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Evaluation of Naltrexone as a Treatment for Amphetamine Dependence

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Pa, my therapy patient died this afternoon from complications of alcohol overdose...its really unfair! He was working so hard to quit.
It all seems so pointless. I don't ever want to work with this population of patients anymore!

You just said, he was trying to quit...that to me, sounds like hope and a good enough reason for you to continue working...

- Conversation with my father
May 15th, 1998

ABSTRACT

Amphetamine addiction is a disease that affects millions of people worldwide and lacks effective treatment. An estimated 35 million persons are reported to abuse amphetamines, which is more than the total number of cocaine and heroin abusers combined. A majority of intravenous drug users in Sweden abuse amphetamine, pushing this disorder to the forefront of psychiatric problems. At present, there is no approved pharmacotherapy for amphetamine dependence.

Several lines of evidence point towards involvement of the endogenous opioid system in the pathophysiology of stimulant addiction. The opioid antagonist naltrexone has shown to modulate some of the behavioral and neurochemical effects of amphetamine in animal models. The aim of this thesis was to investigate naltrexone in humans as a potential pharmacotherapy for the treatment of amphetamine dependence.

In the first study, we examined the effect of an acute dose of naltrexone in drug-naïve individuals. Structured batteries of subjective, physiological and behavioral measures were systematically administered to investigate the interaction effect of naltrexone and amphetamine. The results demonstrated that pre-treatment with naltrexone significantly reduces the subjective effects of amphetamine. Pre-treatment with naltrexone had no effect on the physiological and behavioral measures.

In the next study, we examined the effect of an acute dose of naltrexone on the subjective, physiological and biochemical effects of amphetamine in dependent individuals, using a double-blind placebo controlled design. Pre-treatment with naltrexone significantly attenuated the subjective effects of amphetamine. In addition, craving for amphetamine was blunted by naltrexone. This data provide the proof-of-concept that naltrexone not only dampens the subjective effect of amphetamine in the event of drug use, but also decreases the likelihood of additional drug consumption

Thereafter, we investigated the effect of chronic treatment with naltrexone in amphetamine dependent individuals, in an open-label design. The aim was to assess the tolerability and compliance to naltrexone in this new population. Twelve weeks of treatment with naltrexone led to a reduction in both frequency and quantity of drug consumption. Overall, the results showed that naltrexone was well tolerated with minimal side effects.

Finally, we investigated naltrexone for the treatment of amphetamine dependence in a randomized placebo-controlled trial. Patients either received 12-weeks of treatment with naltrexone or placebo. Twice-weekly urine toxicology tests were performed and in addition patients received weekly relapse prevention therapy. The results indicate that treatment with naltrexone reduced the percentage of amphetamine-positive urine samples in patients with chronic amphetamine dependence. Continued treatment with naltrexone also led to a reduction in craving as compared to placebo. In addition, the medical safety of naltrexone was further confirmed in this population.

In conclusion, naltrexone pharmacotherapy significantly reduces the reinforcing effects of amphetamine in acute and chronic dosing models. Taken together, this thesis provides support for the potential use of naltrexone as a treatment for amphetamine dependence.

LIST OF PUBLICATIONS

I. Jayaram-Lindstrom N., Wennberg P., Hurd YL., Franck J. Effects of naltrexone on the subjective response to amphetamine in healthy volunteers. *Journal of Clinical Psychopharmacology* 2004, 24: 665-669.

II. Jayaram-Lindstrom N., Konstenius M., Eksborg S., Beck O., Hammarberg A., Franck J. Naltrexone attenuates the subjective Effects of amphetamine in patients with amphetamine dependence. *Neuropsychopharmacology* 2007, published online, October 27th.

III. Jayaram-Lindstrom N., Wennberg P., Beck O., Franck J. An open clinical trial of naltrexone for amphetamine dependence: Compliance and tolerabilty. *Nordic Journal of Psychiatry*. 2005, 59: 167-171.

IV. Jayaram-Lindstrom N., Hammarberg A., Beck O., Franck J. Naltrexone for the treatment of amphetamine dependence: A randomized placebo controlled trial. *Submitted,* 2007.

CONTENTS

LIST OF ABBREVIATIONS

ADHD	Attention deficit hyperactivity disorder
AE	Adverse events
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CBT	Cognitive behavior therapy
CM	Contingency management
C^{11}	Carbon-11
DA	Dopamine
D_2	Dopamine receptor
DSM IV	Diagnostic and Statistical Manual of mental disorders
δ	Delta
FDA	Food and Drug Administration
GABA	Gamma-aminobutyric acid
HPA	Hypothalamic pituitary axis
I.V.	Intravenous
κ	Kappa
MAO	Monoamine oxidase
MDA	Methylenedioxiamphetamine
MDMA	Methyenedioximetaamphetamine
μ	Mu
Nacc	Nucleus accumbens
NIDA	National Institute of Drug Abuse
NE	Norephinephrine
PET	Positron emission tomography
POMS	POMS
RCT	Randomized controlled trial
TLFB	Time-Line Follow-Back
UNODC	United Nations Office of Drug and Crime
UN	United Nations
VAS	Visual analog scale
5-HT	Serotonin

