CHAPTER 9

GENERAL DISCUSSION
The sensitivity to the reinforcing effects of drugs of abuse constitutes a likely vulnerability factor for drug dependence. The overall objective of the studies described in this thesis was to investigate the role of μ-opioid receptors in cocaine reinforcement and the underlying neurobiological mechanisms. In this chapter the findings of this thesis will be discussed accordingly.

μ-OPIOID RECEPTORS AND COCAINE REINFORCEMENT

The studies described in Chapters 3 and 5 of this thesis demonstrate that μ-opioid receptor knockout mice were impaired in cocaine self-administration while, on the other hand, no differences between μ-opioid receptor knockout mice and wild-type controls were observed in cocaine-induced locomotor activity or cocaine-induced behavioural sensitization. These findings show (1) that μ-opioid receptors are specifically involved in cocaine reinforcement, and (2) that the mechanisms involved in cocaine reinforcement are divergent from those involved in acute cocaine-induced locomotor activity and sensitization to the locomotor stimulant effects of cocaine, at least with regard to μ-opioid receptor involvement.

Previous studies demonstrated, by local administration of the opioid antagonist naltrexone, that particularly opioid receptors in the ventral tegmental area (VTA) account for opioid modulation of cocaine reinforcement (Ramsey et al., 1999). In the VTA of wild-type mice, μ-opioid receptors are present on GABAergic neurons (Figure 1A; Garzon & Pickel, 2001; Garzon & Pickel, 2002). Opioids can activate μ-opioid receptors in the VTA, which involves intracellular signalling through the ERK1/2 pathway (Chapter 4), causing hyperpolarization of the GABAergic neurons (Johnson & North, 1992a; Johnson & North, 1992b). These GABAergic neurons are thought to be local interneurons, although GABAergic projection neurons from the VTA to amongst others the nucleus accumbens and prefrontal cortex have also been described (Van Bockstaele & Pickel, 1995; Steffensen et al., 1998; Carr & Sesack, 2000). The local GABAergic interneurons in the VTA synapse onto dopaminergic neurons that form a major output pathway from the VTA (Johnson & North, 1992a). In μ-opioid receptor knockout mice, the inhibitory GABAergic input onto dopamine neurons in the VTA was increased in a cocaine free state, as is evident from increased spontaneous inhibitory postsynaptic currents (IPSC’s) measured from dopamine neurons (Chapter 3, Figure 1C).

Interestingly, actively self-administered, but not passively administered, cocaine was positively correlated with pro-opiomelanocortin (POMC) mRNA levels in the arcuate nucleus (Chapter 3), that is POMC mRNA levels increased as total active cocaine intake increased. POMC is the precursor of the μ-opioid receptor selective endogenous opioid peptide β-endorphin. Indeed, β-endorphin levels increase in response to cocaine and also after administration of amphetamine and alcohol, at least in the nucleus accumbens (Olive et al., 2001; Roth-Deri et al., 2003; Marinelli et al., 2003). Also, in vivo autoradiography revealed that opioid levels are increased after cocaine self-administration (Gerrits et al., 1999). Active self-administration is a measure for cocaine reinforcement, which requires μ-opioid receptor activation (Chapter 3). The positive correlation of active cocaine self-administration with
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POMC mRNA, the precursor of β-endorphin, therefore suggests that β-endorphin, through interactions with µ-opioid receptors, may account for opioid modulation of cocaine reinforcement (Figure 1B). However, other endogenous opioids such as the novel µ-opioid receptor selective endomorphins 1 and 2 (Zadina et al., 1997) or enkephalins, which also have affinity for µ-opioid receptors, may also be involved. It is through inhibition of the GABAergic neurons that µ-opioid receptors cause disinhibition of dopamine neurons in the VTA, thereby presumably contributing to augmented dopamine release from the nucleus accumbens in response to different drugs of abuse (Di Chiara & Imperato, 1988a) and to burst

**Figure 1**

Proposed mechanism for µ-opioid receptor mediated cocaine reinforcement in the VTA. The cocaine-free state of the VTA of wild-type (+/+) and knockout (-/-) mice, obviously with differences in µ-opioid receptors and consequent changes in GABAergic transmission are shown in (A) and (C), respectively. Addition of cocaine causes endogenous opioid peptides to be released, which through activation of µ-opioid receptors cause hyperpolarization of GABA neurons and consequently disinhibition of dopamine neurons, facilitation of burst firing of dopamine neurons and enhancement of dopamine output. As a result, cocaine is reinforcing to wild-type (+/+) mice (B). In contrast, endogenous opioid peptides, released in response to cocaine, can not activate µ-opioid receptors in the µ-opioid receptor knockout (-/-) animals. As a consequence, disinhibition of dopamine neurons does not occur, dopamine output is not enhanced and cocaine is not reinforcing in µ-opioid receptor knockout mice (D).
firing of dopamine neurons (Schultz et al., 1997; Cooper, 2002), which may ultimately lead to cocaine reinforcement (Figure 1B).

