Cannabinoid and opioid modulation of impulsive behavior and drug addiction

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Chapter 6
General discussion

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The aim of this thesis was two-fold. First, by employing two different translational models of impulsivity, we intended to gain more knowledge on the role of the endogenous cannabinoid (eCB) and opioid neurotransmitter systems in impulsive behavior. Previous studies in humans as well as rodents had already suggested that both neurotransmitters may modulate impulsive behavior. The research described in Chapters 2-4 extended those findings by demonstrating that cannabinoid CB1 and μ-opioid receptors in the brain 1) have divergent roles in regulating distinct types of impulsive behavior (motor impulsivity vs. impulsive choice), and 2) regulate impulsivity under different conditions ('states'). In a parallel research line, we addressed the role of the eCB system in relapse to reward seeking during abstinence. Since cannabinoid CB1 receptor antagonists are known to suppress relapse behavior, it was generally assumed that the eCB system becomes activated during a bout of relapse. In Chapter 5, it was confirmed that unlike conditioned food seeking, relapse to heroin seeking is indeed accompanied by robust eCB (2-arachidonyl glycerol; 2-AG) release in the dorsal medial prefrontal cortex (dmPFC), a brain region that is critically involved in mediating this behavior. Thus, our data support the idea that the eCB and opioid neurotransmitter systems may be interesting targets for novel pharmacotherapies against hyper-impulsivity and heroin addiction. Below, we will discuss our findings more elaborately in the context of data from other studies.

6.1. An update on cannabinoids, opioids, and impulsive behavior

Impulsive behavior can be defined as "*actions which are poorly conceived, prematurely expressed, unduly risky or inappropriate to the situation and that often result in undesirable consequences*" (Durana and Barnes, 1993). Acting on an impulse is not necessarily disadvantageous (Williams and Taylor, 2006; Winstanley et al., 2010a). Doing so on a regular basis, however, becomes maladaptive. This endophenotype is closely associated with a variety of psychiatric and neurological disorders including Attention–Deficit/Hyperactivity Disorder (ADHD), Parkinson's disease, bipolar disorder, borderline personality disorder, and substance use disorders (American Psychiatric Association, 2000). Hence, having effective treatments for deficient impulse control is of utmost importance. In search of adequate anti-impulsivity therapies, scientists should realize that there are multiple forms of impulsive behavior, generally ranging from impulsive actions to impulsive choices (Evenden, 1999; Moeller et al., 2001; Winstanley et al., 2006a; Pattij and Vanderschuren, 2008; Dalley et al., 2011). Largely distinct neurobiological mechanisms may underlie each of these aspects of impulsivity. In this thesis, two specific aspects of impulsivity were studied: motor impulsivity (action restraint or inhibitory response control) and impulsive choice (delay aversion, i.e. intolerance to delayed gratification). These forms of impulsivity

were assessed in rats using a 5-choice serial reaction time task (5-CSRTT) (Carli et al., 1983; Robbins, 2002; Bari et al., 2008) and a delayed reward task (DRT) (Evenden and Ryan, 1996; Cardinal, 2006), respectively.

In line with the long-known therapeutic efficacy of the psychostimulant drugs amphetamine and methylphenidate to alleviate impulse control-related problems (Heal et al., 2012), most impulsivity research has traditionally been directed at monoaminergic neurotransmission (dopamine, norepinephrine, and serotonin (5-HT)) (Pattij and Vanderschuren, 2008; Winstanley, 2011; Dalley and Roiser, 2012). However, the efficiency rate of current monoaminergic anti-impulsivity pharmacotherapies ranges between 50-70%. Moreover, these pharmacological agents can cause side-effects such as weight loss, sleep disturbances, and nausea (Heal et al., 2012). Moreover, particularly with stimulant medication there is an abuse potential. Hence, there is a need for novel anti-impulsivity pharmacotherapies that perhaps target non-monoaminergic neurotransmission. Two closely-interacting modulatory neurotransmitter systems in the brain that may be relevant in this regard are the cannabinoid and opioid systems, in particular cannabinoid CB1 and μ-opioid receptors (e.g. Madden et al., 1997; McDonald et al., 2003; Kieres et al., 2004;

Table 6.1. Summary of the role of cannabinoid CB1 and μ-opioid receptor activity in motor impulsivity and impulsive choice.