1 INTRODUCTION

1.1 CLINICAL FEATURES OF ADDICTION

According to the definition stated in the DSM IV (Diagnostic and Statistic Manual of Mental Disorders) drug use refers to the harmful use of a drug leading to social and/or/ personal problems for the individual (i.e. recurrent use resulting in failure to fulfill major roles at work, school, home, repeated substance-related legal problems, substance use in situations in which it is physiologically hazardous and/or continuing use despite having problems caused or exacerbated by the effects of drug). Substance dependence is a state in which a person needs a drug to function within normal limits and is often associated with tolerance, withdrawal and relapse (American Psychiatric Association 1994)**.**

Table 1: DSM IV criteria for substance dependence.

The DSM-IV defines alcohol and other drug addiction as "substance dependence" and describes the diagnostic criteria as a maladaptive pattern of substance use leading to clinically significant impairment or distress, as manifested by three or more of the following occurring at any time in the same 12-month period:

1. Tolerance, as defined by either of the following
    - The need for markedly increased amounts of the substance to achieve intoxication or desired effect
    - Markedly diminished effect with continued use of the same amount of the substance
2. Withdrawal, as manifested by either of the following
    - The characteristic withdrawal syndrome for the substance
    - Use of the same (or closely related) substance to relieve or avoid withdrawal symptoms
3. The substance often taken in larger amounts or over a longer period than was intended
4. A persistent desire or unsuccessful efforts to cut down or control substance use
5. A great deal of time spent in activities necessary to obtain or use the substance or to recover from its effects
6. Important social, occupational, or recreational activities given up or reduced because of substance use
7. Continued substance use despite knowledge of having had a persistent or recurrent physical or psychological problem that was likely to have been caused or exacerbated by the substance.

An individual is diagnosed of substance dependence if he/she fulfils 3 or more of the 7 DSM IV symptoms. Among the diagnostic criteria, the biologically measurable criteria in humans are tolerance and withdrawal. Tolerance and physical dependence are two prominent features accompanying addiction but not prerequisites. The phenomenon of tolerance, like withdrawal, tends to abate within days or weeks and does not account for the persistent nature of addiction. Further, the feature of physical dependence *per se* is neither necessary nor sufficient to cause addiction and importantly not all drugs of abuse cause physical dependence. The 4th criterion in the DSM IV includes elements of cognition and memory, referred to as craving, which is less accessible to biological measurements. However with recent advances in neuroimaging techniques, there is a better understanding of the neuroanatomical substrates involved in craving and its role in relapse. Finally the last 3 criteria involve occupational, social and health consequences and can be further measured via interviews with the individual and family with the aid of structured questionnaires.

Despite the fact that addiction is a classified disorder, the stigma associated with substance use and dependence often prevent the individual from seeking treatment. A WHO study of attitudes of 18 disabilities in 14 countries found that "substance-addiction" ranked at or near the top in terms of social disapproval or stigma (Room 2001) The developments made in neuroscience however, have now clarified that drug addiction is a chronically relapsing disorder requiring treatment. Within context, the DSM IV criteria can serve as a very important tool in psychiatry, as it presents a disease classification within a medical framework for the physician and patient to work with, devoid of aspects of social stigma.

1.2 THEORETICAL MODEL OF ADDICTION

The disease of addiction is embedded in highly complex sociological and psychological contexts. Not only does acute drug use modify brain functions in some critical ways but prolonged drug use causes pervasive changes in the brain, that persist long after the individual has stopped using. The transition that takes place from controlled use to compulsive drug use is accompanied by several drug-induced changes in the brain, in addition to changes in psychological functions (Robinson and Berridge 2000). Advances in neuroscience have now made it possible to evaluate the effects of chronic drug use and dependence on many levels: molecular, cellular, functional and structural (Hyman 1996; Nestler, Berhow et al. 1996). The findings from these studies have

clearly highlighted that the addicted brain is fundamentally different from the "normal" brain as manifested by changes in e.g., brain metabolic activity, receptor availability and responsiveness to environmental cues.

The knowledge that addiction is fundamentally tied to changes in brain structure and function justify the use of a biomedical model in developing treatment. The main tenants of this theoretical model of addiction are (a) addiction is a medical disorder, similar to cardiovascular diseases or diabetes (Fig1), (b) it's a chronic disease with a relapsing nature, (c) there is a biological predisposition towards addiction, (d) environmental factors can have a strong mediating role between existing biological vulnerability and the exposure to the drug and (e) the vulnerability to the effects of the disease is expressed in the form of loss of control. Viewing addiction as a chronic relapsing disorder means that a good treatment outcome and perhaps the most reasonable expectation, is a significant decrease in drug use with sustained periods of abstinence with occasional relapses. In other words, a realistic expectation of a standard for treatment success is the management of the illness, not a cure (O'Brien and McLellan 1996).