POMC mRNA levels were not different between μ-opioid receptor knockout mice and wild-type controls (Zhou et al., 2002), suggesting that similar levels of β-endorphin, the major endogenous μ-opioid receptor ligand, are released in response to cocaine in both genotypes. However, in case of μ-opioid receptor knockout mice, this or other endogenous opioids obviously cannot activate a μ-opioid receptor. Consequently, the GABA neurons cannot be hyperpolarized and disinhibition of dopamine neurons cannot occur as in wild-type mice. We propose that impaired disinhibition of dopamine neurons together with the increased GABAergic inhibitory input onto the dopamine neurons contributes to impaired cocaine self-administration by μ-opioid receptor knockout mice (Chapter 3). There is no evidence for altered basal firing frequency of dopamine neurons in vitro in the VTA of μ-opioid receptor knockout mice (Chapter 3) nor are there indications for altered dopamine release from the nucleus accumbens, both under basal conditions and in response to alcohol (Tang et al., 2002). Rather, the threshold for burst firing of dopamine neurons might be augmented in these mice, which is yet to be investigated (Figure 1D).

**DRUG REINFORCEMENT**

**μ-Opioid receptors and drug reinforcement**

This thesis provides evidence for an important role of μ-opioid receptors in cocaine reinforcement. Previous studies substantiate the involvement of the μ-opioid receptor in drug reinforcement across pharmacological classes. For instance, μ-opioid receptor knockout mice do not self-administer morphine (Becker et al., 2000) and consume less alcohol (Roberts et al., 2000; Hall et al., 2001; Becker et al., 2002).

Thus, drugs of abuse from different pharmacological classes have μ-opioid receptor mediated modulation of their reinforcing efficacy in common. This is interesting considering the different primary targets that are used by different drugs of abuse. Opiates interact with opioid receptors (Snyder & Pasternak, 2003), cocaine acts as a dopamine transporter blocker (Ritz et al., 1987), amphetamine interacts with the vesicular monoamine transporter (Pifl et al., 1995), alcohol is considered to act through interactions with ligand-gated ion channels (Soderpalm et al., 2000), nicotine acts through nicotinic acetylcholine receptors (Corrigall et al., 1992; Picciotto et al., 1998) and cannabinoids act through cannabinoid receptor interactions (Gardner & Vorel, 1998; Childers & Breivogel, 1998). It is likely that the actions of different classes of drugs converge to a common system. μ-Opioid receptors in the VTA may form part of such a common system, which is relevant for drug reinforcement.

How does μ-opioid receptor modulation of drug reinforcement relate to the mesolimbic dopamine system? It appears likely, that μ-opioid receptors modulate drug reinforcement by affecting dopamine output of the mesolimbic system. For, as outlined previously in this
chapter, µ-opioid receptors in the VTA cause, by disinhibition of dopamine neurons, enhanced dopamine output of the mesolimbic system.

Yet, the importance of the mesolimbic dopamine system in drug reinforcement is a matter of debate. For example, opiate reinforcement does not require intact dopamine input to the nucleus accumbens (Gerrits & Van Ree, 1996) and haloperidol or the D1 receptor antagonist SCH23390 could not abolish the initiation of heroin self-administration (Pettit et al., 1984; Van Ree & Ramsey, 1987; Gerrits et al., 1994, see Chapter 1). Apparently, opiates can support self-administration independent of dopamine. There is an interesting parallel in the effects of opiates and dopamine upon GABAergic medium spiny neurons in the nucleus accumbens, which form the main output neurons of the nucleus accumbens (Tzschentke & Schmidt, 2000). Opiates, dopamine and also psychostimulants depress excitatory postsynaptic currents (EPSC’s) in the nucleus accumbens as measured from medium spiny neurons in the nucleus accumbens (Pennartz et al., 1992; Harvey & Lacey, 1996; Nicola et al., 1996; Martin et al., 1997; Hoffman & Lupica, 2001). Inhibition of these GABAergic medium spiny neurons, and not so much dopamine, may therefore be an important common effect of drugs of abuse that may be required for drug reinforcement. The relevance of medium spiny neuron inhibition in the nucleus accumbens for reinforcement could be subject of future research. Obviously, the model outlined here is simplified; other brain regions, such as the ventral pallidum, are also likely involved in drug reinforcement.

