the involvement of dopamine1 and dopamine2 receptors

Several lines of evidence indicate that the DA-ergic system is crucially involved in various reward-related behaviors including conditioning, yet there may be some differences between the psychomotor stimulants in view of the involvement of DA<sub>1</sub> and DA<sub>2</sub> receptors (see section 2.4.1). With regard to methylphenidate in particular, which is considered to be a DA uptake blocker (White and Wolfe 1991), it is quite perplexing that various DA-ergic manipulations tested thus far have failed to affect its rewarding properties. For instance, methylphenidate can induce place preference in 6-hydroxyDA lesioned rats or DAT knockout mice (Martin-Iverson et al. 1985, Sora et al. 1998). Furthermore, DA<sub>2</sub> receptor blockade by haloperidol has been similarly ineffective, with the exception of the dose of 1 mg/kg that was considered unspecifically high (Martin-Iverson et al. 1985, Mithani et al. 1986). In our study, DA<sub>1</sub> receptor blockade by SCH 23390 prevented methylphenidate-induced place preference, which suggests that the DA-ergic system plays a role in the conditioning of methylphenidate-reward. It should be noted that at the doses used in our study, the possible affinity of SCH 23390 for 5-HT<sub>2</sub> receptors appears to be insignificant (Bischoff et al. 1986). Previous studies have shown the importance of DA<sub>1</sub> receptors in place preference induced by other psychomotor stimulants (Tzschentke 1998), and thus methylphenidate appears to be no exception. Instead, methylphenidate-induced place preference remained unaffected by the DA<sub>2</sub> receptor antagonist raclopride in our study, which thus extends the previous findings with haloperidol. Again, it should be noted that many DA<sub>2</sub> antagonists, including raclopride, have some affinity for DA<sub>3</sub> receptors (Cooper et al. 1996), which may have affected the results. Nevertheless, in terms of conditioned psychomotor stimulant-reward, a primary distinction appears to be that DA<sub>2</sub> antagonists can prevent place preference-induced by amphetamine but not cocaine. Thus, given the observed inefficacy of raclopride in our study, it could be suggested that DA-related neural mechanisms that mediate conditioning of methylphenidate-reward might be more closely correlated with those of cocaine- than those of amphetamine-reward.

#### 6.2.4 Morphine and the involvement of potassium channels

K<sup>+</sup> channels appear to have a crucial role in mediating the effects of opioid receptor activation by morphine. Most neurochemical data indicate the involvement of inward rectifier K<sup>+</sup> channels, yet some evidence also indicate the involvement of voltage- and calcium activated K<sup>+</sup> channels (see section 2.5.1). Activation of these channels by morphine would ultimately result in hyperpolarization, a dysfunction of action potential propagation, and consequently inhibition of the neuron. In line with neurochemical data, behavioral data quite uniformly have shown that ATP-sensitive K<sup>+</sup> channels, a subgroup of inwardly rectifier K<sup>+</sup> channels, supraspinally mediate the analgesic properties of morphine. There have been fewer studies concerned with other K<sup>+</sup> channels, but existing evidence favor the involvement of some voltage-sensitive K<sup>+</sup> channels (Galeotti et al. 1997, Clark and Tempel 1998).