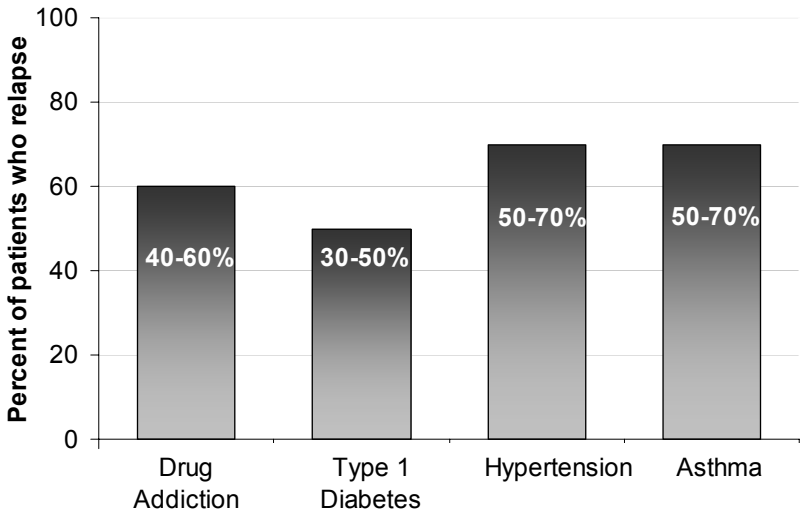


Figure 1.
Relapse rates for drug addicted are compared with those suffering from diabetes, hypertension and asthma. Relapses are common and similar across these illnesses. *Source: McLellan et al., JAMA, 284:1689-1695, 2000*

The studies in the present thesis are based on the premises of this model and consequently the recruitment of patients, outcome measures and assessments has focused on: (a) chronic amphetamine abusers displaying motivation to quit or reduce drug consumption, (b) use of systematic assessments to elucidate the factors that lead to relapse, such as craving (drug-induced and cue-induced), (c) combining pharmacotherapy and psychotherapy as the model treatment to address and treat both biological, psychological and sociological aspects of the disease (d) the end point of treatment being amphetamine-abstinence and (e) to finally gain some insight into the feasibility of treating this rather difficult patient population.

1.3 AMPHETAMINES

1.3.1 Historical perspective on amphetamine abuse

The use of stimulant compounds dates back in history. Chinese physicians have been using the drug Ma-Huang for more than 5000 years. The active agent in Ma-Huang was found to be ephedrine, which was used for its ability to dilate bronchial passages and for symptomatic relief of asthma. As supplies of ephedra became more difficult to obtain, pharmacological companies sought to identify synthetic substitutes. In 1887, amphetamine proper was first synthesized as part of a programme to manufacture aliphatic amines. As early as 1919, a Japanese chemist synthesized methylamphetamine (commonly referred to as methamphetamine, and a more potent version of amphetamine) and in 1927, Gordon Alles a chemist in Los Angeles, suggested that amphetamine could serve as a cheap alternative to ephedrine. It wasn't until 1932 that amphetamine was marketed as Benzedrine, an over-the-counter inhaler to treat nasal congestion (King 1997).By 1939, amphetamine was classified by the Food and Drug Administration (FDA) as a scheduled drug. During World War II amphetamines were administered to American soldiers and pilots to prevent fatigue. It is estimated that over 200 million amphetamine tablets were supplied to the U.S. troops over the course of the war. One of the major factors involved in the increase of illicit use of amphetamine after the war is the continued use by soldiers upon their return. Following the war, the misuse of amphetamine became common concern in a number of countries, notably the USA, Japan and Sweden, thereby increasing the knowledge of the potential dangers of amphetamine. The 1980s however saw a rapid growth of amphetamine use, largely due to decreases in price, increased routes in drug trafficking and an increase in concomitant physical and social problems.

1.3.2 The current amphetamine epidemic

Today, only dextroamphetamine and methylphenidate are available for medical use (for the treatment of attention deficit hyperactivity disorder and narcolepsy). All other amphetamines (e.g., amphetamine sulphate and methamphetamine) are made in illicit/clandestine laboratories. These drugs go by the street names of "speed", "crystal" and "meth".

In the last decade (1996-2006) one of the fastest growing drug problems around the world has been the use and abuse of amphetamine-type stimulants (Rawson and Condon 2007). Globally, 35 million adults are reported to be using amphetamine-type stimulants, this figure is more than the number of heroin and cocaine users combined (UNODC 2005). The only illicit drug that is used more often than amphetamine is cannabis (UNODC 2005). Violence associated with amphetamines is also a major social and health concern. The recent United Nations report (UN 2003) stated that in the past 12 months, 34 million people worldwide have abused amphetamine-type stimulants and this number exceeds the number of cocaine and heroin abusers combined.

