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ROLE OF BRAIN DOPAMINE IN PSYCHOMOTOR STIMULATION INDUCED BY MORPHINE AND COCAINE IN ALCOHOL-PREFERRING AND ALCOHOL-AVOIDING RATS

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ACADEMIC DISSERTATION

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To Anu and Liisa

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ABSTRACT

Drugs of abuse share a common ability to increase dopamine release in the mesolimbic and nigrostriatal dopamine pathways. Furthermore, drugs of abuse generally increase the locomotor activity of animals, which is believed to result, at least partly, from increased dopamine release in the nucleus accumbens. After repeated treatment, the effects of these drugs, both on locomotor activity as well as on mesolimbic dopamine release, are enhanced, i.e. these drugs induce neurochemical and behavioural sensitisation. This phenomenon of sensitisation is believed to be involved in the reinforcing effects of drugs of abuse.

The aim of this study was to explore whether acute and repeated treatment with morphine and cocaine differentially activates the locomotor activity and mesolimbic and nigrostriatal dopaminergic mechanisms in alcohol-preferring AA (Alko Alcohol) and alcohol-avoiding ANA (Alko Non-Alcohol) rats.

An initial dose of morphine was found to increase locomotor activity to a greater extent in AA than in ANA rats. This increase of locomotor activity after acute morphine was associated with increased release and metabolism of nigrostriatal dopamine in AA rats but not in ANA rats. Repeated 4-day treatment with morphine (1 mg/kg) induced behavioural sensitisation in AA but not in ANA rats. However, no sensitisation occurred either in mesolimbic or nigrostriatal dopamine release, suggesting that an additional, non-dopaminergic component, may be involved in morphine-induced behavioural sensitisation. Sensitisation of dopaminergic mechanisms was, however, seen in AA rats, where sensitisation of rotational behaviour was observed when the rats were challenged with morphine 8 days after withdrawal from repeated 4-day morphine treatment. Acute administration of morphine enhanced brain 5-HT metabolism in AA rats but not in ANA rats, this was in contrast to repeated treatment, where morphine was found to induce no significant enhancement of 5-HT metabolism in either AA or ANA rats.

In naive AA and ANA rats an acute dose of cocaine increased the locomotor activity as well as mesolimbic and nigrostriatal dopamine release. After repeated treatment, behavioural sensitisation was seen with a smaller dose of cocaine in AA rats than in ANA rats. Furthermore, on examintion of mesolimbic dopamine release, only AA rats showed sensitisation to repeated doses of cocaine, whereas the effect of cocaine on nigrostriatal dopamine release was not sensitised in rats from either line.

Thus, AA rats show both psychomotor and/or neurochemical sensitisation to repeated doses of morphine and cocaine more easily than ANA rats. This difference may also be involved in the different alcohol preference between these rats.

Keywords: Dopamine, serotonin, reinforcement, morphine, cocaine, caudate-putamen, nucleus accumbens, nigrostriatal, mesolimbic, locomotor activity, AA rats, ANA rats

ABBREVIATIONS

3-MT	3-Methoxytyramine
5-HIAA	5-Hydroxyindoleacetic acid
5-HT	5-Hydroxytryptamine, serotonin
β-EPLPs	β-Endorphin-like peptides
AA	Alko Alcohol rat line
ANA	Alko Non-Alcohol rat line
COMT	Catechol-O-methyl transferase
DA	Dopamine
DOPA	3,4-Dihydroxyphenylalanine
DOPAC	3,4-Dihydroxyphenylacetic acid
HAD	High Alcohol-Drinking rat line
HVA	Homovanillic acid
IP	Intraperitoneal
LAD	Low Alcohol-Drinking rat line
MAO	Monoamine oxidase
MEAP	(Met)enkephalinArg^{6}Phe^7
mRNA	Messenger ribonucleic acid
NP	Non-Preferring rat line
P	Preferring rat line
POMC	Proopiomelanocortin
SNP	Sardinian Non-Preferring rat line
SP	Sardinian Preferring rat line
VTA	Ventral tegmental area

LIST OF ORIGINAL PUBLICATIONS

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

I   A. Honkanen, J. Mikkola, E.R. Korpi, P. Hyytiä, T. Seppälä, L. Ahtee (1999) Enhanced morphine- and cocaine-induced behavioural sensitization in alcohol-preferring AA rats. Psychopharmacology 142:244-252[a]

II   J.A.V. Mikkola, A. Honkanen, T.P. Piepponen, K. Kiianmaa, L. Ahtee (2000) Effects of repeated morphine on cerebral dopamine release and metabolism in AA and ANA rats. Pharmacology, Biochemistry & Behavior 67:783-791[b]

III   J.A.V. Mikkola, A. Honkanen, T.P. Piepponen, K. Kiianmaa, L. Ahtee (2001) Effects of repeated morphine on metabolism of cerebral dopamine and serotonin in alcohol-preferring AA and alcohol-avoiding ANA rats. Alcohol & Alcoholism in press

IV   J.A.V. Mikkola, A. Honkanen, T.P. Piepponen, K. Kiianmaa, L. Ahtee (2001) Effects of repeated cocaine treatment on striatal dopamine release in alcohol-preferring AA and alcohol-avoiding ANA rats. Naunyn-Schmiedeberg´s Archives of Pharmacology 363:209-214[a]

V   J.A.V. Mikkola, S. Janhunen, P. Hyytiä, K. Kiianmaa, L. Ahtee (2001) Rotational behaviour in the AA and the ANA rats after repeated administration of morphine and cocaine. European Journal of Pharmacology submitted

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1. INTRODUCTION

The main goal of alcoholism research is to explain why people drink, why some drink to excess, and why some are unable to stop drinking even when they understand that drinking has extremely harmful consequences (see McBride and Li 1998). Although environmental effects undoubtedly have an important role in alcohol use (Light et al. 1996), inherited characteristics seem to play an important role in determining the susceptibility of an individual to become drug dependent or an alcoholic (for reviews see Ball and Murray 1994; Cloninger 1987; Crabbe et al. 1994). For instance, studies examining twins and adoptions have found moderate to strong genetic influences on alcoholism among men, with heritability estimates of 40 – 60 % (see Prescott and Kendler 1999).

Selective breeding for alcohol preference is the process whereby systematic mating of animals that exhibit the most extreme levels of high and low alcohol preference from a heterogeneous stock, over many generations, yields two lines of animals that exhibit high and low alcohol drinking (see McBride and Li 1998). Theoretically, these lines should then have either a high or low frequency of genes that impact on alcohol preference, whereas the frequency of irrelevant genes in this respect would remain randomly distributed. Thus, rat lines selectively bred for differential alcohol preference are a useful tool in research of alcohol consumption, because all differences between rats of these lines should be related to differential alcohol consumption.

Drugs of abuse have reinforcing effects, which may be positive or negative, and are major reasons for drug abuse. If a drug (e.g. alcohol) is used, for example, to reduce anxiety or to alleviate the withdrawal symptoms after alcohol drinking, then the states of withdrawal or anxiety serve as negative reinforcers. Negative reinforcing effects are not the only reason why many people use alcohol or other drugs of abuse. Alcohol and other drugs of abuse have positive reinforcing effects, and people drink or use drugs because these substances have some properties that make people want to drink

or use the drug again and again. A growing body of evidence supports the role of cerebral dopaminergic mechanisms in drug reinforcement (for reviews see Koob 1992; Koob et al. 1998a; Robinson and Berridge 1993; Spanagel and Weiss 1999; Wise and Bozarth 1987). Alcohol, opioids, such as morphine, psychostimulants (in this thesis the term psychostimulant is used to represent both cocaine and amphetamine) along with a variety of other drugs of abuse, all share a common ability to increase dopamine release in the terminal areas of mesolimbic and nigrostriatal DA pathways (Di Chiara and Imperato 1988a). These pathways, especially the mesolimbic pathway, seem to play an important role in the reinforcing properties of drugs of abuse (for reviews see Koob 1992; Robinson and Berridge 1993). Changes in cerebral dopaminergic mechanisms, for example, polymorphisms in the dopamine metabolising enzyme, catechol-O-methyl transferase (COMT) (Kauhanen et al. 2000; Tiihonen et al. 1999) and changes in dopamine transporters (Repo et al. 1999; Tupala et al. 2000), have also been associated with high alcohol consumption in humans.

The alcohol-preferring AA (Alko Alcohol) and alcohol-avoiding ANA (Alko Non-Alcohol) rats have been selectively bred for high and low alcohol consumption, respectively (Eriksson 1968). In addition to alcohol, AA rats consume more cocaine and etonitazene, an opioid agonist, when offered in drinking fluid, than ANA rats (Hyytiä and Sinclair 1993). This suggests that the reinforcing effects of these drugs may be stronger in AA than in ANA rats. Given that alcohol drinking activates brain dopaminergic mechanisms in AA rats (Honkanen et al. 1997a), possibly mediating reinforcement, it is possible that differences in the dynamics of dopaminergic transmission contribute to the different alcohol-preferences between rats of these lines.

The aim of the present series of experiments was to study possible differences in cerebral dopaminergic mechanisms between the AA and ANA rats. This was done by measuring motor activity and cerebral dopamine release and metabolism after acute and repeated treatment with morphine and cocaine, two drugs that by diverse primary mechanisms increase cerebral dopamine release.

2. REVIEW OF THE LITERATURE

2.1. Dopamine and drug addiction

2.1.1. Drug addiction

Drug (or alcohol) addiction is a chronically relapsing disorder that is characterised by the compulsion to seek and take the drug, followed by loss of control over drug intake and subsequent emergence of a negative emotional state when access to the drug is prevented (see Koob et al. 1998b). Typically, the daily activities of individuals addicted to drugs are centred on obtaining and consuming the drug at the expense of social and occupational commitments and despite the knowledge of the related adverse medical consequences. Moreover, there are usually attempts to abstain from drug or alcohol use.

Abused drugs have rewarding and reinforcing effects, which are important in maintaining drug intake. In the present context reward is used as a synonym for euphoria, pleasure and liking. Reinforcement is a more neutral term and may be roughly divided into two main classes; positive and negative reinforcement. A positive reinforcer may be defined as a stimulus that increases the frequency of behaviour upon which it is contingent (for reviews see Altman et al. 1996; Stolerman 1992). For example, most drugs of abuse that are self-administered by humans serve as positive reinforcers when used in operant paradigms in rats by reinforcing lever-pressing to obtain intravenous infusion of the drug. Negative reinforcers, in turn, are stimuli or events, the omission or termination of which increases the probability of the response upon which it is contingent (for reviews see Altman et al. 1996; Stolerman 1992). Thus, the use of the drug is maintained because the aversive symptoms associated with withdrawal are alleviated by the drug.

2.1.2. Life cycle of dopamine

The major routes for synthesis and metabolism of dopamine are presented in figure 2.1. The synthesis of all catecholamines, including dopamine, originates from the amino acid tyrosine, which is converted to 3,4-dihydroxyphenylalanine (DOPA) by the enzyme tyrosine hydroxylase. This is the rate-limiting step in the synthesis of catecholamines. DOPA is subsequently converted to dopamine by aromatic amino acid decarboxylase (see Cooper et al. 1996).

Newly synthesized dopamine is stored in synaptic vesicles, from where it is released in a calcium dependent manner upon arrival of neuronal stimuli. Dopamine may also be released from extravesicular pools, for instance by amphetamine, when vesicular stores have been depleted by reserpine (see Bartholini et al. 1989). In addition to transmitter release into the synaptic cleft, transmitters may also be released non-synaptically by non-synaptic varicosities (see Vizi 2000).

After its release, dopamine can interact with its receptors, diffuse out of the synaptic cleft and/or may be removed by uptake or metabolism by monoamine oxidase (MAO) or catechol-O-methyl transferase (COMT). MAO is located both in dopaminergic nerve endings and in extraneuronal compartments, e.g. glial cells (Schoepp and Azzaro 1983) for review see (Wood and Altar 1988). It is known that the MAO enzyme exists in two isoforms, MAO-A and MAO-B. There seems to be little species variation in the distribution of these isoforms in the brain, but MAO-A is predominantly found in catecholaminergic neurons whereas MAO-B is the form most abundant in serotonergic and histaminergic neurons and in glial cells (see Shih et al. 1999). Dopamine is a substrate for both isoforms, but MAO-A seems to be the isoform responsible for the oxidative metabolism of dopamine in the rat striatum (Fornai et al. 2000; see Cesura and Pletscher 1992). In contrast to MAO, there seems to be no significant COMT activity in presynaptic dopaminergic neurons, but some activity is present in postsynaptic neurons and substantial activity has been found in glial cells (Kaakkola et al. 1987; Kastner et al. 1994; see Männistö and Kaakkola 1999). COMT itself can be found in two active forms, one being soluble whilst the other is membrane-bound. The soluble form has been suggested to be located in glial

cells and the membrane bound form in postsynaptic neurons and extraneuronal cells (Kaakkola et al. 1987; Rivett et al. 1983). However, as there are no specific soluble COMT or membrane-bound COMT antisera available, it has not been possible to separately analyze the tissue distribution of these two forms of the enzyme (see Männistö and Kaakkola 1999). Dopamine, as mentioned previously, can also be metabolised intraneuronally in dopaminergic nerve endings by MAO to 3,4-dihydroxyphenylacetaldehyde which is then converted to 3,4-dihydroxyphenylacetic acid (DOPAC) by aldehyde-dehydrogenase (Fornai et al. 2000; see Wood and Altar 1988). Subsequently, DOPAC can be metabolised to homovanillic acid (HVA) by COMT. Dopamine may also be metabolised by COMT to 3-methoxytyramine (3-MT), which may be further metabolised to HVA. Changes in 3-MT levels have been suggested to reflect dopamine release, changes in DOPAC levels may signify
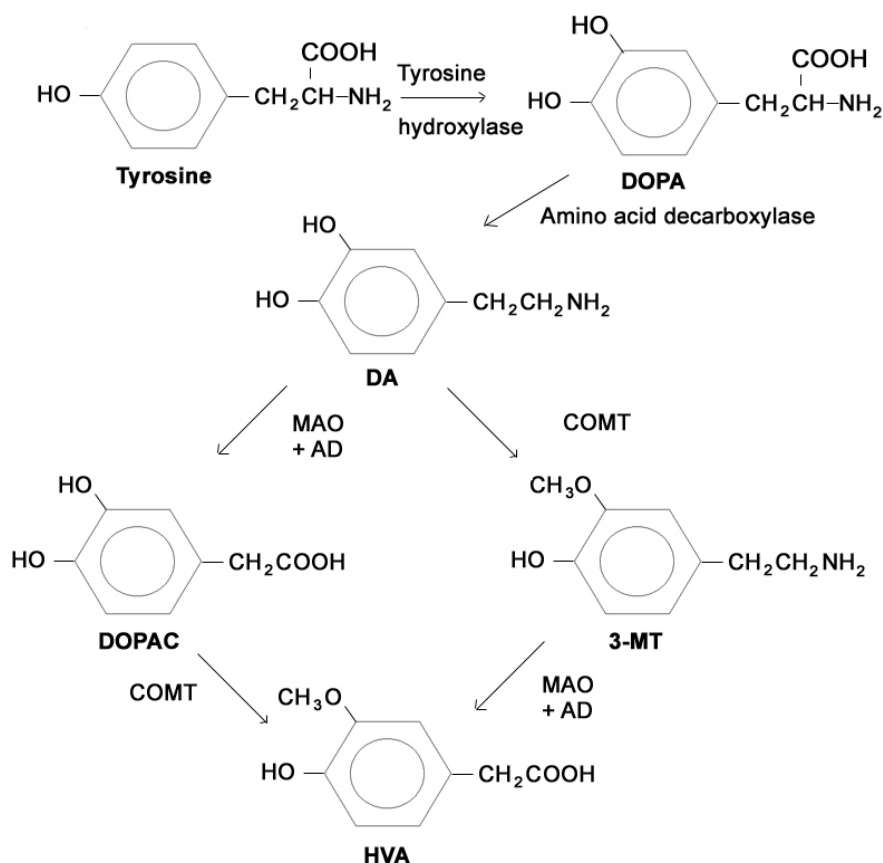
Figure 2.1. Major routes for synthesis and metabolism of dopamine. Abbreviations: DOPA = 3,4-dihydroxyphenylalanine, DA = dopamine, DOPAC = 3,4-dihydroxyphenylacetic acid, HVA = homovanillic acid, 3-MT = 3-methoxytyramine, MAO = monoamine oxidase, COMT = catechol-O-methyl transferase, AD = aldehyde-dehydrogenase.

dopamine synthesis and metabolism, whereas changes in HVA levels seem to be secondary to the efflux of DOPAC from nerve terminals (Brown et al. 1991; Kuczenski and Segal 1992; see Wood and Altar 1988) as well as dopamine release.