Since morphine's analgesic and rewarding properties most likely share some common overlapping neuronal mechanisms, it may be assumed that certain K<sup>+</sup> channels could also participate in morphine-reward. There is currently, however, only indirect evidence for such an effect: two unselective K<sup>+</sup> channel blockers, quinine and 4-aminopyridine, have inhibited both an increase in accumbal DA and motor stimulation induced by morphine (Pei et al. 1993). In our study, the efficacy of these blockers to modify morphine-reward was investigated using the conditioned place preference method: only quinine, but not 4-aminopyridine attenuated place preference induced by morphine. Instead, when assessed in separate experiments both drugs were able to attenuate secondary locomotor hyperactivity following high dose morphine administration, which indicates that the ineffectiveness of 4-aminopyridine was not due to an inappropriate dosage, and further highlights the distinguishability between the motor stimulating and conditioned rewarding properties of opiates, as described previously (Cunningham and Kelley 1992). At present, no comprehensive interpretations for the observed difference between quinine and 4-aminopyridine in our study can be given, particularly considering that both drugs were previously shown to attenuate morphine-induced increase in accumbal DA, which may be critical for conditioned opioid-reward (Van Ree et al. 1999). There is, however, a diversity of the K<sup>+</sup> channels distributed differentially over the CNS, and they may respond differently depending on blocking agent. Furthermore, quinine and 4-aminopyridine quite are rather unselective drugs with different pharmacological profiles. While both drugs block various voltage-sensitive K<sup>+</sup> channels, quinine can also block ATP- and calcium sensitive K<sup>+</sup> channels (Fatherazi and Cook 1991, Shieh et al. 2000). They even have some actions not directly related to K<sup>+</sup> channels, such as modulation of sodium channels (Smith and Levinson 1989, Mei et al. 2000).

Despite some shortcomings, however, the results of the present study imply that K<sup>+</sup> channels can modify conditioning of morphine-reward, warranting further studies with more specific K<sup>+</sup> channel blockers. Also other behavioral methods less dependent on classical conditioning might be applied to characterize the role of K<sup>+</sup> channels across different reward-related behaviors.

### 6.3 Sensitization to the rewarding properties of psychomotor stimulants

The sensitization phenomenon, that allegedly occurs in humans during repeated drug intake, has been suggested to contribute to the development of drug addiction. Animal studies have shown that motor behaviors induced by the two prototype psychomotor stimulants cocaine and amphetamine can be sensitized by prior exposure to the drug, and similar information concerning their rewarding properties is also accumulating. A very recent study suggests that, when assessed in terms of Pavlovian approach behavior, an enhancement in stimulus-reward learning and impaired inhibitory modulation of approach are significant processes underlying the development of sensitization to motivational properties of cocaine and amphetamine (Taylor and Jentsch 2001).

There has been some concern with methylphenidate about whether its repeated intake can induce sensitization that would predispose to addictive behavior, because long-term methylphenidate treatment is used for hyperactivity disorder in children (Rang et al. 1999). Unfortunately, in animal studies assessing sensitization by means of measuring locomotor activity, somewhat inconsistent results have been obtained. Yet a majority of studies has favored the conclusion that sensitization do occur with repeated drug administration (see section 2.6). In the present study it was shown that, like the motor stimulating effects, the rewarding properties of methylphenidate can be sensitized by prior exposure to the drug. This would suggest that in a similar manner to cocaine and amphetamine, repeated methylphenidate treatment can increase sensitivity to its rewarding properties thus predisposing to addictive behavior. This is further supported by a recent finding that exposure to methylphenidate during adolescence enhances the acquisition of cocaine self-administration in adult rats (Brandon et al. 2001).

**Relief from withdrawal or tolerance of aversive properties.** Although not relevant to studies assessing sensitization by means of motor behavior, ‘sensitization-like’ enhancement of place preference or self-administration may be argued to result from re-administration-induced attenuation of withdrawal symptoms, or development of tolerance to aversive properties of a drug, instead of true sensitization. Yet typically studies concerning with sensitization to reward have not controlled these aspects. The results of the additional experiments in our study, however, revealed that sensitization to the rewarding properties of a drug can occur at doses possessing no aversive properties, and without withdrawal symptoms being present, thereby excluding the involvement of these alternative explanations.