In most parts of Europe (including Sweden), amphetamine sulphate was and remains the most common amphetamine derivative used in the drug scene. In contrast, methamphetamine has been dominant in countries such as Japan and the USA. An epidemiological study in Sweden in 2005 reported that amphetamine was identified in about 50-60% of all drunken driving suspects, alone or in combination with other controlled substances (Jones 2005). Further, half of the heavy intravenous (i.v.) drug user's abuse amphetamine, pushing this disorder to the forefront of psychiatric problems in Sweden.

In the face of the current amphetamine epidemic, the lack of effective treatment for amphetamine users has far reaching health ramifications, both in terms of the consequences from continued drug use and from the potential risk of increased HIV transmission. As a result, the development of an effective treatment for amphetamine dependence is a pressing concern and needs to be addressed.

1.3.3 Mechanism of action

Amphetamines are indirect catecholamine agonists and exert its effects by increasing concentrations of dopamine (DA), serotonin (5-HT) and to a lesser extent norepinephrine (NE) via a combination of enhanced release and uptake inhibition (Azzaro and Rutledge 1973). Similar to other psychostimulants, the behavioural correlates associated with amphetamines rewarding effects appear to be mediated principally by DA (Di Chiara and Imperato 1988). That amphetamine mediates its action through elevated DA concentrations has been demonstrated in numerous studies, and may be exemplified by a study in which amphetamine increased extracellular DA levels in the nucleus accumbens (Nacc) of rats 10-fold (Di Chiara and Imperato 1988). Amphetamine also acts both inside and outside the nerve terminal to inhibit the action of monoamine oxidase (MAO), an enzyme normally involved in the breakdown of NE and DA. Inhibition of this enzyme allows the released transmitters to remain active longer, to further exaggerate the action of these transmitters.

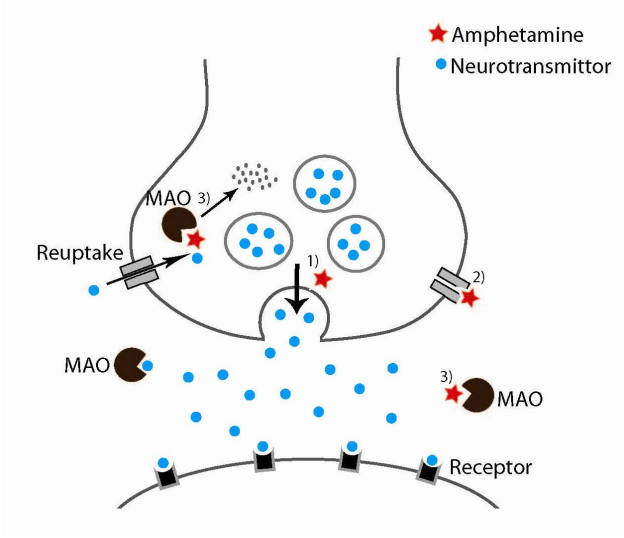


Figure 2. Mechanism of action. Amphetamine enhances the action of endogenous neuro-transmitters by (1) enhanced release of transmitters (2) reduced reuptake and (3) metabolism by MAO

The effects of amphetamine are mediated through the mesolimbic DA system, which projects from the ventral tegmental area (VTA) to the Nacc, with projections to other areas of the limbic system and the orbitofrontal cortex, also known as the reward system (Koob 2000). Evidence that amphetamine is rewarding was initially demonstrated in pre-clinical studies, showing that rats will self-administer

amphetamine under a variety of conditions (Ramsey and van Ree 1991). In humans, stimulant users continue to self-administer amphetamines despite negative consequences (Hart, Ward et al. 2001)

In the last decade there has been growing evidence that long term abuse of amphetamine leads to significant brain changes involving dopaminergic (McCann, Wong et al. 1998) and serotonergic systems (Sekine, Ouchi et al. 2006), glucose metabolism (Volkow, Chang et al. 2001) and neurometabolite levels (Nordahl, Salo et al. 2005). Imaging studies have shown consistent patterns of reduced dopamine D_2 receptors in the striatum of amphetamine abusers (Volkow, Chang et al. 2001) .This low level of D_2 dopamine receptors is associated with a lower level of glucose metabolism in orbitofrontal cortex, suggesting that D_2 receptor–mediated dysregulation of the orbitofrontal cortex could underlie a common mechanism for loss of control and compulsive drug intake in drug addicted subjects. A functional dopamine transporter (DAT) is required to promote DA efflux and to mediate the action of amphetamine (Amara and Sonders 1998).It has been shown that humans with a history of amphetamine abuse have reduced density of DAT (McCann, Wong et al. 1998). The reduction in DAT density is either caused by internalisation (Saunders, Ferrer et al. 2000) or by toxic damage to DA axons as a consequence of long term amphetamine abuse (McCann, Wong et al. 1998).