It has been estimated that about 70 to 80 % of the released dopamine is removed from the synaptic cleft by re-uptake into dopaminergic nerve terminals (see Bartholini et al. 1989). Dopamine uptake transporters in the nucleus accumbens seem to be located extrasynaptically rather than in the synapses themselves (Garris et al. 1994; Nirenberg et al. 1997a). However, in the VTA, the transporters are mainly located in the perikarya and dendrites of dopaminergic neurons (Nirenberg et al. 1997b).

2.1.3.   The ascending dopamine pathways

The ascending dopamine systems are long projections originating from the VTA (A10), the substantia nigra (A9) and the retrorubral nucleus (A8). The dorsal component of the mesostriatal pathway (the nigrostriatal pathway) mainly stems from the substantia nigra, in particular from the zona compacta (group A9) and not only innervates the entire caudate nucleus and putamen, but also the subthalamic nucleus and globus pallidus (Fig. 2.2.). The ventral component of the mesostriatal dopamine pathway (the mesolimbic pathway) is derived mainly from the VTA, but also from the A9 area, and innervates the nucleus accumbens, olfactory tubercle and nucleus interstitialis striae terminalis (Fig. 2.2.). The mesolimbocortical dopamine system originates primarily from the VTA with minor projections from the A9 group. These fibres innervate the septum, hippocampus, amygdala and many limbic cortical regions. The medial forebrain bundle is formed by noradrenergic and dopaminergic pathways (for reviews see Björklund and Lindvall 1984; Fallon and Loughlin 1995; Fuxe et al. 1985).
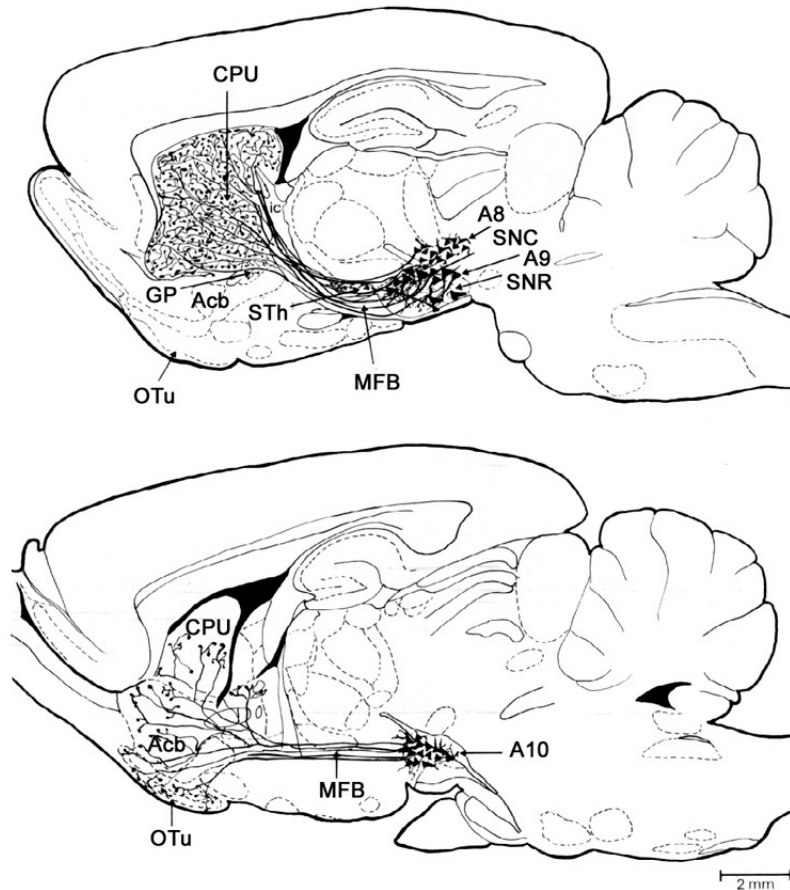
Fig. 2.2. A schematic drawing illustrating the nigrostriatal (upper panel) and the mesolimbic (lower panel) dopamine pathways. Abbreviations: Acb = nucleus accumbens, CPU = caudate-putamen, GP = globus pallidus, MFB = medial forebrain bundle, OTu = olfactory tubercle, STh = subthalamic nucleus, SNC = substantia nigra pars compacta, SNR = substantia nigra pars reticulata (modified from Fuxe et al. 1985).

2.1.4. Dopamine receptors

Dopamine receptors belong to the family of G-protein coupled receptors and can be divided into 2 subfamilies, namely D1- and D2-like receptors (for reviews see Cooper et al. 1996; Vallone et al. 2000). The D1-subfamily comprises of D1- and D5-receptors, and the D2-subfamily of D2-, D3- and D4 receptors. The D1-like receptors are positively coupled to adenylyl cyclase, whereas the D2- like receptors are negatively coupled to adenylyl cyclase, and thus, are inhibitory in nature. Both somatodendritic and nerve terminal autoreceptors controlling the activity of dopamine neurons and dopamine release seem to be D2-like, whereas postsynaptic dopamine receptors can be classified as either D1- or D2-like receptors (see Cooper et al. 1996). Recently it has been shown that D2-receptor has two isoforms, the long D2L-receptor and the short D2S-receptor, which are generated by alternative splicing (Picetti et al.

1997). The D2L-receptor is mainly located at postsynaptic sites wherese the D2S-receptor is a presynaptic autoreceptor (Usiello et al. 2000).

2.1.5. Dopamine and drugs of abuse

Virtually all drugs of abuse, including psychostimulants, opioids, alcohol as well as nicotine share a common ability to increase the release of dopamine in the nucleus accumbens and in the caudate-putamen (Bassareo et al. 1996; Cadoni and Di Chiara 1999; Di Chiara and Imperato 1988a; Di Chiara and Imperato 1988b; Honkanen et al. 1997a; Honkanen et al. 1994b; Hurd et al. 1989; Kalivas and Duffy 1990; Kiianmaa et al. 1995; Liljequist and Ossowska 1994; Pei et al. 1993; Piepponen et al. 1999a; Pontieri et al. 1995; Pontieri et al. 1996; Spanagel et al. 1993). Cocaine prevents the uptake of dopamine by acting as an inhibitor of dopamine transporters, and thus, increases the extracellular concentrations of dopamine (Koe 1976; Kuhar et al. 1991; see Reith et al. 1997). Alcohol has been shown to increase the firing rate of dopamine neurons in the VTA (Brodie and Appel 1998; Brodie et al. 1999a; Brodie et al. 1999b; Brodie et al. 1990) which results in increased dopamine release in the nucleus accumbens. The increase in activity of dopamine neurons may result from action of alcohol directly on dopaminergic neurons (Brodie et al. 1999a) or indirectly via GABAergic (see Grace 2000) or glutamatergic (Rossetti et al. 1999) mechanisms. Opioids, such as morphine, have been suggested to increase the release of dopamine in the nucleus accumbens indirectly via the VTA, by hyperpolarizing γ-amino butyric acid neurons that tonically inhibit the activity of dopaminergic neurons (Gysling and Wang 1983; Johnson and North 1992). In addition to elevated dopamine release, μ-opioids increase the synthesis and metabolism of dopamine (Ahtee and Kääriäinen 1973; Attila and Ahtee 1984; Di Chiara and Imperato 1988b; Honkanen et al. 1994b; Piepponen and Ahtee 1995). The effects of drugs of abuse on brain dopaminergic mechanisms seem to be stronger in the nucleus accumbens than in the caudate-putamen (Attila and Ahtee 1984; Di Chiara and Imperato 1988a; Di Chiara and Imperato 1988b). Furthermore, it has been determined that acute morphine, nicotine, cocaine and amphetamine can all increase dopamine release preferentially in the shell subdivision of the nucleus accumbens as compared with the core subdivision (Cadoni and Di Chiara 2000; Cadoni et al. 2000; Pontieri et al. 1995). Besides well known

stimulatory effect on dopamine release, microdialysis studies suggest that morphine may also have a local inhibitory effect on dopamine release in the caudate-putamen (Piepponen et al. 1999b; Rossetti et al. 1990).

After repeated treatment, the effects of opioids and psychostimulants on brain dopamine mechanisms seem to be augmented. This sensitisation may be perceived, for instance, as enhanced depletion of dopamine in the nucleus accumbens, in the limbic forebrain or in the striatum after inhibition of tyrosine hydroxylase (Attila and Ahtee 1984; Kalivas and Duffy 1987) or alternately as increased dopamine release in the nucleus accumbens or striatum (Acquas and Di Chiara 1992; Ahtee et al. 1989; Cadoni and Di Chiara 1999; Honkanen et al. 1994b; Kalivas and Stewart 1991; Spanagel et al. 1993) induced by morphine challenge after repeated morphine treatment. Similarly, the ability of psychostimulants to elevate the concentration of extracellular dopamine in the nucleus accumbens is enhanced after repeated treatment (Cadoni et al. 2000; Kalivas and Duffy 1990; Kalivas and Duffy 1993). It also seems that the sensitisation to opioids, nicotine and psychostimulants may be seen particularly in the core subdivision of the nucleus accumbens but not in the shell subdivision (Cadoni and Di Chiara 1999; Cadoni and Di Chiara 2000; Cadoni et al. 2000). Alcohol may differ from opioids and psychostimulants, in that respect, in that there may be no sensitisation of its effect on cerebral dopamine after repeated treatment. When AA and ANA rats were administered alcohol repeatedly intragastrically, and thereafter given i.p. injections of alcohol, similar increases in accumbal dopamine concentrations occurred to those found previously with acute IP administration of alcohol (Kiianmaa et al. 1995; Nurmi et al. 1996). Indeed, prior alcohol drinking would seem to induce even tolerance to the effect of IP alcohol on accumbal dopamine release in AA rats (Nurmi et al. 1996).

2.1.6. Dopamine and addiction theories

The pivotal role of dopamine in the reinforcing or rewarding properties of drugs of abuse has been known for several years, and there has been a lot of discussion on the exact role of dopamine in drug addiction during recent years (for reviews see Bechara et al. 1998; Berridge and Robinson 1998; Di Chiara 1995; Di Chiara 1999; Ikemoto

and Panksepp 1999; Koob et al. 1997; Robinson and Berridge 1993; Spanagel and Weiss 1999; Wise 1996; Wise and Bozarth 1987; Wise and Rompré 1989). The incentive-sensitisation theory of addiction by Robinson and Berridge (1993) suggests that after repeated use of drugs of abuse, the feeling of "wanting" drugs transforms itself into excessive drug craving. The sensitisation of the mesolimbic dopamine system plays a critical role in this phenomenon. Moreover, they suggest that dopamine is necessary only for "wanting" the incentives, but not for "liking" them or learning new "likes" or "dislikes" (see Berridge and Robinson 1998). On the other hand, the opponent process model of addiction suggests that in addition to positive reinforcement processes, the motivation for maintenance of compulsive drug use requires negative reinforcement processes (see Koob et al. 1997). Thus, abstinence from drugs of abuse, e.g. psychostimulants, results in negative motivational states, so called hedonic homeostatic dysregulation, associated with dysphoria, anxiety and irritability (for reviews see Koob et al. 1997; Koob and Le Moal 1997; Koob et al. 1998b), and the drug alleviates this aversive state. This state of withdrawal is associated with decreased levels of dopamine and 5-HT and increased levels of corticotrophin-releasing factor in the nucleus accumbens, as well as increase in intracranial self-stimulation reward thresholds (Koob et al. 1997; Koob and Le Moal 1997). Drug addiction has also been suggested to be a dopamine-dependent associative learning disorder (see Di Chiara 1999). Thus, both drugs of abuse and natural rewards increase dopamine transmission in the shell subdivision of the nucleus accumbens. The difference between natural rewards and drugs of abuse is that the enhancement of DA transmission habituates rapidly with natural rewards, but slower with drugs of abuse. The effect of repeated stimulation of dopamine transmission in the nucleus accumbens shell with drugs of abuse results in abnormal associative learning, which leads to the expression of excessive control over behaviour (see Di Chiara 1999).

2.1.7. Dopamine and behaviour

Locomotor activity. In addition to enhancing cerebral dopamine release, drugs of abuse increase the locomotor activity of animals (for reviews see Kalivas and Stewart 1991; Koob 1992). It has been suggested that mesolimbic dopaminergic neurons

mediate both the rewarding and locomotor activity stimulating effects of the various drugs of abuse (for reviews see Wise and Bozarth 1987; Wise and Rompré 1989). It seems that, in the case of psychostimulants, the stimulation of locomotor activity results primarily from an interaction with dopamine transporters (for reviews see Di Chiara 1995; Pierce and Kalivas 1997; Wise and Bozarth 1987; Woolverton and Johnson 1992). The terminal areas of dopaminergic neurons in the striatum, especially in the nucleus accumbens, seem to play an important role in psychomotor stimulation induced by psychostimulants. An example of this is that local infusion of cocaine into the VTA did not enhance the locomotor activity of rats (Chen and Reith 1994) whereas local microinjections of cocaine into the nucleus accumbens or into the striatum did promote an increase in locomotor activity, with the effect of cocaine being noticeably intence when injected into the nucleus accumbens (Delfs et al. 1990). Opioids have also been shown to exert and effect on locomotor activity, both dependently and independently of dopamine. Dopamine dependent locomotion seems to result from opioid-action in the VTA (Di Chiara 1995; Stinus et al. 1992), whereas dopamine independent locomotion seems to derive from the nucleus accumbens (Kalivas et al. 1983; Pert and Sivit 1977; Stinus et al. 1985). However, when administered intra-nigrally, opiates elicit stereotypical behaviour, that is sensitive to low doses of dopamine D1-receptor antagonists (Morelli et al. 1989). The effect of opioids on horizontal locomotor activity also depends on the dose of the drug used, that is, smaller doses of morphine increase the locomotor activity of the animals, whereas larger doses cause initial depression, followed by delayed excitation (Babbini and Davis 1972).

The role of central catecholamines on alcohol-induced euphoria and stimulation was shown by studies that took place in the early 1970's, when a group of Swedish scientists took placebo or α-methyl-*p*-tyrosine, a tyrosine hydroxylase inhibitor, prior to drinking alcohol (Ahlenius et al. 1973). They found that those subjects who took α-methyl-*p*-tyrosine demonstrated significant reduction in alertness, talkativeness, elation and happiness as compared to placebo taking controls. Although alcohol increases dopamine release, it has sedative-hypnotic effects, probably resulting from its action on $GABA_A$ receptors (Grobin et al. 1998; Korpi 1994). Thus, experimenter-administered alcohol does not generally increase locomotor activity in rats (Criswell et al. 1994; Cunningham et al. 1993; Frye and Breese 1981; Masur et al. 1986;

Päivärinta and Korpi 1993). Low doses of alcohol (≤ 0.25 g/kg, IP) were shown to increase spontaneous motor activity in alcohol-preferring P rats and Maudsley Reactive rats (Waller et al. 1986), but this could not be replicated later with P rats (Criswell et al. 1994). However, voluntary alcohol drinking seems to have a permissive effect on the locomotor activity of at least some lines of alcohol-preferring rats (Colombo et al. 1998; Päivärinta and Korpi 1993). In addition, several studies have also shown that alcohol increases the locomotor activity in some strains of mice (Carlsson et al. 1972; Cunningham et al. 1993; Liljequist 1991; Liljequist et al. 1981; Liljequist and Karcz-Kubicha 1993; Liljequist and Ossowska 1994; Masur and Boerngen 1980). This effect of alcohol described above seem to be, at least partly, dopamine dependent, since several types of dopamine receptor antagonists have been found to suppress the alcohol-induced locomotor stimulation in mice (Broadbent et al. 1995; Cohen et al. 1997; Le et al. 1997; Liljequist et al. 1981; Risinger et al. 1992).

