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# IMAGING OF THE BRAIN OPIOID SYSTEM IN AMPHETAMINE DEPENDENCE

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Front cover:  $^{11}\text{C}$ -carfentanil PET images, coronal slices at the level of the striatum.

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# Imaging of the brain opioid system in amphetamine dependence

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*Nichts ist unergründlicher, als das System von Triebfedern unserer Handlungen.*

G.C. Lichtenberg



## ABSTRACT

Amphetamine dependence is a global health problem, often giving rise to severe medical and social complications in affected individuals. Unfortunately, there is still limited evidence for any specific treatment that would help amphetamine dependent patients to avoid relapse. One of the most promising treatments is the opioid antagonist naltrexone, which has been shown to attenuate the subjective effects of amphetamine and in some randomized clinical trials also reduce the risk of relapse. The aim of this thesis work was to investigate the mechanism of action of naltrexone for amphetamine dependence, in order to better understand the neurobiology involved and facilitate further treatment development. We used the neuroimaging techniques positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) to study these processes in the human brain.

In Study I, we tested the hypothesis that an amphetamine injection causes a release of endogenous opioids in the brain, which might explain why an opioid antagonist such as naltrexone attenuates the subjective effects of amphetamine. However, using PET and the  $\mu$  opioid radioligand  $^{11}\text{C}$ -carfentanil, we found no evidence of such an amphetamine-induced opioid release in healthy human subjects without any previous experience of amphetamine.

Study II investigated whether naltrexone pre-treatment affects the dopamine release that occurs in the brain after amphetamine intake, an effect that some previous studies have found to correlate with the subjective effects of amphetamine. If naltrexone were to attenuate this dopamine release, it might help to explain why it affects the subjective effects of amphetamine. In a first experiment, we used PET and the radioligand  $^{11}\text{C}$ -raclopride, but found no evidence that naltrexone affected amphetamine-induced dopamine release in healthy, previously amphetamine-naïve human subjects. We proceeded with experiments using *in vivo* microdialysis in rats, where similar results were found: pre-treatment with naltrexone did not affect the dopamine release caused by an acute amphetamine dose in rats without previous exposure to amphetamine. However, in rats that had been treated with amphetamine for a longer time period, naltrexone did attenuate the dopamine release when amphetamine was reinstated, suggesting that the brain opioid system might be involved in the adaptations to chronic amphetamine exposure.

In Study III, we investigated the effects of naltrexone on cue reactivity, i.e. the reaction of substance dependent patients to environmental stimuli reminding them of drug use. This process is interesting as it can be an important trigger of relapse. For this study, we included 40 men with severe, intravenous amphetamine dependence, who received one oral dose of naltrexone or placebo and then underwent an fMRI examination including exposure to drug-related and neutral film clips. The hypothesis was that the drug-related films would cause a subjective craving reaction and increase the activity of a number of motivationally relevant brain regions, and that naltrexone would attenuate this reactivity. We found that the films did cause strong craving and wide-spread fMRI activations, but there was no evidence of any effect of naltrexone on these measures.

Study IV investigated the proposed phenomenon of subliminal cue reactivity, where the brains of substance dependent patients have been reported to react specifically to drug-related pictures, even when the pictures are presented very fast and with a backward mask, so that they never reach conscious awareness. In our study, which used the same patient sample as Study III and 30 healthy controls, we found no evidence of any subliminal drug cue reactivity. Upon closer examination of the earlier studies, we found that the reliability of their statistical inferences could be questioned, which together with our negative results suggest that there is no strong evidence for subliminal cue reactivity in addiction.

In summary, the studies of this thesis have not corroborated the hypotheses we started out with regarding the mechanisms behind naltrexone's effects in amphetamine dependence. Instead, the results have inspired new hypotheses, for example regarding how the interplay between the brain dopamine and opioid systems may change with long-term amphetamine use. These studies have also highlighted methodological challenges that may help to improve future neuroimaging studies of addiction.



## LIST OF SCIENTIFIC PAPERS

- I. **Guterstam J**, Jayaram-Lindström N, Cervenka S, Frost JJ, Farde L, Halldin C, Franck J: Effects of amphetamine on the human brain opioid system - a positron emission tomography study. *International Journal of Neuropsychopharmacology*. 2013 May;16(4):763-9
- II. Jayaram-Lindström N, **Guterstam J**, Häggkvist J, Eriksson M, Malmlöf T, Schilström B, Halldin C, Cervenka S, Saijo T, Nordström AL, Franck J: Naltrexone modulates dopamine release following chronic, but not acute amphetamine administration – a translational study. *Translational Psychiatry*. 2017
- III. **Guterstam J**, Jayaram-Lindström N, Berrebi J, Petrovic P, Ingvar M, Fransson P, Franck J: Cue reactivity and opioid blockade in amphetamine dependence: a randomized, controlled fMRI study. (Manuscript)
- IV. **Guterstam J**, Jayaram-Lindström N, Berrebi J, Petrovic P, Ingvar M, Fransson P, Franck J: Subliminal cue reactivity in amphetamine dependence. (Manuscript)

## LIST OF ADDITIONAL PAPERS CITED IN THE TEXT

Bramness JG, Gundersen ØH, **Guterstam J**, Borger Karlsen E, Konstenius M, Løberg EM, Medhus S, Tanum L, Franck J: Amphetamine-induced psychosis - a separate diagnostic entity or primary psychosis triggered in vulnerable individuals? *BMC Psychiatry*. 2012. Dec 12(1): 221

Konstenius M, Jayaram-Lindström N, **Guterstam J**, Beck O, Philips B, Franck J: Methylphenidate for ADHD and drug relapse in criminal offenders with substance dependence: a 24-week randomized placebo-controlled trial. *Addiction*. 2014 Mar;109(3):440-9

Khemiri L, Steensland P, **Guterstam J**, Carlsson A, Franck J, Jayaram-Lindström N: The effects of the monoamine stabilizer (-)-OSU6162 on craving in alcohol dependent individuals: a human laboratory study. *European Neuropsychopharmacology* 2015 Dec;25(12):2240-2251

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## LIST OF ABBREVIATIONS

AC-PC	anterior commissure-posterior commissure
ADHD	attention-deficit hyperactivity disorder
ANOVA	analysis of variance
BOLD	blood oxygen level dependent
BP <sub>ND</sub>	non-displaceable binding potential
DA	dopamine
DSM	diagnostic and statistical manual of mental disorders
FD	framewise displacement
fMRI	functional magnetic resonance imaging
FWE	family-wise error
ICD	international statistical classification of diseases and related health problems
MR	magnetic resonance
OFC	orbitofrontal cortex
PET	positron emission tomography
PFC	prefrontal cortex
ROI	region of interest
VAS	visual analog scale
VS	ventral striatum
VTA	ventral tegmental area



# 1 INTRODUCTION

This thesis is based on four studies, which will be discussed alongside each other throughout the text. First, we will look at the background to the research project as a whole, including the diagnosis, epidemiology and pathophysiology of amphetamine dependence. Special attention is paid to previous research on the pathophysiologic role of the opioid system and the use of the opioid antagonist naltrexone as a treatment for amphetamine dependence. I will also introduce the main neuroimaging approaches currently used in the study of substance use disorders.

The third chapter articulates the scientific aims of the thesis. Chapter four describes the methods used in the four studies, in particular the study designs and imaging technologies employed. After that, the main findings of each study are described in chapter five.

In the sixth chapter, the results are discussed in relation to the current state of the research field. Specifically, I will discuss the role of the opioid system in two different parts of the so-called cycle of addiction: the acute effects of amphetamine intake and the longer-term consequences of craving and relapse.

The discussion is summed up in a chapter with conclusions and future perspectives, followed by acknowledgements and references. The four individual manuscripts on which the thesis is based are provided as attachments.



## 2 BACKGROUND

### 2.1 AMPHETAMINE DEPENDENCE: HISTORY, EPIDEMIOLOGY AND CLINICAL PICTURE

Amphetamine was first synthesized in Berlin in the end of the 19<sup>th</sup> century, but was never put to any medical use until it was rediscovered by Gordon Alles in the United States in 1927 (1). In the 1930s and 40s, amphetamine became one of the best selling drugs in the world, marketed for the treatment of asthma, obesity and depression but also used for conditions like headache, alcoholism and general fatigue (2,3). Especially during the Second World War, amphetamine and the closely related compound methamphetamine were widely used as powerful stimulants that enabled soldiers to stay awake for long periods of time.

After the war, when amphetamine use became even more widespread in the general population, the adverse consequences of the drug became apparent. In particular, the risk of addiction to amphetamines was recognized. During the 1950s and 60s, the role of amphetamine changed from being a legitimate pharmaceutical product to becoming one of the dominating “recreational” drugs of the emerging youth culture. From over-the-counter sales it became strictly regulated and eventually banned internationally by the United Nations in the 1970s (4).

In Sweden and the other Nordic countries, amphetamine came to be the dominating drug among injecting drug users, a pattern that is still apparent today. The latest major survey of illegal drug use in Sweden was conducted in 1998 and concluded that approximately 26 000 people were “heavy drug users” and 73% of them used amphetamine regularly, although many of them also used cannabis or other drugs (5). Several minor surveys have been conducted since then and most indicators suggest that there has been a rise in the number of heavy drug users during the recent years. For instance, the amount of amphetamine seized by Swedish police and customs has been increasing steadily for decades; however, such indicators can be hard to interpret since they are affected by changes in laws, resources and priorities in the involved authorities (6).

In the 2017 “World Drug Report” from the United Nations Office on Drugs and Crime, the number of people that have used amphetamine in the last year is estimated to 35 million, equivalent to a global prevalence of 0.8% in the population aged 15-64 (7). However, many of these users do not suffer from amphetamine dependence: although the “World Drug Report” offers no specific estimate, earlier epidemiological surveys in the United States have found that about 20% of stimulant users eventually go on to develop addiction (8,9). This proportion is likely to exhibit significant regional variation, depending on a number of different factors such as the socio-cultural context of the drug use and the typical route of administration, which is intravenous in the Nordic countries as opposed to oral in many other parts of the world.

Long-term use of amphetamine may cause a number of medical problems. Because of its vasoconstrictive and sympathomimetic properties, with strong elevations of heart rate and

blood pressure, amphetamine use increases the risk of cardiovascular disease and death (10). Amphetamine may cause xerostomia and bruxism, which along with poor oral hygiene contributes to the high prevalence of dental problems among amphetamine users (11). If the drug is injected, there is an increased risk of infections such as endocarditis, HIV and viral hepatitis; indeed, a majority of injection drug users in Sweden are infected with hepatitis C virus and users of amphetamine show particularly high levels of injection risk behavior (12,13). Amphetamine users often exhibit neurological symptoms such as choreiform movements and ataxia, and in some cases these symptoms remain even after many years of abstinence (14). A number of less common medical complications, such as rhabdomyolysis, hepatotoxicity and seizures, have also been described in the literature (15). The severe social problems associated with amphetamine use often complicate the management of these medical conditions.

Within psychiatry, the most dramatic complication is amphetamine-induced psychosis, an acute psychotic state often characterized by delusions of persecution, hallucinations and agitation (16). Typically, amphetamine-induced psychotic symptoms remit within a couple of days of abstinence. Whether such episodes increase the risk of chronic psychotic syndromes such as schizophrenia is unclear, although a recent register study from Finland found that as much as 30% of the patients diagnosed with amphetamine-induced psychosis received the diagnosis of schizophrenia within eight years of follow-up (17). Interestingly, psychotic reactions to methamphetamine have been described in the Japanese medical literature for decades, and in these studies about 10-30% of the patients continue to experience psychotic symptoms even after more than one month of abstinence (18). Importantly, neither the Finnish nor the Japanese studies on this topic have taken genetic and other possible confounders into account, and it is possible that some of these patients would have developed long-term psychotic syndromes even if they had never used amphetamine.

In contrast to the dramatic symptoms that might be seen after amphetamine intake and intoxication, the amphetamine withdrawal syndrome is typically not very severe, with mild to moderate dysphoria and excessive sleep for a couple of days after a longer period of amphetamine intake.

A common psychiatric co-morbidity that has gained a lot of attention in recent years is attention-deficit hyperactivity disorder (ADHD). By definition, ADHD is a neurodevelopmental disorder that is present from childhood and therefore cannot be considered a complication of amphetamine use, which typically starts in teenagers or young adults (19). ADHD is a strong risk factor for substance use disorders and the prevalence rate among patients seeking treatment for addiction is estimated to be 20-25% (20). It has long been known that ADHD can be symptomatically treated with stimulants, and recent studies have shown that this can be the case even in many patients with stimulant use disorders if the dose is high enough (21,22). Of course, the risks of medication diversion and misuse also have to be taken into account when prescribing stimulants.

As mentioned above, amphetamine addiction was recognized as a growing problem in the



1960s and “stimulant dependence” (with amphetamine as the prototypical substance) was included as a disorder in the American Psychiatric Associations Diagnostic and Statistical Manual for Mental Disorders-III (DSM-III) in 1980, applying the same criteria as for other substance dependence syndromes (e.g. alcohol and opioid dependence) (23). The revised manual DSM-III-R of 1987 made a distinction between the compulsive drug use of substance dependence and the supposedly less severe condition of substance abuse, a residual category for patients that did not meet criteria for dependence but still exhibited social complications and risky drug intake. This distinction was preserved in the DSM-IV but abandoned in the DSM-5, the currently used version that was released in 2013 (19). The so-called stimulant use disorder of DSM-5 combines the earlier diagnostic criteria for both stimulant dependence and abuse, with the exception of the removal of a legal complications criterion and the addition of craving as a new criterion. The following, abbreviated DSM-5 list of diagnostic criteria for stimulant use disorder also gives an idea of the problems amphetamine dependent patients may present with:

1. The stimulant is often taken in larger amounts or over a longer period than was intended.
2. Persistent desire or unsuccessful efforts to cut down.
3. A great deal of time is spent in order to obtain, use and recover from use of the stimulant.
4. Craving, or a strong desire or urge to use the stimulant.
5. Recurrent stimulant use resulting in a failure to fulfill major role obligations at work, school, or home.
6. Continued use despite persistent or recurrent social problems caused or exacerbated by the use.
7. Important activities are given up or reduced because of stimulant use.
8. Recurrent stimulant use in situations in which it is physically hazardous.
9. Stimulant use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the stimulant.
10. Tolerance, i.e. a need for markedly increased amounts of the stimulant to achieve the desired effect.
11. Withdrawal.

A new feature of DSM-5 is the grading of severity based on the number of diagnostic criteria fulfilled, such that mild = presence of 2-3 symptoms, moderate = 4-5 symptoms, and severe = 6 or more symptoms.

In parallel to the DSM, the World Health Organization has its own International Classification of Diseases (ICD), currently in its tenth edition (24). Their categorization of substance use disorders is quite similar to the DSM-IV, with two diagnostic categories (‘dependence’ and ‘harmful use’) for each drug. Notably, the ICD-10 lists craving as a symptom of substance dependence, which was not included in the DSM before its current,

fifth edition. At present, it is still unclear how ICD-11, due to be released in 2018, will classify these disorders.