Taken together, these results confirm that amphetamine dependence is a disease of the brain, emphasising the need for development of a treatment for amphetamine abuse that can shorten the time of active drug abuse and prolong the time of abstinence from drug abuse.

1.3.4 Pharmacokinetics

The pharmacokinetics of amphetamines is similar to those of ephedrine: amphetamines show high bioavailability and long duration of action. Amphetamine is easily absorbed via the gastrointestinal tract and the nasal mucosa and freely passes the blood brain barrier. For a single oral dose of amphetamine consumed, peak plasma levels are achieved in 1 to 3 hours. The biological half-life of different forms of amphetamine varies, e.g., the half-life of dexamphetamine is 10.5 hours, while that of methamphetamine is 4-5 hours (Derlet and Heischober 1990),). Following i.v.

administration, methamphetamine for example, is eliminated with a t ½ of 12 ± 3.2 hours, highlighting the long duration of action and effects of amphetamines.

1.3.5 Metabolism

Some of the amphetamine in the body is eliminated by the liver, while a significant percentage is excreted unchanged (approximately 38%) in the urine (Mendelson, Jones et al. 1995) with an excretion rate that increases with the acidity of the urine. Clinically, amphetamines can be detected in the urine for upto 7 days. This depends on both the dose consumed and the acidity of the urine (if the urine is highly acidic, upto 60% is filtered from the blood and excreted unchanged in the urine). With regard to metabolic and elimination rates, in clinical treatment trials, twice weekly urine toxicology tests are deemed sufficient for amphetamine dependent individuals and three times weekly for cocaine dependent individuals to ensure that all new drug use is detected.

1.3.6 Clinical aspects of amphetamine use

When amphetamine is synthesized, two mirror image molecules are formed, a "d" (*dextro* or "right") form and an "l" (*levo* or "left") form. The "d" form (dextroamphetamine) acts more on the brain while the "l" (levoamphetamine) form acts more on the cardiovascular system. Many chemical modifications of amphetamine have been synthesized, including Methylenedioxiamphetamine (MDA) and Methyenedioximetaamphetamine (MDMA). In Sweden, the racemic compound (containing *levo*- and *dextro*-form in equal amounts) is the most commonly abused amphetamine. Methamphetamine, one of the other derivatives, is significantly more widespread in the United States and United Kingdom.

Amphetamines are usually a whitish powder, also available in the form of tablets and capsules. The dose of amphetamine commonly abused ranges from low to high dose (15 mg-1000 mg) and from infrequent to chronic binge use. The abuse of amphetamine follows two different patterns: 1) Chronic dosing, with periodic self-administration throughout the day, when the drug is taken as often as every 30 minutes, or 2) Multiple dosing for a defined period of time, labelled as "binge" or "run", whereby increasing doses are taken for several days in a row, ending in a "crash" when the abuser sleeps for 2-3 days. The common routes of administration of illicit amphetamine are oral (rolled in paper or in drinks), intravenous or nasal (Cho and Melega 2002). The route of administration is known to influence the rewarding effects of the drug and in majority

of the users there is usually a rapid transition from oral to intravenous use to achieve a faster onset of euphoria. The onset of action for an oral dose of amphetamine is 20-30 minutes (Seigel 1991), while the effects are almost instantaneous for an intravenous dose. The duration and magnitude of its effects are dose-related, and the reinforcing effects are more accentuated in humans when the drug is taken intravenously. With increased dosage and duration of administration, a state of mental delirium can occur. In addition, there is a risk for related medical complications such as cardiac arrest, seizures and stroke. During a high-dose binge episode, individuals can experience amphetamine-induced psychosis, characterized by paranoia, delusions and compulsive behaviors. Withdrawal from amphetamine occurs following cessation of drug use and can produce a wide range of dysphoric symptoms. In general amphetamines are used in combination with marijuana and /or sedating drugs to manage the negative effects. Alcohol abuse and/or dependence may also co-occur but users seldom report concomitant use due to an unpleasant taste associated with the combination.

1.3.7 Effects of amphetamine

Amphetamine is a powerful psychostimulant and even in small doses it can increase wakefulness, attention and physical activity and decrease fatigue and appetite. With regard to its long-term effects, it is important to mention that existing clinical data are confirmed by imaging studies, showing damage to dopaminergic and serotonergic neurons with a concomitant increase in glial cells in subjects with a history of methamphetamine abuse long after they have stopped using (Ernst, Chang et al. 2000).

Table 2. Short and long-term effects of amphetamine abuse.