Repeated treatment with opioids or psychostimulants has also been shown to induce behavioural sensitisation in rats, which is manifested as an enhancement of the effect of drug on horizontal locomotor activity and has been associated with increased dopamine release in the nucleus accumbens (Acquas and Di Chiara 1992; Cadoni and Di Chiara 1999; Cadoni and Di Chiara 2000; Cadoni et al. 2000; Kalivas and Duffy 1990; Spanagel et al. 1993). The sedative and cataleptic effects of acute administration of opioids may be converted, after repeated treatment, into stereotypies (Ahtee 1974; Babbini and Davis 1972; Fog 1970), which have been associated with enhancement of the effect of the drug on nigrostriatal dopamine mechanisms (Ahtee 1974; Ahtee and Attila 1987; Bloom et al. 1989; Patrick et al. 1991). It seems, however, that behavioural sensitisation is not always associated with increased dopamine release (Acquas and Di Chiara 1992; Heidbreder et al. 1996; Johnson et al. 2000; Kalivas and Duffy 1993; Segal and Kuczenski 1992). The length of the withdrawal period between repeated drug treatment and the test session appears to play important role in the expression of neurochemical sensitisation. Thus, even if behavioural sensitisation occurs, sensitisation of accumbal dopamine may not be seen if cocaine challenge is given in the early stages of withdrawal (1 or 2 days after repeated treatment) (Heidbreder et al. 1996; Johnson et al. 2000; Segal and Kuczenski 1992), but may be detected after prolonged withdrawal (22 days) (Heidbreder et al.

1996). The dose of drug used may also play an important role in this phenomenon. For instance, repeated treatment with 15 mg/kg of cocaine for 4 or 5 days resulted in behavioural sensitisation associated with increased dopamine release in the nucleus accumbens when the rats were challenged with the same dose of cocaine on the following day (Kalivas and Duffy 1990; Kalivas and Duffy 1993), whereas when the rats were pretreated with 30 mg/kg of cocaine for 5 days and on the following day challenged with 15 mg/kg of cocaine, behavioural sensitisation was again present, but the increase in extracellular DA was reduced when compared with naive rats (Kalivas and Duffy 1993). However, when the rats were challenged with cocaine (15 mg/kg) 17-21 days after discontinuing the repeated cocaine treatment (15 or 30 mg/kg for 5 days), both sensitisation of locomotor activity and mesolimbic dopamine release occurred (Kalivas and Duffy 1993). Generally, both neurochemical and behavioural sensitisation may be seen in rodents when they are challenged with the drug 3 or more days after discontinuation of the daily drug treatment (Acquas and Di Chiara 1992; Cadoni and Di Chiara 1999; Cadoni and Di Chiara 2000; Cadoni et al. 2000; Spanagel et al. 1993; see Kalivas and Stewart 1991). Behavioural sensitisation associated with sensitisation of dopaminergic mechanisms has been shown to last for at least 3-4 weeks (Kalivas and Duffy 1993; Spanagel et al. 1993) even after a single exposure to amphetamine (Vanderschuren et al. 1999).

Rotational behaviour. Dopaminergic neurons can be lesioned by injecting 6-hydroxydopamine (6-OHDA) into the dopaminergic pathways (Ungerstedt 1971b). Unilateral lesions of, for example, the nigrostriatal dopaminergic pathways, induce motor disturbances, and the effects of drugs on the nigrostriatal dopaminergic system may be investigated by examining the rotational behaviour of the rat (Ungerstedt and Arbuthnott 1970). The dopamine receptors in the denervated striatum become hypersensitive after 6-OHDA lesion, and, thus, drugs that activate presynaptic dopamine receptors directly (like apomorphine or L-DOPA) induce contralateral circling, i.e. circling away from the denervated striatum (Costall et al. 1983; Ungerstedt 1971a). In turn, drugs that activate the remaining dopamine pathways presynaptically, like amphetamine, cocaine or morphine, induce ipsilateral rotation, i.e. rotation towards the lesioned side (Browman et al. 1998; Crombag et al. 1999; Guan et al. 1985; Kimmel and Holtzman 1997; Robinson 1984; Silverman 1990; Ungerstedt 1971c; Volpicelli et al. 1999). Repeated drug treatment with morphine,

cocaine or amphetamine also induces behavioural sensitisation in this animal model, which can be seen as increases in the rotational behaviour of the rats (Browman et al. 1998; Crombag et al. 1999; Guan et al. 1985; Robinson 1984; Silverman 1990; Ungerstedt 1971c; Volpicelli et al. 1999).

2.1.8. Neuronal adaptations underlying sensitisation

Repeated injections of opioids or psychostimulants into the VTA, according to several studies, induce behavioural and biochemical sensitisation to subsequent intra-accumbal or systemic administration of drugs (Cador et al. 1995; Hooks et al. 1992; Kalivas and Weber 1988; Perugini and Vezina 1994; Vezina 1993; Vezina 1996; Vezina et al. 1987; Vezina and Stewart 1984; Vezina and Stewart 1989; Vezina and Stewart 1990). This sensitisation does not occur if these drugs are administered repeatedly into the nucleus accumbens (Cador et al. 1995; Hooks et al. 1992; Vezina et al. 1987; Vezina and Stewart 1990). This evidence seems to indicate that the critical locus in the initiation of sensitisation may be the VTA.

Chronic drug treatments have been shown to provoke both functional and structural changes in mesolimbic dopamine systems (for reviews see Pierce and Kalivas 1997; Self and Nestler 1995). Chronic cocaine or amphetamine treatments, for example, produce transient increases in the spontaneous firing rate of VTA dopamine neurons in the early stages of withdrawal (Ackerman and White 1990; Henry et al. 1989), which, however, turns into decrease after prolonged withdrawal (10-14 days) (Ackerman and White 1992). Chronic cocaine and morphine also increase the levels and activity of tyrosine hydroxylase in the VTA (Beitner-Johnson et al. 1992a; Masserano et al. 1996). In addition to neurochemical changes, structural changes also occur in the VTA after repeated drug treatment, and these may include decreased levels of neurofilament proteins and increased levels of glial fibrillary acidic proteins (Beitner-Johnson et al. 1992b; Beitner-Johnson et al. 1993). The neurofilament proteins are a major element of the neuronal cytoskeleton and are involved in the axonal caliber and transport (see Self and Nestler 1995). Interestingly, alcohol-preferring P rats as well as alcohol- and other drugs-preferring Lewis rats have lower

levels of neurofilaments in the VTA than their non-preferring counterparts, NP and Fisher 344 rats, respectively (Guitart et al. 1992; Guitart et al. 1993).

In the nucleus accumbens, enhancement of the release of dopamine may involve changes in calcium transduction, dopamine uptake mechanisms or autoreceptor sensitivity (see Pierce and Kalivas 1997). The expression of behavioural sensitisation was found to be blocked by L-type calcium channel antagonists (Karler et al. 1991b; Martin-Iverson and Reimer 1994). In studies concerning dopamine uptake mechanisms there are some inconsistencies, but generally, the dopamine transporter mechanisms seem to be upregulated during early withdrawal from repeated psychostimulant administration, but in contrast, after a week or more of withdrawal the dopamine transporter density may be reduced in the shell of the nucleus accumbens (see Pierce and Kalivas 1997). Concerning dopamine autoreceptor function, increases (Dwoskin et al. 1988), decreases (Yi and Johnson 1990) or no changes (Fitzgerald and Reid 1991; Gifford and Johnson 1992) in the dopamine D2-autoreceptor function have been reported after repeated psychostimulant administration.

There may be also changes in the postsynaptic neurons that contribute to the expression of behavioural sensitisation. Repeated morphine and cocaine treatments have been shown to induce long lasting hypersensitivity in postsynaptic dopamine D1-receptors (Henry and White 1991; Tjon et al. 1994). However, it seems that the density of D1 or D2 receptors is not altered after repeated psychostimulant administration (see Pierce and Kalivas 1997). Thus, there may be changes at the signal-transduction level in postsynaptic dopamine receptors. In addition, chronic cocaine and morphine treatments increase the activity of adenylyl cyclase (Terwilliger et al. 1991) and decreases it the levels of G protein subunit G_i in the nucleus accumbens have been brought about by both chronic morphine and cocaine administration (Nestler et al. 1990; Terwilliger et al. 1991). Thus, the inhibition of adenylyl cyclase arising from G_i coupled receptors may be reduced (Pierce and Kalivas 1997). As D1 receptors mediate their action via activation of adenylyl cyclase, this may contribute to the hypersensitivity of D1-receptors after repeated drug treatment.

The antagonists of the N-Methyl-D-aspartate (NMDA) subtype of glutamate receptor have been shown to block the development of behavioural sensitisation to psychostimulants (Druhan and Wilent 1999; Gaytan et al. 2000; Kalivas and Alesdatter 1993; Karler et al. 1991a; Li et al. 1999; Li and Wolf 1999; Pudiak and Bozarth 1993; Stewart and Druhan 1993; Wolf and Jeziorski 1993; Wolf and Khansa 1991), morphine (Jeziorski et al. 1994; Wolf and Jeziorski 1993) and apomorphine (Druhan et al. 1993; Voikar et al. 1999), supporting the role of glutamatergic mechanisms in behavioural sensitisation. In addition to NMDA receptors, the α–amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) subtype of ionotropic glutamate receptors as well as metabotropic glutamate receptors seem to be involved in this process (Carlezon et al. 1999; Kim and Vezina 1998; Li et al. 1997). Repeated administration of morphine and psychostimulants also increases glutamate receptor subunit levels in the VTA (Churchill et al. 1999; Fitzgerald et al. 1996), increases the responsiveness of VTA dopamine neurons to glutamate (Zhang et al. 1997), and moreover, the induction of behavioural sensitisation to psychostimulants can be blocked by intra-VTA administration of glutamate antagonists (Cador et al. 1999; Kalivas and Alesdatter 1993; Kim and Vezina 1998). This indicates that glutamatergic transmission especially in the VTA may be critically involved in the sensitisation process.

2.2. The endogenous opioid system

The endogenous opioid system is involved in several physiological processes, including pain relief, reward, mood, ingestive behaviour, motor behaviour, release of hormones and neurotransmitters, gastrointestinal transit and respiration. Opioid receptors were initially discovered in the early 1970´s (Pert and Snyder 1973; Simon et al. 1973; Terenius 1973) and soon thereafter it was found that the brain contains endogenous peptides with opiate-like activities (Bradbury et al. 1976; Hughes et al. 1975; Lord et al. 1977; Pasternak et al. 1975). Opioid receptors can be divided into three major classes of receptors, namely the μ-, δ- and κ- receptors, which are differentially distributed throughout the central nervous system (Mansour et al. 1995; Mansour et al. 1988; see Loughlin et al. 1995) Morphine will bind to all opioid

receptors, but its relative affinity for μ-receptors is far stronger than its affinity for δ- or κ- receptors (see Corbett et al. 1993), and it is, thus, considered to be somewhat selective for μ-opioid receptors. Endogenous opioid peptides are derived from three major precursor proteins. Pro-opiomelanocortin is a precursor molecule that may be converted into the opioid peptide β-endorphin, as well as other peptides with no opioid receptor activity, including adrenocorticotrophic hormone (for reviews see Loughlin et al. 1995; Young et al. 1993). β-Endorphin has similar affinities for μ- and δ- opioid receptors but is much less active at the κ-binding site (see Corbett et al. 1993). Another family of peptides are the enkephalins, which are all derived from proenkephalin. These include the opioid peptides Met-enkephalin and Leu-enkephalin as well as several related peptides such as Met-enkephalin-Arg-Gly-Leu and Met-enkephalin-Arg-Phe (for reviews see Loughlin et al. 1995; Rossier 1993). The Met- and Leu-enkephalins are relative selective for δ-opioid receptors (see Corbett et al. 1993). Finally, the dynorphins, like dynorphin A and dynorphin B, are derived from prodynorphin and have the highest affinity for κ-opioid receptors (for reviews see Corbett et al. 1993; Day et al. 1993; Loughlin et al. 1995).

Alcohol has been found to stimulate release of endogenous opioid peptides both in humans and rodents (De Waele and Gianoulakis 1994; de Waele et al. 1994; De Waele et al. 1992; Gianoulakis 1989; Gianoulakis 1990; Gianoulakis 1996; Gianoulakis et al. 1996; Keith et al. 1986) and additionally, alcohol may also affect opioid receptor sensitivity (for reviews see Herz 1997; Ulm et al. 1995). Enhanced opioid activity might lead to enhancement of mesolimbic dopamine release, possibly through the action of μ-opioid receptors in the ventral tegmental area (VTA) or δ-opioid receptors in the nucleus accumbens (for reviews see Gianoulakis 1996; Herz 1997). As cerebral dopaminergic mechanisms seem to be important in reinforcing effects of drugs of abuse (see section 2.1.), the stimulation of endogenous opioid mechanisms by alcohol may also be involved in the rewarding or reinforcing effects of alcohol (see Herz 1997).

2.3. Serotonin

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter found throughout the central nervous system. The cell bodies of brain 5-HT neurons are mostly located in the medial and dorsal raphe nuclei, from where the 5-HT fibres project to the forebrain and terminate in several cortical areas as well as in the striatum, nucleus accumbens, VTA, amygdala and hippocampus. To date, 7 main families of 5-HT receptors have been found, 5-HT_1 to 5-HT_7 . The 5-HT_1 receptor family can be subclassified into 5-HT_{1A}, $5\text{-HT}_{1B/D}$, 5-HT_{1E} and 5-HT_{1F} subtypes, the 5-HT_2 receptor family into 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2c} subtypes and the 5-HT_5 receptor family into 5-HT_{5A} and 5-HT_{5B} subtypes (see Ciccocioppo 1999).

The synthesis of 5-HT begins with the amino acid tryptophan, which is derived primarily from the diet. Tryptophan is first hydroxylated to 5-hydroxytryptophan by enzyme tryptophan hydroxylase. 5-Hydroxytryptophan is subsequently converted to 5-HT by amino acid decarboxylase. 5-HT may be metabolized by monoamine oxidase and aldehyde dehydrogenase to 5-hydroxy indole acetic acid (5-HIAA), which is the main metabolite of 5-HT. In the pineal gland, 5-HT may also be metabolized to N-acetyl serotonin, by 5-HT N-acetylase, which is then further converted to melatonin by 5-hydroxy indole-O-methyl transferase (see Cooper et al. 1996).

5-HT is involved in the function several physiological processes (including gastrointestinal transit, cardiovascular system, nociception, sleep-wake rhythm) and clinical disorders such as migraine, anxiety, depression, psychosis, eating disorders, emesis as well as alcoholism and drug addiction. Alcohol, morphine and cocaine have all been shown to increase 5-HT release in the nucleus accumbens (Parsons et al. 1995; Tao and Auerbach 1994; Yoshimoto et al. 1992). Withdrawal from alcohol or the abused drug may be associated with a reduction in accumbal 5-HT release (Parsons et al. 1995; Weiss et al. 1996), suggesting that 5-HT mechanisms are also involved in drug dependence. A deficit in brain 5-HT function may contribute to the loss of control associated with drug craving (Ciccocioppo 1999) and type 2 alcoholism in humans (characterised by antisocial, often violent behavioural traits) is

also related to low cerebrospinal fluid 5-HIAA concentrations (for reviews see Cloninger 1987; Linnoila et al. 1994; Virkkunen and Linnoila 1997).

2.4. AA and ANA rats

2.4.1. Rat lines selected for differential alcohol consumption

One of the oldest alcohol-preferring/alcohol non-preferring rat lines in experimental use is the AA (Alko Alcohol) and ANA (Alko Non-Alcohol) rats bred in Helsinki. The breeding of these lines of rats started in 1960´s in the Research Laboratories of the State Alcohol Monopoly (ALKO), Helsinki, Finland, by Kalervo Eriksson, when he discovered a wide range of individual variation and a rather high average preference for alcohol among rats of Wistar origin (Eriksson 1968). Systematic mating of rats exhibiting high and low alcohol consumption selected from a large population resulted in two outbred rat lines, the AA and ANA rats, that differed in their levels of voluntary alcohol consumption (Eriksson 1968; for reviews see Eriksson 1969; Eriksson 1971; Eriksson and Rusi 1981; Sinclair et al. 1989). Line difference in alcohol consumption was found already in the F_8 generation of these rats (see Eriksson 1969). Even before the AA and ANA rats, another breeding project was started in the 1940´s in Chile, that yielded inbred UChA (alcohol non-preferring) and UChB (alcohol-preferring) rat lines (see Mardones and Segovia-Riquelme 1983). Other selectively bred rat lines have been produced later on, namely the alcohol-preferring (P), non-preferring (NP), high alcohol-drinking (HAD) and low alcohol-drinking (LAD) rats from Indianapolis (for reviews see Li et al. 1993; Li et al. 1987) and the Sardinian alcohol-preferring (SP) and non-preferring (SNP) rats from Sardinia (Fadda et al. 1989). All these rat lines show clear differences in their voluntary alcohol consumption between the "alcoholic" and "non-alcoholic" rats (Table 2-1). It has been estimated that, taking in to account the different rate of drug metabolism in rats and humans, the amount of ethanol (about 6-7 g/kg/day) these "alcoholic" rats consume would correspond to about 5 measures of whisky per day for humans (see Colombo 1997).