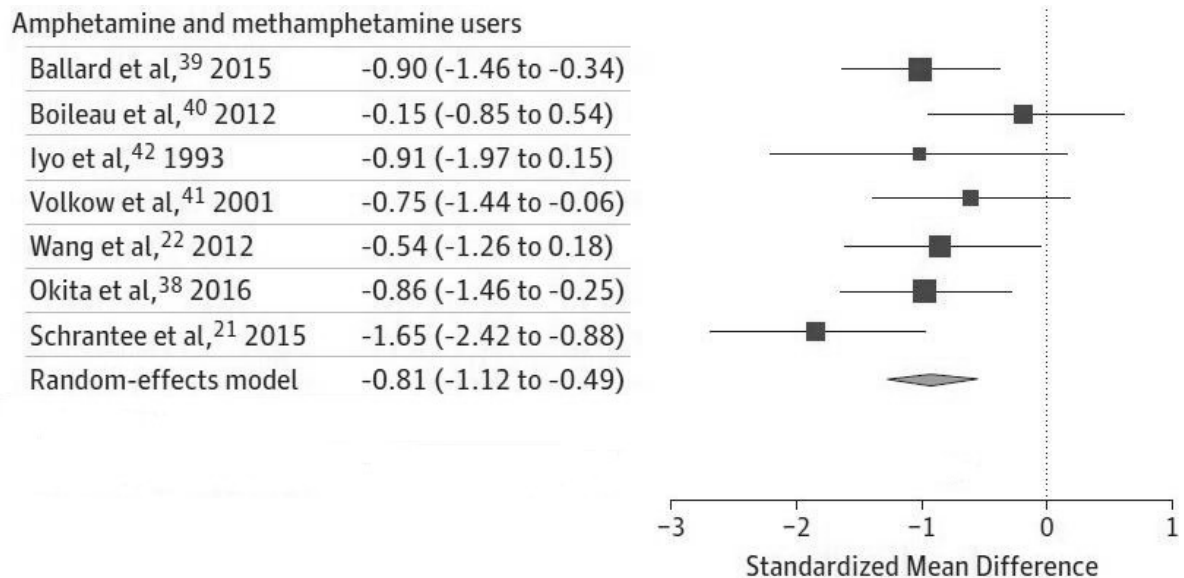
The patients included in Study III of this thesis were all diagnosed with amphetamine dependence according to DSM-IV. Since we only recruited patients with long histories of intravenous amphetamine use, they would all qualify for a diagnosis of severe amphetamine use disorder according to DSM-5. In this thesis, the terms amphetamine dependence and addiction will be used as synonyms, along with the somewhat wider DSM-5 category of amphetamine use disorder.

## **2.2 PATHOPHYSIOLOGY OF AMPHETAMINE DEPENDENCE**

Amphetamine is often considered the prototypical stimulant drug because of its strongly activating physiological and psychological effects. It typically reduces the urge to sleep and eat food and causes sympathomimetic effects such as mydriasis and heightening of heart rate and blood pressure. Amphetamine may also cause a strong euphoria, in particular when it is administered intravenously, as is common among Swedish users (25–27).

Early research on the brain monoamine systems revealed that amphetamine stimulated dopamine (DA) release (28). These effects, particularly the strong elevation of extracellular DA levels in the ventral striatum, were quantified in rats using *in vivo* microdialysis in the 1980s (29) and in humans using <sup>11</sup>C-raclopride positron emission tomography (PET) in the 1990s (30,31). The magnitude of amphetamine-induced mesolimbic DA release can be several times higher than that produced by “natural” rewards such as sex (32). While some early studies found a correlation between DA release and subjective euphoria (25), other studies have indicated a relationship to drug wanting rather than euphoria or liking (33,34).

A number of case-control PET studies, particularly from the laboratory of Nora Volkow, have found that amphetamine dependent patients, as compared to healthy controls, have low densities of striatal DA D<sub>2</sub> receptors and blunted DA responses to stimulants (35–37)(Figure 1). In some studies, such abnormalities in the DA system have been associated with increased risk of relapse, although the studies are small and the conclusions therefore highly tentative (36). There is some evidence for the involvement of DA in stimulant craving reactions: in two PET studies with cocaine dependent patients, drug cues activated mesolimbic and mesocortical DA systems (38,39).



**Figure 1. Meta-analysis of molecular imaging studies with abstinent amphetamine or methamphetamine users, showing decreased dopamine receptor availability in the striatum, as compared with healthy controls. Adapted from Ashok et al. JAMA Psychiatry 2017**

There is also a huge body of preclinical research on the role of DA in reward and the formation of habits. An influential theory developed by Kent Berridge is that DA encodes “incentive salience”: in other words, that DA release is not necessary for us to like a particular stimulus, but is essential for causing us to want that stimulus again (40). Of course, we often want what we like and vice versa, and these two components of reward might be hard to differentiate clinically, although they may be quite distinct at the neurochemical level.

Based on these findings, the DA system has been the primary focus of many neurobiological theories of amphetamine addiction, and indeed of addictive disorders in general, although the evidence is weaker for the involvement of DA in for instance opioid or cannabis use disorders (41). Disappointingly, no primarily dopaminergic medications have been found effective for the treatment of substance use disorders. This could be related to the fact that medications that raise DA levels may themselves be addictive or trigger relapse, while DA receptor antagonists such as antipsychotic medications often have severe side effects and worsen the low DA activity seen in at least some categories of substance users (42). Theoretically, DA partial agonists or stabilizers could avoid these problems and be more therapeutically useful, but further clinical studies of such compounds are needed (43).

However, the mesolimbic DA system is not the single mediator of amphetamines effects. Surprisingly, repeated laboratory studies have shown that pretreatment with DA D<sub>2</sub> antagonists, even in high doses that effectively block most striatal DA receptors, does not attenuate the subjective effects of amphetamine, such as drug liking and wanting more of the drug (44,45). This suggests that amphetamine also affects other brain signaling systems, and one of these is the endogenous opioid system.

### 2.3 THE BRAIN OPIOID SYSTEM

The brain opioid system comprises four different types of G-protein coupled receptors:  $\mu$ ,  $\delta$ ,

$\kappa$ , and the more recently discovered NOP receptor. Corresponding to these are four different genes coding for propeptides that are processed into endogenous opioid ligands with agonist properties at their receptor: proopiomelanocortin ( $\beta$ -endorphine), proenkephalin (met-enkephalin, leu-enkephalin), prodynorphin (dynorphin), and prepronociceptin (nociceptin). These ligands are not completely selective, but have differential affinities for each receptor type (46). Opioid receptors are inhibitory, attenuating the excitability of the neuron when activated.

The  $\mu$  receptor was the first opioid receptor to be characterized in the early 1970s and was named after its first known ligand, morphine (47–49). It has received great scientific attention because of its importance in pain regulation and addiction, and  $\mu$  receptor agonists such as morphine are very useful in health care as powerful analgetics (50). While there is only one known gene coding for the  $\mu$  receptor, there are several versions of the receptor since alternative splicing gives rise to different intracellular C terminals, which impacts the second messenger effects of receptor activation. The distribution of different splicing variants of the  $\mu$  receptor might therefore be one explanation for individual variability in the pharmacological response to opioids (51). A single nucleotide polymorphism (rs1799971, A118G) in the first exon of the  $\mu$  opioid receptor gene is also a source of functional variation, since it strongly increases the affinity of  $\beta$ -endorphine to the receptor (52). Interestingly, this genetic variant has also been associated with better therapeutic outcomes when treating alcohol dependence with the opioid antagonist naltrexone (53).

$\mu$  opioid receptors can be found in the intestines, in peripheral nerves and in all parts of the central nervous system (except the occipital lobes of the brain), with a particularly high density in the striatum, thalamus, and brain stem.

The brain opioid system is phylogenetically old, probably dating back around 450 million years, and has been evolutionary well preserved so that the same types of opioid receptors and ligands can be found in all vertebrates (54). This indicates that the system has an important fitness value. It also means that it is possible for experiments involving the opioid system in rodents to have translational validity, although there are several challenges in translating such research, especially on the behavioral level, to the human condition (55).

A number of scientific methods have been used to investigate the brain opioid system, particularly in animal models. A radical method is to use knockout animals (most often mice), where a particular gene has been inactivated and the resulting physiological effects are studied. In  $\mu$  opioid receptor knockout mice, self-administration of opioids is blocked and several addictive behaviors are attenuated also for other substances such as alcohol and nicotine (56). However, only few studies have looked at the responses of these animals to stimulant drugs, and the results are inconclusive (56). A problem with knockout studies is that an animal born without a particular gene might compensate for this by some other mechanism, thereby clouding the physiological role of the gene in a normal animal.

Another way to study neurotransmitter systems is microdialysis, an invasive technique where a probe with a semipermeable membrane is inserted into the tissue of interest. The probe is

perfused with a fluid, allowing for molecules to pass over the membrane by diffusion and the contents of the resulting dialysate can then be analyzed. This technique may be used to measure the concentration of neurotransmitters, such as endogenous opioids, in rodents *in vivo*. One problem is that the dialysis probe needs to be carefully inserted into a specific anatomical spot in the brain, which entails a risk of misplacement and also means that neurotransmitter changes in the rest of the brain are not measured. The temporal resolution is also somewhat limited, since there needs to be a time interval between every analytic sampling, but microdialysis is still a very valuable tool when evaluating for instance neurotransmitter release in a specific region in response to different stimuli. In Study II of this thesis, microdialysis is used to analyze amphetamine-induced DA release in the nucleus accumbens after pre-treatment with naltrexone or vehicle.

So far, the technique that allows for the most direct study of the opioid system in living humans is PET with specific radioligands (see also section 2.6).

## **2.4 THE ROLE OF THE OPIOID SYSTEM IN AMPHETAMINE DEPENDENCE**

Since the opioid system has long been known to be involved in addictive behaviors, a number of studies have investigated its possible role in the pathophysiology of amphetamine dependence.

One study using microdialysis in rats found that amphetamine injections gave rise to  $\beta$ -endorphin release in the nucleus accumbens (57). The aim of Study I of this thesis was to investigate if that finding could be translated to humans.

Looking instead at possible long-term consequences of drug use, a series of PET studies from the US National Institute of Drug Abuse has found that abstinent cocaine dependent patients as compared to healthy controls have elevated binding of the  $\mu$  opioid receptor radioligand  $^{11}\text{C}$ -carfentanil, which can be interpreted as an increased density of opioid receptors and/or lower concentrations of endogenous opioids (58). This elevated binding is seen both in subcortical structures and in the frontal lobes, and its magnitude is positively correlated with craving, increased risk of relapse and worse treatment outcome (59–61). Results in the same direction have been obtained in some neurochemical studies of rodents exposed to cocaine, but the pathophysiological mechanism behind this phenomenon remains unclear (62,63). Unfortunately, there have not yet been any studies of how amphetamine exposure influences brain opioid receptor levels.

A more indirect way of studying the opioid system is through the use of drugs like naloxone and naltrexone, which both work as antagonists at the three classic opioid receptors  $\mu$ ,  $\delta$ , and  $\kappa$ . Early research on rodents found that pretreatment with naloxone attenuated the locomotor effects of amphetamine (64). In a study from the 1990s using several behavioral paradigms and microdialysis in rats, naloxone attenuated behavioral effects and the DA-release caused by amphetamine (65). More recent studies have shown differential effects on a number of amphetamine-induced behaviors: for example, locomotor sensitization was attenuated (66), but conditioned place preference was unaffected by naltrexone pre-treatment (67).

A consistent finding from a number of human laboratory studies is that naltrexone pre-treatment attenuates the subjective effects of amphetamine, both in healthy individuals and in amphetamine dependent patients (68–71)(Figure 2). In these studies, naltrexone weakened the subjective ratings of several amphetamine effects (e.g. euphoria, liking of the drug, wanting more, etc.), but did not affect its sympathomimetic properties, such as elevation of blood pressure and pulse. These findings were also replicated in Study II of this thesis. Since naltrexone does not alter the pharmacokinetics of amphetamine, this effect must be explained at a pharmacodynamic level, which was the rationale for Study I and II.

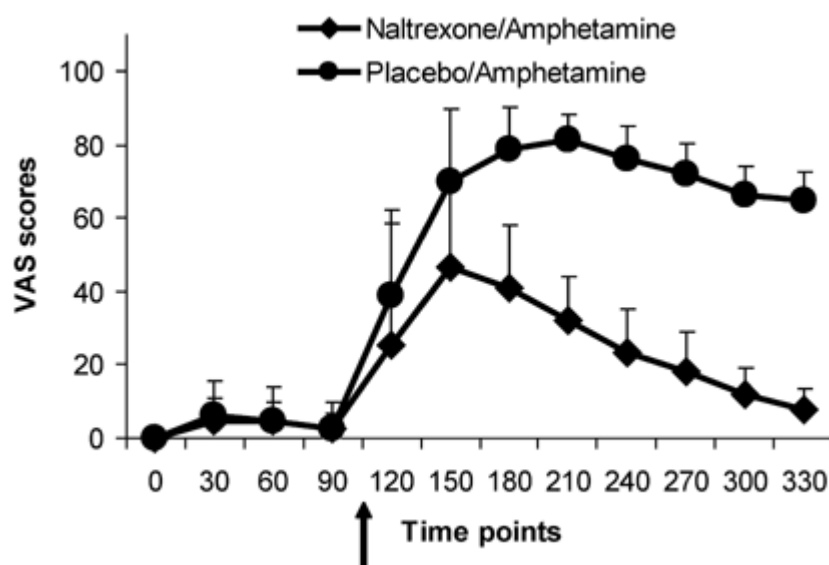


Figure 2: Effect of naltrexone pre-treatment on subjective high from an oral dose of amphetamine (30 mg), given at the time point indicated by the arrow, in amphetamine dependent patients. Naltrexone significantly reduced the subjective effects of amphetamine in this sample, a finding that has subsequently been replicated a number of times. From Jayaram-Lindström et al. *Neuropsychopharmacology* 2008.

## 2.5 NALTREXONE AS A TREATMENT FOR AMPHETAMINE DEPENDENCE

Naltrexone has been approved by the United States Food and Drug Administration for the treatment of alcohol dependence since 1992, when two placebo-controlled, randomized clinical trials were published that found naltrexone to reduce the risk of relapse in alcohol dependent patients (72,73). Since then, more than 50 clinical trials have been published on naltrexone for alcohol dependence and there is strong evidence for its efficacy and safety, with relatively mild side effects (74). More recently, naltrexone has been approved in the US for the treatment of opioid dependence, as an alternative to the more established agonist maintenance treatments for this condition. In particular, an extended-release injectable formulation of naltrexone has been used successfully in the treatment of some cases of opioid dependence (75,76), although this formulation is not yet available for clinical use in the European Union.

As mentioned above, a number of laboratory studies have found that naltrexone attenuates the subjective effects of amphetamine, both in healthy controls and in addicted patients. Some clinical trials have also investigated if it reduces the risk of relapse in amphetamine

dependence. In the first clinical trial, 80 amphetamine dependent patients were randomized to naltrexone or placebo and followed for 12 weeks (77). The results showed that naltrexone treated patients were less likely to relapse and also experienced less craving (Figure 3).

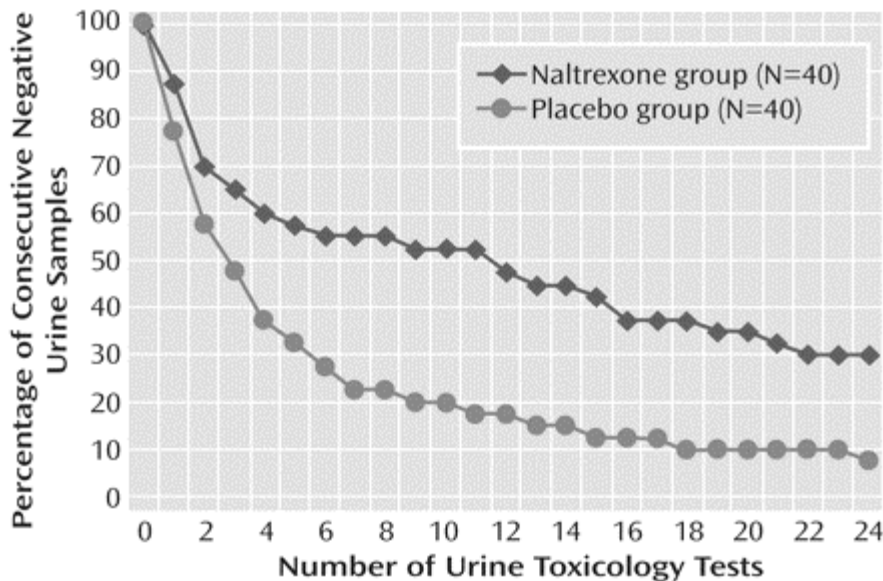


Figure 3: Survival analysis, showing the percentage of amphetamine dependent patients with consecutive negative urine samples when treated with oral naltrexone or placebo. From Jayaram-Lindström et al. *Am J Psychiatry* 2008.

Similar results were obtained in a 2010 trial, although this finding was less clear since the study was underpowered and investigated the effects of naltrexone and N-acetylcysteine combined versus placebo (78). A third study included patients with concurrent amphetamine- and heroin dependence and found that long-acting naltrexone improved retention in treatment and increased the proportion of drug-free urine samples as compared to placebo (79). In a more recent trial of naltrexone depot vs placebo for amphetamine dependence, there was very little amphetamine use regardless of treatment, and no advantage of naltrexone could be found (80). Another study of naltrexone depot for methamphetamine dependent patients did not find any reduction in methamphetamine use in the naltrexone group, as compared to placebo (81).