#### 6.3.1 Involvement of dopamine1 and dopamine2 receptors

Investigations largely based on alterations in motor behavior has shown quite conclusively that the DA-ergic neurotransmission is intricately, yet crucially, involved in sensitization of psychomotor stimulants, with a notable exception that the development of sensitization to cocaine can occur irrespective of the DA-ergic system (see section 2.6.1). However, fewer studies have applied more direct measures of drug-reward, such as the conditioned place preference or self-administration methods, in investigating the role of the DA-ergic system. In these studies, it has been shown that prior exposure to the specific DA uptake blocker GBR12783 can enhance place preference induced by the blocker or cocaine (Le Pen et al. 1998). This indicates that the activation of the DA-ergic system alone is sufficient to induce

the sensitization. Furthermore, DA1 receptor blockade has prevented the enhancement of cocaine-induced place preference, or acquisition of amphetamine self-administration, induced by prior exposure to the psychomotor stimulant (Shippenberg and Heidbreder 1995, Pierre and Vezina 1998). Thus, the development of sensitization to the rewarding properties of both cocaine and amphetamine can be inhibited by DA1 antagonism, despite the previous findings that DA1 antagonists have generally been ineffective in modulating the development of sensitization to cocaine's motor effects. Nevertheless, the importance of DA1 receptors is further emphasized by our present finding that the enhancement of methylphenidate-induced place preference can be prevented by co-administering the DA1 receptor antagonist SCH23390 with the stimulant during the prior exposure.

While the three aforementioned studies uniformly indicate the importance DA1 receptors, the role of DA2 receptors is less well documented. In our study co-administration of the DA2 receptor antagonist raclopride with methylphenidate during the prior exposure prevented the enhancement of methylphenidate-induced place preference, suggesting that DA2 receptors also play an important regulatory role. In fact, compared to DA1 receptors DA2 receptors appeared to be more specifically involved, since SCH23390 but not raclopride was able to directly interfere with conditioning of methylphenidate's place preference. Using roughly identical experimental setup with our study, however, it has been previously shown that the development of sensitization to cocaine-reward is not prevented by raclopride at similar or higher doses, although some modulation could be observed (Shippenberg and Heidbreder 1995). Thus, comparing those results with ours, it may be suggested that neural mechanisms mediating reward-related sensitization of various psychomotor stimulants might not be totally identical, as previously observed between cocaine and amphetamine in terms of the development of sensitization to their motor stimulating properties (see section 2.6.1).

Taken together, the existing data including the present study quite uniformly favor the view that DA1 receptors are essential for the development of sensitization to psychomotor stimulant-reward. Less support is available for DA2 receptors, but our results suggest their involvement at least in the development of sensitization to methylphenidate-reward. Furthermore, although not directly addressed in the present study, our results are compatible with the assumptions that both the local DA1 heteroreceptor activation and desensitization of DA2 autoreceptors in the VTA might be important in initializing the neuronal cascade leading to sensitization (see section 2.6.1).

#### 6.4 Clinical implications

The present study was primarily addressed at evaluating neuropharmacology of conditioning and sensitization of psychomotor stimulant- or morphine-reward. However, because the phenomena of conditioning and sensitization are both suggested to be important elements in the development and maintenance of addictive behavior (see section 2.1), drugs that would interfere with them might be also expected to possess some potential as anti-addictive therapeutics. Consequently, the drugs that were effective in the present study are considered below from a clinical point of view as anti-craving agents. Furthermore, some clinical implications of our finding with sensitization to methylphenidate are briefly discussed.