Table 2-1. Voluntary alcohol consumptions (g/kg/day, mean ± SD) in rat lines selected for differential alcohol-preference.

Rat line	"Alcoholic" ♂	"Alcoholic" ♀	"Non-Alcoholic" ♂	"Non-Alcoholic" ♀
AA/ANA (F_{55})[a]	7.6 ± 2.1	8.0 ± 2.3	0.4 ± 0.5	0.3 ± 0.2
sP/sNP[b,c]	6-7		<1	
P/nP (S-31)[d]	5.7 ± 0.16	6.6 ± 0.19	0.5 ± 0.08	0.4 ± 0.08
HAD-1/LAD-1 (S-13)[d]	5.6 ± 0.34	4.5 ± 0.45	0.3 ± 0.06	0.7 ± 0.12
HAD-2/LAD-2 (S-12)[d]	4.7 ± 0.43	7.0 ± 0.58	0.3 ± 0.04	1.5 ± 0.36

(Sinclair et al. 1989)[a], (Colombo et al. 1995; Lobina et al. 1997)[c,d], (Li et al. 1993)[d] . F and S indicate the generations of the rat lines.

2.4.2.   Drinking behaviour

One of the proposed criterias for an animal model of alcoholism is that ethanol should be positively reinforcing, and animals should be willing to work for alcohol and overcome obstacles to obtain it (see McBride and Li 1998). AA rats learn to press a lever to get a dose of alcohol even in the absence of water or food, or special training (Hyytiä and Sinclair 1989). Furthermore, AA rats respond significantly more for alcohol than water, and if the fixed-ratio schedule to obtain a measure of alcohol is increased from 1 to 2 or 4, the lever pressing of AA rats increases (Hyytiä and Sinclair 1990; Ritz et al. 1989). Thus, alcohol seems to serve as a reinforcer in AA rats. Using a sucrose-substitution procedure, a training procedure that initially involves sucrose which is subsequently replaced little by little by alcohol (Samson 1986), ANA rats can also be taught to press lever for alcohol (Files et al. 1997). If the rats are given a liquid, alcohol containing diet as the only source of energy, even ANA rats can be made to drink significant amounts of alcohol. In a study by (Winkler et al. 1999), using the aforementioned liquid alcohol containing diet, ANA rats drank 9.3 ± 0.3 g/kg/day of alcohol while AA rats drank 8.5 ± 0.2 g/kg/day.

When AA and ANA rats were given alcohol solutions intraorally with intraoral fistulas, AA rats produced more ingestive responses (mouth movements, tongue protrusions) to alcohol than ANA rats (Badia-Elder and Kiefer 1999). This occurred both before and after the rats had had 3-weeks free access to a 10 % alcohol solution. There were no differences in the aversive responses to alcohol prior to continuous alcohol access between rats of these lines. However, after 3-weeks of alcohol consumption the AA rats were shown to have decreased aversive responses to alcohol, particularly at higher (> 20 %) alcohol concentrations. These results suggest that the AA rats respond to alcohol solutions in a highly ingestive manner (Badia-Elder and Kiefer 1999). However, line difference in voluntary alcohol consumption is probably not completely related to oral factors, since AA rats self-administer more alcohol intravenously than ANA rats (Hyytiä et al. 1996). AA rats have also been found to consume more concentrated solutions of quinine, saccharin, citric acid and salt than ANA rats (Hyytiä and Sinclair 1993; Sinclair et al. 1992), suggesting that AA rats may be less sensitive to strong or aversive tastes than ANA rats. However, when quinine was given intraorally, AA rats showed more aversive responses than ANA rats (Badia-Elder and Kiefer 1999).

Opioid receptors and endogenous brain opioid systems (see chapter 2.2.) seem to be involved in alcohol drinking behaviour (see Herz 1997). The involvement of opioids in drinking behaviour has been shown both in experimental animals as well as in humans (for reviews see O. Brien et al. 1996; Ulm et al. 1995; Volpicelli et al. 1995). Of the opioid receptor antagonists, naloxone, a non-selective antagonist, and CTOP [D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH$_2$], a selective μ-opioid receptor antagonist have been shown to reduce alcohol consumption in AA rats (Hyytiä 1993; Wegelius et al. 1994). Furthermore, naloxonazine, a selective μ$_1$-opioid receptor antagonist initially reduced alcohol consumption of AA rats on the first day of drug treatment, but was without effect on the 2nd and 3rd days of drug treatment (Honkanen et al. 1996). δ-Opioid receptor antagonists were found to have no significant effect on voluntary alcohol consumption in AA rats (Honkanen et al. 1996; Hyytiä 1993).

In addition to alcohol, AA rats consume more aqueous solutions of etonitazene, a μ-opioid receptor agonist, and cocaine than ANA or non-selected Wistar rats (Hyytiä

and Sinclair 1993). AA rats have also been shown to self-administer initially higher amounts of heroin intravenously than ANA rats (Hyytiä et al. 1996). Thus, cerebral mechanisms mediating reward may be more sensitive in AA than in ANA rats. No difference has been found between AA and ANA rats when examining alcohol-, morphine- or stress-induced analgesia (Honkanen et al. 1995), which suggests that there is no general enhanced sensitivity of AA rats to opioids.

2.4.3. Blood and brain alcohol levels

The levels of blood alcohol in AA rats measured after voluntary alcohol drinking with continuous access to alcohol have been shown to be in the intoxicating range, the highest levels being 25 mmol/l (= 1.15 ‰) (Aalto 1986). With the limited access method, in which the rats are only allowed to drink alcohol solution for one hour daily, brain alcohol concentrations as high as 33.6 mmol/l (=1.55 ‰) have been measured in AA rats (Nurmi et al. 1999). In one study, AA rats have been found to have lower concentrations of blood alcohol than ANA rats after intraperitoneal (IP) injection of 2 or 4 g/kg doses of alcohol but not after a 1.5 g/kg dose of alcohol (Hilakivi et al. 1984). However, in a later study after IP injection of alcohol, no significant differences between AA and ANA rats were found in the levels of alcohol, either in the blood (Kiianmaa et al. 1995; Koivisto et al. 1993) or in the brain (Nurmi et al. 1994) after IP injection of alcohol.

2.4.4. Alcohol metabolism

The elimination rate of alcohol has been shown to be faster in female AA than female ANA rats (Eriksson 1973; Forsander and Sinclair 1992), which may allow higher alcohol consumption. However, no such difference in alcohol metabolism was found between male AA and ANA rats and therefore it can-not account for the difference in alcohol consumptions between the rat lines. One difference, which may account for the low alcohol intake of ANA rats is that these rats accumulate higher levels of blood acetaldehyde during alcohol metabolism than AA rats. This difference was first reported in the F_{17} generation (Eriksson 1973) and has been later confirmed in the F_{40},

F_{43} and F_{60} generations (Hilakivi et al. 1984; Koivisto et al. 1993). The activity of liver alcohol dehydrogenase was found to be higher in ANA than in AA rats (Koivisto and Eriksson 1994; Koivula et al. 1975) or equivalents (Forsander and Sinclair 1992), whereas the activity of liver aldehyde dehydrogenase was found to be lower in ANA than in AA rats (Koivisto and Eriksson 1994; Koivula et al. 1975). The higher activity of alcohol dehydrogenase and lower activity of aldehyde dehydrogenase may account for the higher acetaldehyde levels during alcohol metabolism in ANA rats as compared with AA rats. Further support for the hypothesis that acetaldehyde plays a role in the low alcohol consumption of ANA rats comes from a study, in which AA rats were transplanted with livers of ANA rats (Eriksson et al. 1997). Those AA rats, who received livers from ANA rats, showed very low drinking, whereas those AA rats, who were transplanted with livers of AA rats showed heavy drinking.

2.4.5. Neurotransmitters

Among the first differences found between AA and ANA rats were findings by Ahtee and Eriksson in the early 1970´s, that AA rats have about 15-20 % higher cerebral concentrations of 5-HT and 5-HIAA than ANA rats (Ahtee and Eriksson 1972; Ahtee and Eriksson 1973). This has later been confirmed several times in different brain regions (Honkanen et al. 1999; Korpi et al. 1991; Korpi et al. 1988). AA rats were also found to have higher (up to 33 % depending on brain area studied) cerebral levels of dopamine than ANA rats (Ahtee et al. 1980; Ahtee and Eriksson 1975; Honkanen et al. 1999; Kiianmaa et al. 1991; Kiianmaa and Tabakoff 1984; Korpi et al. 1988). In contrast to higher tissue levels of dopamine in AA rats, basal dopamine release, when measured as levels of 3-methoxytyramine (3-MT), seems to be slower in the caudate-putamen and nucleus accumbens of AA than ANA rats (Honkanen et al. 1999). However, no significant differences have been found in basal extracellular dopamine levels in the nucleus accumbens between these rats when measured with *in vivo* microdialysis (Kiianmaa et al. 1995; Nurmi et al. 1996). Noradrenaline concentrations have been found to be lower (up to 15 %) in AA than in ANA rats in whole brains, including hemispheres, diencephalon, cerebral cortex, frontal cortex, and hippocampus (Ahtee et al. 1980; Kiianmaa et al. 1991). Whereas, higher levels (by

about 12 %) of noradrenaline were found in the limbic forebrain of AA rats when compared to levels from ANA rats.

Another neurochemical difference is that the activity of tyrosine hydroxylase has been found to be higher in whole brains of AA than ANA rats (Pispa et al. 1986). As this enzyme is the rate-limiting step in biosynthesis of dopamine, the higher enzyme activity in AA rats may contribute to the higher concentrations of dopamine in brains of AA rats.

AA rats have been found to have a higher content of proopiomelanocortin (POMC) messenger RNA (mRNA) than ANA rats in the hypothalamus and in the anterior and intermediate lobes of the pituitary (Gianoulakis et al. 1992; Marinelli et al. 2000). Proenkephalin content has been found to be higher in AA rats in the prefrontal cortex and prodynorphin content in the mediodorsal nucleus of the thalamus (Marinelli et al. 2000), whereas (Met)enkephalinArg^{6}Phe^7 (MEAP) levels, a marker of the proenkephalin system, were lower in the nucleus accumbens of AA rats than in that of ANA rats (Nylander et al. 1994). Similarly, levels of the markers of the prodynorphin system, dynorphin A and dynorphin B, have been found to be lower in the nucleus accumbens and (Leu)enkephalin^6 levels lower in the VTA of AA than of ANA rats (Nylander et al. 1994). β-Endorphin-like immunoreactivity has been found to be higher in AA rats than ANA rats in the septum and the anterior lobe of the pituitary, but lower in the amygdala and the periaqueductal grey matter, whereas no difference was found between the rat lines in the arcuate nucleus plus median eminence, the nucleus accumbens, the caudate, the hippocampus and the cortex (Gianoulakis et al. 1992). Spontaneous *in vitro* release of β-endorphin-like peptides (β-EPLPs) has been found to be raised in the hypothalamus of ANA when compared to that of AA rats (de Waele et al. 1994) but ethanol-stimulated increases of release of β-EPLPs did not differ between the rats of these lines. However, the β-EPLPs released by ethanol in ANA rats were predominantly in the acetylated forms, which are devoid of opioid activity (de Waele et al. 1994). This might lead to lower reward after alcohol in ANA than in AA rats, and may contribute to the low alcohol consumption of ANA rats. Voluntary alcohol consumption in turn increased the content of MEAP in the nucleus accumbens of AA rats (Nylander et al. 1994), which may be important in maintaining

the alcohol consumption in AA rats. Furthermore, lower concentrations of dynorphin peptides in the nucleus accumbens of AA rats compared with ANA rats, may indicate less inhibitory modulation of dopamine release by these peptides acting on κ-opioid receptors on dopaminergic terminals in the nucleus accumbens, which may also account for the differential alcohol preference of these rats (Nylander et al. 1994).

Microdialysis studies have shown that IP administered alcohol increases the extracellular concentrations of dopamine in the nucleus accumbens of AA and ANA rats to either the same extent (Kiianmaa et al. 1995; Nurmi et al. 1996) or more in ANA rats (Sällström Baum et al. 1999). When dopamine release was measured *post mortem* using 3-MT as indicator of dopamine release, no elevation of 3-MT could be detected in rats of either line (Honkanen et al. 1994a). Thus, studies where alcohol is administered IP suggest that the difference in the voluntary alcohol intake between these rats is not related to differences in the alcohol-induced cerebral dopamine release. Voluntary alcohol drinking has been shown to increase dopamine release in the nucleus accumbens, the caudate-putamen and the olfactory tubercle of AA rats, when measured *post mortem*, whereas water drinking increased dopamine release only in the caudate-putamen, indicating selective activation of the mesolimbic dopaminergic pathway by alcohol. (Honkanen et al. 1997a). However, when measured with *in vivo* microdialysis, the dopamine elevating effect of voluntarily consumed alcohol was very low and of short duration (Nurmi et al. 1998).

Morphine increased dopamine release (*post mortem* 3-MT concentration) to a similar extent in the nucleus accumbens of AA and ANA rats. In the caudate-putamen, the effect of morphine on dopamine release was more pronounced in AA than in ANA rats (Honkanen et al. 1999). Additionally, morphine increased the tissue concentrations of 5-HIAA more in the nucleus accumbens and caudate-putamen of AA than in those of ANA rats (Honkanen et al. 1999). However, no significant differences were found in the effects of nicotine on extracellular accumbal concentrations of dopamine between AA and ANA rats (Kiianmaa et al. 2000).

2.4.6. Receptors

AA rats were found to have slightly fewer D2-receptors in the striatum as compared with ANA rats (Korpi et al. 1987). However, this finding could not be replicated later on, where no baseline difference was found in the D1- or D2-receptor binding or in the D2-receptor gene expression between AA and ANA rats (Syvälahti et al. 1994). In all probability, the differences in dopamine receptor densities between AA and ANA rats are marginal and are not likely to contribute to the differential alcohol intake between these rats (Syvälahti et al. 1994). No difference between AA and ANA rats could be detected when binding of serotonergic ligands to $5\text{-}HT_1$-, $5\text{-}HT_2$- or $5\text{-}HT_3$-receptors was examined in any of the brain areas studied (Ciccocioppo et al. 1997; Ciccocioppo et al. 1998; Korpi et al. 1992).

Distribution and density of opioid receptors in AA and ANA rats have both been studied using autoradiography with opioid receptor agonist (de Waele et al. 1995; Marinelli et al. 2000; Soini et al. 1999) or antagonist (Soini et al. 1998) ligands. However, the studies do not support fully each other. De Waele et al. (1995) found a higher density of μ-opioid receptors in AA than in ANA rats in the medial nucleus accumbens, the caudate, the ventral tegmental area, the central grey matter, the septum, the hippocampus, the interpeduncular nucleus and the superior colliculus. Soini et al. (1998) found a higher density of μ-opioid receptors in AA than in ANA rats in the substantia nigra and in superior colliculus, whereas in the hippocampus the μ-receptor density was lower in AA than in ANA rats. Soini et al. (1999) found a higher density of μ-opioid receptors in AA than in ANA rats in the substantia nigra pars reticulata, the globus pallidus, the basolateral amygdala and the medial preoptic area. Marinelli et al. (2000) found a higher density of μ-opioid receptors in AA rats than in ANA rats in the shell region of the nucleus accumbens and the prefrontal cortex. These results, when taken together, suggest that AA rats seem to have higher density of μ-opioid receptors in the substantia nigra pars reticulata and in the superior colliculus than ANA rats with a similar trend occurring in the nucleus accumbens shell and in the caudate-putamen.