In summary, there is strong evidence that naltrexone reduces the subjective effects of amphetamine and some evidence that it might reduce the risk of relapse in amphetamine dependence. While the trial-based evidence is mixed, it remains one of the most promising candidate drugs for the treatment of amphetamine addiction and further clinical trials are currently ongoing (82,83). At the same time, experimental research is targeting the mechanisms behind the effects of naltrexone, how the opioid system is involved in amphetamine dependence and in what ways pharmacologic manipulations of this system could have therapeutic potential, perhaps even beyond that of the non-selective opioid receptor antagonism of naltrexone. This thesis tackles some of these questions with the use of two different neuroimaging methods, which I will now introduce.

## 2.6 NEUROIMAGING IN ADDICTION RESEARCH

Detailed structural imaging of the living human brain has been possible since the introduction of computerized tomography in the early 1970s. *Functional* neuroimaging is an even younger research field, but has grown rapidly since the first studies of the early 1980s. In the works of this thesis, we make use of PET and functional magnetic resonance imaging (fMRI). These are also the two functional imaging techniques that have been most widely used in addiction research so far.

As mentioned earlier, PET is an imaging modality that, among other things, allows for neurochemical mapping and quantification of neurotransmitter release in the living human brain. This is made possible by the use of radioligands that are injected into the subject, pass the blood-brain-barrier and bind to targets of interest. The radioligand emits positrons, which collide with electrons in the tissue and give rise to photons detectable by a PET system. This data can then be used to calculate the binding of the radioligand in different parts of the brain, using kinetic models specific for each radioligand. For both of the radioligands used in this thesis,  $^{11}\text{C}$ -carfentanil in Study I and  $^{11}\text{C}$ -raclopride in Study II, a reference-tissue model can be used, where the radioligand density in the regions of interest (ROIs) are compared with a reference region with no (or very few) receptors of the type studied. Using such reference-tissue models, we can calculate the non-displaceable binding potential ( $\text{BP}_{\text{ND}}$ ), which represents the ratio of specifically bound to that of non-displaceable radioligand (84).

For most ligands, the extent of specific binding depends not only on the density of receptors in the ROI, but also the levels of endogenous neurotransmitters that compete for the receptor binding sites. This is important to keep in mind when interpreting PET studies, particularly with case-control designs, where for instance cases with lower receptor density and reduced neurotransmitter levels may display similar  $\text{BP}_{\text{ND}}$  values as healthy controls, since these changes affect the binding in opposite directions. This ambiguity is less of a problem with experimental designs, such as Study I and II of this thesis, where each participant serves as his or her own control. Receptor densities typically change very slowly within the same individual, as compared to changes in neurotransmitter concentrations that occur on a timescale of seconds to minutes. Therefore, a lower  $\text{BP}_{\text{ND}}$  value in an experimental condition compared to baseline can often be interpreted as evidence that neurotransmitters have been released, occupying the relevant receptor sites so that the radioligand does not bind to the same extent. However, in some cases, differential internalization of receptors may constitute a remaining confounder working at a similar timescale as neurotransmitter release, and it is therefore of great value if PET findings can be validated with other techniques.

PET has been of great importance in addiction research, not least in the study of the neurochemical effects of drugs of abuse, which up until the 1980s could not be studied in the living human brain. As mentioned above (Section 2.2), the mesolimbic DA system has received particular attention, but a steadily growing number of radioligands has continued to expand the possibilities of PET, which can now be used to study a large number of targets in the brain (85). As far as the opioid system is concerned, many different radioligands have



been proposed but only two of them have been more widely used and validated:  $^{11}\text{C}$ -carfentanil and  $^{11}\text{C}$ -diprenorphine (86).  $^{11}\text{C}$ -carfentanil is a selective  $\mu$  receptor ligand, while  $^{11}\text{C}$ -diprenorphine has a similar affinity to all three classic opioid receptors. Since  $^{11}\text{C}$ -carfentanil is more suitable for measuring endogenous opioid release in vivo, this was the ligand we chose in Study I to investigate the effects of an amphetamine injection on the brain opioid system (87).

While PET can be very useful as a research tool, obstacles to its wider use include the potentially harmful health effects of ionizing radiation and the high costs associated with radioligand production. Because of this, many PET studies have very few participants and low statistical power. While the spatial resolution is steadily improving with new generations of PET systems, the temporal resolution is still relatively poor and a single measurement typically takes about an hour.

Another imaging method that has been increasingly used since the 1990s is fMRI. While structural magnetic resonance imaging had already been in use for several years, the principles of BOLD (blood oxygen level-dependent) contrast were discovered in 1990 (88,89). Using a strong magnet and a number of so-called radiofrequency coils, it was shown that an MR system can detect changes in the ratio of oxygenated to deoxygenated hemoglobin, since they have differing magnetic properties. A local increase in neural activity and metabolism increases oxygen extraction from the blood, raising the levels of deoxygenated hemoglobin. Because of the so called neurovascular reflex, neural activations are followed by increased local blood flow within a few seconds, effectively washing out the deoxygenated hemoglobin and instead raising the levels of oxygenated hemoglobin. This response can be detected with an MR system as an increased BOLD signal, which therefore is interpreted as an indirect measure of neural activity (90).

The cellular mechanisms behind the neurovascular reflex are still being investigated and debated (91,92). The most important contributor to the changes in cerebral blood flow relevant for fMRI seems to be glutamatergic synaptic activity, but several other neurotransmitter systems and also different cell types may be involved. This means that the relationship between neural activity and vascular response can vary between different brain regions, but also between different individuals and different developmental stages (90). Our understanding of this variability is still limited and it is therefore hard to draw detailed conclusions about underlying neural activity based on BOLD responses alone.

fMRI has several important advantages: it does not require any ligand or contrast injections and does not use ionizing radiation, which means that the same research subject can be studied on many occasions or for an extended period of time. Compared to PET, fMRI is cheaper and has superior temporal and spatial resolution. The ability to study the activity of the whole brain over time also allows for comparisons of the patterns of spontaneous signal change in different brain regions, which of course is not feasible with single-cell recordings or techniques with inferior resolution. This has led to one of the major findings so far in the fMRI field, namely the characterization of networks of functional connectivity, consisting of

brain regions with highly correlated spontaneous fluctuations in BOLD signal strength (93). Such networks are often studied with resting state fMRI, which does not mean that the brain is resting (its total metabolic demands actually change very little depending on what the person is doing), but only that the subject is at rest in the camera during data acquisition, not involved in any particular task.

In addiction research, fMRI has often been used to study the neural correlates of different neuropsychological functions, such as impulsivity (94). It has also been important in the study of craving, since it allows the researcher to repeatedly expose the subject to, for instance, drug-related stimuli, and detect the neural reactivity to such cues (95). A paradigm of this kind was used in Study III and a modified version of it also in Study IV.

A problem with fMRI is the complex relation of the BOLD signal to neural activity, as discussed above, which means that the results can be hard to interpret in neurophysiological terms. In contrast to PET, fMRI cannot map specific neurochemical systems or detect neurotransmitter release, which means that it cannot answer some of the critical questions in neuropsychopharmacology. There are also problems that arise from the huge amounts of data generated by fMRI, in particular the statistical problem of multiple comparisons that often result in underpowered studies, considering the small sample sizes typically used (96). The rapid development of different analytic methods without proper validation, combined with weak traditions of study pre-registration and data-sharing also contribute to current problems of inconsistency and bias in the fMRI literature (97–99).

Finally, a more practical problem is the sensitivity of fMRI to artifacts, particularly head movements during image acquisition, which may cause so much noise that the signal of interest is lost (100). When preprocessing the data, movement artefacts can be corrected for up to a certain degree, but if the participant has moved a lot, the data may not be interpretable at all.

## **2.7 CRAVING AND CUE REACTIVITY IN STIMULANT DEPENDENCE**

Intense craving for drugs has long been recognized as a common symptom in addictive disorders. As noted above, it serves as a diagnostic criterion for addiction in both DSM-5 and ICD-10, and is a central concept in almost all theoretical accounts of these disorders (101,102). Craving is also often the focus of clinical attention, both in psychological and pharmacological treatment (103). Despite all this, the concept of craving has been notoriously hard to define and measure in a valid and reliable way (104). In the year 2000, the prominent craving researcher Stephen Tiffany paraphrased the consensus statements from international meetings held on craving by the World Health Organization and others in the following way:

“Although we do not know what craving is and we can establish no consensus about the best way to measure it or manipulate it, we certainly believe that more research should be conducted on this possibly, but not necessarily, important construct.” (104)

While craving is still hard to define and measure, Tiffany and others have helped to advance

the field and craving has been the focus of intense research during the years since 2000, with growing use of neuroimaging technology to elucidate the biological mechanisms of craving. Because of the difficulties in obtaining a valid and reliable subjective measure of craving, a goal of these approaches is to develop a biomarker that would correlate with addiction severity and the risk of subsequent relapse (105). Such a biomarker could be useful both in the clinic to improve diagnostic accuracy and in research as a surrogate outcome when assessing new therapies. However, much research remains before these goals are fulfilled (105).

Three principal factors eliciting craving in addicted subjects has been identified: stress (106), conditioned cues (102) and priming doses of the addictive drug (107). In study III, amphetamine dependent patients were exposed to drug-related cues and we will now briefly look at previous research using similar paradigms of cue-induced craving.

Many studies have used fMRI to investigate the neural mechanisms of drug cue reactivity (108). Since most studies have been done in the United States, where cocaine is the dominating stimulant drug, only a few studies have included amphetamine users, and then mainly patients using methamphetamine through smoking, oral or intranasal administration rather than injecting amphetamine as is common in Sweden (109–111). The three studies published so far on methamphetamine users have all employed still pictures of drug taking or paraphernalia and contrasted this with exposure to neutral pictures. It is worth noting that two of these three studies did not have a DSM diagnosis of amphetamine dependence as an inclusion criterion and the cases represent mild or moderate addiction severity.

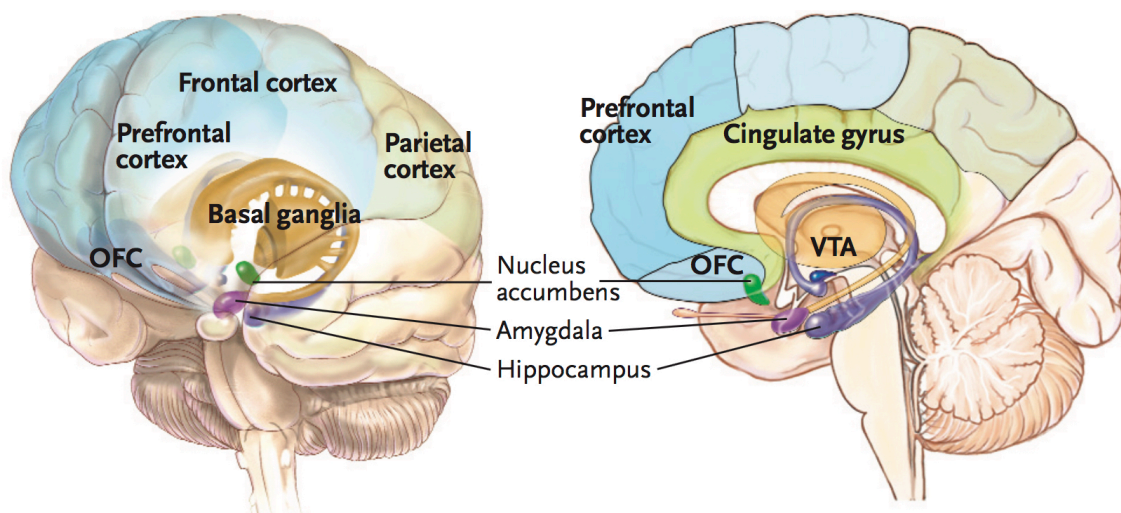


Figure 4. Schematic picture of some of the neuroanatomical regions involved in cue reactivity in addiction. OFC = orbitofrontal cortex, VTA = ventral tegmental area. Adapted from Fowler et al. 2007 (112).

Imaging studies of cue-induced stimulant craving (i.e. amphetamine, methamphetamine or cocaine) typically show activations of the nucleus accumbens/ventral striatum (VS), prefrontal cortex (PFC), amygdala and cingulate gyrus (see Figure 4)(95). However, the

results are quite heterogeneous, with activations of different brain regions reported in different studies. Several issues might contribute to explain this, not least the fact that early studies often were underpowered, used suboptimal study designs and inadequate statistical procedures for obtaining their results (113–115). However, all the variability is not caused by noise, since reviews of the literature have identified important patterns in the results. An interesting finding is that participants not seeking treatment for their addiction seem to react differently from participants who are active treatment-seekers (116). The former group is more likely to show activations of the PFC, a part of the brain involved in executive functions. It has been suggested that this could reflect the intention of the participants to seek out and take drugs after having been exposed to drug-related cues, while treatment-seeking individuals may not have such intentions (116).

A more recent attempt at a quantitative meta-analysis of the field also concluded that prefrontal activations, particularly of the orbitofrontal cortex (OFC), differed between treatment seekers and non-treatment seekers (95). The authors also discuss alternative interpretations of this result, for example that treatment seekers might have diminished frontal functions as part of the adverse effects of drug intake that led them to seek treatment in the first place. To decide between these different alternative explanations, they need to be tested empirically, preferably with experimental manipulation of variables like expectancies or self-control (95). So far, few studies have coupled their neuroimaging results to longitudinal data on the subsequent course of the participants disease (e.g. risk of relapse), which would be needed in order to establish cue reactivity as a valid biomarker for addiction (105).

Some studies have investigated the effects of pharmacological treatments on cue reactivity. Of particular interest in this context are two fMRI studies of alcohol dependent patients which both found that naltrexone, as compared to placebo, attenuated cue-induced BOLD activations of prefrontal areas, and in one of the studies also the striatum (117,118). Also, one of the studies of methamphetamine users mentioned above also tested the effects of naltrexone on cue reactivity; while they did not find any effect on subjective measures of cue-induced craving, naltrexone pre-treatment attenuated cue-induced activations of primary sensory and motor areas (110). The latter finding is somewhat surprising and of unclear significance, since these areas are not typically implicated in drug cue processing (95). In other words, the effects of naltrexone on cue-induced craving and neural reactivity in amphetamine dependence are still quite unclear.

## **2.8 SUBLIMINAL CUE REACTIVITY**

As mentioned above, exposure to drug-related conditioned cues is a common cause of craving, but patients quite often report that strong feelings of craving “just came over me” for no apparent reason. An intriguing hypothesis is that such reactions can be elicited by cues that are so subtle that they are only perceived subliminally, i.e. without reaching conscious awareness (119).

Subliminal processing of visual stimuli has been studied with several different methods, one of them being backward visual masking. In this paradigm, a very brief visual stimulus (the

target) is immediately followed by another visual stimulus (the mask), which disturbs the perception of the target stimulus so that it does not reach conscious awareness (120). Within emotional psychology, visual masking has been of particular importance in studies of fear, where the amygdala has been shown to play a central role in a subcortical network that is able to detect fear-relevant stimuli and elicit fear responses in a fast and nonconscious manner (121). This system has probably been of great evolutionary importance as a quick way of detecting and reacting to the presence of predators, without the need for more elaborate, cortical processing of warning signs in the environment.

There are many studies on subliminal fear processes, but a number of studies have also investigated positive motivational systems, not least in addictive disorders. In recent years, visual masking has been employed in some fMRI studies of substance dependent patients. In 2008, a pilot study of cocaine patients reported activation of several limbic structures when cocaine patients were exposed to masked visual cocaine cues (119). In 2014, the same research group published two more studies: one suggesting that the GABA<sub>B</sub> receptor agonist baclofen could block this subliminal cue reactivity (122) and another where cannabis patients were found to have similar levels of subliminal cue reactivity as the cocaine patients (123). So far, the only published attempt at replicating these results in another laboratory is a study of nicotine dependent patients. In the latter study, the right amygdala showed a decreased BOLD signal in response to masked smoking-related images, a reaction opposite to that expected (124). Prior to Study IV, there were no published studies on subliminal cue reactivity in amphetamine dependent patients.