**Antagonism of conditioning and sensitization of psychomotor stimulant- or morphine-reward.** The results with the drugs used in our study, in the conjunction with the results of previous place preference studies, are summarized in Table 6.1. As the variability in the results imply, several factors may determine the efficacy of a drug. Neurochemical mechanisms mediating and regulating reward of various drugs of abuse are not identical, not even in the class of psychomotor stimulants (see section 2.4), as exemplified with our finding that the 5HT3 antagonist MDL 72222 attenuated conditioning of cocaine- and mazindol-, but not methylphenidate-reward. Similarly, the mechanisms of various reward-related behaviors may differ. For instance, the DA2 antagonist raclopride prevented the development of sensitization but not conditioning of methylphenidate-reward, or MDL 72222 prevented acquisition but not expression of conditioned cocaine-reward. In fact, it needs to be realized that conditioning and sensitization are only two processes involved in only partly understood complexity of the elements contributing to the development and maintenance of addictive behavior. When speculating about the eligibility of a drug for clinical use, the drug might be required to show quite non-selective attenuation of different reward-related neurochemical and particularly behavioral effects induced by drugs of abuse. Furthermore, unlike in animal experiments usually, this efficacy of the drug should also be confirmed using chronic administration, as this is most often applied in clinical practice. Finally, the drug should also be safe and well-tolerated by patients.

TABLE 6.1. Antagonism of conditioning and sensitization of psychomotor stimulant- or morphine-reward with ligands binding to DA, GABA A, 5-HT3 receptors and K+ channels, when assessed in the conditioned place preference method. 'Yes' indicates that evidence incline toward the antagonism, 'no' against it, and 'yes/no' that there is roughly equivalent evidence. Empty cells indicate that no corresponding experiment has been conducted. The data are assembled from Tzschentke (1998), Leri and Franklin (2000a), and the present study.

Drug of abuse	Conditioning of reward				
	DA1 antagonists	DA2 antagonists	GABA A agonists	5-HT3 antagonists	K+ channel blockers
Cocaine	yes	no	yes*	yes/no	
Amphetamine	yes	yes	yes	no	
Methylphenidate	yes*	no		no*	
Mazindol				yes*	
Morphine	yes	no	no	yes	yes/no*

Drug of abuse	Sensitization to reward	
	DA1 antagonists	DA2 antagonists
Cocaine	yes	no
Methylphenidate	yes*	yes*

\* Based solely on the present study.

Unfortunately, none of the drugs that were effective in the present study may fulfill the criteria described above. For instance, the clinical usability of diazepam and other benzodiazepines is

apparently restricted by their abuse potential and capabilities for inducing withdrawal symptoms following abrupt cessation of drug intake. Also the development of tolerance, another characteristics of diazepam-like drugs, may further hinder their emergence as a pharmacological treatment option. On the other hand, when the effects of 5-HT<sub>3</sub> receptor antagonists on various neurochemical and behavioral correlates of psychomotor stimulant-reward has been assessed, the data is somewhat ambiguous. This may be partly due to their limited role only in certain reward-related behaviors, such as the acquisition of conditioning. Thus, given their restricted efficacy in reward-related behaviors, it may be that 5-HT<sub>3</sub> antagonists might be useful only in selected cases as a supplementary treatment combined with other therapy. Considering DA receptor antagonists, both animal and human data, that employed short-term treatment of antagonist, suggest that they may modify some reward-related effects of psychomotor stimulants (Sherer et al. 1989, Romach et al. 1999). In contrast, in human studies with somewhat more repeated administration DA antagonists have failed to affect, or even enhanced, such effects (Ohuoha et al. 1997, Evans et al. 2001, Haney et al. 2001, Nann-Vernotica et al. 2001). The reason for the inefficacy is not known, but in case of DA<sub>1</sub> antagonists some authors have suggested that it might be related to tolerance or alterations in receptor sensitivity resulting from more chronic treatment (Haney et al. 2001, Nann-Vernotica et al. 2001). In accordance with this, animal data have shown that a DA<sub>2</sub> antagonist with short-term treatment attenuates, whereas long-term treatment enhances cocaine-reward (Kosten et al. 1996). Finally, should the preliminary finding with quinine on conditioned morphine-reward receive support from future studies, the drug nevertheless possesses a range of side-effects in humans, including the syndrome of 'cinchonism' consisting of nausea, tinnitus, headache and blurred vision (Rang et al. 1999), that may restrict its clinical usefulness.