	CB1 receptor		μ-opioid receptor	
	activation	**inactivation**	**activation**	**inactivation**
Motor Impulsivity				
Baseline (ITI = 5 s)	⇔	⇓[1]	⇧	⇔
Long ITI (ITI = 7 s)	⇔	⇓	N.D.	⇔
Amphetamine	N.D.	⇓	N.D.	⇓
GBR 12909	N.D.	⇔	N.D.	⇓
Nicotine	N.D.	⇓	N.D.	⇔
Impulsive Choice				
Baseline	⇓⇔[2]	⇔	⇧	⇔
Amphetamine	N.D.	⇧	N.D.	⇔

Arrows indicate the direction of the effects of cannabinoid CB1 and μ-opioid receptor (ant)agonists on motor impulsivity (5-CSRTT) and impulsive choice (DRT), relative to baseline or (non)drug-induced changes in impulsivity. N.D. not determined; [1] Effects have been found to be baseline-dependent (see Chapter 3); [2] Effects have been found to be compound-specific (see Chapter 3).

Ehlers et al., 2007; Pattij et al., 2008; Love et al., 2009).

There is a wealth of literature on functional interactions between the cannabinoid CB1 and μ-opioid receptors, and even the formation of receptor heteromeres has been suggested (Schoffelmeer et al., 2006; Robledo et al., 2008; Parolaro et al., 2010). Nonetheless, previous studies in our laboratory revealed that cannabinoid CB1 and μ-opioid receptors may play distinct roles in regulating motor impulsivity as well as impulsive choice (Table 6.1; Pattij et al., 2007a, 2009). Specifically, tonic CB1 receptor activity was found to regulate motor impulsivity, but not impulsive choice, under baseline conditions. Further receptor activation by the exogenous CB1 receptor agonist WIN55,212-2 appeared to be ineffective in both the 5-CSRTT and DRT. In contrast, whereas the endogenous opioid systems may not modulate motor impulsivity or impulsive choice under baseline conditions, the μ-opioid receptor agonist morphine increased both premature responding in the 5-CSRTT as well as impulsive decision making in the DRT. These data on baseline (trait) impulsivity raised our interest in the putative roles of cannabinoid and opioid receptors in state-dependent changes in impulsivity. This thesis focused particularly on psychostimulant-induced changes in impulsive behavior.

6.1.1. μ-Opioid receptor-mediated motor impulsivity and impulsive choice

As discussed in Chapter 1 (section 2.3), most previous studies on the role of opioids in impulsivity have indicated a role of μ- rather than δ- or κ-opioid receptors (e.g. Kieres et al., 2004; Pitts and McKinney, 2005; Love et al., 2009; Olmstead et al., 2009; Pattij et al., 2009). Accordingly, the experiments discussed in Chapter 2 showed that activation of μ-opioid receptors is necessary for amphetamine-induced motor impulsivity in a 5-CSRTT, while no evidence was obtained for a role of δ- or κ-opioid receptors in baseline or amphetamine-induced impulsivity in this task.

Although multiple brain regions may be involved, the intracranial infusion experiments in Chapter 2 pointed toward the nucleus accumbens (NAc) shell subregion, but not the NAc core subregion, as an anatomical locus for the μ-opioid receptor effects on motor impulsivity. Those experiments also demonstrated that NAc shell μ-opioid receptor activation on itself is sufficient to induce inhibitory control deficits. Interestingly, the effects of NAc shell μ-opioid receptor activation on motor impulsivity seem to resemble the effects of electrical stimulation of this brain region on this behavior (Sesia et al., 2008, 2010). In contrast, lesioning of the NAc shell has been shown to result in suppression of amphetamine-induced impulsivity in the 5-CSRTT (Murphy et al., 2008). The intracranial administration data are consistent with previous studies showing that amphetamine induces endogenous opioid release (Olive et al., 2001; Colasanti et al., 2012), which may facilitate amphetamine-induced dopamine release in the NAc (Hooks et al., 1992; Schad et al., 1995, 2002). Moreover, μ-opioid receptor agonist are known to induce dopamine release