### **3 AIMS**

The aim of this thesis is to investigate the role of the opioid system in amphetamine dependence and thereby elucidating the therapeutic mechanism of action of the opioid antagonist naltrexone. Specifically, the four studies aimed to test the following hypotheses:

Study I: That an amphetamine injection gives rise to an endogenous opioid release in healthy human subjects.

Study II: That naltrexone attenuates amphetamine-induced striatal DA release in rodents and healthy human subjects.

Study III: That naltrexone attenuates craving and neural cue-reactivity in amphetamine dependent patients.

Study IV: That subliminal drug cues as compared to neutral cues activate motivationally relevant brain structures in amphetamine dependent patients, but not in healthy controls, and that naltrexone attenuates this reaction.

## 4 METHODS

In the studies included in this thesis, we made use of the neuroimaging methods PET and fMRI, which have already been introduced above. In Study II, we also employed in vivo microdialysis to measure DA release in rats. In this chapter, I will present in more detail the methods used in each of the studies, and also discuss ethical issues relevant to the studies. All studies were approved by the Stockholm Ethics Review Board and the Swedish Medical Products Agency, and each subject provided written informed consent before any study specific procedures were made. Since Study I and II used ionizing radiation, they were also approved by the Radiation Safety Committee of the Karolinska University Hospital Solna.

### 4.1 STUDY I: EFFECTS OF AMPHETAMINE ON THE ENDOGENOUS OPIOID SYSTEM: A <sup>11</sup>C-CARFENTANIL PET STUDY

In Study I, we investigated if amphetamine activates the brain opioid system. As mentioned above, the opioid antagonist naltrexone has been shown to attenuate the effects of amphetamine, and a possible explanation for this could be that amphetamine activates the opioid system, an activation that of course would be inhibited by an opioid antagonist. Some rodent studies have suggested such a mechanism (57) and we aimed to test the hypothesis that an intravenous dose of amphetamine would cause an endogenous opioid release in the brain of healthy human subjects.

*Subjects:* Ten healthy men, aged 23-31, were recruited via flyers and by word-of-mouth at Karolinska Institutet. Before inclusion in the study, all participants went through a screening procedure. Exclusion criteria included (1) DSM-IV diagnosis of major Axis-1 psychiatric disorder including any history of substance use disorder (including nicotine), (2) use of a psychoactive substance within the past 30 days, (3) history of serious medical conditions, (4) positive result on alcohol breath analyzer at the test sessions, (5) traces of opiates, cannabis, amphetamines or benzodiazepines in the urine at screening or during test days. The subjects also underwent a structural MR scan to exclude intracranial pathology and obtain anatomical references for definition of ROIs to be used when analyzing the subsequent PET examinations (see below). The participants were paid an equivalent of €500 for their participation.

*Methods:* Study I was designed as a cross-over randomized controlled trial, where each participant went through three PET examinations with <sup>11</sup>C-carfentanil: at baseline, after an injection of amphetamine (0.3 mg/kg body weight) and after an injection with placebo. The order of amphetamine and placebo was randomized and double-blind, to avoid confounding with expectation effects. The examinations for each subject were done approximately one week apart, although three of the examinations were delayed for technical reasons and instead performed after 20-40 days.

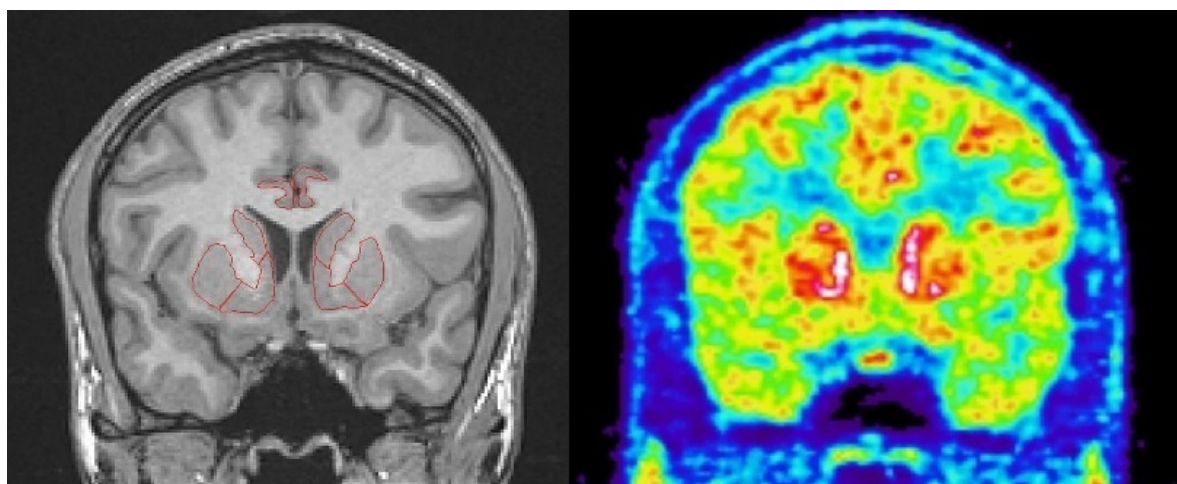
To study endogenous opioid release, we used PET with <sup>11</sup>C-carfentanil, a radioligand specific for  $\mu$  opioid receptors (125). This ligand has been used extensively since the 1980s and has been shown to have excellent test-retest reliability, which makes it well suited for studies

with repeated measures, such as cross-over experiments (126). There is also a validated method for estimating its binding with a simplified reference-tissue model, which means that there is no need for arterial blood sampling to calculate its binding potential (127). In several earlier studies,  $^{11}\text{C}$ -carfentanil has been used successfully to detect changes in endogenous opioid release in response to behavioral or pharmacological challenges (126,128–130).

All PET examinations were performed using a high-resolution research tomograph (Siemens Molecular Imaging, Germany). For each subject, an individual helmet was made and attached to a holder on the couch to minimize head movement. After a six-minute transmission scan using a single  $^{137}\text{Cs}$  source, the study drug (amphetamine/placebo) was injected into an intravenous catheter and flushed with saline. Two minutes later,  $^{11}\text{C}$ -carfentanil was administered as a rapid bolus and flushed with saline. List-mode data were acquired for 69 min, starting at the time of ligand injection.

*Data analysis:* PET images were reconstructed from a series of 16 time frames, including modelling of the point spread function, after correction for attenuation, randoms and scatter. This reconstruction procedure yields a spatial resolution of 1.5 mm (131). PET images were corrected for head movement using frame-by-frame realignment (132) using the first frame as reference.

When analyzing the data, ROIs were delineated on the MR images of each individual subject using the Human Brain Atlas software (133)(Figure 5). Ventral striatum was chosen as the primary ROI, while a secondary analysis included other brain regions involved in drug abuse and reward, i.e. associative and sensorimotor striatum, prefrontal cortex (divided into orbitofrontal, dorsolateral and medial), anterior cingulate cortex, hippocampus and amygdala. The definitions of ROIs were based on previously published guidelines (134–138).



**Figure 5:** T1-weighted magnetic resonance image, coronal slice through the striatum, with regions of interest delineated in red (left), and  $^{11}\text{C}$ -carfentanil positron emission tomography image, corresponding slice (right). The high levels of  $^{11}\text{C}$ -carfentanil binding in the striatum, particularly in its ventral parts, correspond to the high density of  $\mu$  opioid receptors in this region. From Guterstam et al. *Int J Neuropsychopharm* 2013.

PET images were then co-registered to the MR image using SPM 5. Correction for partial volume effects was done with the method described by Meltzer et al. (139). Quantitative analysis was performed using the simplified reference tissue model with the occipital lobe as



reference region, an approach that has previously been validated for  $^{11}\text{C}$ -carfentanil (126,127).  $\text{BP}_{\text{ND}}$  was the parameter of interest, representing the ratio at equilibrium of specifically bound to that of non-displaceable radioligand (84).  $\text{BP}_{\text{ND}}$  measures from the baseline, placebo and amphetamine conditions were analyzed with repeated measures analysis of variance (ANOVA) for effect of treatment.

## **4.2 STUDY II: EFFECTS OF NALTREXONE ON AMPHETAMINE-INDUCED DOPAMINE RELEASE**

In study II, we aimed to explore the effects of naltrexone pre-treatment on amphetamine-induced DA release in the striatum. Since naltrexone weakens the subjective effects of amphetamine, and some of these effects have been correlated with striatal DA release, our hypothesis was that naltrexone as compared to placebo would attenuate the DA-release caused by amphetamine.

In order to study DA release in humans, we used PET and the DA  $\text{D}_2$  receptor radioligand  $^{11}\text{C}$ -raclopride. The sensitivity of this radioligand to stimulant-induced changes in brain DA concentration is well-established (140,141). Since DA release might be sensitive to expectations of amphetamine (142), we included a placebo condition in the study. For ethical reasons, only a limited strength and number of doses of amphetamine may be given to human subjects in an experimental setting. Therefore, we used a rat model to study both the acute and the more long-term effects of amphetamine, using in vivo microdialysis to analyze brain DA levels.

### **4.2.1 $^{11}\text{C}$ -raclopride PET**

A cross-over randomized, placebo-controlled, double-blind design was used to test the hypothesis that pre-treatment with naltrexone would attenuate the brain DA release induced by amphetamine.

*Subjects:* Seven healthy males aged 20-45 years were recruited via flyers posted at Karolinska Institutet. The sample size was based on previous studies demonstrating significant effects of amphetamine on  $^{11}\text{C}$ -raclopride binding in healthy controls (25,33,142), as well as earlier work from our own group on naltrexone and amphetamine (68,69). Exclusion criteria were similar to those in Study I and included (1) DSM-IV diagnosis of major Axis-I psychiatric disorder including any history of substance use disorder (including nicotine), (2) use of a psychoactive substance within the past 30 days, (3) history of serious medical conditions, (4) positive result on alcohol breath analyzer at the test sessions, (5) traces of opiates, cannabis, amphetamines or benzodiazepines in the urine at screening or during test days. All participants provided written informed consent and were paid an equivalent of €500 for their participation.

*Methods:* Prior to the PET measurements, all subjects underwent a structural MR scan (1.5 T) to exclude intracranial pathology and obtain anatomical references for definition of ROIs. In total, each subject underwent three PET examinations with  $^{11}\text{C}$ -raclopride, approximately one week apart: at baseline, after placebo plus amphetamine administration, and after naltrexone

plus amphetamine administration (denoted here as baseline, placebo+amphetamine, and naltrexone+amphetamine, respectively). The order of the two latter examinations was randomized.

On test days, subjects arrived at the laboratory at 8:00 am and received a standardized breakfast. Subjective and physiological measures were evaluated throughout the experimental procedure. At 9:00 am, the participants received either a capsule of naltrexone (50 mg) or placebo. One hour post ingestion of study medication, they underwent a PET examination with  $^{11}\text{C}$ -raclopride, using the ECAT HR 47 (CTI/Siemens, Knoxville, TN) PET system run in 3D mode. Prior to each emission scan, a transmission scan was performed for attenuation correction. The subjects received an intravenous dose of amphetamine (0.3 mg/kg bodyweight), immediately followed by a saline solution of  $^{11}\text{C}$ -raclopride (223–268 MBq, specific radioactivity 193–1131 GBq/ $\mu\text{mol}$ ) injected as a bolus. The cannula was then flushed with 10 ml saline. Immediately following  $^{11}\text{C}$ -raclopride administration, PET emission data was obtained for 51 min (143). To minimize movement artifacts, an individual plastic helmet was made for all participants and used together with a head fixation system. The reconstructed data were displayed as 47 horizontal sections with a center-to-center distance of 3.125 mm.

A visual analog rating scale (VAS) was administered to describe the subjective drug effects. The VAS comprised four scales: ‘feel the drug’, ‘like the effect’, ‘feel aroused’ and ‘want more’, providing a composite measure of subjective effects. The subjects rated their experiences starting at the time of naltrexone/placebo administration and continuing at designated time points. To measure the physiological effects of amphetamine, heart rate and blood pressure were recorded manually at the same time points as the subjective measures.

*Data analysis:* ROIs were manually delineated on individual structural MR images, based on previously published guidelines (137,144) in which the striatum is divided into limbic, associative and sensorimotor subregions based on their differential connectivity (145). The same ROIs were used for all three examinations and all ROIs were combined to create a ROI for the whole striatum. The MR images were reoriented to the AC-PC plane and then used for co-registration to PET images using SPM 2. The average values of right and left ROIs were used to increase the signal-to-noise ratio for the quantification. A ROI for cerebellum was drawn below the appearance of the petrosal bone in five slices, corresponding to a thickness of 10 mm. ROIs were applied to the PET images using the co-registration parameters to extract regional time activity curves.  $^{11}\text{C}$ -raclopride  $\text{BP}_{\text{ND}}$  was calculated using the simplified reference tissue model (146) with cerebellum as a reference region.

Statistical evaluation of  $\text{BP}_{\text{ND}}$  data for each ROI was conducted using two-way repeated-measures ANOVA. Three comparisons of binding potential values were estimated by the ANOVA: (1) baseline vs. amphetamine, (2) baseline vs. naltrexone+amphetamine, (3) placebo+amphetamine vs. naltrexone+amphetamine. Condition by region interactions in the ANOVA were investigated further with post hoc t-tests. All statistical tests were two-tailed with a significance level conventionally set at  $p < 0.05$ . The secondary outcome of subjective

measures was defined as the mean score of the four VAS items for the various time points during each test day, comparing the naltrexone+amphetamine and placebo+amphetamine conditions. A group composite score was calculated as an aggregate of the mean scores for each time point. This score was compared between the two conditions with repeated measures ANOVA.

#### **4.2.2 Microdialysis**

We used in vivo microdialysis to investigate the effects of naltrexone on amphetamine-induced DA release in freely moving rats. First, two different acute amphetamine doses were tested. In a second experiment, we investigated the effects of amphetamine reinstatement, i.e. a challenge dose of amphetamine after a period of chronic treatment followed by abstinence.

*Methods:* Male Wistar rats (250-380 g) were housed four per cage in a temperature ( $\pm 21$  °C) and humidity ( $\pm 40$ -50%) controlled environment on a 12 h light/dark cycle (lights on 7:00 AM). Food and water were available ad libitum. All experiments were conducted during the light phase of the cycle. Animals were handled in accordance with the guidelines of the Swedish National Board of Laboratory Animals and the study was approved by the Ethical Review Board of Stockholm (acute experiment) or Gothenburg (chronic experiment), Sweden.

Dexamphetamine sulphate and naltrexone were dissolved in physiological saline (sodium chloride 0.9% (w/v)). All drugs were administered intraperitoneally (i.p.), and injected at a volume of 1 or 2 ml per kilogram of body weight.

For the surgical procedure, rats were anaesthetized with a mix of fentanyl citrate (0.39 mg/kg) and fluanisone (12.5 mg/kg) and midazolam (6.25 mg/kg) diluted in distilled water (1:1:2; 5 ml/kg i.p.) or by isoflurane and mounted in a stereotaxic frame. Dialysis probes were implanted in the nucleus accumbens with stereotaxic coordinates anteriorposterior: +1.6 mm: mediolateral - 1.4 mm: dorsoventral -8.2 mm relative to bregma and the dural surface, in accordance with an anatomical atlas (147). After surgery, animals were individually housed and allowed 2 days of recovery before initiation of the experiment.

The microdialysis experiments were conducted approximately 48 h after surgery. Dialysis occurred through a semi-permeable membrane with an active surface length of 2-2.25 mm. The dialysis probe was perfused with a physiological solution at a rate of 2 or 2.5  $\mu$ l/min set by a micro infusion pump. Dialysate was collected over 15 min intervals (37.5  $\mu$ l) in the two acute dialysis studies and over 20 min intervals (40  $\mu$ l) in the chronic dialysis study, after which the samples were injected into a high-performance liquid chromatography system. On-line quantification of DA in the dialysate was accomplished by electrochemical detection. After baseline measurements in the acute dialysis studies, rats were treated with either naltrexone (3 mg/kg i.p) or saline (1 ml/kg i.p) 30 minutes before given an amphetamine (0.5 or 2 mg/kg i.p) or saline injection (1 ml/kg i.p). In the chronic dialysis study, rats were conditioned to amphetamine using a protocol which induces robust locomotor sensitization to amphetamine (66). Briefly, rats received daily injections of either saline or amphetamine (2

mg/kg) for ten consecutive days after which the animals were left untreated for another ten days. Surgery was performed eight days into the drug free period. In the following microdialysis experiment, the rats received an injection with naltrexone or vehicle, followed 40 min later by a saline injection for the previously saline treated rats and amphetamine (0.5 mg/kg i.p.) for the previously amphetamine treated rats. Dialysate was collected for 180 min after the last drug administration.