Cannabinoid CB1 receptors and drug reinforcement

In Chapter 8 of this thesis the involvement of cannabinoid CB1 receptors in behavioural effects of cocaine was investigated. No effects were observed of the CB1 receptor antagonist SR141716A upon cocaine reinforcement nor was cocaine-induced behavioural sensitization affected by SR141716A treatment. When reviewing the available literature dealing with CB1 receptor involvement in reward-related effects of drugs of abuse, in combination with the present findings, a discrepancy between CB1 involvement in opiate and alcohol but not psychostimulant reinforcement is apparent.

Other studies also suggest that the endogenous cannabinoid system is not involved in cocaine reinforcement (Fattore et al., 1999; Cossu et al., 2001; De Vries et al., 2001) or amphetamine reinforcement (Cossu et al., 2001), although cannabinoid CB1 receptor blockade impaired cocaine-induced CPP (Chaperon et al., 1998) and reinstatement of cocaine self-administration (De Vries et al., 2001). In contrast to these findings, other studies reported CB1 receptor mediated modulation of opiate reinforcement (Ledent et al., 1999; Cossu et al., 2001; Navarro et al., 2001; Solinas et al., 2003; De Vries et al., 2003) and CB1 modulation of alcohol reinforcement (Arnone et al., 1997; Serra et al., 2001; Lallemand et al., 2001), although others found no effect of SR141716A upon alcohol preference or intake (Colombo et al., 2002). With respect to drug-induced behavioural sensitization, CB1 receptor knockout mice were impaired in morphine-induced behavioural sensitization (Martin et al., 2000a), although behavioural sensitization to morphine was not affected by SR141716A co-administration (Norwood et al.,
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Thus, the reinforcing effects of and sensitized responses to depressant drugs such as opiates and alcohol are sensitive to cannabinoid modulation while CB1 receptors appear not to modulate psychostimulant reinforcement or behavioural sensitization.

Little is known about the mechanisms, which may explain the discrepancy in CB1 receptor involvement in the effects of different drugs of abuse. A likely site of CB1 receptor mediated involvement in drug reinforcement is the VTA. Interestingly, strikingly similar mechanisms of action of CB1 receptors in the VTA were observed as for µ-opioid receptors: CB1 receptors inhibit GABA neurons in the VTA resulting in disinhibition of dopamine neurons and causing enhanced dopamine output to the nucleus accumbens (Szabo et al., 2002). Tanda and co-workers however described non-reciprocal cannabinoid-opioid interactions in dopamine release from the nucleus accumbens. They reported that the µ-opioid receptor antagonist naloxonazine, administered in the VTA, reduced both the cannabinoid and heroin-induced increase in dopamine release from the nucleus accumbens. In contrast, intra-VTA administered SR141716A only inhibited the effects of cannabinoids upon dopamine release but failed to affect the response to heroin. It thus appears that CB1 receptors are not required for µ-opioid receptor mediated effects in the VTA, thus suggesting that VTA CB1 receptors might not be involved in CB1 receptor effects upon opiate reinforcement. Future studies could use local injections of antagonists to pinpoint the site and mechanism of interaction between µ-opioid and CB1 receptors in modulation of drug self-administration and explore the differential involvement of CB1 receptors in opiate/alcohol but not psychostimulant reinforcement.

µ-OPIOID RECEPTORS AND VULNERABILITY FOR DRUG DEPENDENCE

What are the implications for human drug addiction of the important role of µ-opioid receptors in drug reinforcement? Drug reinforcement is a key factor in vulnerability for drug dependence. Therefore, involvement of µ-opioid receptors in the sensitivity to drug reinforcement across pharmacological classes suggests that variations in µ-opioid receptors, either genetic or environmental in nature, may contribute to an individual’s vulnerability for drug dependence.

Genetic variation in the human µ-opioid receptor gene

Single nucleotide polymorphisms (SNP’s) make up for genetic variations between individuals. They occur everywhere in the genome and can affect the expression or function of genes. A number of SNP’s in the human µ-opioid receptor gene have been identified and studied for association with drug addiction in humans. An example is the relatively extensively studied nucleotide substitution at position 118 (A118G), predicting an Asp40Asn amino acid change (Bergen et al., 1997; Bond et al., 1998). Different studies suggested a significant association of the A118G variant with opiate dependence (Szeto et al., 2001; Tan et al., 2003) or with alcohol dependence (Town et al., 1999; Schinka et al., 2002). The frequency of the A118G
variant is lower in drug dependent subject groups. However, other studies failed to show a significant association of the A118G variant of the µ-opioid receptor gene with either opiate (Bond et al., 1998; Franke et al., 2001; Shi et al., 2002) or alcohol dependence (Bergen et al., 1997; Sander et al., 1998; Gelernter et al., 1999; Franke et al., 2001; Rommelspacher et al., 2001). Such genetic studies may define genetic predisposition and associated risk for drug addiction in individuals. Moreover, they can contribute to delineate the importance of specific genes in the neurobiological mechanisms of addiction.