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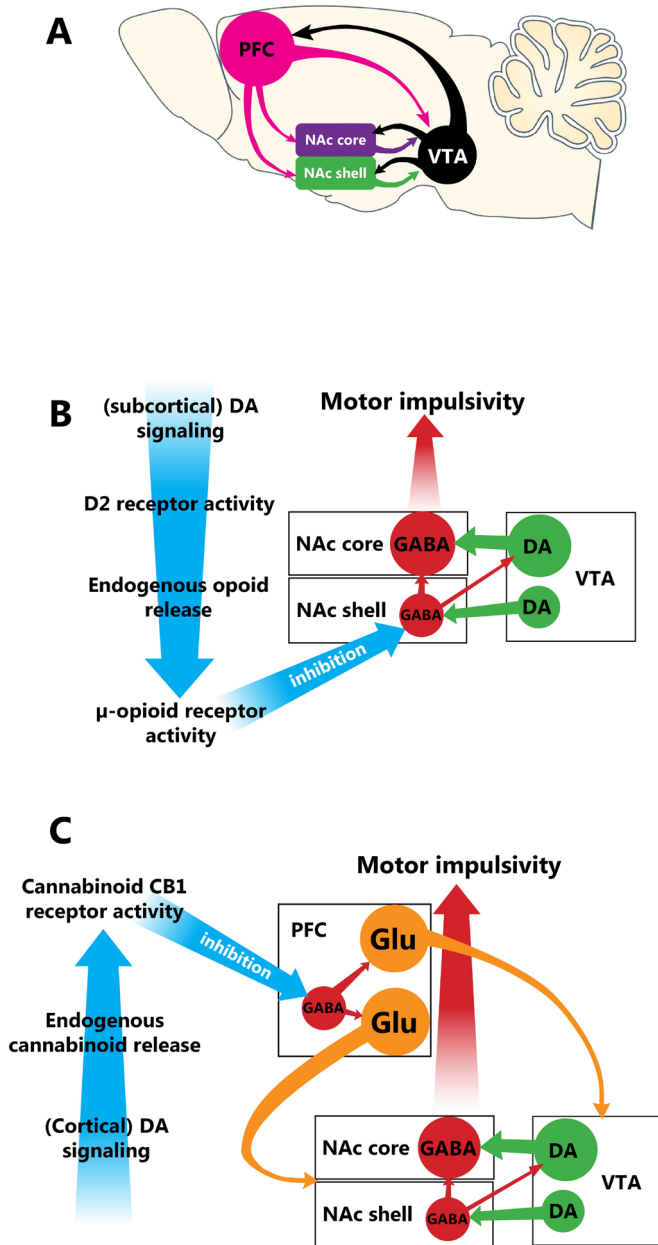
Figure 6.1. Tentative working hypothesis for μ-opioid receptor and cannabinoid CB1 receptor modulation of motor impulsivity. Depicted in A is a saggital midline section of the rat brain showing the ventral tegmental area (VTA), the nucleus accumbens (NAc) core and shell subregions, and the prefrontal cortex (PFC). Depicted in B is a simplified model showing how a rise in DA levels in specific (subcortical) projections could indirectly (most likely via stimulation of dopamine D2 receptors outside the NAc shell) result in μ-opioid receptor stimulation in the NAc shell. Subsequently, μ-opioid receptor activity may alter motor impulsivity by potentiating NAc core dopamine release via (1) direct or (2) indirect (involving feedbackloops to the VTA) GABAergic mechanisms, or (3) via effects on GABAergic output neurons targeting downstream brain regions mediating behavioral responding. Depicted in C is a simplified model showing how a rise in dopamine (DA) levels in specific (cortical) projections could indirectly induce cannabinoid CB1 receptor stimulation. Via net inhibition of local GABAergic activity and the consequent increase in glutamatergic (Glu) output from the PFC, CB1 receptor activity may alter motor impulsivity by directly or indirectly (via the VTA) potentiating NAc neuronal activity. Based in part on data from others; opioid model e.g. (Johnson et al., 1996; Svingos et al., 1997; Haber et al., 2000; Olive et al., 2001; Aono et al., 2008), cannabinoid model e.g. (Pistis et al., 2001, 2002; Egerton et al., 2006). See sections 1.1 and 1.2 for additional discussion.

in the NAc (Di Chiara and Imperato, 1988; Spanagel et al., 1992). Thus, it is conceivable that it is a rise in dopamine levels that induces NAc shell μ-opioid receptor activation within motor impulsivity-related neural circuitries. Accordingly, we found that motor impulsivity induced by the selective dopamine transporter inhibitor GBR 12909, which selectively increases dopamine levels in subcortical brain regions (Elsworth et al., 1993; Weikop et al., 2007), depends on opioid signaling. However, the opioid antagonist naloxone did not reduce premature responding when the intertrial interval (ITI) was either 5 s (baseline) or 7 s (long ITI sessions), or following acute administration of the psychostimulant nicotine. Even though premature responding under these conditions is also known to be dopamine-dependent (Chapter 4; van Gaalen et al., 2006a). Thus, apparently not all dopamine-dependent forms of motor impulsivity require NAc shell μ-opioid receptor activation. Instead, this particular mechanism may be brain region-specific (occurring in subcortical rather than cortical dopamine projections) and/or dependent on the quantitative and temporal properties of dopamine release (Figure 6.1a,c). Interestingly, while dopamine D1 receptors seem to be tonically activated to mediate motor impulsivity, dopamine D2 receptors have been found to mediate amphetamine-induced impulsivity, but not baseline impulsivity (van Gaalen et al., 2006a; Pattij et al., 2007b), much alike μ-opioid receptors. Hence, activation of dopamine D2 receptors might be responsible for impulsivity-related opioid signaling in the NAc shell. Indeed, the dopamine D2 receptor antagonist eticlopride has been shown to prevent cocaine-induced NAc μ-opioid receptor activation (Soderman and Unterwald, 2009). Irrespective of the subtype of dopamine receptors involved, the location of those receptors is likely outside the NAc shell (possibly in the NAc core), because dopamine signaling within the NAc core rather than NAc shell has been found to be critical for psychostimulant-induced premature responding in the 5-CSRTT (Pattij et al., 2007b; Economidou et al., 2012). Upon activation, NAc shell μ-opioid receptors may induce inhibitory control deficits by potentiating NAc dopamine signaling (Yoshida et al., 1999; Hirose et al., 2005), either via local GABAergic mechanisms (van Dongen et al., 2005; Aono et al., 2008) or via effects on the reciprocal spiraling projections that are thought to connect the ventral tegmental area and NAc along a ventromedial to dorsolateral axis (Heimer et al., 1991; Johnson et al., 1996; Haber et al., 2000; Ikemoto, 2007). Additionally, μ-opioid receptor stimulation might alter activity in GABAergic output neurons targeting brain regions downstream of the NAc (Svingos et al., 1997).