*Data analysis:* DA levels were expressed and statistically analyzed as percent of baseline levels. Baseline was defined as the average of the four dialysate samples collected immediately before the first injection. The mean percent changes from baseline were then calculated for each 15/20 min sample for all rats in each group. Data were analyzed by one- or two-way ANOVA followed by planned comparison using the STATISTICA software.

### **4.3 STUDY III: EFFECTS OF NALTREXONE ON CUE REACTIVITY IN AMPHETAMINE DEPENDENCE: AN FMRI STUDY**

This study was designed as a randomized, double-blind clinical trial with two parallel groups of amphetamine dependent patients. Each patient received one oral dose of naltrexone (50 mg) or placebo and then went through an fMRI examination, investigating the effect of naltrexone on drug cue reactivity and craving. The study was preregistered in the EU Clinical Trials Register (EudraCT 2010-021384-33) and performed according to ICH guidelines for Good Clinical Practice, with external monitoring by the Karolinska Trial Alliance.

*Subjects:* 40 male, non-treatment-seeking amphetamine users aged 20-65 were recruited via advertisement and word of mouth at the needle exchange program and in shelters in the Stockholm region. All participants were screened by a study physician, including psychiatric assessment with the Structured Clinical Interview for DSM-IV, Axis 1 (SCID-1), and detailed assessment of substance use history and other variables with the Addiction Severity Index. Inclusion criteria included DSM-IV diagnosis of amphetamine dependence since at least two years, history of intravenous amphetamine use, amphetamine use for minimum of 12 times in the last 12 weeks, and having been drug free 1-30 days (minimum 24 hours). Exclusion criteria were other ongoing substance dependence (except nicotine), schizophrenia or bipolar disorder I, left-handedness, clinical signs of amphetamine intoxication at the day of testing, traces of cannabis, opiates, cocaine or benzodiazepines in the urine at the day of testing, traces of alcohol as measured by breathalyser at the day of testing or presence of severe somatic disorder (e.g. renal or hepatic failure). Patients with contraindications to MRI (e.g. cardiac pacemaker, severe claustrophobia) or the study medication (e.g. regular opioid use or known hypersensitivity) were also excluded. All patients were compensated for their participation with food coupons to the value of €150.

*Study procedures:* After a first screening visit, eligible patients were scheduled for a test day. Having arrived in the clinic on the test day, the patients were asked for a urine test to exclude current use of drugs other than amphetamine. If the result was negative and a breathalyzer test showed no trace of alcohol, the patient was included in the study and randomized to a capsule of 50 mg naltrexone or an identical capsule with placebo. After an interval of at least

60 minutes, the MRI procedures were started.

The experiment described here consisted of the patients seeing film clips depicting drug related scenes (i.e. people preparing and taking drugs) or neutral scenes (e.g. old people drinking coffee or chatting). Nine film clips of each type, lasting 16 seconds each, were shown in a pseudo-randomized order. After every clip, the participants were asked to rate with a trackball their level of amphetamine craving on a visual analog scale on the screen, with 0 in one end representing no craving at all and 100 in the other end representing the maximum level of craving imaginable. In total, the experiment lasted for 7 minutes.

After the examination, the patient was debriefed, asked about lingering craving and possible adverse events. All patients were asked if they were interested in clinical follow-up at the Stockholm Centre for Dependence Disorders.

*Magnetic resonance imaging:* MRI examinations were performed with a 3 T instrument (GE MR750 Discovery) at the Karolinska MR Research Center. Each subject went through a total of four fMRI paradigms and structural imaging, lasting for about 50 minutes in total. The experiment described here as Study III was the last to be performed before the structural imaging and was preceded by a resting state examination and two different paradigms with still pictures.

*Data analysis:* Data was analyzed with SPM 12. Using a standard preprocessing approach, the functional images were realigned, co-registered to the participant's structural image, segmented and smoothed with a 7 mm FWHM Gaussian kernel. The images were then normalized to an MNI template. Since movement artefacts represent a major problem in fMRI, particularly for patient populations, we decided to set a limit for the framewise displacement (FD), such that no more than 25% of the volumes were allowed to have a FD >0.3.

The functional data were analyzed as a block design using the general linear model and random field theory as implemented in SPM 12. In the first-level analysis, we defined separate regressors for the intervals that represented the drug and neutral movies, and defined linear contrasts in the general linear model to test our hypotheses. The resulting contrast images from all patients were entered into a random effects group analysis to accommodate intersubject variability and to compare the naltrexone vs. placebo groups.

For the statistical inference, we applied corrections for multiple comparisons within the appropriate search space using Family-Wise Error (FWE) correction with level of significance set at  $p < 0.05$ . We pre-specified ROIs based on the prior literature on cue reactivity in stimulant dependence (95). In these regions, which included the VTA, striatum, anterior cingulate cortex, OFC and medial prefrontal cortex, we corrected for multiple comparisons within small volumes. For areas outside our ROIs, we corrected for multiple comparisons using the whole brain as search space.

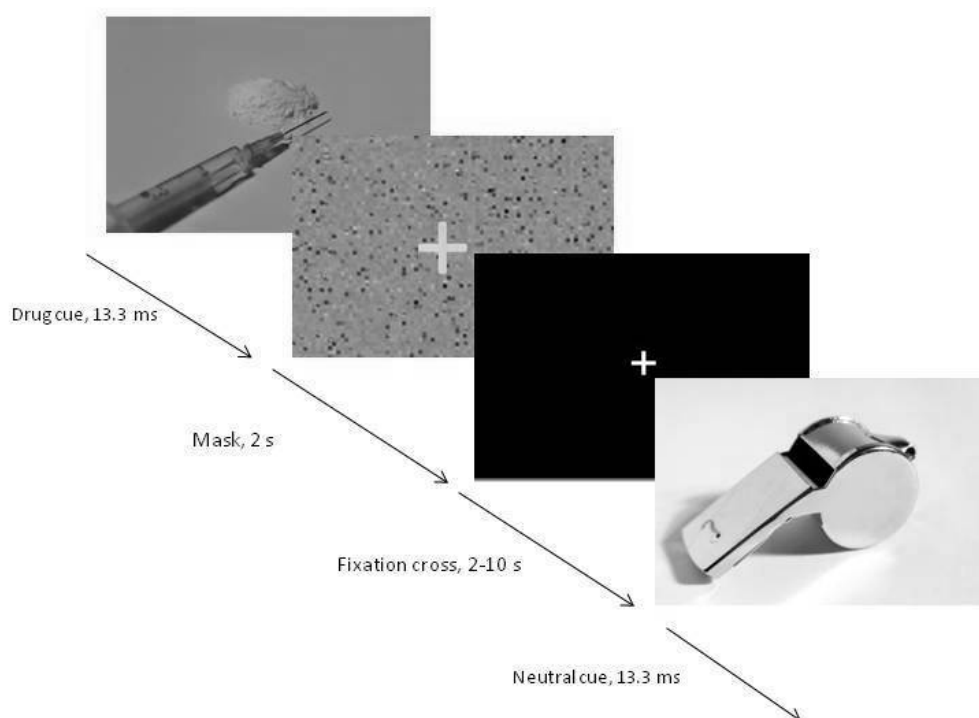
#### 4.4 STUDY IV: SUBLIMINAL CUE REACTIVITY

This study was part of the same project as Study III and used the same cohort of amphetamine dependent patients. For Study IV, we also included 30 healthy controls. Both patients and controls received one oral dose of naltrexone (50 mg) or placebo before the fMRI examination, which in this case aimed to investigate if subliminal drug cues would activate motivational systems of the brain.

*Subjects:* 40 male, non-treatment-seeking amphetamine users aged 20-65 were included, as described above for Study III. In addition, we included 30 healthy controls, who were also male, aged 20-65. Exclusion criteria for the healthy controls included any history of substance use disorder or other psychiatric diagnoses, current use of any medication, left-handedness or contraindications for MRI or the study medication. All participants were compensated for their participation with food coupons to the value of €150.

*Study procedures:* The basic procedures were similar to Study III. After a first screening visit, eligible participants were scheduled for a test day. After inclusion, each participant was randomized to a capsule of 50 mg naltrexone or an identical capsule with placebo. After an interval of at least 60 minutes, the MRI procedures were started.

The experiment of Study IV consisted of exposing the participants to drug-related or neutral pictures for a very brief interval, 13.3 ms. This was then followed by another picture, the “mask”, either immediately or after an interval of 94 ms with a black screen (this interval would presumably attenuate the masking effect and could therefore be used as a supraliminal perceptual control). After the mask, a fixation cross was shown on the screen for a variable interval of 2-10 s. An overview of the paradigm is shown in Figure 6.



**Figure 6: Subliminal cue paradigm of Study IV: each trial consisted of a very brief cue, with either drug-related or neutral content, followed by a masking picture and then a fixation cross until the appearance of the next cue.**

*Magnetic resonance imaging:* MRI examinations were performed with a 3 T instrument (GE MR750 Discovery) at the Karolinska MR Research Center. Each subject went through a total of four fMRI paradigms and structural imaging. The experiment described here was the first to be performed, preceded only by a resting state examination.

*Data analysis:* The preprocessing procedures were identical to those of Study III. In Study IV, however, the functional data were analyzed in an event-related design using the general linear model, where the primary contrast was Active with immediate mask > Neutral with immediate mask. The statistical approach was identical to Study IV, with FWE correction for multiple comparisons and level of significance set at  $p < 0.05$ .

#### **4.5 ETHICAL ASPECTS**

The studies described in this thesis involve several potential ethical problems that need to be discussed. Before the start of each study, we described and analyzed these ethical issues in the applications to the Ethics Review Board. Here, I will briefly discuss the most important points.

In Study I and II, we gave intravenous injections of dexamphetamine to healthy participants, which of course could be seen as problematic since amphetamine is an addictive substance that might cause adverse effects. This dilemma was handled by making sure that all participants were carefully screened by a study physician, ruling out somatic or psychiatric conditions that could increase the risk of adverse effects. All substance use disorders, both in the participants and in their first-degree relatives, were considered exclusion criteria. The risks of amphetamine intake were also minimized by giving a quite low dose, 0.3 mg/kg body weight, which is lower than a normal dose of stimulant medication for ADHD. Since the drug was administered in conjunction with PET examinations, the participants were carefully monitored for more than one hour post-injection by qualified staff in a research center in the middle of the Karolinska University Hospital, where possible adverse reactions could be handled effectively. With these precautions, our conclusion was that the risks involved in giving amphetamine to healthy persons were very small. In the end, no serious adverse events were seen in Study I or II.

In Study II and III, we gave a single, oral dose of naltrexone (or placebo) to healthy volunteers and amphetamine dependent patients. This could hardly be seen as ethically problematic, since naltrexone is a well-known medication that has been in clinical use for many years and the risk for any serious adverse events from a single dose is minimal. The only major side-effect that could be of importance is the risk of inducing acute opioid withdrawal symptoms, if someone with ongoing opioid use would take naltrexone. Although not dangerous in a medical sense, such a reaction could be highly unpleasant. Therefore, all participants were asked about current opioid use and also asked to leave a urine sample to objectively exclude this before randomization.

Study II also included work on rats, that were exposed to study drugs and examined with microdialysis. Although this might be seen as ethically problematic, we saw no feasible way

of answering the relevant research questions without the use of such validated animal models. All animals were housed and treated in accordance with current national guidelines, and the procedures were approved by the regulatory board.

Study III and IV included amphetamine dependent individuals, which in itself raises certain ethical problems. A point of discussion in medical ethics has been if drug dependent patients are competent and autonomous enough to leave a truly informed consent, for example to participate in research projects (148,149). But the fact that drug addicted patients may have lost control over certain parts of their lives (i.e. the substance use) does not entail that they do not know their best interest and are able to act accordingly in other situations. Study III and IV also did not involve the administration of any addictive substance, a situation that could be seen as particularly problematic (148). When meeting the potential participants, we made sure they understood the full meaning of participation before they were allowed to sign the consent form.

One part of Study III that could be seen as controversial is that we exposed amphetamine dependent subjects to drug-related cues in order to induce craving, a procedure which might increase the risk of relapse to drug abuse. However, earlier studies addressing this issue have not found any elevated risk of relapse in stimulant dependent patients exposed to drug cues in experimental settings (150,151). This is also in accordance with our experience from earlier studies of cue-induced craving: in an experimental setting, craving vanishes rather quickly and is often completely gone before the participants leave the research facility (43,152). The clinical milieu probably attenuates the feelings of craving and in case any adverse reactions of this nature would occur, an experienced physician was always present to support the patient. To make sure that we did not increase the risk of relapse in stable, drug-free individuals, we chose only to include people with long-term, active amphetamine dependence (at least 12 amphetamine intakes during the last 12 weeks and not more than 30 days of continuous abstinence).

The participants in the study had at least one free visit to the study physician for assessment of physical and mental health and problems related to substance use. Although the patients were not actively treatment-seeking, a visit of this kind can sometimes be of help, constituting a positive first contact with organized health care. Having participated in the study, the patients were offered a referral to their local substance dependence clinic.

In conclusion, we estimated the risks for the participants in Study III and IV to be small and the moderate expected benefits for them to be bigger. Since we could not see any relevant arguments against the study in a larger context, we concluded that it was ethically defensible. No serious adverse events were seen in Study III or IV.



## 5 RESULTS

### 5.1 STUDY I

In Study I, all 10 participants included in the study performed all examinations according to the protocol (apart from the time delay in performing three of the examinations, as already mentioned in the methods chapter, section 4.1). As expected, within 1-2 minutes the amphetamine injection caused strong subjective effects in all the subjects. Ratings of how much the subjects “felt” the drug effect ranged from 50 to 100 (mean  $88 \pm 16$ ), feeling of “high” from 30 to 90 (mean  $66 \pm 22$ ) and how much they wanted more of the drug from 0 to 100 (mean  $64 \pm 33$ ). Placebo ratings were between 0 and 10 for all three questions. No serious adverse events occurred during the study.

When analyzing the PET data, repeated measures ANOVA revealed no significant effects of amphetamine compared to placebo on  $^{11}\text{C}$ -carfentanil  $\text{BP}_{\text{ND}}$  in the ventral striatum (Figure 7). The mean difference in  $\text{BP}_{\text{ND}}$  in this region was  $-0.037$ , with a 95% confidence interval of  $-0.186$  to  $0.111$  (or  $-6.4$  to  $3.8\%$ ). Similar results were found in the other regions of the striatum, prefrontal cortex, amygdala and hippocampus.