Short term effects	**Long term effects**
Enhanced mood and body movement	Confusion
Increased wakefulness, physical activity	Paranoia
Increased respiration	Hallucinations, Delirium
Euphoria	Weight loss
Insomnia	Tremors and convulsions
Increased heart rate	Damage to nerve cells, stroke
Increased blood pressure	Cardiovascular collapse, death
Reduced appetite	
Cardiovascular collapse	
Dilated pupils	

1.4 PRINCIPLES IN TREATMENT OF AMPHETAMINE DEPENDENCE

It is evident from the previous sections that amphetamine dependence is a biological disorder affecting brain regions involved in reward and motivation. However an effective pharmacotherapy is yet to be identified for amphetamine dependence. More specifically, a Cochrane review showed that there has been a lack of controlled clinical trials for amphetamine dependence pharmacotherapy (Srisurapanont, Jarusuraisin et al. 2001). The treatment of amphetamine abuse and dependence till date has mainly focused on 2 areas; 1) treatment of overdose, and 2) treatment of withdrawals. It is however clear that detoxification is only the first step at the beginning of treatment and the more critical issue is prevention of relapse. Thus a third and more important area is treatment of chronic amphetamine abuse. The next section provides an overview of the principles involved and the available treatments targeting the three mentioned areas.

1.4.1 Treatment of amphetamine overdose

Clinically the management of acute amphetamine intoxication and its related medical complication is quite distinct from the management of the underlying disorder of abuse and dependence. Cases of acute amphetamine-induced agitation and psychosis usually presents at the emergency with a range of symptoms such as hyperexcitibility, hypervigilance, psychomotor vigilance, delirium and psychosis. Usually the presented symptoms are managed with tranquilizing agents such as benzodiazepines and/or antipsychotic agents such as haloperidol, olanzapine or risperidone. It however remains unclear, whether benzodiazepines or neuroleptics should be preferred. This is an important issue, as the long-term effects of these medications are unknown, especially in cases in which treatment needs to be extended (Ling, Rawson et al. 2006). Most often amphetamine-induced psychosis and paranoia maybe related to the dose and duration of amphetamine administration, but it may also be related to a psychiatric predisposition and then continued treatment with anti-psychotics is justified (King 1997). Acute amphetamine intoxication can also result in more serious medical challenges such as stroke, cardiac arrhythmia, and hyperthermia. Treatment for amphetamine intoxication remains symptomatic and thus far, there have been no controlled clinical trials to document the relative efficacy of any of the medications for this condition (Vocci and Ling 2005). A recent line of enquiry in medication

development has been directed towards the availability of a vaccine to counteract the acute toxic effect of amphetamine, in the case of emergency (Vocci and Ling 2005).

1.4.2 Treatment of amphetamine withdrawal

Amphetamine withdrawal is less studied (e.g., compared to opiates) although it is a common problems with a prevalence rate of 87% among amphetamine users (Cantwell and McBride 1998). Although withdrawal from amphetamines are less dramatic than experienced with alcohol and opioids, its symptoms, particularly that of intense craving maybe a critical factor in relapse, to amphetamine use (King 1997). The abrupt cessation of amphetamine, following binge use leads to the experience of "crash" which often presents clinically as a state of hyperarousal, with symptoms of craving, agitation and vivid dreams (Markou, Kosten et al. 1998). The reversed vegetative symptoms include a cluster of depression-related symptoms including dysphoria, anhedonia, and fatigue, which are usually marked during the first week and then resolve by the end of the acute phase of abstinence (McGregor, Srisurapanont et al. 2005). Although the symptoms occurring during amphetamine-withdrawal may abate in four or five days, some continue for weeks or months. Symptoms prompting consideration for use of medication include sleep-deprivation and agitation, which may respond to short acting benzodiazepines (Ling, Rawson et al. 2006).

Amphetamine withdrawal has been studied in animal models (Stadler, Caul et al. 1999), however a majority of the human studies have either been retrospective in nature (Cantwell and McBride 1998), had too small sample sizes (Watson, Hartmann et al. 1972) or included subjects withdrawing from multiple substances (Gillin, Pulvirenti et al. 1994).

Failure in addressing or in managing amphetamine withdrawal during treatment may contribute to high rates of relapse during the first days or week, post drug cessation (Brecht, von Mayrhauser et al. 2000).As symptoms of depressions are most common during the early phase of abstinence, antidepressants have been one of the first interventions studied in controlled trials. Such a strategy has met with mixed results mainly due to its delayed onset of action and also that stimulants themselves may induce depression. These secondary or drug-induced depressions are then a less clear target for such an intervention (Kosten, Markou et al. 1998). Recent studies have

highlighted the importance of assessing the nature and severity of the withdrawal symptoms at the start of treatment, as the severity of symptoms seem to be correlated with better treatment response (Kampman, Alterman et al. 2001).

1.4.3 Treatment of chronic amphetamine abuse

Till date, much of the clinical progress in the field of addiction has come from improving methods of treating physical dependence and severe withdrawal symptoms (i.e. improved methods of detoxification). As our understanding of the complex nature of addictive disorders has developed, it as become apparent that treatment should however be based on a more chronic disease model. There has been a substantial initiative undertaken by the National Institute of Drug Abuse (NIDA), over the last decade with the intent to stimulate research in the prevention and treatment of amphetamine abuse (Rawson and Condon 2007). Table 3, presents a summary of different medications tested so far in the treatment of amphetamine dependence. Although there is evidence of some promising drugs in the initial phases (Phase I & II), till date none have shown clear efficacy in randomised placebo controlled trials (Phase III) as pharmacotherapy for amphetamine dependence.