Our current understanding of the role of opioids in impulsive choice remains rather limited. In Chapter 2, the lack of effect of various opioid receptor antagonists on impulsive choice indicated that endogenous activation of neither μ- nor δ- or κ-opioid receptors is involved in impulsive decision making in a DRT under baseline conditions or following an amphetamine challenge. Nonetheless, we and others have previously shown that activation of μ-opioid receptors can under certain conditions increase impulsive choice in both rats and humans (Kieres et al., 2004; Mitchell et al., 2007; Boettiger et al., 2009; Pattij et al., 2009). Thus, as discussed above for motor impulsivity, it is conceivable that μ-opioid receptors can modulate neuronal activity within the neural circuitry mediating impulsive choice. The neurochemical mechanisms underlying opiate-induced changes in motor impulsivity and

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impulsive choice may differ, involving dopaminergic- and non-dopaminergic pathways, respectively. Accordingly, preliminary experiments in our laboratory have suggested that morphine-induced motor impulsivity, but not morphine-induced impulsive choice, depends on dopamine (D2 receptor) activity (Wiskerke and Pattij, unpublished observations). It remains to be determined whether morphine can provoke impulsive decision making by inhibiting cortical norepinephrine release, as we have previously suggested (Pattij et al., 2009).

6.1.2. Opposite effects of cannabinoid CB1 receptor activity on motor impulsivity and impulsive choice

This thesis also describes our recent studies on the role of cannabinoid CB1 receptors in impulsivity. Previous studies have already implicated CB1 receptor activity in several amphetamine-induced behaviors, including locomotor activity, reward and motivation, and relapse to drug seeking (for review, see Wiskerke et al., 2008). The work described in Chapter 3 extended those findings by demonstrating that acute blockade of CB1 receptors prevents the opposite effects of amphetamine on motor impulsivity and impulsive choice. With respect to the cannabinoid system, however, one problem is the rather complex pharmacological profile of CB1 receptor agonists and antagonists. An illustration of this complexity are the differential effects of the CB1 receptor agonists Δ9-tetrahydrocannabinol (Δ9-THC) and WIN55,212-2 in the DRT, with only Δ9-THC decreasing impulsive choice (Chapter 3). Therefore, it is important that the effects of the CB1 receptor antagonist SR141716A on baseline and amphetamine-induced impulsivity could be replicated with the neutral CB1 receptor antagonist O-2050 (Chapter 3; Pattij et al., 2007a). In contrast to SR141716A, O-2050 is devoid of inverse agonistic effects at the CB1 receptor, i.e. does not affect constitutive CB1 receptor activity (Pertwee, 2005; Canals and Milligan, 2008; Wiley et al., 2010). These findings argue that impulsivity-related behavioral effects of CB1 receptor antagonists are not compound-specific and are due to blockade of eCB-induced CB1 receptor activation. Together, these data indicate that transient, drug-induced CB1 receptor activation reduces impulsive choice, while tonic endocannabinoid-induced CB1 receptor activity mediates motor impulsivity.