AA rats were found to have a higher density of δ-opioid receptors than ANA rats in the caudate, the cortex, the thalamus and the nucleus accumbens (de Waele et al. 1995), whereas Soini et al. found lower density of δ-opioid receptors in the cingular cortex, the hippocampus, the basolateral amygdala, the thalamus, the interpeduncular nucleus and the colliculus (Soini et al. 1998) or no differencies in δ-opioid receptor density in any of the brain areas studied (Soini et al. 1999). AA rats also seem to have a higher density of κ-opioid receptors in the limbic areas and the basal ganglia (Soini et al. 1999).

2.4.7. Behaviour

Alcohol reduces anxiety, and one of the main reasons that humans use alcohol is due to its anxiolytic action (see Nutt 1999). Several studies have been conducted to investigate possible differences in levels of anxiety and fearfulness between AA and ANA rats (Table 2-2). In the early generations of AA and ANA rats, there was a tendency that AA rats were more active than ANA rats in the open field test (see Sinclair et al. 1989). However, in later generations, the activities of AA and ANA rats in the open field were found to be similar (Badishtov et al. 1995; Overstreet et al. 1997) or lower activity in AA rats was observed (Fahlke et al. 1993). Reduced activity in the open field test is usually interpreted as a sign of fearfulness (see Archer 1973). Furthermore, AA rats were found to respond to a strong auditive stimulus with a more sustained freezing reaction than ANA rats, which presumably reflects a stronger fear response in threatening situations (Fahlke et al. 1993). On the other hand, ANA rats defecated more than the AA ones in the open field, a behaviour suggestive of enhanced emotionality/anxiety in ANA rats (Badishtov et al. 1995; Fahlke et al. 1993). ANA rats emitted more ultrasonic vocalizations after aversive but non-painful head/neck-focused air-puff than AA rats, which again may indicate a greater level anxiety or fear in ANA rats (Knapp et al. 1997). ANA rats were found to be more anxious (Möller et al. 1997) or as anxious as AA rats in elevated plus maze test (Tuominen et al. 1990; Viglinskaya et al. 1995). AA rats showed longer latencies in burying in shock prod defensive burying test, and less stomach ulceration in 75-min water immersion test compared with ANA rats (Sandbak et al. 1998). AA rats were also found to be calmer also in 2-min forced swim test and in escapable electric shock

test (Korpi et al. 1988). ANA rats were also found to show defective habituation to nociception as compared with AA rats (Honkanen et al. 1995). These results suggest that AA rats may be less fearful and less sensitive to aversive stimuli.

Taken together, the experiments described above do not support the idea that AA rats are more anxious than ANA rats, and that consequently AA rats would drink alcohol to relieve anxiety. In contrast, these behavioural studies suggest generally more anxiety/emotionality in ANA rats than AA rats. However, the lower sensitivity to aversive stimulation in AA than in ANA rats may also indicate lower sensitivity to aversive properties of alcohol in AA rats, which in turn might allow higher alcohol consumption in AA than in ANA rats.

Table 2-2. Comparison of AA and ANA rats in behavioural studies measuring experimental anxiety/fearfulness.

Test	Result	Conclusion
Open field test	AA rats less active AA and ANA rats as active AA rats defecate less	AA rats more fearful [a] No difference in fearfulness [b,c] AA rats less anxious [a,b,c]
Strong auditive stimulus	More sustained freezing in AA rats	AA rats more fearful [a]
Head/neck-focused air-puff	Less ultrasonic vocalisations in AA rats	AA rats less anxious or fearful [d]
Elevated Plus Maze	Times spent in open arm similar AA rats longer and more often in open arm	No difference in anxiety [f,g] AA rats less anxious [h]
2-min forced swim test 75-min water immersion test	AA rats more calm Less stomach ulcers in AA rats	AA rats less anxious [e] AA rats less anxious or fearful [i]
Shock prod defensive burying test	Longer latency in burying in AA rats	AA rats less anxious or fearful [i]
Punished drinking test	More punished drinking episodes in AA rats	AA rats less anxious or fearful [h]

(Fahlke et al. 1993)[a], (Badishtov et al. 1995)[b], (Overstreet et al. 1997)[c], (Knapp et al. 1997)[d], (Korpi et al. 1988)[e], (Tuominen et al. 1990)[f], (Viglinskaya et al. 1995)[g], (Möller et al. 1997)[h], (Sandbak et al. 1998)[i]

AA rats have been found to express more offensive and defensive behaviour than ANA rats, and these findings support the observation that high alcohol-preference is related to elevated levels of aggression (Tuominen et al. 1990; Virkkunen and Linnoila 1997).

Voluntary alcohol drinking increases the locomotor activity in AA rats, but acute IP injection of alcohol (0.6 or 1.0 g/kg) does not alter the locomotor activity either in AA or ANA rats (Päivärinta and Korpi 1993). Nicotine increased the locomotor activity equally in AA and ANA rats (Kiianmaa et al. 2000) and repeated nicotine treatment progressively enhanced the locomotor activity in rats of both lines, indicating sensitisation, but there was no difference in the magnitude of sensitisation between rats of these lines (Kiianmaa et al. 2000).

3. AIMS OF THE STUDY

The main aim of this study was to explore the effects of repeated morphine and cocaine treatments on striatal dopaminergic mechanisms and dopamine-mediated behaviour in alcohol-preferring AA and alcohol avoiding ANA rats in order to study the role of cerebral dopaminergic mechanisms in alcohol drinking behaviour and drug addiction. AA rats were used as a model for a rat line vulnerable to addictive behaviour.

Specific goals of this work were

1) To examine whether alcohol, cocaine and morphine differentially enhance locomotor activity in AA and ANA rats treated acutely or repeatedly with these drugs in order to clarify the role of drug-induced psychomotor sensitisation in addictive behaviour.

2) To biochemically examine whether the responses of striatal dopaminergic mechanisms to acute or repeated treatment with drugs of abuse differ between AA and ANA rats in order to study the importance of striatal dopaminergic mechanisms in addictive behaviour. This was done by using 2 drugs i.e. morphine and cocaine that activate the cerebral dopamine pathways by different primary mechanisms.

3) To examine whether the effects of acute or repeated morphine or cocaine treatments on rotational behaviour differ between AA and ANA rats. Rotational behaviour was used as a model for dopamine dependent behaviour to investigate functional differences in the ascending dopamine pathways between AA and ANA rats.

4. MATERIALS AND METHODS

4.1. Animals

Adult male Wistar (Laboratory Animal Centre, University of Helsinki, I), AA and ANA (from generations F_{68}-F_{75}, F_{78}-F_{80}, Department of Mental Health and Alcohol Research, National Public Health Institute, Helsinki, I-V) rats (3-6 months old weighing 250-500 g) were used. The rats were housed in groups of 3 to 6 rats of each line per cage, except as described below, under 12/12 h light/dark cycle (lights on at 6 a.m.) at an ambient temperature of 22-23 °C. Tap water and standard laboratory food [Altromin 1324, Chr. Petersen A/S, Denmark (I, III-V) or RM1 (E) SQC pellets from SDS, Witham, UK (II)] were available *ad libitum*. The rats were weighed and accustomed to handling for at least 2 consecutive days before the experiments. The animal experiments were approved by the local institutional animal care and use committee and the chief veterinarian of the county administrative board, and were conducted according to the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes".

4.2. Drugs and their administration

Alcohol (ethanol 96 %, purchased from Alko Ltd.) solution was given IP as a 12 % (m/V) solution prepared in saline (0.9 % NaCl). Morphine and cocaine hydrochloride were obtained from the University Pharmacy (Helsinki, Finland). Morphine was administered SC and cocaine IP, in a volume of 1 ml/kg, dissolved in saline. 6-Hydroxydopamine (6-OHDA) and desipramine were obtained from Sigma Chemical Co. (St. Louis, MO). Desipramine was administered IP, 15 mg/kg, 1 ml/kg, dissolved in purified water. 6-OHDA was dissolved in saline containing 0.02 % ascorbic acid.

4.3. Measurement of locomotor activity (I)

The horizontal locomotor activity of the animals was registered daily in transparent Macrolon III cages (18x33x15 cm) by means of computer controlled photocells. Test cages were placed on top of a sensor (area:18x33 cm) containing 40 photocells under a transparent cover. Interruptions of light beams to the photocells during horizontal movement of the animals were registered by computer. Animals were habituated to the locomotor activity cages for 120 min on the day preceding the first experimental day, and subsequently for 40 or 45 min on each day before experimental treatments.

In some of the cocaine experiments, the behaviour of the rats was videotaped at 5 min intervals for 10 s during the 60 min following the first and fourth cocaine injections. From these recordings behaviour of the animals was later rated using a 9-point scale described by Ellinwood and Balster (Ellinwood and Balster 1974). On this scale, 1 = asleep; 2 = inactive; 3 = normal in place activity; 4 = normal, alert, active; 5 = hyperactive; 6 = slow patterned stereotyped behaviour; 7 = fast patterned stereotyped behaviour; 8 = restricted stereotyped behaviour, 9 = dyskinetic-reactive behaviour.

4.4. Determination of dopamine, DOPAC and HVA from microdialysis samples (II and IV)

The system used for determination of the extracellular concentrations of dopamine, DOPAC and HVA, consisted of an ESA Coulochem II detector (ESA Inc., MA, USA) equipped with a model 5014A microdialysis cell, a Pharmacia LKB model 2248 HPLC pump (Pharmacia LKB, Sweden) and a SSI model LP-21 pulse damper (Scientific Systems Inc., PA, USA). The column (Spherisorb ODS2, 3 μm, 4.6 x 100 mm or Spherisorb ODS 2, 3 μm, 2.0 x 100 mm) was kept at 40 °C with a column heater (Croco-Cil, France). The mobile phase consisted of 0.1 M NaH_2PO_4 buffer, pH 4.0 (adjusted with 1.0 mM citric acid), 0.1-0.2 mM octane sulfonic acid, 16 % methanol and 1.2 mM EDTA. Twenty microlitres of the dialysate sample was injected with a CMA/200 autoinjector (CMA, Stockholm, Sweden). Dopamine was reduced

with an amperometric detector (potential - 80 mV) and DOPAC and HVA were oxidized with a coulometric detector (+ 300 mV). The flow rate of the HPLC pump was set at 0.3 ml/min (with 2.0 x 100 mm column) or 1.0 ml/min (with 4.6 x 100 mm column) and the chromatogram was processed with a Hitachi D-2000 chromato-integrator.

4.5. Determination of dopamine, 3-MT, HVA, 5-HT and 5-HIAA from brain samples (III and V)

Samples were homogenized in 1 ml of 0.2 M $HClO_4$ after which 25 µl KOH/HCOOH buffer was added to the homogenates to adjust the pH to 2.4. Samples were centrifuged at 5,500 x g for 45 min. The supernatants were purified using a method described earlier (Haikala 1987) with slight modifications. In brief, a 950 µl sample of supernatant was pipetted onto Sephadex G-10 columns and washed with 3.0 ml of 0.01 M HCl. Dopamine and 3-MT were collected by washing the columns with 1.5 ml 0.01 M HCl and 1.0 ml 0.02 M NH_3. HVA, 5-HT and 5-HIAA were collected by subsequent washing of the columns with 1.0 ml of 0.02 M NH_3 and 4.0 ml of 0.01 M KOH. Thirty microlitres of 2.6 mM sodium pyrosulfite and 5.7 mM ascorbic acid (in 0.01 M HCl) were added into the tubes containing dopamine/3-MT and HVA/5-HT/5-HIAA, respectively. The samples were assayed for the concentration of dopamine and its metabolites by using HPLC with electrochemical detection as described earlier (Honkanen et al. 1994a).

4.6. Measurement of plasma and brain morphine concentrations (I)

After sacrifice of the rats trunk blood was collected into chilled heparinized test tubes and samples centrifuged for 15 min. Morphine and its metabolites, morphine 3-glucuronide and morphine 6-glucuronide, in rat plasma and whole brain, were determined according to Svensson and associates (Svensson 1986; Svensson et al. 1982) by HPLC (HP 1081 B, Hewlett-Packard, USA; 4.6 mm x 25 cm Zorbax ODS

C18 Column, Du Pont, France) method, utilizing both electrochemical and UV (PU 4020, Pay Unicam, England) detection in the same run. Before analysis, the brains were homogenized mechanically (Ultra Turrax, Janke & Kunkel, Germany) in 0.1 M HClO4 (Merck, Germany). Oxymorphone was used as an internal standard. Extraction and other details were carried out according to the original papers (Svensson 1986; Svensson et al. 1982).

4.7. Measurement of rotational behaviour (V)

Rotational activity of the rats was measured in circular metal bowls (35 cm diameter and 15 cm high) with a transparent Plexiglas cylinder (40 cm high) surrounding the bowls. The rat was attached to a rotation sensor by means of a spring tether connected to a plastic belt around the neck of the rat. The rotation sensor detected full (360°) clockwise and counter clockwise turns.

4.8. Implantation of the microdialysis guide cannula (II and IV)

The rats were implanted with guide cannulae (CMA/11 or BAS MD-2250) under halothane anaesthesia (3.5 % during induction for 5 min and then 2.5-1 % during surgery). The location of the guide cannulae were calculated relative to bregma and were aimed at the point above the nucleus accumbens (NAC), A/P = + 1.7, L/M = - 1.2, D/V = - 6.8, or the caudate-putamen (CPU), A/P = + 1.0, L/M = + 2.7, D/V = - 4.0, according to the atlas by Paxinos and Watson (Paxinos and Watson 1986). The cannula was fastened to the skull with dental cement (Aqualox, Voco, Germany) and three stainless steel screws. After the surgery the rats were placed into individual test cages (30x30x40 cm) and allowed to recover for at least 4 days before the experiment. The rats were weighed and handled for at least 2 days before the beginning of the microdialysis experiments.

After completion of the experiments, the positions of the probes were verified by fixing the brain in formalin which was then sliced into frozen 100 μm coronal sections, stained with thionine, and the placement examined microscopically.

4.9. 6-OHDA lesion (V)

Unilateral lesions of the right nigrostriatal tract were produced by injection of 6-hydroxydopamine (6-OHDA, 8 μg, 2 μg /μl, 1 μl/min for 4 min) into the medial forebrain bundle of rats under halothane anaesthesia (3.5 % during induction and 2 % during surgery) with a 30 gauge needle. Upon completion of the injection the needle was kept in place for an additional minute to minimize backflow of the solution. The coordinates used were A/P − 4.4 mm, L/M + 1.3 mm, D/V − 8.2 mm relative to bregma according to the atlas of Paxinos and Watson (1986). Prior to surgery, desipramine (15 mg/kg, IP, 1 ml/kg) was administered to the rats to prevent the uptake of 6-OHDA into noradrenergic nerve endings and thus to protect these nerve terminals from denervation. After the surgery, the rats were placed into individual cages for 1 to 2 days and thereafter returned to the group cages and were allowed to recover for 2 weeks after the surgery before any further experimental procedures were performed.

6-OHDA lesion was verified by measuring the depletion of striatal DA concentration. One to two weeks after the experiments, the rats were decapitated, brains removed from the skull, placed on a glass plate and the striata dissected as described previously (Ahtee et al. 1989). The dissected samples were frozen immediately on dry ice and stored at −80 °C until assay. Samples were homogenized and purified as described earlier (Haikala 1987) and were assayed for the concentration of dopamine by using HPLC with C-18 reverse-phase column (Spherisorb ODS2, 4.6 x 250 mm) and electrochemical detection (+780 mV, Waters Model 464 detector, Millipore, MA, USA). Only rats with a dopamine depletion of more than 95% in the lesioned right striatum as compared to the intact left striatum were included in the final data analysis. The mean dopamine depletion % ± S.D. of rats included in results were: AA 98.8 ± 1.6 %, ANA 99.7 ± 0.8 %.