Table 3. Overview of medications tested to treat amphetamine dependence (+ = positive finding; – = negative finding.) Importantly, in the four studies listed for positive findings, there was no overall significant effect of medication, only a subgroup analysis was indicative of a positive outcome e.g., in low consumers.

Mechanism	Reference	Medication	Sample size	Result
Dopamine transporter inhibitor	(Elkashef, Rawson et al. 2007)	Bupropion	151	+ (only in a sub group)
	(Tiihonen, Kuoppasalmi et al. 2007)	Methylphenidate	53	+ (secondary finding)
GABAergic	(Brodie, Figueroa et al. 2005)	Gammavinyl-GABA	30	+ (but strong side-effect profile
	Johnson, Roache et al. 2007)	Topiramate		–
	(Heinzerling, Shoptaw et al. 2006)	Baclofen and Gabapentin	340	+ (only for baclofen)
Selective serotonin reuptake inhibitors	(Batki 1999)	Fluoxetine	60	–
	(Shoptaw, Huber et al. 2006)	Sertraline	414	
Calcium channel blockers	(Batki 2001)	Amlodipine	77	–
Tricyclic antidepressant	(Galloway 1996)	Imipramine	32	–
5HT-3 antagonist	(Johnson, Ait-Daoud et al. 2007)	Ondansetron	150	–

1.5 EVALUATION OF A PHARMACOTHERAPY FOR AMPHETAMINE DEPENDENCE

As evident from Table 3, a number of approaches may be considered in treatment of chronic amphetamine dependence: 1) substitution treatment, with the goal of suppressing the negative effect of drug abstinence (withdrawal and craving). For example, methadone treatment for heroin dependence or nicotine replacement therapy for tobacco dependence; 2) an antagonist medication that blocks the site where the drug binds. The rationale being that this will lead to extinction of the drug taking behavior as the drug no longer serves to be rewarding. For example using a dopamine antagonist for amphetamine dependence; 3) a medication that might indirectly antagonize the effects of the drug by acting on other sites; and 4) the use of a medication that targets the secondary clinical symptoms that arise from long term substance use, such as depression.

The studies in this thesis have utilized the third approach, i.e., testing a medication which would indirectly modulate the rewarding effects of amphetamine. The medication under study in this thesis, is naltrexone hydrochloride (Revia®, Du Pont). Although amphetamine does not act directly on the opioid receptors, its effects are influenced by the endogenous opioid activity. In the following section, some background and evidence from pre-clinical and clinical studies is presented to motivate the examination of the opioid antagonist, naltrexone in amphetamine dependence.

1.6 THE OPIOID ANTAGONIST, NALTREXONE AS A POTENTIAL PHARMACOTHERAPY FOR AMPHETAMINE DEPENDENCE

1.6.1 The endogenous opioid system

Opioid receptors are widely distributed throughout the brain and in the peripheral nervous system and have been implicated in many diverse physiological functions, such as endocrine, cognitive, affective, immune and respiratory. These receptors mediate physiological effects of three families of endogenous opioid peptides, namely endorphins, enkephalins and dynorphins. Evidence from behavioral and pharmacological studies have demonstrated the existence of 3 classes of opioid receptors in the central nervous system, the mu (μ) delta (δ) and kappa (κ) opioid receptors (Terenius 1973; Martin, Eades et al. 1976).

These receptors have some selectivity for the endogenous opioid ligands, with μ receptors having highest affinity to β endorphin, δ receptors for enkephalins and κ receptors for dynorphins. Many peptides and alkaloid compounds that show high selectivity for the various opioid receptors have became available in recent years (Herz 1997). In addition, several opioid receptor antagonists have been synthesized. Naltrexone and naloxone are "non-selective" opioid antagonists, in that they have an affinity for all three opioid receptors and in turn block the actions of endogenous opioids as well as morphine-like drugs (Rang HP 2003). Under normal physiological conditions, the GABAergic neurons tonically inhibit the release of DA in the VTA. Blockage of the receptors by opioid antagonists activate the GABAergic neurons. (Gysling and Wang 1983). Treatment with naltrexone thus prevents endogenous opioids from hyperpolarizing GABA neurons leaving the inhibitory influence on DA neurons intact (Schad, Justice et al. 1996). Given alone, opioid antagonists have almost no effect at all, but they rapidly reverse the effects of opioids when given together, a fact underlying the use of naloxone to treat respiratory depression following opioid overdosing (Gysling and Wang 1983).