Environmental factors, endogenous opioids and reinforcement

In contrast to genetic, intrinsic factors, environmental influences may also affect the functionality of the endogenous opioid system and could thereby contribute to the reinforcing efficacy of drugs of abuse and hence, the vulnerability for drug dependence. In this section, prenatal morphine, emotional stress and play deprivation will be outlined briefly as examples of such environmental factors, which facilitate drug reinforcement, presumably through changes in endogenous opioid systems.

(1) Prenatal morphine treatment leads to increased µ-opioid receptor binding in rats (Vathy et al., 2003). Interestingly, prenatal morphine treatment also causes rats, as adults, to be more sensitive to the reinforcing effects of both heroin and cocaine (Ramsey et al., 1993). (2) Another example is emotional stress, which is a witness stress: the emotional stressed animal can see, hear, smell but not touch the physically stressed subject, that receives uncontrollable footshocks (Takahashi et al., 1987). Cocaine and morphine self-administration was facilitated by emotional stress in rats and mice (Ramsey & Van Ree, 1993; Kuzmin et al., 1996) and also intracranial self-stimulation was facilitated by emotional stress (Bespakov, unpublished data). By means of autoradiography and naloxone administration, changes in the endogenous opioid system were demonstrated after emotional stress (Van den Berg et al., 1998; Pijlman, unpublished data). (3) Further, isolation of rats during postnatal weeks 4-5 in which rats normally display high levels of play behaviour, which involves increased endogenous opioid peptide release (Panksepp & Bishop, 1981), has been associated with adaptations in opioid peptide and opioid receptor levels (Vanderschuren et al., 1995; Van den Berg et al., 1999). Recent findings revealed, that play deprivation caused facilitation, although modest, of the acquisition of cocaine self-administration in adults rats (Gerrits, unpublished data).

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

This study revealed that µ-opioid receptors have an important role in cocaine reinforcement, suggesting that variations in µ-opioid receptors might contribute to vulnerability to develop drug dependence. Factors that affect the function of the µ-opioid receptor should be considered to influence drug dependence. The genetic make-up of the µ-opioid receptor gene and developmental and environmental factors affecting its expression could be part of a neurobiological process underlying vulnerability for drug dependence. An important next step
will be to confirm that variations in µ-opioid receptors indeed contribute to liability to develop drug dependence.

Repeated and extended self-administration sessions in models that mimic human addiction more are required in preclinical studies. Such models of extended access, described by different groups, are for example characterized by escalating and irregular patterns of drug intake and would be valuable tools in this respect (Ahmed & Koob, 1998; Tornatzky & Miczek, 2000; Mantsch et al., 2001). Important to consider in this context is the notion that, based on previous studies which for instance described a rightward shift in cocaine self-administration after naloxone treatment (Kuzmin et al., 1997a), µ-opioid receptors probably modulate drug reinforcement but may not be required as such for drugs to act as reinforcers. Therefore, µ-opioid receptor knockout mice may eventually acquire cocaine self-administration. If true, it will also be interesting to investigate the reinstatement behaviour of µ-opioid receptor knockout mice, considering the reduction in dopamine D3 receptor levels observed for these mice (Chapter 6).

Clearly the present findings suggest further human research to determine the role of µ-opioid receptor variations in susceptibility to drug dependence. Since variations in µ-opioid receptors may be genetic in nature or may be induced by environmental factors, such as traumatic life events, attempts should be made to differentiate between variations in µ-opioid receptors caused by genetic and environmental factors. Moreover it is interesting to consider gene × environment interactions in connection to vulnerability for drug dependence.

Although the main output of the VTA is formed by dopamine projections to amongst others the nucleus accumbens, GABA projections to nucleus accumbens and prefrontal cortex have also been described to originate in the VTA (Steffensen et al., 1998). These projections have been poorly studied in relation to addiction processes and it will be interesting to focus more on these projections and to investigate their role in drug reinforcement. Also, it is interesting to consider in future studies the role of nucleus accumbens medium spiny neurons in drug reinforcement.

Finally, future studies could also focus on cannabinoid CB1 receptors, which appear to differentially modulate opiate and alcohol as opposed to psychostimulant reinforcement. Knowledge of the mechanisms through which cannabinoid CB1 receptors interfere with opiate and alcohol reinforcement may provide more insight into the distinct mechanisms of action of depressant and psychostimulant drugs.

The findings described in this thesis suggest a role of µ-opioid receptors, and neurobiological mechanisms associated to µ-opioid receptors, in vulnerability for drug dependence. This knowledge may lead to improvement of strategies in prevention and treatment of addiction.