4.10. Drug treatments

4.10.1. Effects of repeated alcohol, cocaine and morphine on locomotor activity (I)

Animals were habituated to the locomotor activity cages for 120 min on the day preceding the first experimental day, and subsequently for 40 or 45 min on each day before experimental treatments. On the first experimental day all rats were given saline, and thereafter they were treated with alcohol (0.4 or 1.0 g/kg) on 6 consecutive days, or cocaine (5 or 20 mg/kg, as base) on 4 consecutive days. On experimental day 8 (alcohol) or on experimental day 6 (cocaine), all rats were given again saline. Locomotor activity was measured daily for 30 or 60 min following the saline and drug treatments. The control animals were given an equivalent volume of saline daily for 8 and 6 days, respectively. Since there was no rat line differences in the effects of saline injections on motor activity, and because responses to saline did not change in any rat line during repeated treatment, no separate control groups were included in further experiments, in which the effects of morphine (0.3, 1.0 and 3.0 mg/kg, as base) and a 10 mg/kg dose of cocaine were studied. In these experiments all rats were treated with saline on the first experimental day and from day 2 to 5, the rats were treated with morphine or cocaine, and on experimental day 6, all animals were again given saline injections. Locomotor activity was measured for 120 or 180 min following treatments and the locomotor activity after saline treatment was used as a control.

4.10.2. Effects of repeated morphine or cocaine on striatal neurotransmitters (II-IV)

Microdialysis studies (II and IV). Rats were treated with morphine HCl (1 or 3 mg/kg as base, II), cocaine HCl (5 or 10 mg/kg, IV) or saline once daily for 4 consecutive days. Microdialysis experiments were performed on days 1 and 4. In the morning of the experiment days, a microdialysis probe (CMA/11, 2 mm membrane, o.d. 0.24 mm or BAS, MD-2200, 2 mm membrane) was inserted into the guide cannula. Modified Ringer solution (147 mM NaCl, 1.2 mM CaCl$_2$, 2.7 mM KCl, 1.0 mM MgCl$_2$ and 0.04 mM ascorbic acid) was infused through the probe at a flow rate of 1.5 μl/min. The collection of microdialysis samples (every 15 min, 22.5 μl/sample) was started

2.5-3 hours after probe insertion. The samples were discarded until a stable baseline was achieved; the average concentration of the first 3-4 stable samples was used as basal level. Thereafter, rats were given morphine (II), cocaine (IV) or saline, and the samples collected for the next 3-4 hours. The probes were subsequently removed and inserted again on day 4. It has been shown that extracellular DA concentrations in the striatum remain constant even after 10 dialysis experiments (i.e. 10 probe insertions and removals) over a 23 day period, and thus, repeated microdialysis may be used in this brain region (Martin-Fardon et al. 1997). In nucleus accumbens experiments reported in paper II conducted by inserting the probes on day 1, the average extracellular accumbal concentrations of DA declined during the experiments to approximately 50 % of basal levels, and surprisingly, morphine was without effect on DA. Therefore, these experiments (paper II) were conducted by inserting a probe into the guide cannula 4 days before the actual microdialysis experiment, perfusing the probe with a Ringer solution for 6 hours and then removing the probe. Four days after that the actual accumbal microdialysis experiment was conducted as described above, either in naive control rats receiving morphine for the first time or in rats which had received morphine on the three previous days. The rats were kept individually in the same cages throughout the experiments and received morphine or saline repeatedly in the same environment.

Metabolism of DA in tissue samples (III). Forty AA and ANA rats were given repeatedly morphine (1 mg/kg) or saline for 4 days. The rats were kept overnight in groups of 4 to 5 rats of either line. The rats were placed in individual cages 20 min before morphine or saline injection and were kept in these cages for 2 h post-injection, after which they were moved back to the original cages. Rats of both lines were divided into four experimental groups that received different treatments. Group 1: morphine once daily for 4 days. Group 2: saline once daily for 4 days. Group 3: morphine once daily for 3 days and saline on day 4. Group 4: saline once daily for 3 days and morphine on day 4. Each cage housed rats given different treatments.

Sixty minutes after the last administration of morphine or saline (on day 4), each rat was taken from its cage to another room, killed with head-focused microwave irradiation (7 kW for 1.4 s) using model NJE 2603-10kW microwave instrument (New Japan Radio Inc., Japan), and decapitated. The brains were removed from the

skull and placed on a brain mold (RBM-4000C, ASI Instruments, USA) cooled on ice, and sectioned coronally with razor blades at 2.7 and - 0.3 mm from bregma (Paxinos and Watson 1986). The caudate-putamen and nucleus accumbens were dissected from the 2nd slice by using needles with inner diameters of 2 and 3 mm, respectively. Tissue ventral to the nucleus accumbens was dissected as the olfactory tubercle. The tissues were immediately frozen on dry ice and stored at − 80 °C until assayed for DA, serotonin and their metabolites (see section 4.4.).

4.10.3. Effects of repeated cocaine and morphine on rotational behaviour (V)

Rats were taken from cages housing 3-5 rats, given saline (1 ml/kg, SC or IP), placed individually into test chambers, and allowed to habituate to the chamber for 30 min. Thereafter, rats were given morphine (1 or 3 mg/kg), cocaine (10 mg/kg) or saline and rotations counted for 2, 3 or 3.5 h (cocaine, morphine 1 mg/kg and morphine 3 mg/kg, respectively). After the sessions, the rats were returned to the home cages. The procedure was repeated on 4 consecutive days with an additional challenge session 8 days after the fourth session. In the challenge session all rats, including those that had previously received saline were given either morphine or cocaine.

4.11. Statistical analysis

Locomotor activity data (paper I) was analysed with two- or three-way analysis of variance (ANOVA) for repeated measures. Between factors were rat line, treatment and session (experimental day). When appropriate, comparisons between groups or sessions were conducted using contrast analysis with Bonferroni adjustment or Tukey's compromise post-hoc test (when separate control group was included). The behavioural rating scores were analyzed with Kruskall-Wallis statistics, after which comparisons between groups or sessions were performed with the Mann-Whitney U-test. Two-way ANOVA (rat line, dose) was used to test significance of differences in plasma morphine concentrations. Microdialysis data (papers II and IV) was analysed using two- or three-way ANOVA (rat line, treatment, day) for repeated measures.

When appropriate, the effect of different doses of morphine or cocaine were compared with the corresponding saline group with the Student-Newman-Keuls post-hoc test (paper II) or contrast analysis (paper IV). Differences in the basal concentrations of DA between rat lines and days were tested with two-way ANOVA (paper IV). Possible differences in the histologically verified coordinates of probes implanted in the nucleus accumbens between the rat lines were investigated with Mann-Whitney U-test in rats that received morphine or cocaine. When the concentrations of DA, 5-HT and their metabolites were measured in tissue samples (paper III), differences in the basal concentrations of DA, 5-HT and their metabolites between the rat lines were tested with Student's t-test in rats treated repeatedly with saline. Effects of drug treatments and the interactions between drug pretreatments and drug treatments within rat lines were tested with two-way ANOVA (treatment, pretreatment) followed by Tukey´s compromise –test. The effects of morphine and cocaine on rotational behaviour were tested with two- or three-way ANOVA (rat line, treatment, day) followed by Tukey´s compromise –test (paper V).

5. RESULTS

5.1. Effects of acute and repeated treatments with alcohol, morphine or cocaine on locomotor activity (I)

Locomotor activities of AA and ANA rats did not differ following acute or repeated treatment with saline. Furthermore, acute or repeated treatment with alcohol did not produce any change in the locomotor activity in AA or ANA rats at either dose (0.4 or 1 g/kg, IP) studied.

Acute administration of morphine induced a greater degree of locomotor stimulation in AA than in ANA or Wistar rats. Furthermore, during repeated 4-day treatment with 1 mg/kg of morphine AA rats, but not ANA or Wistar rats, showed sensitisation to morphine (Fig. 5.1.). Repeated treatment with 0.3 or 3 mg/kg of morphine did not induce sensitisation of locomotor response in rats of these three lines.
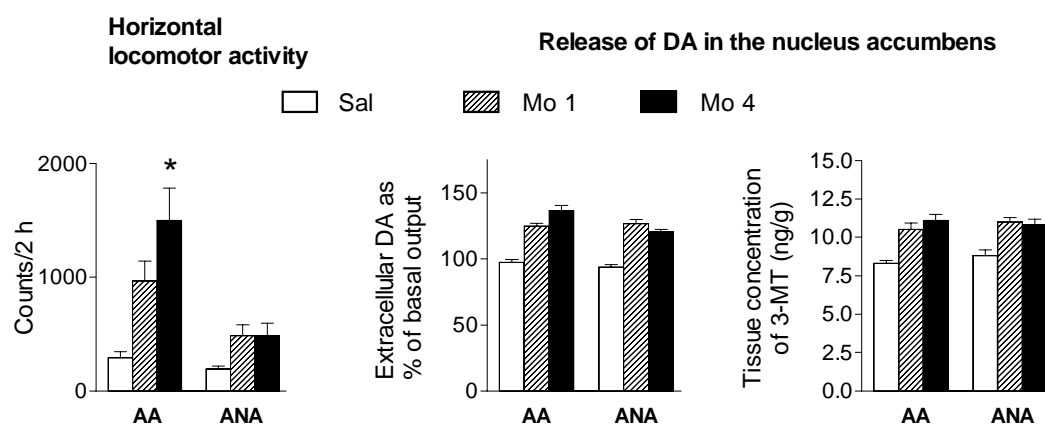

Figure 5.1. Effects of saline (Sal), acute (Mo 1) and repeated 4-day treatment (Mo 4) with morphine (1 mg/kg) on horizontal locomotor activity and release of dopamine (DA) in the nucleus accumbens in AA and ANA rats. (Means ± S.E.M., DA values are means of 13 samples collected during 180 min after morphine injection, 3-MT concentrations were estimated at 1 h after morphine injection, n=6-10) * $P < 0.05$, in comparison with acute morphine (2-way ANOVA).

Acute administration of cocaine increased the locomotor activity to a similar level in AA and ANA rats and to an lesser extent in Wistar rats. AA rats, but not ANA or Wistar rats, were sensitized to cocaine after 4-day treatment with 10 mg/kg of cocaine (Fig. 5.2.). Rats of all lines were sensitised to a dose of 20 mg/kg of cocaine, which was seen as enhanced stereotyped behaviour, although the ambulatory activity of AA and ANA rats was even reduced after repeated treatment with this dose of cocaine.
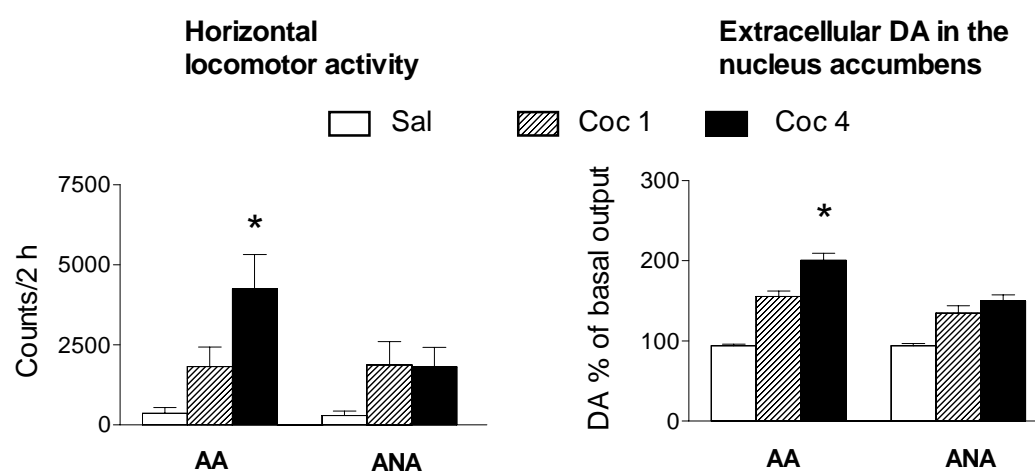

Figure 5.2. Effects of saline (Sal), acute (Coc 1) and repeated 4-day treatment (Coc 4) with cocaine (10 mg/kg) on horizontal locomotor activity and extracellular dopamine (DA) concentration in the nucleus accumbens in AA and ANA rats. (Means ± S.E.M., DA values are means of 13 samples collected during 165 min after cocaine injection, n=6-10) * P < 0.05 in comparison with acute drug treatment (2-way ANOVA).

5.2. Plasma and brain morphine concentrations (I)

One week after the end of the four-day treatment with morphine, the AA and ANA rats were given morphine as before and killed 30 min (1 mg/kg) or 60 min (3 mg/kg) later. The plasma concentrations of morphine (μg/ml) were 0.29 ± 0.03 and 0.36 ± 0.03 in AA rats and 0.33 ± 0.03 and 0.38 ± 0.06 in ANA rats after 1 or 3 mg/kg of morphine, respectively. These values did not differ significantly between AA and ANA rats. The concentrations of morphine in brains were below the detection limit (5 ng/g) of the analysis method used.

5.3. Basal levels of DA, 5-HT and their metabolites (II-IV)

In the nucleus accumbens, the concentrations of 5-HT and 5-HIAA, and in the olfactory tubercle, the concentrations of DA, 5-HT and 5-HIAA were higher (by about 10-15 %) in AA rats than in ANA rats (paper II). In the caudate-putamen, the concentration of 3-MT was lower (by about 25 %) in AA rats than in ANA rats (paper II). No significant differences were found in the extracellular levels of DA, DOPAC or HVA between AA and ANA rats (papers II and IV).

5.4. Effects of acute and repeated morphine and cocaine treatments on DA release and metabolism (II-IV)

No significant differences were found on examination of the effects of acute or repeated morphine administration on DA release or metabolism in the nucleus accumbens between AA and ANA rats (papers II and III; see also Fig. 5.1.). In contrast to this, acute morphine increased the release (Table 5.1.) and metabolism (Table 5.2.) of dopamine in the caudate-putamen of AA but not ANA rats. After repeated morphine treatment, the morphine induced release of DA was not enhanced in AA or ANA rats. However, the effects of morphine on DA metabolism was enhanced in rats of both lines treated with the larger dose (3 mg/kg) of morphine but not in those treated with the lower dose (1 mg/kg) of morphine (Tables 5.1. and 5.2.). Results from the olfactory tubercle were found to be comparable with those obtained from the nucleus accumbens, in that no significant differences were found in the metabolism or release of DA between the AA and ANA rats (III).

Acute cocaine administration (5 and 10 mg/kg) increased DA release equally in AA and ANA rats in both the nucleus accumbens and in the caudate-putamen (IV). After repeated treatment, the effect of cocaine on dopamine release was enhanced in the nucleus accumbens of AA rats but not in that of ANA rats (IV; see also Fig. 5.2.). Neither was any enhancement of DA release observed in the caudate-putamen in rats of either line (IV; Table 5.1.).

Table 5.1. Effects of acute (day 1) and repeated 4-day treatments (day 4) with morphine (1 or 3 mg/kg) or cocaine (10 mg/kg) on dopamine release in the caudate-putamen of AA and ANA rats.

Treatment	Microdialysis (dopamine)			Tissue sample (3-MT)
	Morphine 1	Morphine 3	Cocaine 10	Morphine 1
AA day 1	NS	↑	↑↑	↑↑
AA day 4	NS	↑↑	↑↑	↑
ANA day 1	NS	NS	↑↑	NS
ANA day 4	↑	↑	↑	NS

NS = not significant, ↑ P < 0.05 and ↑↑ P < 0.01, in comparison with corresponding rats treated with saline acutely and repeatedly (microdialysis experiments) or saline on day 4 instead of morphine (3-MT experiment) (Contrast analysis, Student-Newman Keuls or Tukey's compromise post-hoc test).

Table 5.2. Effects of acute (day 1) and repeated 4-day treatments (day 4) with morphine (1 or 3 mg/kg) on dopamine metabolism in the caudate-putamen of AA and ANA rats.

Treatment	Microdialysis				Tissue sample
	Morphine 1 DOPAC	Morphine 3 DOPAC	Morphine 1 HVA	Morphine 3 HVA	Morphine 1 HVA
AA day 1	↑↑	↑↑	↑↑	↑↑	↑↑
AA day 4	↑↑	↑↑ ***	↑	↑↑ **	NS
ANA day 1	NS	NS	NS	NS	NS
ANA day 4	NS	↑↑ ***	NS	↑↑ **	↑

NS = not significant, ↑ P < 0.05 and ↑↑ P < 0.01, in comparison with corresponding saline treated rats. For treatments see subtitle for Table 5.1. (Student-Newman Keuls or Tukey's compromise post-hoc test). ** P < 0.01 and *** P < 0.001, in comparison with day 1 (2-way ANOVA).