Despite these disadvantages that may limit the direct application of these drugs to clinical practice, nevertheless, these results still provide information on mechanisms involved in psychomotor stimulant- and morphine-reward, that may eventually contribute to the development of novel approaches to pharmacological intervention strategies for the treatment of addiction.

**Sensitization to methylphenidate.** An important finding of our study is that the rewarding properties of methylphenidate can be sensitized by prior exposure to the drug. Thus long-term administration of the methylphenidate may predispose to addictive behavior, which is of particular significance considering its use for the treatment of hyperactivity syndrome in children and adolescents. The underlying neurochemical mechanisms in this syndrome, however, may be such that addiction will not readily develop, since in controlled use the abuse of methylphenidate is considered to be quite rare. Nevertheless, its illicit use has been recognized by several reports indicating that methylphenidate can possess abuse potential (Rappley 1997). Considered in light of our results, it appears prudent that methylphenidate would be used only after a careful consideration of pros and cons on a case-by-case basis, until it is fully evaluated whether long-term treatment with methylphenidate during childhood and adolescence truly increases reactivity to psychomotor stimulants and other drugs of abuse in later life.

## 7. CONCLUSIONS

1. Both GABA A and 5-HT<sub>3</sub> receptor mediated neurotransmission are involved in regulatory activity over conditioning of psychomotor stimulant reward. Only the GABA A receptor agonist diazepam, but not another GABA A receptor agonist zolpidem that has an distinct binding profile from that of diazepam, was able to prevent place preference induced by cocaine and amphetamine. Hence it appears that the GABA A receptor-mediated inhibitory control may involve 'zolpidem-insensitive' receptors, such as receptors composed of  $\alpha 1 \beta 1,2 \text{ or } 3 \gamma 3$ , or  $\alpha 5 \beta 1 \text{ or } 3 \gamma 2$  subunits. Place preference induced by cocaine and mazindol, two psychomotor stimulants with similar mechanisms of action, was likewise attenuated by the 5-HT<sub>3</sub> receptor antagonist MDL 72222; this was, however, not evident in animals that had already established the conditioned cocaine effect, suggesting a quite limited role for 5-HT<sub>3</sub> receptors in the process. Also cocaine- and mazindol-induced increase in accumbal DA levels and motor activity was attenuated by the 5-HT<sub>3</sub> antagonist. However, the 5-HT<sub>3</sub> antagonism failed to affect the responses to methylphenidate, which unlike cocaine and mazindol does not enhance the 5-HT neurotransmission, thereby suggesting that the ability of a drug to increase the 5-HT-ergic transmission would be a prerequisite for the drug to be susceptible to the 5-HT<sub>3</sub> antagonism.
2. Methylphenidate-induced place preference was enhanced by prior exposure to the drug, i.e. the rewarding properties of methylphenidate appeared to be sensitized. This was not due to attenuation of withdrawal induced by the prior exposure, or development of tolerance to methylphenidate's aversive properties. While the DA<sub>1</sub> receptor antagonist SCH 23390 but not DA<sub>2</sub> receptor antagonist raclopride was able to directly prevent methylphenidate-induced place preference, both antagonists co-administered with methylphenidate during the prior exposure prevented the enhancement of place preference. Thus, this suggests that both DA<sub>1</sub> and DA<sub>2</sub>, the latter possibly more specifically, are involved in the sensitization processes of methylphenidate-reward.
3. Quinine but not 4-aminopyridine was able to attenuate morphine-induced place preference, whereas morphine-induced secondary hyperactivity was attenuated by both drugs. Despite the limited selectivity of these K<sup>+</sup> channel blockers, our results imply that at least some K<sup>+</sup> channel-related mechanisms of morphine's action might be involved in conditioning of its reward, warranting further studies with more selective antagonists. Furthermore, the results with 4-aminopyridine exemplifies the dissociability between the motor stimulating effects and rewarding properties of morphine.

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