As it stands now, we can only speculate about the neurochemical mechanism(s) via which CB1 receptor activity can modulate (psychostimulant-induced) impulsivity. As stated before, baseline motor impulsivity and impulsive choice, as well as the opposite effects of amphetamine on impulsivity in the 5-CSRTT and DRT, are known to critically depend on dopamine signaling (Cole and Robbins, 1987, 1989; Winstanley et al., 2003, 2005; van Gaalen et al., 2006a,b, 2009; Pattij et al., 2007b). Nicotine-induced premature responding in the 5-CSRTT has also been demonstrated to be dopamine-dependent (van Gaalen et al., 2006). Moreover, there is a wealth of data on behaviorally relevant, reciprocal cannabinoid-dopamine interactions throughout the brain (El Khoury et al., 2012; Solinas et al., 2008; Tanda et al., 1997). In fact, using *in vivo* microdialysis, we recently demonstrated that

blockade of CB1 receptors by SR147176A (1-3 mg/kg) may abolish amphetamine-induced dopamine release (Kleijn et al., 2012). This SR141716A effect was found to be specific for dopamine over norepinephrine and 5-HT. Moreover, the effect appeared to be brain region-specific as it was observed in the NAc shell, but not in the NAc core or mPFC. Previously, it had already been found that SR141716A in a similar dose-range can also block nicotine-induced dopamine release in the NAc shell (Cohen et al., 2002). Since the acute effects of psychostimulant drugs on impulsive behavior in both the 5-CSRTT (amphetamine and nicotine) and DRT (amphetamine) could here be blocked by prior administration of a CB1 receptor antagonist, it is conceivable that the mechanisms underlying CB1 receptor modulation of both motor impulsivity and impulsive choice involve interactions with the dopaminergic system. However, assuming that cannabinoid-dopamine can regulate impulsivity, it was surprising that premature responding in the 5-CSRTT induced by a rise in subcortical dopamine levels (due to administration of the selective dopamine transporter GBR 12909) was not prevented by CB1 receptor blockade.

Overall, the behavioral effects of CB1 receptor antagonists in the 5-CSRTT would fit with two possible explanations. The eCB system either modulates motor impulsivity via non-dopaminergic mechanisms. This explanation is not unlikely in view of the widespread effects that CB1 receptor activity is known to have on the release of various other neurotransmitters, including that of norepinephrine, 5-HT, and glutamate (Schlicker and Kathmann, 2001; Egerton et al., 2006; Lopez-Moreno et al., 2008). Alternatively, assuming that there is an interaction with the dopamine system, this interaction may be restricted to specific forms of dopamine release (in terms of quantitative and temporal properties) (Patel et al., 2003; Kleijn et al., 2012), or the interaction may be confined to cortical brain regions (e.g. mPFC or orbital frontal cortex). In cortical brain regions, dopamine-induced activation of cannabinoid CB1 receptors may lead to a *net* decrease in local GABAergic signaling and a concomitant increase in activity of glutamatergic projection neurons (for review, see e.g. Egerton et al., 2006). In subcortical areas targeted by those neurons, including the ventral tegmental area and NAc, this may further augment impulsivity-related dopaminergic activity (Figure 6.1a,c).

6.1.3. Perspectives on the role of cannabinoids and opioids in impulsivity

This thesis work provides a rather complex picture of the conditions under which cannabinoid CB1 and μ-opioid receptors are involved in regulating impulsivity and the distinct roles of these receptors in motor impulsivity as well as impulsive choice (Table 6.1.). Our data do lend further support to the idea that impulsivity can be subdivided into behavioral constructs with largely distinct underlying neural mechanisms (Evenden, 1999; Moeller et al., 2001; Winstanley et al., 2006a; Pattij and Vanderschuren, 2008; Dalley et al., 2011; Broos et al., 2012). It should be noted here that we only studied two specific aspects of impulsive behavior. For future studies, it may be imperative to additionally examine

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the role of eCBs and opioids in other aspects of impulsivity, including response inhibition, which is an important aspect of impulsive action that we have not studied (e.g. Evenden, 1999; Pattij and Vanderschuren, 2008; Winstanley et al., 2010a).

Also, even though the 5-CSRTT and DRT are generally considered to be valid rodent models for motor impulsivity and impulsive choice, respectively, these paradigms are experimental models with certain limitations (see Chapter 1; Winstanley, 2011). As already mentioned in Chapter 1, one concern with specifically the 5-CSRTT are the paradoxal impulsivity-related effects of psychostimulant drugs such as amphetamine and methylphenidate. In line with their prescription as anti-impulsivity treatment, these drugs in humans generally decrease motor impulsivity in a continuous performance task (CPT) or Go-/NoGo-paradigm (Winstanley, 2011). In contrast, although opposite effects of low doses of methylphenidate were recently reported (Pattij et al., 2012), psychostimulants generally increase premature responding in the 5-CSRTT (Winstanley, 2011). This discrepancy may be attributed to differences in the experimental designs of the 5-CSRTT and CPT. For example, it may be relevant that stimulus presentations to which rats should respond in the 5-CSRTT generally occur at a predictable moment in time (at least with the commonly used fixed intertrial interval design), whereas humans can never predict the presentation of the target stimulus in the CPT (Hayton et al., 2012). A more important factor may be that most human studies involved experiments on cohorts of psychiatric patients, often young subjects diagnosed with ADHD, while the vast majority of rodent studies examined drug effects in adult, otherwise healthy, subjects. If anything, this psychostimulant-issue in a broader perspective highlights our poor understanding of the role of dopamine in impulsivity, and the possible contribution of individual differences in dopamine signaling to impulsivity. For humans as well as rodents, dopamine signaling (particularly at dopamine D2 receptors) has been shown to be different in high vs. low impulsive individuals (e.g. Dalley et al., 2007; Diergaarde et al., 2008; Buckholtz et al., 2010). Obviously, this may affect the behavioral effects that psychostimulants will have in both types of individuals. Indeed, recent studies have reported differential effects of several other dopaminergic compounds on motor impulsivity in high and low impulsive rats (Besson et al., 2010; Winstanley et al., 2010b; Fernando et al., 2012). Moreover, a study comparing two mouse strains that differ with respect to baseline 5-CSRTT impulsivity only found amphetamine- and GBR 12909-induced inhibitory control deficits in the low impulsive strain (Loos et al., 2010). It may thus be informative to further characterize the behavioral effects of amphetamine in high vs. low impulsive rats.