5.5. Effects of acute and repeated morphine treatments on 5-HT and 5-HIAA (III)

Morphine did not significantly affect the concentrations of 5-HT in any of the brain areas studied in either saline or morphine pretreated rats of either line (III).

Morphine significantly increased the concentration of 5-HIAA only in AA rats in the caudate-putamen and olfactory tubercle (III).

5.6. Locations of the accumbal microdialysis probes (II and IV)

No significant differences were found in the histologically verified coordinates (A/V, L/M, D/V) of dialysis probes between AA and ANA rats, indicating that there were no systematic differences in the placements of the probes between AA and ANA rats, which might affect the responses measured.

5.7. Effects of acute and repeated morphine and cocaine on rotational behaviour in AA and ANA rats (V)

Acute administration of morphine (3 mg/kg) induced a significant elevation of ipsilateral rotational behaviour in AA but not in ANA rats (Fig. 5.3.).
Furthermore, morphine treated AA rats significantly showed more rotational behaviour over the 4-day treatment period than morphine treated ANA rats. In the challenge session when all rats received morphine, morphine pretreated AA rats showed more rotational behaviour than saline pretreated AA rats. No such sensitisation was seen in ANA rats.

The smaller dose of morphine (1 mg/kg) did not induce any significant rotation over the 4-day repeated treatment session or in the challenge session, 8 days after repeated morphine treatment (V).

Acute administration of cocaine significantly increased ipsilateral rotations only in AA rats, and cocaine-induced rotational behaviour was more pronounced in AA rats over the 4-day treatment period than in ANA rats (Fig. 5.3.). In the challenge session, significant sensitisation of the effect of cocaine could not be detected in rats of either line.
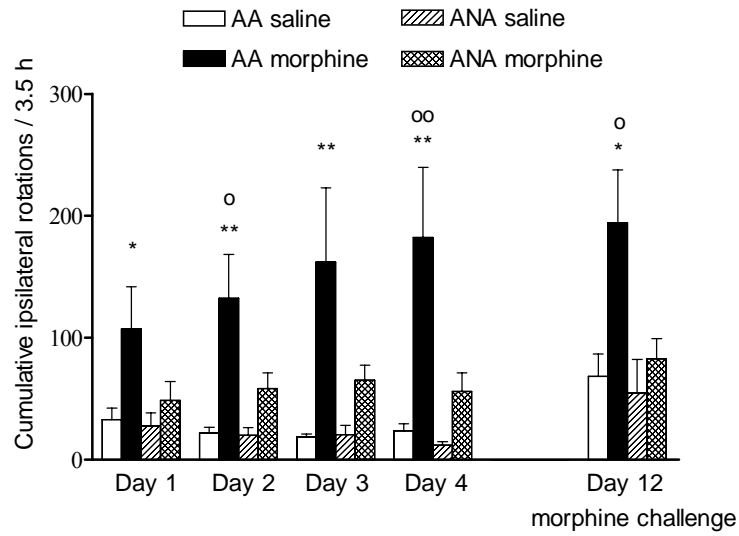
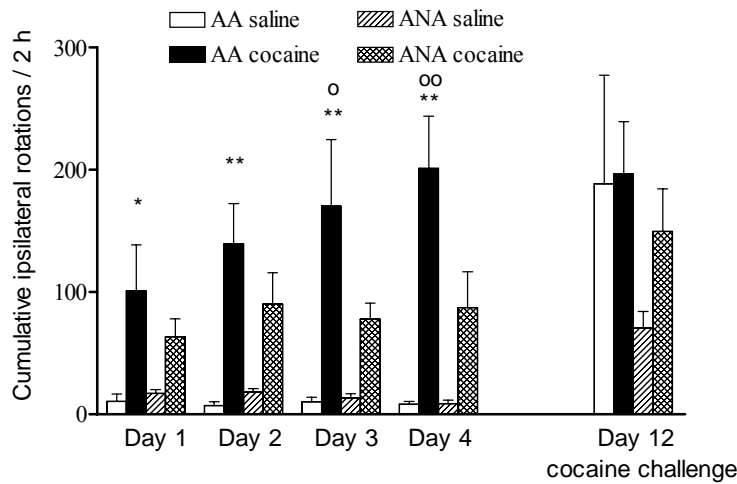**Figure 5.3. Effects of acute and repeated treatments with morphine (3 mg/kg) or cocaine (10 mg/kg) on rotational behaviour in AA and ANA rats. In the challenge session all rats received morphine or cocaine.** Columns represent means ± S.E.M. (n=8-14). * P < 0.05 and ** P < 0.01 in comparison with corresponding saline treated rats, ° P < 0.05 and °° P < 0.01 in comparison with corresponding ANA rats.

6. DISCUSSION

6.1. Effects of acute and repeated treatments with alcohol, morphine and cocaine on the locomotor activity of AA and ANA rats

6.1.1. Effects of alcohol

Alcohol did not have any effect on locomotor activity either in AA or in ANA rats (I). Although there are some reports that low doses of alcohol (≤ 0.25 g/kg, i.p.) can increase the spontaneous motor activity, at least in some lines of rats (Waller et al. 1986), experimenter-administered alcohol does not generally increase locomotor activity in rats (Criswell et al. 1994; Cunningham et al. 1993; Frye and Breese 1981; Masur et al. 1986; Päivärinta and Korpi 1993). Furthermore, Masur et al. (1986) have shown that non-selected rats do not become sensitized to alcohol even after extensive chronic intraperitoneal alcohol administration. However, voluntary alcohol drinking may enhance locomotor activity, an effect that has been seen also with AA rats (Colombo et al. 1998; Päivärinta and Korpi 1993). In agreement with these results are studies showing that dopamine release is increased in the nucleus accumbens of AA rats after voluntary alcohol drinking (Honkanen et al. 1997a) but not after acute intraperitoneal alcohol (Honkanen et al. 1994a). From these observations one can conclude that experimenter-administered alcohol and voluntarily consumed alcohol seem to induce diverse behavioural and neurochemical effects.

6.1.2. Effects of morphine and cocaine

Acute morphine enhanced the horizontal locomotor activity more in AA than in ANA or Wistar rats (I). Furthermore, AA rats become behaviourally sensitized to morphine, an effect that did not occur in ANA or Wistar rats. The greater efficacy of morphine in AA rats as compared with ANA rats is apparently not related to different pharmacokinetics of the drug in these rats, since there was no difference in the plasma morphine concentrations after administration of morphine (I). Furthermore, two

additional studies have demonstrated that AA and ANA rats also differ in response to the μ-agonist DAMGO given repeatedly intracerebroventricularly (Honkanen et al. 1997b) and that analgesic effects of morphine do not differ between these rat lines (Honkanen et al. 1995). This implys that the different behavioural effects of morphine found in AA and ANA rats do not result from differences in the entry of morphine into the brain.

Acute cocaine increased the locomotor activity to a similar extent in both AA and ANA rats. However, AA rats developed behavioural sensitisation to smaller doses of cocaine than ANA rats. The concentrations of cocaine in brain or plasma of these rats were not assessed, and thus, the possibility that the variations in the sensitisation to cocaine in AA and ANA rats are due to different pharmacokinetics can not be completely excluded. This is unlikely, however, since between AA and ANA rats there were no differences in the acute effects of cocaine on locomotor activity (I) or on mesolimbic or nigrostriatal DA release (IV), and, moreover, the differential behavioural sensitisation emerged with one dose only. Furthermore, if bioavailability of cocaine after repeated treatment differed between AA and ANA rats, one would also expect differences, not only in the mesolimbic, but also in nigrostriatal dopamine release, which was not the case. Thus, it seems that AA rats develop behavioural sensitisation to morphine and cocaine more easily and with smaller doses of these drugs than ANA rats.

6.2. Effects of morphine and cocaine on dopamine release and metabolism

6.2.1.   Basal levels of dopamine and its metabolites

The AA rats have been found to have more dopamine in the whole brain as well as in the caudate-putamen than the ANA rats (Ahtee and Eriksson 1975; Honkanen et al. 1999; Kiianmaa et al. 1991). A similar difference was found in the olfactory tubercle (paper III). As found previously (Honkanen et al. 1999), the 3-MT concentrations were smaller in the caudate-putamen of AA than in that of ANA rats. In microdialysis

studies no significant differences between AA and ANA rats were found on examination of extracellular accumbal levels of dopamine, DOPAC or HVA, which is in agreement with results from previous microdialysis studies (Kiianmaa et al. 1995; Nurmi et al. 1996). Additionally, the extracellular concentrations of dopamine, DOPAC and HVA were similar in the caudate-putamen of rats from both lines.

6.2.2. Effects of acute morphine in the nucleus accumbens

Acute doses of morphine increased dopamine release and metabolism similarly in AA and ANA rats in the nucleus accumbens (II and III). This is in line with the findings of Honkanen et al. (Honkanen et al. 1999) showing no differences in the effect of morphine on accumbal dopamine release or metabolism between AA and ANA rats. Therefore, it seems that dopaminergic mechanisms in the nucleus accumbens are not involved in the differences seen in locomotor activity after acute morphine administration between the rats from these lines (see paper I). Morphine and opioid peptides have been shown to induce locomotor activity independently of DA when administered directly into the nucleus accumbens (Kalivas et al. 1983; Pert and Sivit 1977). Thus, there may be differences between AA and ANA rats in the postsynaptic mechanisms in the nucleus accumbens, or other brain areas, e.g. the caudate-putamen (see 6.2.4.), or other brain transmitters, such as serotonin (see 6.3. and Honkanen et al. 1999), may be involved.

6.2.3. Effects of repeated morphine in the nucleus accumbens

The effect of morphine on accumbal dopamine release and metabolism remained similar during 4-days of repeated morphine administration in rats of both lines (II and III). Sensitisation of locomotor activity after repeated morphine treatment has been found to be associated with enhanced dopamine release in the nucleus accumbens (Kalivas and Stewart 1991; Spanagel et al. 1993). In my experiments, sensitisation of dopaminergic mechanisms could not be detected either when the animals received morphine in their home environment (II) or even when the drug administration was paired to a distinct context (III), which should increase the probability of sensitisation

occurring (Di Chiara 1995). Thus, the results reported in papers I, II and III do not support the dopamine hypothesis of behavioural sensitisation, at least where enhanced dopamine release is concerned. However, sensitisation of mesolimbic dopamine release has usually been seen after 3 or more days of withdrawal after repeated morphine administration and also using higher doses of morphine than those used in the present study (Acquas and Di Chiara 1992; Cadoni and Di Chiara 1999; Kalivas and Stewart 1991; Spanagel et al. 1993). Acquas and Di Chiara (1992) reported a tolerance rather than a sensitisation of dopamine release after 1-day withdrawal from repeated high dose morphine treatment and sensitisation of dopamine release after 3 days of withdrawal (Acquas and Di Chiara 1992). Thus, the results of the present series of experiments suggest that the sensitisation of locomotor activity after repeated four-day treatment with relatively low doses of morphine may not be associated with increased dopamine release in the nucleus accumbens, at least after only one day of withdrawal. On the other hand, it has recently been shown that the effect of repeated morphine treatment on accumbal dopamine release may differ between the two subdivisions of the nucleus accumbens, the core and the shell (Cadoni and Di Chiara 1999). Thus, dopamine release was only context-independently sensitised in the core of the nucleus accumbens after repeated morphine treatment, whereas in the shell, tolerance developed to the morphine-induced dopamine release. In the experiments reported in paper III the subdivisions of the nucleus accumbens could not be differentiated due to dissection technique. This raises the possibility that the possible sensitisation of dopamine release in the core could be masked by a tolerance in the shell. However, no differences were found in the locations of dialysis probes between the AA and ANA rats (paper II), thus, the placements of dialysis probes cannot explain the lack of difference in dopamine release between these rats. To summarise, the results of these studies suggest that differential opioid regulation of accumbal dopamine mechanisms is not critically involved in the differences in opioid-induced locomotor activity between these rat lines.

6.2.4.   Effects of acute morphine in the caudate-putamen

AA rats seem to be more sensitive to acute morphine-induced dopamine release and metabolism in the caudate-putamen than ANA rats (papers II and III). When

dopamine release and metabolism were estimated from *post mortem* tissue samples (paper III), acute doses of morphine elevated the concentrations of 3-MT by 43 % and 21 % and the concentrations of HVA by 49 % and 19 % in the caudate-putamen of AA and ANA rats, respectively; with these effects only significant in AA rats. In the microdialysis study (paper II), significant elevation of dopamine release only occurred with the higher (3 mg/kg) dose of morphine and only in the AA rat line. Furthermore, the first morphine injection elevated the striatal extracellular concentrations of dopamine metabolites (DOPAC and HVA) significantly more in AA than in ANA rats. The same difference has also been found in a previous study, where concentrations of DOPAC, HVA and 3-MT, were measured from *post mortem* tissue samples (Honkanen et al. 1999). Together, these findings suggest that morphine activates the nigrostriatal dopaminergic pathway more easily in AA than in ANA rats. One explanation for this is that AA rats have been found to have a higher density of μ-opioid receptors in the substantia nigra, especially in the pars reticulata, and the striatal patches containing μ-opioid receptors are larger than in ANA rats (Soini et al. 1999; Soini et al. 1998). This may contribute to the greater effect of morphine on dopamine in the caudate-putamen of AA rats. It is unclear as to whether this enhancement of sensitivity of the nigrostriatal dopamine system in AA rats contributes to the increases observed in locomotor activity after acute morphine treatment, since increased dopamine release in the caudate-putamen is usually linked to stereotyped behaviour, and not to horizontal locomotor activity (see Bloom et al. 1989). On the other hand, there is some evidence in the literature that μ-opioids may also induce locomotor activity by acting on the substantia nigra (Morelli et al. 1989). Connected with this, it has recently been found, that a selective μ-opioid receptor agonist, [D-Ala[2], MePhe[4], Gly-ol[5]]enkephalin (DAMGO; 0.05 μg), when administered bilaterally into the substantia nigra of Wistar rats enhanced locomotor activity, in addition to stereotypic gnawing (Honkanen et al., unpublished results). Therefore, it is possible that the nigrostriatal dopamine pathway plays a role in the morphine-induced enhancement or modulation of locomotor activity in AA rats.

6.2.5. Effects of repeated morphine in the caudate-putamen

After repeated treatment, the effect of 1 mg/kg dose of morphine on striatal dopamine or metabolite concentrations was not significantly altered in rats of either line when compared with the first dose (papers II and III). As regards the larger, 3 mg/kg, dose of morphine (III), its effects on striatal dopamine metabolite concentrations were similarly enhanced in rats of both lines, but no enhancement of elevation of extracellular dopamine concentration was seen in either rat line after repeated treatment with this dose. In the microdialysis study, morphine elevated the concentrations of DOPAC and HVA more in AA than in ANA rats on both days 1 and 4, but the ratio of the enhancement of metabolite concentrations between days 1 and 4 did not differ between rats of these lines. Therefore, these results suggest that the effects of morphine on nigrostriatal dopamine function are more prominent in AA than in ANA rats, but there is no difference in the sensitisation of nigrostriatal dopamine metabolism to morphine between these rats. Thus, the effects of morphine on nigrostriatal dopamine do not, apparently, explain the differential behavioural sensitisation in these rats.

6.2.6. Effects of acute cocaine in the nucleus accumbens

In agreement with previous studies conducted using microdialysis (Cadoni et al. 2000; Di Chiara and Imperato 1988a; Kalivas and Duffy 1990; Kalivas and Duffy 1993; Pontieri et al. 1995) acute administration of cocaine increased the extracellular levels of dopamine in the nucleus accumbens of AA and ANA rats (paper IV). The locomotor activity enhancing effect of acute cocaine seems to be derived predominantly from the nerve terminal areas of mesolimbic dopamine neurons (Chen and Reith 1994; Delfs et al. 1990). The effect of cocaine on dopamine release did not differ between AA and ANA rats, which is in accordance with the results of the locomotor activity study (paper I). Taking into account the mechanism of action of cocaine (Chen and Reith 1994; Delfs et al. 1990; Koe 1976; Kuhar et al. 1991; Reith et al. 1997), these results suggest that there are no significant differences in accumbal dopamine transporter mechanisms between AA and ANA rats after acute cocaine administration.