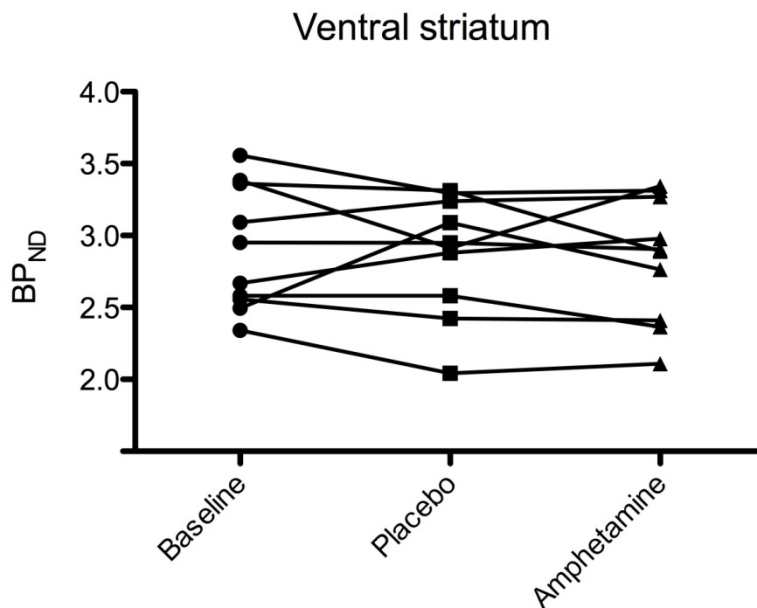


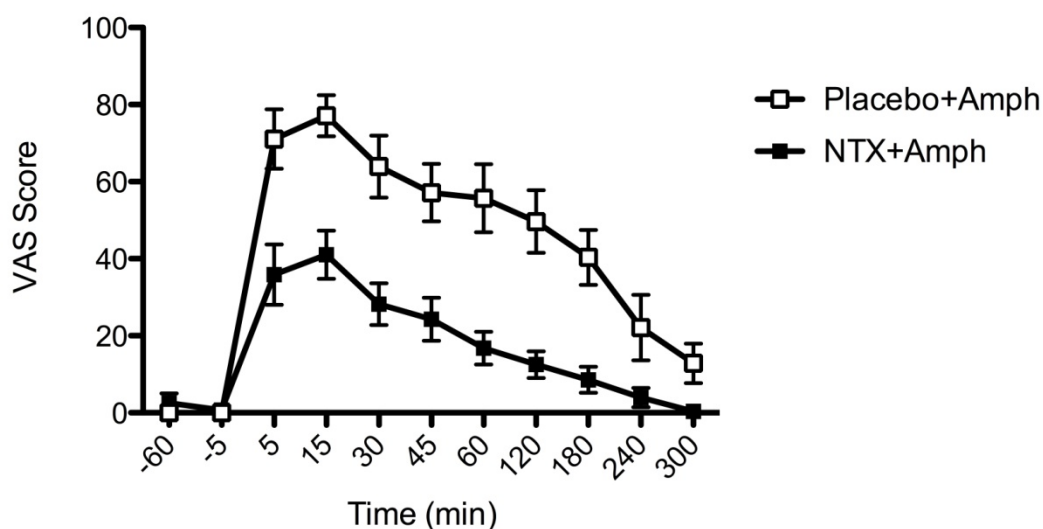
Figure 7: Individual measures of  $^{11}\text{C}$ -carfentanil binding potential ( $\text{BP}_{\text{ND}}$ ) in the ventral striatum at baseline, after placebo and after amphetamine injection.

The  $\text{BP}_{\text{ND}}$  values in the different regions were comparable to the ones obtained in a recent test-retest-study of  $^{11}\text{C}$ -carfentanil (126). However, as can be seen in Figure 6, there was some variability in  $\text{BP}_{\text{ND}}$  between the conditions for several participants. In an exploratory analysis, we examined if these individual differences were correlated with subjective ratings of amphetamine effects, but no such correlation was found. Neither was there any effect of order between the amphetamine and placebo examinations, which means it did not matter if you had the amphetamine first and then placebo at the next occasion or vice versa.

## 5.2 STUDY II

### 5.2.1 <sup>11</sup>C-raclopride PET

*Subjective effects:* The amphetamine injections caused strong subjective effects in all of the participants. Repeated measures ANOVA revealed a main effect for treatment condition ( $F = 482.1$ ;  $p < 0.001$ ), such that the placebo+amphetamine condition produced a significantly stronger subjective drug effect than the naltrexone+amphetamine condition. In other words, naltrexone reduced the subjective effects of amphetamine (Figure 8), just as previously shown in several other studies.



**Figure 8:** Subjective effects of an amphetamine injection (0.3 mg/kg bodyweight) at time point=0, after pretreatment with naltrexone or placebo. Values denote mean  $\pm$  standard error of the mean for the composite score at each time point, defined as the average values of the four visual analog rating scales: ‘feel the drug’, ‘like the effect’, ‘feel aroused’ and ‘want more’. NTX=naltrexone, Amph=amphetamine.

*Effects on dopamine release:* Two-way repeated measures ANOVA revealed main effects of condition ( $F = 9.76$ ,  $p = 0.015$ ), brain region ( $F = 67.76$ ,  $p < 0.001$ ) and a condition-by-region interaction ( $F = 4.21$ ,  $p = 0.024$ ) on <sup>11</sup>C-raclopride BP<sub>ND</sub>. Post hoc paired t-tests demonstrated significantly decreased BP<sub>ND</sub> in all striatal ROIs for both placebo+amphetamine and NTX+amphetamine as compared to baseline, indicating increased DA levels in the striatum after the amphetamine injection, as was expected. However, there was no significant difference in BP<sub>ND</sub> between placebo+amphetamine and naltrexone+amphetamine (Figure 9). The results were similar for all subregions of the striatum.

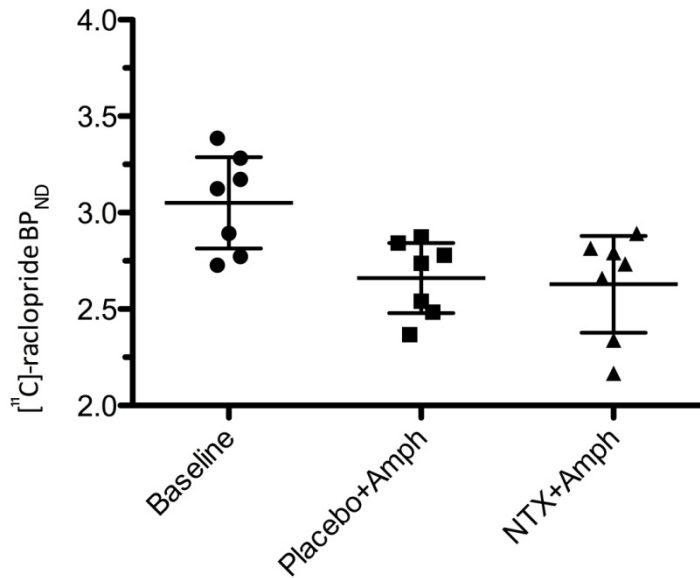


Figure 9:  $^{11}\text{C}$ -raclopride binding potential ( $\text{BP}_{\text{ND}}$ ) in the ventral striatum at baseline, after placebo+amphetamine and naltrexone+amphetamine. Figures represent mean and 95% confidence intervals. Amph=amphetamine, NTX=naltrexone.

## 5.2.2 Microdialysis

We investigated the effects of naltrexone pretreatment on the dopamine release induced by amphetamine, using in vivo microdialysis in Wistar rats. All measurements were made in the nucleus accumbens. We tried two different doses of amphetamine in a model of acute amphetamine intake and one dose in model with chronic amphetamine exposure.

*Acute model:* Two-way ANOVA of the data from the acute amphetamine (0.5 mg/kg) experiment revealed significant time, treatment and interaction effects ( $F_{\text{interaction}(42,252)} = 4.498, p < 0.0001$ ). Amphetamine as compared to saline caused a significant increase in DA concentrations after administration (Figure 10). Pre-treatment with naltrexone (3 mg/kg) did not significantly influence amphetamine-induced DA release.

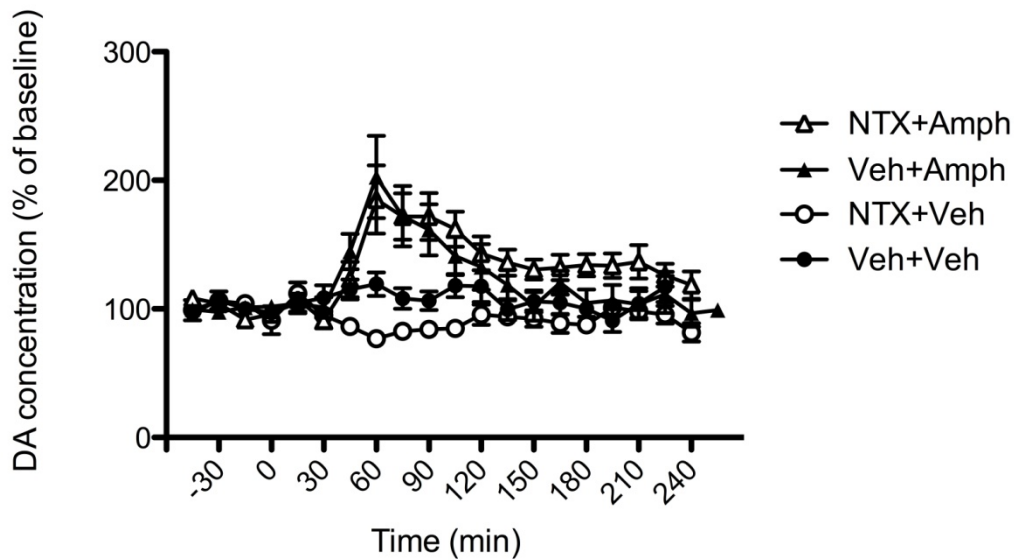


Figure 10: Effects of an acute amphetamine injection (0.5 mg/kg) on dopamine concentrations in the nucleus accumbens, as measured with microdialysis in Wistar rats. Naltrexone/vehicle pretreatment was given at time point=0, while amphetamine/vehicle was given at time point=30 min. Amphetamine increased dopamine levels but naltrexone pretreatment had no effect as compared to vehicle. NTX=naltrexone, Veh=vehicle, Amph=amphetamine.

When amphetamine was administered at a dose of 2.0 mg/kg, two-way ANOVA revealed similar effects as with the lower dose ( $F_{\text{interaction}(42,248)} = 23.39, p < 0.0001$ ). At this higher dose, amphetamine caused a robust increase of DA output compared to saline ( $p < 0.001$ ), an effect that lasted up to two hours (Figure 11). NTX (3 mg/kg) pre-treatment did not affect the amphetamine-induced DA output at any time point.

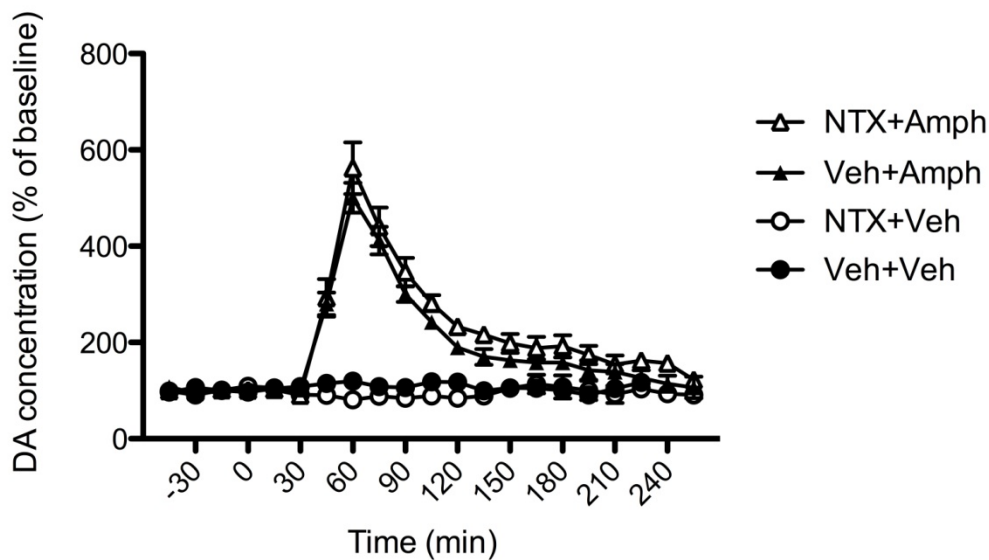
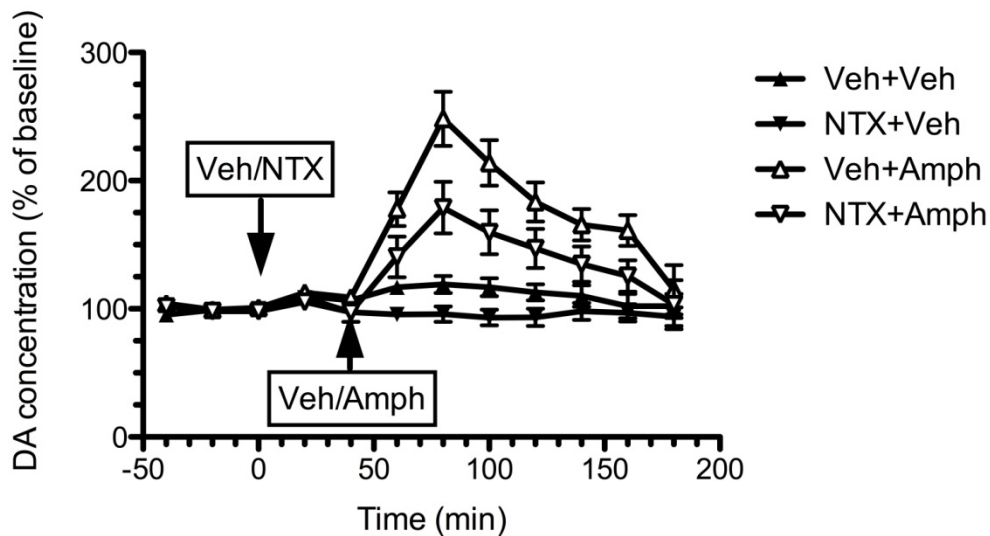


Figure 11: Effects of an acute amphetamine injection (2.0 mg/kg) on dopamine concentrations in the nucleus accumbens. Naltrexone/vehicle pretreatment was given at time point=0, while amphetamine/vehicle was given at time point=30 min. Amphetamine caused a highly significant increase in dopamine levels, but naltrexone pretreatment had no effect as compared to vehicle. NTX=naltrexone, Veh=vehicle, Amph=amphetamine.

*Chronic model:* In the chronic model, with daily administration of amphetamine (2 mg/kg) for ten days, followed by a drug free period of ten days and then reinstatement of

amphetamine (0.5 mg/kg), two-way ANOVA revealed significant time, treatment and interaction effects ( $F_{\text{interaction}}(27,234) = 8.365, p < 0.0001$ ). Post hoc analysis revealed that amphetamine significantly increased DA output 20 minutes after administration compared to baseline (Figure 12). There was also a significant difference between naltrexone+vehicle and naltrexone+amphetamine treatment ( $p=0.014$ ) and between vehicle+amphetamine and naltrexone+amphetamine treatment ( $p=0.030$ ). Effectively, naltrexone pre-treatment attenuated the amphetamine-induced DA elevation by approximately 50% in this model with chronic amphetamine administration.



**Figure 12:** Dopamine concentrations in the nucleus accumbens after reinstatement with amphetamine (0.5 mg/kg), following previous chronic amphetamine administration and 10 days of abstinence before the experiment. Naltrexone significantly reduced amphetamine-induced dopamine release in this model.

### 5.3 STUDY III

This study included 40 male patients with amphetamine dependence. They were on average 44 years old, with long histories of intravenous amphetamine use. Most were unemployed and had a criminal history, having spent a mean of 4.5 years incarcerated. There were no significant differences in important baseline variables between the naltrexone and placebo groups. For more details on demographics, see Table 1.

**Table 1.** Demographics and drug use histories of patients included in Study III. Continuous variables are presented as mean  $\pm$  standard deviation.

	Naltrexone (n=20)	Placebo (n=20)
Age (years)	43 $\pm$ 10	45 $\pm$ 10
Years of amphetamine use	16 $\pm$ 9	21 $\pm$ 10
Days of amphetamine use during last month	21 $\pm$ 10	23 $\pm$ 11

Chronic viral hepatitis	75%	70%
HIV	10%	0%
Nicotine dependence	80%	90%
Living alone	95%	75%
Years in school	11 ± 2	11 ± 4
Months incarcerated	48 ± 81	58 ± 102

All 40 patients were randomized, but some were not able to perform the fMRI examination because of suspected adverse reactions to the medication (n=3; two in the naltrexone and one in the placebo group), claustrophobia (n=1) or technical problems (n=3). One patient was lost to follow-up for unclear reasons and never took part in the fMRI examination: after randomization and having ingested the study medication at the research clinic, he disappeared during the un-supervised 50 m walk from the clinic to the MR center. In total, 32 patients contributed data for analysis. No serious adverse events occurred during the study.

The participants' craving ratings were consistently higher after the drug-related as compared to the neutral film clips (mean scores of  $68 \pm 31$  vs.  $16 \pm 24$ ,  $p < 0.001$ ), which confirms that the films worked as intended and that the participants were paying attention. However, the craving ratings did not differ between the naltrexone and placebo groups ( $p > 0.1$ ).