Finally, with regard to the clinical application of cannabinoid- and opioid-based pharmacotherapies in the treatment of impulse control problems, it may be important to focus on individual characteristics of patients (genotype, personality, psychiatric disorder) that may determine treatment outcome. For example, there is evidence that the impulsivity-suppressing effects of the preferential μ-opioid receptor antagonist naltrexone may be genotype- and/or personality-dependent (Mitchell et al., 2007; Boettiger et al., 2009; Love et al., 2009). Intriguingly, we found the role of cannabinoid CB1 and μ-opioid receptors in

impulsivity to be at least partly incongruent with that of dopamine and norepinephrine, the two neurotransmitters that are targeted by all current anti-impulsivity pharmacotherapies. Thus, further research on the role of cannabinoids and opioids in impulsivity may eventually result in the development of entirely novel classes of anti-impulsivity pharmacotherapies. Such an expansion of treatment options may aid the more personalized approach that, as we proposed in Chapter 1, is necessary for effective treatment of impulse control-related disorders.

6.2. Release of endocannabinoids during relapse to drug seeking

Rehabilitation of drug addicts is often hampered by their persistent vulnerability to relapse, particularly when exposed to drugs of abuse, drug-associated cues, or stressors (De Wit and Stewart, 1981; Shaham et al., 2003; Koob and Volkow, 2010). Understanding the neural mechanisms underlying this phenomenon is of importance for the development of effective pharmacological relapse prevention strategies. In this thesis, putative eCB-related mechanisms underlying relapse to opiate (heroin) and food (sucrose) seeking were studied.

The eCB system, and particularly cannabinoid CB1 receptor activity, is known to be critically involved in drug- and cue-induced reinstatement of reward (drug or nondrug) seeking (De Vries and Schoffelmeer, 2005; Fattore et al., 2007; Serrano and Parsons, 2011). Despite more than a decade of research following the initial discovery of this role of the eCB system (De Vries et al., 2001), many questions regarding the neurochemical mechanisms underlying the relapse-suppressing effects of CB1 receptor antagonists remained unanswered. For instance, it was unknown if there is a change in eCB release in the brain during a relapse to drug and/or food seeking. Gaining insight into which eCBs mediate relapse behavior is important for the future development of a cannabinoid-based therapy for drug addicts, because traditional CB1 receptor antagonists are nowadays considered to be largely unsuitable as pharmacotherapeutics due to undesired side-effects. Hence, more specific, eCB-targeting drugs will need to be developed and tested.

In Chapter 5, an abstinence-relapse model was employed to study the role of the eCB system in conditioned heroin and sucrose seeking in rats. Similar to what was reported for impulsive behavior (Chapter 3), the effects of the traditional CB1 receptor antagonist SR141716A on relapse behavior could be replicated with the neutral CB1 receptor antagonist (O-2050), thereby strongly arguing against any contribution of inverse agonistic properties of CB1 receptor antagonists in suppressing relapse. The role of fatty acid amide hydrolase (FAAH) activity in relapse to heroin and sucrose seeking was also examined. Since inhibition of FAAH by URB597 did not affect conditioned heroin or sucrose seeking,