6.2.7. Effects of repeated cocaine in the nucleus accumbens

Although three-way ANOVA did not reveal any significant difference between rat lines after repeated cocaine treatment, the effect of cocaine on accumbal dopamine overflow on day 4 was enhanced in AA but not ANA rats, when compared with its effect on day 1 (IV). On day 4, moreover, the effect of cocaine on DA was significantly greater in AA rats than in ANA rats. Thus, our results suggest that AA rats begin to show sensitisation to the effects of cocaine on mesolimbic dopamine more easily than ANA rats.

Statistical analysis did not reveal any significant differences in the locations of microdialysis probes between AA and ANA rats. Thus, the placements of the probes apparently cannot account for the differences observed in the sensitisation of dopamine release between AA and ANA rats.

It is well known that, in addition to increasing the concentration of dopamine in the nucleus accumbens, cocaine increases the locomotor activity of rats. After repeated cocaine treatment, cocaine-induced psychomotor stimulation is enhanced, which has been associated with enhanced extracellular concentrations of dopamine in the nucleus accumbens (Cadoni et al. 2000; Kalivas and Duffy 1990; Kalivas and Duffy 1993). Thus, these results showing sensitisation of mesolimbic dopamine in AA rats together with the locomotor activity study (I) suggest that the sensitisation of locomotor activity of AA rats to cocaine after repeated treatment may be associated with increased release of dopamine in the nucleus accumbens.

The mechanisms underlying possible differences between AA and ANA rats concerning the sensitisation of cocaine-induced accumbal dopamine release are not clear. The proposed mechanisms involved in the sensitisation of psychomotor stimulant-induced dopamine release include changes in uptake mechanisms, autoreceptor sensitivity and calcium transduction (see Pierce and Kalivas 1997). The sensitivity of dopamine D2-like autoreceptor mechanisms has not been studied in AA and ANA rats. There is no basal difference in D2 receptor binding or gene expression in the nucleus accumbens or caudate putamen between these rat lines (Syvälahti et al.

1994), which, however, does not exclude the possibility that D2-receptors might differ between AA and ANA rats after repeated cocaine treatment. Concerning DA uptake mechanisms, the present results (no significant differences in the effects of acute cocaine on extracellular dopamine in the nucleus accumbens or caudate-putamen between AA and ANA rats) suggest that the dopamine transporter mechanisms in these rats are similar. However, differences in DA uptake mechanisms between AA and ANA rats after repeated cocaine treatment cannot be ruled out. It has been suggested (Cass et al. 1993) that there may be alterations in the clearance rate of accumbal dopamine after repeated cocaine treatment, even without any differences in the affinity of the dopamine transporter for cocaine or density of binding sites.

6.2.8. Effects of acute and repeated cocaine in the caudate-putamen

In agreement with previous studies (Di Chiara and Imperato 1988a; Martin-Fardon et al. 1996), acute cocaine increased extracellular dopamine concentrations in the caudate-putamen of AA and ANA rats. The effect of acute cocaine did not differ between AA and ANA rats, and no enhancement in the effect of cocaine on dopamine was seen in rats of either line after repeated cocaine treatment (paper IV). Thus, it appears that the nigrostriatal dopamine is not involved in the sensitisation of locomotor activity to cocaine seen in AA rats, which is in line with the suggestion that the predominant site of action of cocaine for the enhancement of locomotor activity seems to be the nucleus accumbens (Chen and Reith 1994; Delfs et al. 1990).

6.2.9. Methodological points concerning repeated microdialysis

When morphine was given acutely to rats with microdialysis probes implanted for the first time approximately 3.5-4 hours before drug administration, morphine was, surprisingly, without effect on extracellular dopamine in the nucleus accumbens (data not shown). Therefore, the microdialysis studies examining nucleus accumbens with acute and repeated morphine were conducted using different rats, resulting in similar elevations of dopamine after morphine both when the rats received morphine for the first time and when they had received morphine on the three previous days. All other

microdialysis trials (effects of morphine in the caudate-putamen and all cocaine experiments) were performed using the same rat on both days 1 and 4. The use of multiple probe insertions raises the possibility that previous damage to the dialysis area induced by insertion of the probe and possible gliosis may have impaired dopamine overflow. In the cocaine study, the baseline levels of dopamine declined somewhat between days 1 and 4 in the nucleus accumbens in both lines, possibly due to the microdialysis procedure. As pointed out in the materials and methods section (4.10.2.), it has been suggested that repeated microdialysis experiments may be used in the striatum (Martin-Fardon et al. 1997). However, there is some evidence that the effect of amphetamine may be attenuated following the second insertion of the probe into this region of the brain (Camp and Robinson 1992). If such attenuation also occurred in our experiments with cocaine, it might have masked any possible sensitisation in ANA rats. Nevertheless, the effect of cocaine was stronger in AA than in ANA rats after repeated cocaine treatment.

6.3. Effects of morphine on 5-HT and 5-HIAA

6.3.1. Basal levels of 5-HT and 5-HIAA

In contrast to P/NP and HAD/LAD rats, lines in which the alcohol-preferring rats have lower cerebral concentrations of 5-HT and its major metabolite, 5-HIAA, than the alcohol-avoiding rats (McBride and Li 1998; McBride et al. 1993), we found that AA rats have higher cerebral concentrations of 5-HT and its 5-HIAA when compared to ANA rats in the nucleus accumbens and in the olfactory tubercle (III). These findings agree with previous studies starting already in the early generations of these rat lines (Ahtee and Eriksson 1973; Honkanen et al. 1999; Korpi et al. 1988). Interestingly, low concentrations of 5-HIAA in the cerebrospinal fluid has been associated with type 2 alcoholism in humans (Cloninger 1987; Virkkunen and Linnoila 1997). Thus, in respect to 5-HIAA, the AA rats may represent a different type of alcoholism than the P and HAD rats.

6.3.2. Effects of acute and repeated morphine on 5-HT and 5-HIAA

Cerebral serotonergic mechanisms have been suggested to be involved in the regulation of alcohol consumption, locomotor activity and mesolimbic dopamine release (Benloucif et al. 1993; Geyer 1996; Gillies et al. 1996; Koob et al. 1998a; Mylecharane 1996). In study III, it was found that acute administration of morphine increased 5-HT metabolism in AA rats by about 20 % but in ANA rats clearly less. The elevation was most pronounced in the olfactory tubercle, and may contribute to the enhanced locomotor activity of AA rats as compared with ANA rats after acute administration of the drug. However, repeated morphine administration caused tolerance rather than sensitisation in the effects of morphine on tissue concentrations of 5-HT and 5-HIAA. Therefore, it seems that the serotonergic mechanisms are not involved in the differences that occur in morphine-induced locomotor stimulation between AA and ANA rats after repeated administration.

6.4. Effects of acute and repeated morphine and cocaine on rotational behaviour (V)

6.4.1. Effects of morphine on rotational behaviour

Acute administration of a 3 mg/kg dose of morphine induced significant ipsilateral rotational activity in AA rats, but did not enhance the rotational activity in ANA rats. This finding is in agreement with the locomotor activity study (paper I) showing a greater degree of morphine-induced locomotor activity enhancement after acute morphine treatment in AA rats as compared to that found in ANA rats. This suggests that morphine more readily activates the dopaminergic transmission in AA than in ANA rats, agreeing with the neurochemical studies performed in the caudate-putamen (papers II and III and Honkanen et al. 1999). However, in the locomotor activity study already 1 mg/kg dose of morphine increased the locomotor activity of rats of both lines, whereas this dose of morphine was not sufficient to enhance the rotational behaviour in rats of either line. It should be noted that the rotational behaviour

measured in nigrostriatally 6-OHDA lesioned rats mainly reflects the responses of dopaminergic nigrostriatal mechanisms, whereas opioids have also been shown to increase locomotor activity independently of dopamine, when administered directly into the nucleus accumbens (Kalivas et al. 1983; Pert and Sivit 1977). Thus, in addition to the possible role of cerebral DA, nondopaminergic mechanisms might also be involved in the differences of locomotor activity responses to morphine shown by rats of these two lines. This may be related to, for instance, findings that AA rats have a higher density of μ-opioid receptors in the shell subdivision of the nucleus accumbens than ANA rats (de Waele et al. 1995; Marinelli et al. 2000).

On the fourth day of repeated daily treatment with 1 or 3 mg/kg of morphine no significant sensitisation of rotational behaviour was found in rats of either line. In line with the responses uncovered concerning rotational behaviour, no significant sensitisation of the locomotor activity stimulating effects of morphine could be detected in either AA or ANA rats during 4-days treatment with a 3 mg/kg dose of morphine. However, AA rats but not ANA rats were sensitised to the locomotor activity stimulating effects of 1 mg/kg of morphine during 4-day treatment (I). If the sensitisation of locomotor activity enhancing effect of 1 mg/kg of morphine resulted from enhanced dopaminergic transmission, at least some enhancement of rotational behaviour should have been observed in the AA rats after repeated treatment with this dose of morphine. Thus, our behavioural studies (I and V) together with the neurochemical studies (II and III) suggest that morphine-induced cerebral dopamine release is not altered significantly during repeated 4-day treatment with morphine in AA or ANA rats.

When the rats were challenged with a 3 mg/kg dose of morphine 8 days after repeated saline or morphine pretreatment, morphine pretreated AA but not ANA rats showed enhanced rotational behaviour when compared with saline pretreated controls suggesting enhanced dopaminergic transmission. The enhanced dopaminergic transmission in the challenge session in AA rats agrees with several studies showing sensitisation of mesolimbic dopamine release after 3 or more days of withdrawal from repeated morphine administration (Acquas and Di Chiara 1992; Cadoni and Di Chiara 1999; Kalivas and Stewart 1991; Spanagel et al. 1993). This suggests that a

withdrawal period from repeated morphine treatment is needed to reveal the enhancement of cerebral dopaminergic transmission in AA rats, but even so this enhancement is not seen in ANA rats.

6.4.2. Effects of cocaine on rotational behaviour

Cocaine induced rotational behaviour in both AA and in ANA rats, but this effect was more pronounced in AA rats, especially on days 3 and 4 during repeated cocaine treatment. However, no significant differences between AA and ANA rats were found concerning the effect of cocaine on day 1. This is in line with the results showing that the effects of acute cocaine on horizontal locomotor activity (I) or on mesolimbic or nigrostriatal dopamine release (IV) do not differ between rats of these lines.

Repeated cocaine treatment did not induce significant enhancement of ipsilateral rotations in rats of either line. In agreement with these results, sensitisation of the effect of cocaine on the concentration of dopamine in the dorsal striatum was not seen in either AA or ANA rats (paper IV). Only rats that showed 95 % or larger depletion of nigrostriatal dopamine were included in the data. However, as the 6-OHDA lesion was aimed to the medial forebrain bundle, depletion of mesolimbic dopamine may also occur. In fact, we also measured dopamine concentrations in the nucleus accumbens of some rats, and found approximately 90 % depletion of mesolimbic dopamine (data not shown). Therefore, any direct conclusions as to whether the rotational behaviour of the rats results from a depletion of mesolimbic or nigrostriatal dopamine cannot be made on the basis of these experiments. Although mesolimbic dopamine function may not be essential for the initiation of rotational behaviour (Costall et al. 1976), it may be important in mediation of circling seen after unilateral lesions of the nigrostriatal dopaminergic pathways. It has been suggested that imbalance in the nigrostriatal dopamine system after unilateral 6-OHDA lesion causes a postural asymmetry and head turning and determines the direction of rotation, but the mesolimbic dopamine system provides a locomotor component and converts the postural asummetry into active circling behaviour (Pycock 1980). Thus, the findings of the rotational study (V) fit to neurochemical findings (IV), in that where there were

no changes in nigrostriatal dopamine release, no significant sensitisation of rotational behaviour during repeated cocaine treatment occurred in rats of either line. The influence of the sensitisation of mesolimbic dopamine release, previously found in AA rats, can be seen in the more pronounced rotational behaviour in these rats on days 3 and 4 when compared to ANA rats.

In the challenge session, 8 days after repeated cocaine treatment, when all rats were given cocaine acutely, no significant differences between saline or cocaine treated rats were seen in rats of either line. It should be noted that the variation in the saline pretreated group of AA rats is relatively high, which is mainly due to 2 rats out of 9 rotating over 600 rounds. If these 2 rats were excluded from data, the difference in saline and cocaine pretreated AA rats would be statistically significant, with cocaine pretreated rats rotating more than saline pretreated rats.

6.5. Role of brain dopamine in morphine and cocaine-induced behavioural sensitisation

It has been suggested that the mesolimbic dopaminergic neurons mediate both the reinforcing and locomotor activity stimulating effects of various drugs of abuse. Thus, accelerated dopamine release in the nucleus accumbens causes both the psychomotor stimulant and the reinforcing effects of drugs of abuse (Wise and Bozarth 1987; Wise and Rompré 1989). Furthermore, after repeated treatment, the drug induced mesolimbic dopamine release becomes sensitised, an effect associated with enhancement in the reinforcing effects of drugs of abuse (Robinson and Berridge 1993). In my studies, AA rats became sensitised to both the locomotor stimulant and to the mesolimbic dopamine release enhancing effects of repeated cocaine with smaller doses of cocaine than ANA rats (I and IV). Therefore, it seems that psychomotor sensitisation may be associated with sensitisation of dopamine release in AA rats after repeated cocaine, which effect may be involved with the stronger vulnerability to addictive behaviour displayed in AA rats. In contrast to cocaine,

psychomotor sensitisation after repeated morphine treatment (I) was not associated with sensitisation of mesolimbic dopamine release in AA rats (II and III). Therefore, it seems that there is also a non-dopaminergic component included in the morphine-induced psychomotor stimulation/sensitisation. Thus, in that respect, the effects of cocaine and morphine may be different. Sensitisation of ascending dopamine pathways to morphine may, however, also be seen in AA rats if there is a withdrawal period of several days between the repeated treatment and the test session (V). No such sensitisation is seen in ANA rats, which may, again, be involved with the enhanced vulnerability to addictive behaviour shown in AA rats than in ANA rats.

7. CONCLUSIONS

1. Morphine induces more pronounced psychomotor stimulation in naive AA than ANA rats, but the effect of acute cocaine on locomotor activity does not differ between these rat lines. When treated repeatedly, AA rats became sensitised to this locomotor activity enhancing effect with lower doses of morphine and cocaine than ANA rats. Thus, the cerebral mechanisms mediating reinforcement may be particularly sensitive to repeated drug treatment in AA rats, a phenomenon which may be involved in their high alcohol intake as well.

2. The effects of either acute or repeated 4-day treatments with morphine on mesolimbic dopamine mechanisms do not differ between AA and ANA rats, and as such, these findings do not support a role for accumbal dopamine systems in morphine-induced behavioural sensitisation in AA rats. However, the effect of acute morphine on nigrostriatal dopamine mechanisms seems to be more prominent in AA rats when compared with ANA rats. Acute cocaine tretment in AA and ANA rats affects the striatal dopamine mechanisms in a similar manner. However, AA rats already begin to show sensitisation to the effect of cocaine on mesolimbic dopamine after only 4-days treatment with cocaine, with such sensitisation not observed in ANA rats. This sensitisation of mesolimbic dopamine may not only render AA rats more susceptible to alcohol, but also to other drugs of abuse, and might explain the findings that AA rats are more susceptible to psychomotor sensitisation than ANA rats. Furthermore, the sensitisation of locomotor activity after repeated drug treatment was clearly associated with sensitisation of mesolimbic dopamine release in AA rats repeatedly treated with cocaine but not when they were treated repeatedly with morphine. Thus, in this respect, the effects of cocaine and morphine seem to differ, suggesting a non-dopaminergic component in morphine-induced behavioural sensitisation.

3. Both morphine and cocaine induced more rotational behaviour in AA than in ANA rats during 4-days of repeated treatment, suggesting that these drugs induce stronger activation of brain dopaminergic mechanisms in AA than in ANA rats. Neither drug induced significant sensitisation in rotational

behaviour during the 4-day drug treatment in rats of either line. However, in a challenge session 8 days after repeated morphine treatment, morphine induced sensitisation in AA but not in ANA rats, signifying that in AA rats a withdrawal period of several days is needed for the expression of sensitisation of striatal dopaminergic mechanisms, and yet this sensitisation is not seen in ANA rats.

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Janne Mikkola

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