For the fMRI analysis, three participants were excluded from the analysis due to excessive head movements during the examination, quantified as  $>25\%$  of the image volumes above a cut-off of 0.3 FD (see above under Methods, section 4.3). This meant that 29 patients had complete imaging data of sufficient quality to be included in the analysis.

Comparing the BOLD activity when viewing drug-related as compared to neutral scenes, we found statistically significant activations in the occipital, parietal and temporal cortices, the striatum, and cingular cortex (Figure 13 and Table 2). There were no statistically significant differences between the naltrexone and placebo groups in the cue-induced BOLD activity of our regions of interest. The same results were found in the whole-brain analysis.

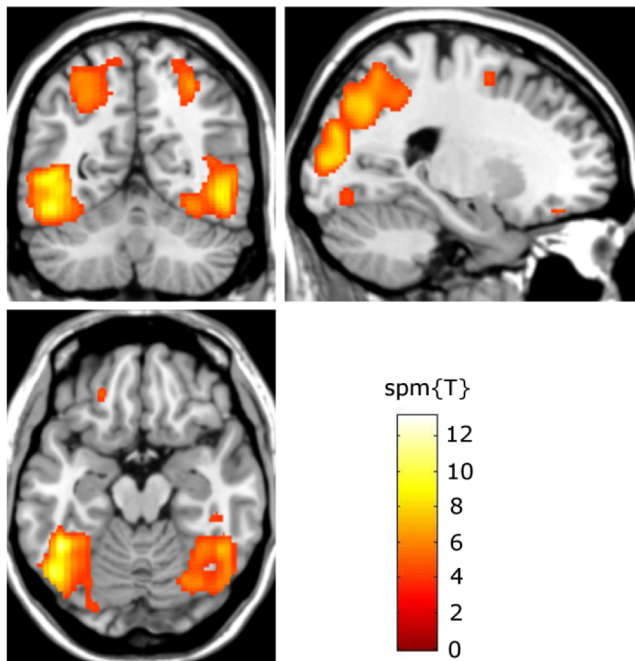


Figure 13. Drug movie > Neutral movie BOLD activations for the whole sample, regardless of treatment condition (n=29). Whole-brain results,  $p < 0.05$ , with Family-Wise Error correction for multiple comparisons.

Table 2. Coordinates and Z-values for the Drug movie > Neutral movie activations.

Anatomical region	Cluster size	X	Y	Z	Peak value	Z-
Middle Occipital Gyrus (R)	4834	32	-82	12	7.36	
Inferior Temporal Gyrus (R)		46	-50	-10	7.15	
Inferior Temporal Gyrus (R)		44	-66	-8	6.76	
Inferior Occipital Lobule (L)	8712	-48	-72	-4	7.35	
Inferior Occipital Lobule (L)		-46	-64	-14	7.11	
Inferior Temporal Gyrus (L)		-46	-48	-18	6.88	
Superior Parietal Lobule (R)	695	26	-64	54	5.84	
Angular Gyrus (R)		28	-64	44	5.70	

Superior Parietal Lobule (R)		36	-52	62	5.29
Fusiform Gyrus (R)	101	38	-34	-28	5.25
Precentral Lobule (L)	85	-46	2	32	5.08
Precentral Lobule (L)		-46	6	20	4.96
Middle Orbitofrontal Lobule (L)	12	-22	38	-18	5.06
Rolandic Operculum (L)	8	-40	-2	16	4.98
Superior Frontal Lobule (L)	31	-22	0	56	4.97
Middle Cingulum (L)	6	-6	-24	42	4.80
Middle Frontal Gyrus (L)	9	-46	40	18	4.77

In secondary analyses conducted post-hoc, we looked at two well-known moderators of cue reactivity. In earlier reviews of the literature, both the length of addiction history and the amount of recent drug use has been linked to increased cue reactivity in addiction (108). When added as a co-variate in the model, the number of amphetamine-use days in the last month did not correlate with neural cue reactivity in our sample. On the other hand, years of drug use showed a positive correlation with cue-induced activation of a large cluster in the left dorsolateral prefrontal cortex (DLPFC), a finding that remained statistically significant at the cluster level after FWE correction (Figure 14).



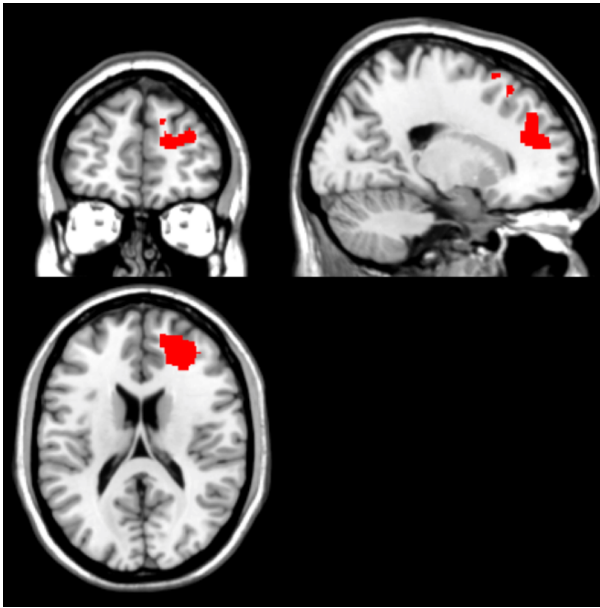


Figure 14. Clusters with cue-induced BOLD activations correlating with years of drug use. Results for the whole sample (n=29) with significance level set at  $p < 0.05$ , with Family-Wise Error correction for multiple comparisons.

#### 5.4 STUDY IV

The patients included in this experiment were the same as in Study III, and their background has been described above in section 5.3. Here, we also had a control group of healthy participants, who were all male and  $40 \pm 8$  years old (not significantly different from the patients). There were no significant differences in baseline variables between the naltrexone and placebo groups in either the patients or the healthy participants.

As described above, all 40 patients were randomized, but nine of them were unable to successfully perform this experiment in the MR scanner due to suspected adverse reactions to the medication, claustrophobia or technical problems. In the group of healthy subjects, three persons could not perform the examination because of technical problems and one interrupted the examination because of claustrophobia. In total, 31 patients and 26 controls contributed data. Head movements were common, particularly in the patient group, and our strict threshold for head movements meant that fMRI data from seven patients and one of the controls were discarded, leaving 24 patients and 25 healthy subjects with complete data and no major movement artifacts.

In our primary contrast of Active with immediate mask  $>$  Neutral with immediate mask, no statistically significant activations were found in our predefined regions of interest. Neither was any significant activation found when extending the analysis to the whole brain. There were no differences between patients and healthy controls and we found no evidence of any effect of naltrexone vs. placebo on these measures, although the sample size may have been too small to reach adequate power for such a comparison. The secondary analysis of Active with delayed mask  $>$  Neutral with delayed mask did not reveal any significant activations either.

## 6 DISCUSSION

### 6.1 ROLE OF THE OPIOID SYSTEM IN THE ACUTE EFFECTS OF AMPHETAMINE

In Study I, contrary to our hypothesis, we did not find any evidence for endogenous opioid release in the brain of healthy participants within an hour after an intravenous amphetamine injection of 0.3 mg/kg bodyweight. While we were preparing the manuscript for this study, a research group at University College London published a paper addressing a similar research question, also with the use of  $^{11}\text{C}$ -carfentanil PET in healthy volunteers (153). In that study, they did find significantly lower  $^{11}\text{C}$ -carfentanil  $\text{BP}_{\text{ND}}$  after amphetamine intake compared to the control condition. Two years later, the same research group published a replication study with the same protocol, obtaining similar results as the first one in a new cohort of participants (154).

These seemingly conflicting results can probably be explained by differences in the experimental procedures. The two studies from London used oral amphetamine dosing of 0.5 mg/kg bodyweight, as opposed to our intravenous injections. Oral amphetamine has a slower, delayed uptake and also quite different behavioral effects, with much less euphoria than after intravenous intake (27). Indeed, the participants in the studies with oral amphetamine did not experience any significant euphoria at all, while our participants consistently reported quite intense euphoria. However, the most important difference between the studies was probably the timing of the PET examinations: instead of starting the examination after a few minutes as in our study, they started three hours after the drug intake. This interval is needed for orally administered dexamphetamine to reach peak plasma concentrations, but it also leaves more time for secondary neurochemical effects to occur (155). Although the exact mechanisms behind amphetamine-induced opioid release remain obscure, an interval of more than one hour after amphetamine intake seems to be needed in order for any opioid release to be measurable with  $^{11}\text{C}$ -carfentanil PET in healthy humans.

The fact that amphetamine-induced opioid release is only measurable after an interval of more than one hour contrasts with the timing of the subjective effects of amphetamine, that are felt strongly within minutes of an intravenous injection. Unfortunately, the limited temporal resolution of PET does not allow for any precise detection of exactly when the opioid release occurs. Therefore, it is hard to say to what extent these findings explain why naltrexone attenuates the subjective effects of amphetamine, an effect that is apparent within the first hour after amphetamine intake, although it is even more evident 2-3 hours after an oral dose (68,69).

In Study II, we examined another hypothesis regarding the mechanism of naltrexone, namely that it would attenuate the DA release caused by amphetamine intake. When testing this in drug-naïve humans and rats, we found no evidence for such an effect. However, in rats previously exposed to amphetamine, naltrexone did attenuate the amphetamine-induced DA increase, as recorded with *in vivo* microdialysis. While not yet experimentally confirmed in humans, this finding opens up for a number of different questions regarding the role of DA

and opioid interactions in the long-term pathophysiology of addiction, as opposed to the acute drug effects at the time of first exposure.

Of course, this does not answer the question why naltrexone attenuates the subjective response to amphetamine in drug-naïve subjects, not only in experienced amphetamine users. When examining the results of earlier studies in patients and healthy volunteers, there seems to be a trend towards a stronger effect of naltrexone in the patient studies, although this has not been formally tested (68,69). Such a difference would be expected given the results above, but it remains to be systematically investigated.

To summarize, amphetamine has acute effects on brain monoamine systems, in particular DA, but also induces a release of endogenous opioids in the brain of healthy humans. The latter mechanism might help explain why the opioid antagonist naltrexone attenuates the subjective effects of amphetamine, but a remaining question is why the opioid release has only been observed three hours after the drug intake, while the subjective effects are apparent within minutes after an amphetamine injection. Since DA release can be detected very soon after amphetamine intake, its timing fits better with the subjective effects of the drug. The level of DA release has also been shown to correlate with some of the subjective effects of amphetamine (see above, section 2.2), and one hypothesis is that naltrexone exerts its effects by interfering with amphetamine-induced DA release. However, we did not find any evidence that naltrexone affects amphetamine-induced DA release in healthy humans or drug-naïve rats, although in rats chronically exposed to amphetamine, naltrexone actually did attenuate the DA release. This might help explain some of the clinical effects of naltrexone in amphetamine dependent patients, but how it attenuates the subjective response to amphetamine in healthy subjects is still somewhat unclear. Most probably, it involves antagonizing the effects of endogenous opioids released after amphetamine intake, but it remains to be investigated to what extent this occurs within the time frame relevant for the acute subjective effects of amphetamine.

## **6.2 ROLE OF THE OPIOID SYSTEM IN CRAVING AND RELAPSE**

While the mechanisms behind the acute effects of addictive drugs are important to study, a more pressing clinical dilemma is how to help patients resist craving and avoid relapse in amphetamine dependence. One way of doing so could be to reduce the rewarding effects of the drug, thereby attenuating the positive reinforcement that may increase the risk of relapse. As we have seen above, naltrexone does seem to have such an effect on amphetamine, and it might be part of the explanation for its suggested clinical efficacy.

However, most contemporary theories of addiction emphasize the role of craving and negative reinforcement, rather than positive reinforcement, in the later stages of the disease (156,157). Clinically, it is also obvious that most long-term amphetamine users do not take amphetamine to get a euphoric “high” every day, but rather to be able to get out of bed, to feel and function “like normal people”. Simply attenuating the effects of the drug that make them feel slightly better for a while might not be a very successful approach, and it would require hard work motivating the patient to comply with such a treatment. This is why it is

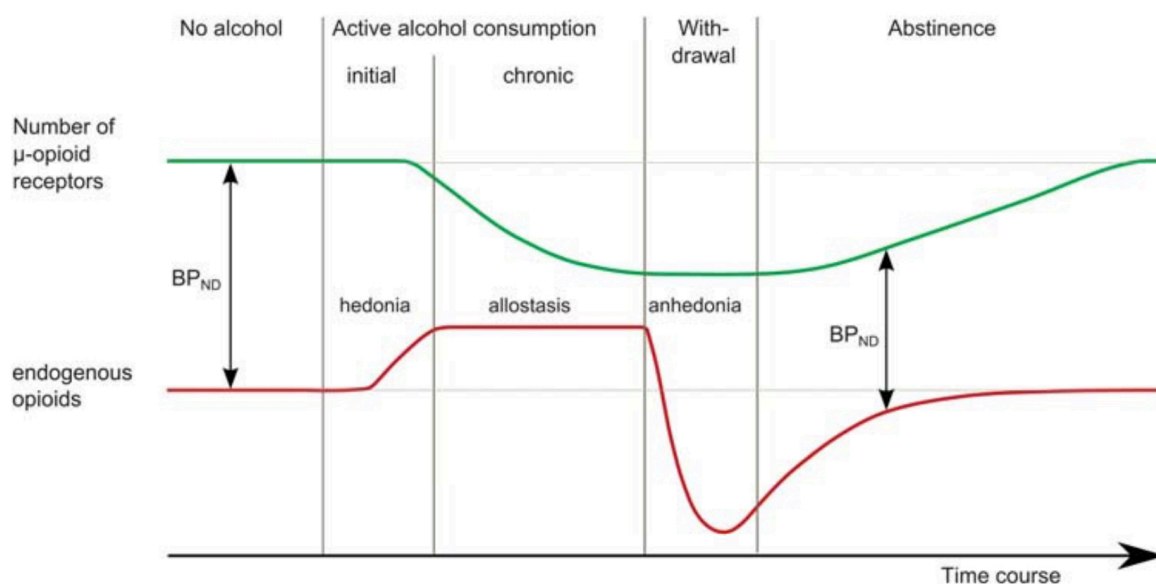
important to understand the mechanisms of craving and develop treatments targeting this phenomenon.

As already discussed in the second chapter of this thesis, some progress has recently been made in this field with the help of neuroimaging techniques that allow us to study the human brain at work, for instance when exposed to drug-related stimuli that may induce craving. Such a cue reactivity paradigm was employed in Study III, which was designed as a randomized controlled study, testing whether naltrexone affected cue reactivity in patients with severe amphetamine dependence. The rationale for the study was based on earlier findings that naltrexone attenuates cue reactivity in alcohol dependent patients (117,118) and may prolong the time to relapse in amphetamine dependence (77), a finding that of course cannot be explained merely by naltrexone attenuating the acute effects of amphetamine. We hypothesized that naltrexone would attenuate cue reactivity as measured with subjective craving ratings and BOLD fMRI activity. We did not find evidence in favor of this hypothesis, although unfortunately a number of patients were not able to perform the experiment or had to be excluded due to movement artifacts, resulting in a somewhat underpowered final sample for the analysis of naltrexone vs. placebo.

While Study III was still ongoing, a research group from University of California, Los Angeles (UCLA) published an fMRI study of the effects of naltrexone on cue reactivity in methamphetamine users (110). Their sample had less severe addiction than our patients, and the cue exposure paradigm employed was also different, but there was still considerable overlap with the neural patterns of cue reactivity found in our study. The UCLA study had a smaller sample but used repeated measurements of cue reactivity in a cross-over randomized study design, allowing for within-subject comparisons of naltrexone vs. placebo. With this design, and minimal data loss due to movement artifacts, they were able to find statistically significant differences between the naltrexone and placebo conditions in cue reactivity in primary sensory-motor areas. This finding is hard to interpret and of unclear relevance, since these regions are rarely activated in studies of cue-induced craving and are not typically considered parts of the brains motivational systems (95,156). In line with our results, they found no difference between the conditions on subjective ratings of craving.