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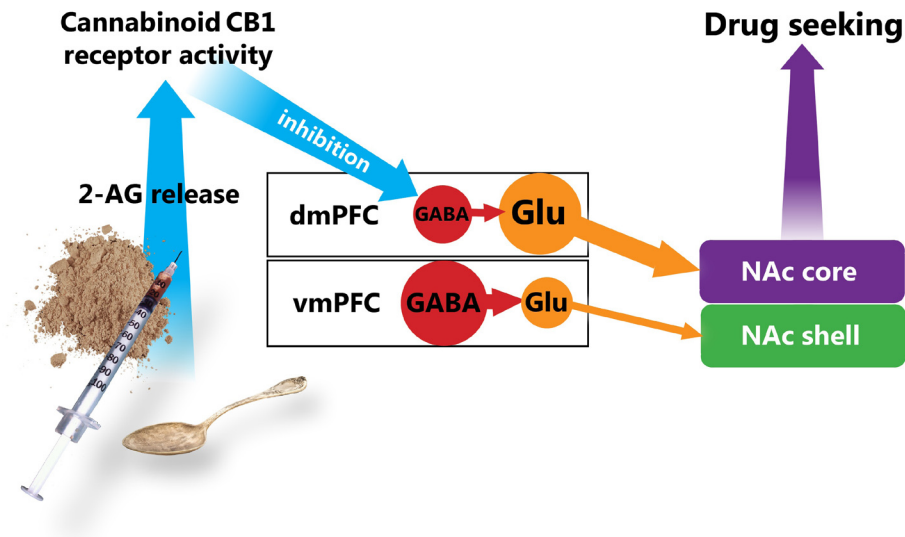
Figure 6.2. Proposed mechanism for 2-AG modulation of prefrontal-accumbal connectivity during drug seeking behavior. Simplified model showing how release of the endocannabinoid (eCB) 2-AG resulting from post-abstinence exposure to heroin-associated cues and/or context could drive drug seeking. This would involve *net* inhibition of local GABAergic activity and consequently an increase in glutamatergic (Glu) output from the dorsal medial prefrontal cortex (dmPFC) towards the nucleus accumbens (NAc) core. Simultaneously, neural activity in ventral mPFC (vmPFC) glutamatergic efferents to the NAc shell, normally functioning to inhibit behavioral responding, declines during relapse to drug seeking, most likely via an eCB-independent mechanism. Based in part on data from e.g. (McFarland and Kalivas, 2001; Pistis et al., 2001; Xi et al., 2006; Van den Oever et al., 2010). See section 2.1 for additional discussion.

this eCB catabolic enzyme may not play an important role during a relapse to drug or food seeking. The URB597-results were in line with the microdialysis data we subsequently obtained, demonstrating a robust release of the eCB 2-arachidonyl glycerol (2-AG) during relapse to heroin seeking, followed by a post-relapse decline in extracellular anandamide (AEA) and N-oleoylethanolamine (OEA) levels. Interestingly, these effects appeared to be largely restricted to the dmPFC. Moreover, no comparable patterns of eCB release were observed in sucrose-trained rats. From this, we hypothesize that aberrant 2-AG signaling in the dmPFC underlies the excessive motivational influence of drug-paired cues on behavior. Future studies should determine to what extent these data generalize to different (non)drug reinforcers and between different experimental paradigms (e.g. extinction vs. abstinence models, cue- vs. drug- vs. stress-induced relapse). Nevertheless, our data suggest that distinct eCB-related mechanisms may underlie conditioned drug and food seeking.

6.2.1. Prefrontal cortical 2-AG release: a targetable upstream-event in the induction of relapse to drug seeking?

Unfortunately, current pharmacological tools did not allow us to demonstrate any causality between mPFC eCB release and relapse to drug seeking. To that end, the development of more selective tools to manipulate eCB synthesis and hydrolysis in rats is required (Wiskerke et al., 2012). Based on the present data and available literature, however, we can speculate about the way eCB release may mediate relapse to drug (heroin) seeking (Figure 6.2). In rats, cue- and drug-induced reinstatement of drug seeking is known to be driven by increased activity of pyramidal neurons in the dmPFC, which will result in increased glutamate release in downstream brain regions such as the NAc core (McFarland and Kalivas, 2001; McLaughlin and See, 2003; LaLumiere and Kalivas, 2008). Neuronal activity in the vmPFC on the other hand is thought to be reduced during reinstatement of drug seeking, presumably resulting in impaired inhibition over behavioral responding (for reviews, see e.g. Jentsch and Taylor, 1999; Peters et al., 2009; Van den Oever et al., 2010). Accordingly, most human imaging studies have found enhanced activity in the PFC during cue-elicited drug craving, including heroin craving (Grant et al., 1996; Childress et al., 1999; Goldstein and Volkow, 2002; Langleben et al., 2008), while there also is a report on reduced activation of the vmPFC in addicts upon re-exposure to cocaine cues (Bonson et al., 2002). The microdialysis data in Chapter 5 fit well with these findings. The robust increase in dmPFC, but not vmPFC, 2-AG levels that was observed for heroin seeking may be expected to result in enhanced CB1 receptor activation in the dmPFC. As mentioned before in section 1.2 of this chapter, activation of mPFC cannabinoid CB1 receptors may overall result in a disinhibition of glutamatergic pyramidal output neurons (for review, see e.g. Egerton et al., 2006). Disinhibition of dmPFC pyramidal neurons will result in enhanced glutamate release in down-stream brain areas including the NAc core, an event that in turn may induce drug seeking. Indeed, it has previously been shown that systemic CB1 receptor blockade attenuates cocaine-induced increases in glutamate in the rat NAc core as well as cue- and cocaine-induced relapse behavior (De Vries et al., 2001; Xi et al., 2006). Finally, the fact that a robust increase in seeking-associated 2-AG release was observed in the mPFC of heroin- but not sucrose-trained rats is in accordance with the minor role of this brain region in conditioned food seeking as compared to drug seeking (McFarland and Kalivas, 2001; Schmidt et al., 2005; Koya et al., 2006; Van den Oever et al., 2008).