In other words, there is very limited evidence regarding the possible role of the opioid system in craving reactions and relapse in amphetamine dependence. However, some hypotheses might be based on findings from other addictive disorders. In this context, the work on cocaine dependence using  $^{11}\text{C}$ -carfentanil PET, as reviewed above in section 2.4, is of particular interest, since the increased opioid receptor binding found in cocaine dependent patients has been correlated both with craving and risk of relapse (60,61). This suggested pathophysiological mechanism has not yet been studied in amphetamine users, but could of course be relevant when trying to explain the mechanism of action of naltrexone. One could also speculate that the variability in  $^{11}\text{C}$ -carfentanil  $\text{BP}_{\text{ND}}$  observed in the patient samples of these studies might correlate with individual differences in the response to naltrexone. A recent study combining PET and a randomized clinical trial with alcohol dependent patients found that a lower striatal  $^{11}\text{C}$ -carfentanil  $\text{BP}_{\text{ND}}$  was associated with a higher risk of relapse,

an effect that seemed to be more pronounced in the naltrexone group, although the study was not powered to detect any significant interaction with treatment allocation (158). In the same paper, the authors also investigated a sample of alcohol patients post mortem using autoradiography with the  $\mu$  opioid receptor ligand  $^3\text{H}$ -DAMGO and found a reduced binding as compared to controls. This suggests that the increased  $^{11}\text{C}$ -carfentanil  $\text{BP}_{\text{ND}}$  found in earlier studies of recently abstinent alcohol patients reflects reduced levels of competing endogenous opioids, rather than increased  $\mu$  opioid receptor densities (159).



**Figure 15.** Model of the dynamic changes of the opioid system during the course of alcohol dependence.  $\text{BP}_{\text{ND}}$  refers to the binding potential for  $^{11}\text{C}$ -carfentanil, which is determined by the density of  $\mu$  opioid receptors and the concentration of endogenous opioids, as indicated by the black arrows. From Hermann et al. 2017 (ref. 158).

The authors propose a model where both  $\mu$  opioid receptors and endogenous opioids undergo dynamic changes during the cycle of addiction, such that the receptors are continuously downregulated during chronic drinking as a reaction to the alcohol-induced endogenous opioid activation, forming an allostatic equilibrium (Figure 15). In early withdrawal, the endogenous opioid levels drop quickly, contributing to anhedonia and other symptoms (158). With prolonged abstinence, the opioid system slowly recovers towards normal function. While this model is proposed specifically for alcohol, it might be relevant also for other forms of addiction, including stimulant dependence. Indeed, PET studies in recently abstinent cocaine patients have consistently found elevated  $^{11}\text{C}$ -carfentanil  $\text{BP}_{\text{ND}}$  (58,59), while a post mortem autoradiography study of cocaine users found reduced  $^{125}\text{I}$ -DAMGE binding as compared to controls (160), in other words the same pattern as in alcohol patients. At present, no relevant studies have investigated these phenomena in amphetamine dependent patients. How these proposed dynamic changes during the addiction cycle may affect the impact of pharmacological treatments acting on the opioid system is also an important topic for further study.

Another factor that has not yet been sufficiently investigated in amphetamine dependent patients is the role of genetic variants, for instance the Asn40Asp (A118G, rs1799971) single nucleotide polymorphism of the  $\mu$  opioid receptor that has been shown to correlate with the

therapeutic response to naltrexone in alcohol dependence (53). A limiting factor is the large number of patients that need to be tested prospectively in order to get adequate statistical power for such comparisons, considering that the 118G variant only has a prevalence of about 20% in people with European ancestry. The only study on this topic published so far, an open-label study of methamphetamine dependent patients treated with depot-naltrexone, only included 11 patients in each group and was therefore underpowered to detect relevant differences (161).

An alternative approach could be to study the effects of Asn40Asp on the response to amphetamine and naltrexone in healthy volunteers. However, a recent study did not find any evidence that this variant affected amphetamine-induced euphoria (162). It is also worth keeping in mind that the results of candidate gene studies in psychiatry so far have been quite unsuccessful, even when applied to seemingly simple and promising endophenotypes such as response to an amphetamine challenge (163).

Turning to another proposed mechanism involved in craving and relapse, we found no evidence of subliminal drug cue reactivity in Study IV, although our patient sample was the largest of any study of this kind. This negative result led us to examine the earlier literature in more detail (see also section 2.8). All fMRI studies published so far on subliminal cue reactivity in addiction have reported significant results, but most studies come from the same laboratory and suffer from several of the common methodological problems mentioned above (section 2.6). A closer look reveals that the risk of Type I errors (false positives) due to multiple comparisons has not been adequately controlled. Despite the small sample sizes and very subtle, subliminal effects under study, statistically significant results in different directions are reported in all studies. The two most recent papers, published in 2014 (122,123), are also examples of a specific problem pointed out by Eklund et al. (98), namely that a particular software (AFNI) overestimates group level smoothness and also until 2015 contained a bug, raising the risk of false positives even further. Since both 2014 studies used AFNI for their statistical analyses, the results must be interpreted with great caution until reanalysed with more stringent methods. At present, there is no convincing evidence for the existence of specific subliminal drug cue reactivity in addiction.

In summary, there is not yet enough evidence to describe in any detail how the opioid system might be involved in craving reactions and relapse in amphetamine dependence. Some clinical trials suggest that the opioid antagonist naltrexone reduces the risk of relapse, but the exact mechanisms behind this possible effect are not yet known. Two fMRI studies have investigated the effect of naltrexone on cue reactivity, but none of them found any effect of naltrexone on subjective ratings of craving and the effects on neural cue reactivity are still unclear.

## 7 CONCLUSIONS AND FUTURE PERSPECTIVES

In short, the studies included in this thesis have come to the following conclusions:

- In Study I, we found no evidence of amphetamine-induced opioid release in healthy volunteers, during the first hour after an amphetamine injection. Other studies using the same technique have found signs of opioid release three hours after an oral amphetamine dose, which suggests that naltrexone attenuates the effects of amphetamine at least in part by blocking the effects of endogenous opioids released by amphetamine intake.
- In Study II, naltrexone pre-treatment was found to attenuate amphetamine-induced dopamine release in rats with a previous history of chronic amphetamine exposure, an effect was not seen in drug-naïve rats or humans. That an opioid antagonist like naltrexone has different effects depending on the level of prior amphetamine exposure might indicate that the opioid system is involved in the long-term changes in brain motivational systems that form part of the disease of addiction.
- Study III found that patients with severe amphetamine dependence exhibited strong cue reactivity, in terms of subjective craving and wide-spread neural activations in response to drug-related film clips. However, there was no indication that naltrexone modulates this response and it is unclear whether the opioid system plays any significant role in cue-induced craving reactions in amphetamine dependence.
- Study IV found no evidence for subliminal cue reactivity in a sample of patients with severe amphetamine dependence and healthy controls. A closer investigation of earlier studies revealed that the evidence for this proposed phenomenon is not convincing.

Karl Popper noted that “every solution of a problem raises new unsolved problems (...) The more we learn about the world, and the deeper our learning, the more conscious, specific and articulate will be our knowledge of what we do not know, our knowledge of our ignorance” (164). While this thesis work has reached few clear solutions, it has hopefully contributed a little bit to our knowledge of our ignorance. The work has taken a number of years, and during this time the field of addiction neuroimaging has also evolved, with a steadily increasing number of studies getting published each month. It could therefore be worthwhile to end this thesis by discussing some of the most important developments and challenges ahead.

Neuroimaging is a field of rapid technical development. For PET, new radioligands are being synthesized, allowing for new research questions to be addressed. In relation to the subject of this thesis, it is particularly interesting to follow the development of specific  $\kappa$ -opioid receptor radioligands, which have recently been validated in humans (165). This could make it possible to disentangle the roles of different parts of the opioid system in physiological and pathological processes in humans. It may also have clinical implications, since  $\kappa$ -opioid antagonists are currently being investigated in clinical trials for psychiatric conditions including addiction (166).

Besides the synthesis of new radioligands, new PET systems and improved methods of analysis will probably allow better spatial resolution and more precise measurements in the future.

In fMRI, new cameras with higher magnetic field strengths will improve the signal-to-noise-ratio. In terms of improving paradigms for the study of cue reactivity, personalized cues are likely to be more powerful (167) and the cues might also be presented in a more effective way by using virtual reality techniques. With more efficient noise reduction, the current standard of visual stimuli could be complemented with good quality audio and perhaps even tactile and olfactory stimuli, in order to make the paradigms more realistic and powerful.

Another promising approach is the use of several imaging modalities in parallel, where the strengths of each method contribute to a fuller understanding of the phenomenon under study: techniques that could complement PET and fMRI include autoradiography for detailed molecular analysis and electroencephalography or magnetoencephalography for superior temporal resolution (168). New MR techniques not relying on BOLD may also contribute important information, like arterial spin labeling for the measurement of cerebral blood flow and diffusion tensor imaging for mapping white matter structure, which can be highly relevant in the study of neural networks (169).

Brain stimulation methods like transcranial magnetic stimulation and deep brain stimulation allow for the modulation of neural activity in specific brain regions. In recent years, there has been a rapid development of these techniques and a growing number of clinical trials are investigating their therapeutic potential in brain disorders, including addiction (170). Knowledge gained from imaging studies on the neurocircuitry involved in processes like craving could be important in designing efficient brain stimulation treatment protocols. Brain stimulation methods might also be highly useful as experimental tools, since they allow the neural processes under study to be manipulated rather than just observed (171). Until recently, the limited anatomical range of transcranial magnetic stimulation and the risks associated with the surgical implant of electrodes for deep brain stimulation have precluded their wider use in addiction research. However, the recent development of magnetic coils that allow for transcranial stimulation of subcortical structures (172), as well as temporal interference techniques for non-invasive deep brain stimulation, although so far only validated in rodents (173), might overcome these problems and open up interesting experimental possibilities.

At the same time, crucial improvements could be made in the application of techniques already in use, with more efficient paradigms in larger samples, and analysis of data with validated statistical methods. The formation of international consortia for collection of samples with adequate power has been successful in genetics, and similar projects such as ENIGMA are now starting up in the field of psychiatric neuroimaging (174). Study designs with control for hereditary factors and/or information on genotype will probably become standard, considering the great influence of genetic factors on brain structure and function (175).



The methodological standards in addiction neuroimaging are far higher today than 15 years ago, when some of the first fMRI studies of addiction were published. Hopefully, the next 15 years will see an even more impressive development. There are however some fundamental problems that will need to be addressed before these methods can reach their full potential.

As mentioned earlier in this thesis, the field of addiction neuroimaging suffers from publication bias and other practices that give rise to inflated rates of positive findings in the literature (176). Some obvious examples are discussed in Study IV, where all of the earlier published studies on the topic might constitute false positives. The same issues seem to be prevalent in many other areas of psychological and medical research (177). There are probably no simple solutions to these problems, since they are imbedded in the publication practices and incentive structures that have dominated medical research for decades. However, in terms of a general direction forward, there are strong arguments for a more open science: for instance, widespread implementation of study preregistration and sharing of data and analytic code are simple measures that could dramatically improve the reliability of the published literature (178). While all these measures were not implemented in this thesis project from the start, we have taken some steps towards a more open scientific practice along the way. Considering the present state of the field, the fact that we have published a number of "negative findings" could actually be something to be proud of. We also aim to contribute and share data for future patient-level meta-analyses.

Returning to the topic of naltrexone, there is a need for more high-quality randomized clinical trials in order to strengthen the evidence for (or against) the efficacy of naltrexone as a treatment for amphetamine dependence. While one study found a significantly prolonged time to relapse (77), not all trials have reported this outcome and data on this has not been made available upon request (personal communication, 2017). It is also possible that naltrexone treatment is beneficial only for a subgroup of amphetamine dependent patients, for instance those with more severe addiction. New clinical trials are needed to settle these and other questions. Future trials might also consider the inclusion of laboratory sessions or neuroimaging procedures to investigate the mechanisms behind the proposed therapeutic effects of naltrexone. Hopefully the results of this thesis work can help inform the design of such studies and thereby play some part in the further growth of knowledge in this field.

## 8 SVENSKSPRÅKIG SAMMANFATTNING

Amfetaminberoende ger upphov till en rad medicinska och sociala problem och inte minst i de nordiska länderna är det relativt vanligt. Trots detta finns endast begränsad evidens för någon specifik behandling som skulle kunna hjälpa patienter att minska risken för återfall. En av de mest lovande behandlingsmetoderna för amfetaminberoende är naltrexon, ett läkemedel som hämmar aktiviteten i hjärnans opioidsystem. Tidigare studier har visat att naltrexon dämpar de subjektiva effekterna av amfetaminintag och vissa kliniska prövningar tyder på att det kan minska risken för återfall i amfetaminberoende. Denna avhandling syftade till att med hjälp av hjärnavbildning undersöka på vilket sätt naltrexon utövar dessa effekter, för att ge bättre förståelse för de neurobiologiska mekanismer som är involverade och underlätta fortsatt behandlingsutveckling.

I Studie I undersökte vi om en amfetamininjektion ger upphov till frisättning av kroppsegna opioider i hjärnan. I så fall skulle det kunna förklara varför ett opioidblockerande läkemedel som naltrexon dämpar amfetamins effekter. Med hjälp av positronemissionstomografi (PET) fann vi dock inga tecken till att amfetamin gav upphov till någon sådan opioidfrisättning hos friska försökspersoner utan tidigare erfarenhet av amfetamin.

I Studie II undersöktes hur förbehandling med naltrexon påverkar den dopaminfrisättning som sker i hjärnan efter amfetaminintag, som i vissa tidigare studier visat sig korrelera med subjektiva effekter av amfetamin. Om naltrexon dämpar dopaminfrisättningen skulle det därför kunna vara en mekanism som förklarar dess dämpning av de subjektiva effekterna. I en första delstudie använde vi PET och fann inga tecken till att naltrexon skulle påverka dopaminsvaret på amfetaminintag. För att kunna undersöka detta närmre och ge upprepade amfetamindoser, gick vi vidare med experiment på råttor, där vi mätte dopaminfrisättning med hjälp av mikrodialys. När vi gav råttor en enstaka dos amfetamin påverkade inte naltrexon dess effekter, men hos råttor som redan behandlats under längre tid med amfetamin fann vi att förbehandling med naltrexon halverade dopaminfrisättningen efter förnyat amfetaminintag.

Studie III syftade till att undersöka hur naltrexon påverkar en annan fas i beroendetillståndet, nämligen det drogsug som inte sällan tycks föregå återfall vid beroendetillstånd. Till denna studie rekryterade vi 40 amfetaminberoende män, som erhöll naltrexon eller placebo och därefter genomgick en undersökning med funktionell magnetresonanstomografi (fMRI). Under själva fMRI-undersökningen fick deltagarna se ett antal filmklipp, där vissa hade ett drogrelaterat innehåll. Hypotesen var att de drogrelaterade filmerna skulle väcka ett drogsug hos patienterna och ge ökad aktivitet i motivationsrelaterade områden i deras hjärnor, samt att naltrexon skulle dämpa denna reaktivitet. Vi fann att filmerna gav ett starkt drogsug och även påtagligt ökad hjärnaktivitet i vissa hjärnregioner, men vi såg inga signifikanta effekter av naltrexon jämfört med placebo.

Studie IV gick ut på att undersöka hur amfetaminberoende patienter reagerar på drogrelaterade bilder som presenteras väldigt snabbt och på ett sådant sätt att de inte ens blir medvetet varseblivna. Vissa tidigare fMRI-studier har nämligen tyckt sig se att

beroendepatienters hjärnor kan reagera även på sådana mycket svaga drogrelaterade stimuli. I vår studie fann vi dock inga belägg för att de amfetaminberoende patienterna skulle reagera annorlunda på drogrelaterade bilder jämfört med neutrala. Vid en närmare granskning av de tidigare studierna fann vi också att deras statistiska metodik var bristfällig, och slutsatsen blir att det för närvarande saknas belägg för att beroendepatienters hjärnor reagerar specifikt på drogrelaterade stimuli som är så svaga att de inte når medvetandet.

Sammanfattningsvis har studierna i denna avhandling inte kunnat bekräfta de hypoteser vi hade från början avseende mekanismerna bakom naltrexons effekter vid amfetaminberoende. Däremot har resultaten gett upphov till nya hypoteser, bland annat om hur samspelet mellan hjärnans dopamin- och opioidsystem förändras vid långvarig amfetaminanvändning. De har också aktualiserat metodologiska frågor som förhoppningsvis kan bidra till förbättrade hjärnavbildningsstudier i framtida beroendeforskning.



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