6.2.2. Perspectives on the role of endocannabinoids in relapse behavior

The previous paragraph discussed how the observed relapse-associated changes in mPFC eCB signaling may trigger known relapse-inducing glutamatergic events in the PFC-NAc pathway. However, there is compelling evidence that disturbances in PFC-NAc glutamate signaling already arise during periods of abstinence, hence, prior to a relapse (Kalivas, 2009; Van den Oever et al., 2010). Thus, a considerable portion of the research on

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relapse-preventing therapies is currently focusing on normalizing the disturbed PFC-NAc glutamate signaling that characterizes addicted individuals during periods of abstinence as well as during subsequent bouts of drug craving and/or relapse (Kalivas and Volkow, 2011). Although preliminary, the latter line of research appears promising. The present research may provide leads for additional non-glutamatergic drug targets that interfere with relapse-related events upstream in the PFC-NAc glutamatergic pathway. Additionally, it would be highly informative to gain more insight into putative (long-lasting) drug-induced adaptations in the eCB system that may impair its normal functioning during periods of protracted drug abstinence, thereby possibly contributing to a subject's propensity to relapse. For example, a recent study found changes in NAc AEA signaling and the levels and phosphorylation state of CB1 receptors in the NAc and basolateral amygdala of rats with a history of extended access to cocaine (Orio et al., 2009).

Drug addiction, and particularly relapse-vulnerability, has been linked to maladaptive display of impulsivity (Jentsch and Taylor, 1999; Perry and Carroll, 2008; Verdejo-Garcia et al., 2008; De Wit, 2009; Winstanley et al., 2010a; Dalley et al., 2011). Furthermore, we proposed that the CB1 receptors involved in (motor) impulsivity may be located in the PFC (Chapter 4), the same part of the brain in which relapse-associated eCB release was demonstrated in Chapter 5. Thus, it could be speculated that the same eCB-dependent mechanisms modulate impulsivity and relapse to drug seeking. On the other hand, the dmPFC, where the most robust relapse-associated eCB release was found, is generally thought to be less important in regulating inhibitory control as compared to the vmPFC (Winstanley et al., 2006a; Pattij and Vanderschuren, 2008; Dalley et al., 2011). Moreover, no study on the occurrence of within-animal correlations of pharmacological effects on impulsivity and relapse to drug seeking is as yet available (in fact, see Broos et al., 2012 for a lack of such a correlation).

6.3. Concluding remarks

This thesis revealed important distinctions between cannabinoid CB1 and μ-opioid receptors in the brain regarding their involvement in regulating two aspects of impulsive behavior, motor impulsivity and impulsive choice. Furthermore, we demonstrated that the mechanisms underlying drug-induced increases in premature responding in the 5-CSRTT (Cole and Robbins, 1987; Olmstead et al., 2009; Pattij et al., 2009; van Gaalen et al., 2006a) may not be identical for all drugs of abuse. Finally, we discovered that conditioned heroin seeking is associated with brain region- and reinforcer-specific patterns of eCB release in the brain. As such, the present work significantly contributed to our knowledge of the neuronal events that mediate impulsivity and drug addiction. Future studies are clearly warranted to further substantiate our findings. The development of adequate positron emission topography (PET)-ligands for imaging of CB1 receptors and μ-opioid receptors in humans and rodents, as well as the ongoing rapid improvements in pharmacological

tools to more selectively manipulate eCB synthesis and hydrolysis will certainly aid such future research. Similarly, the recent development of techniques such as optogenetics – the use of light to precisely control activity in specified neural populations in the brain of a behaving animal (Yizhar et al., 2011) – will likely be important. This thesis may guide future experiments as it identified relevant test conditions as well as brain regions for studying the role of cannabinoids and opioids in impulsivity and relapse to *drug*

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seeking