1.6.2 The endogenous opioid system and addictive behaviors

In the last decade, considerable evidence has accumulated from anatomical, biochemical and behavioral studies suggesting an interrelationship between the opiodergic and dopaminergic systems in the central nervous system. In brain regions known to have a role in appetitive behaviors (such as the VTA and substantia nigra), neurons containing opioids and dopamine are known to co-exist (Moore and Bloom 1978; Johnson, Sar et al. 1980) and opioid receptors are located on dopamine neurons (Llorens-Cortes, Pollard et al. 1979). An increase in the extracellular concentration of dopamine has been observed in the Nacc, following administration of agonists selective for μ, and δ opioid receptor subtypes (Di Chiara and Imperato 1988). This opioid-induced release of DA appears to be secondary to an inhibition of GABAergic interneurons (Koob 1992). Conversely k-opioid receptor agonists decrease the amount of dopamine released into the synapse (Di Chiara and Imperato 1988). All of these opioid receptor-mediated effects on dopamine release can be blocked by the specific opioid receptor antagonist, naloxone or naltrexone(Di Chiara and Imperato 1988; Spanagel, Herz et al. 1990).

As mentioned before, although the reinforcement mechanism differs between drugs, DA release in the NAcc plays a central role in the reinforcement exerted by most drugs of abuse. In addition to the critical role of DA, the endogenous opioids also appear to be modulated by some of the drugs that act via this system. For example, both alcohol and opioids appear to induce dopamine release in the Nacc via activation of the μ-opioid receptors in the VTA (Tomkins and Sellers 2001). The opioid antagonist, naltrexone may interfere with this process by blocking dopamine release and thereby reducing the reinforcing effects of alcohol. In addition to alcohol, the μ-opioid receptor is also involved in the reinforcing effects of heroin (Greenstein, O'Brien et al. 1981), nicotine (Houdi, Pierzchala et al. 1991) and cocaine (Corrigall and Coen 1991). Interestingly, alcohol and cocaine self-administration is reduced in mu-opioid knock out mice (Roberts, McDonald et al. 2000; Becker, Grecksch et al. 2002).

1.6.3 Interaction between opioid antagonists and stimulants

Naltrexone was initially investigated more than 30 years ago for its utility in treating heroin subsequent to which it received FDA approval in 1984 for the treatment of heroin dependence. In the 1970s, preclinical data emerged demonstrating that opioid antagonists such as naltrexone blocked the ethanol induced dopamine release in the brain reward pathway (Harris and Erickson 1979; Altshuler, Phillips et al. 1980). Further naloxone reduced alcohol preference in alcohol dependent rats (Marfaing-Jallat, Miceli et al. 1983) and reduced drinking in rats selectively bred for high alcohol preference (Froehlich, Harts et al. 1990). The results from the animal studies led to the first open dose ranging studies of naltrexone in alcoholic patients, followed by a placebo-controlled trial (Volpicelli 1990; Volpicelli, Alterman et al. 1992). The results of these studies were consistent with the pre-clinical findings, in that naltrexone reduced rates of relapse to heavy drinking compared to placebo, with minimal side effects. These two pivotal clinical trials provided the basis for the Food and Drug Administration (FDA) approval of oral naltrexone in 1994 for the treatment of alcohol dependence in the United States. Naltrexone has also been approved as treatment for alcohol dependence in Australia, Canada and a number of European countries, including Sweden.

Several studies in both humans and animals have also investigated whether naltrexone or naloxone can reduce the abuse and dependence of cocaine. In a human

study with cocaine abusers, it was found that chronic treatment with naltrexone reduced euphoria and the "crash" from an intravenous injection (Kosten, Silverman et al. 1992). In a more recent study, 50mg naltrexone combined with relapse prevention therapy significantly reduced cocaine use in cocaine dependent patients, as measured by urine samples (Schmitz, Stotts et al. 2001). In addition to this, Oslin et al. (1999) showed that naltrexone reduced the use of alcohol and cocaine in patients diagnosed with both cocaine and alcohol dependence. These findings collectively suggest that the endogenous opioid system maybe involved in certain aspects of cocaine addiction. Results from animal studies in which the effect of opioid blockade on cocaine self-administration was studied, seem to confirm such an involvement (Mello and Negus 1996).

With respect to amphetamine, its rewarding action like most other drugs of abuse is via the mesocorticolimbic DA-system, and evidence from several studies also point to the involvement of endogenous opioid systems. For example, animal studies have shown that opioid antagonists reduce some amphetamine-related effects. Pre-treatment with naloxone attenuated both neurochemical effects and the locomotor activity of amphetamine in rats and mice (Dettmar, Cowan et al. 1978; Schad, Justice et al. 1995). Ambulation and rearing in rats are behaviours associated with activation of DA systems. These behaviours that are induced by amphetamine were decreased by treatment with naloxone and naltrexone, a fact that provides additional evidence that opioid antagonists affect DA systems. Furthermore naloxone attenuated the dopaminergic response to amphetamine, indicating that amphetamine is less reinforcing after opioid blockade (Hooks, Jones et al. 1992)

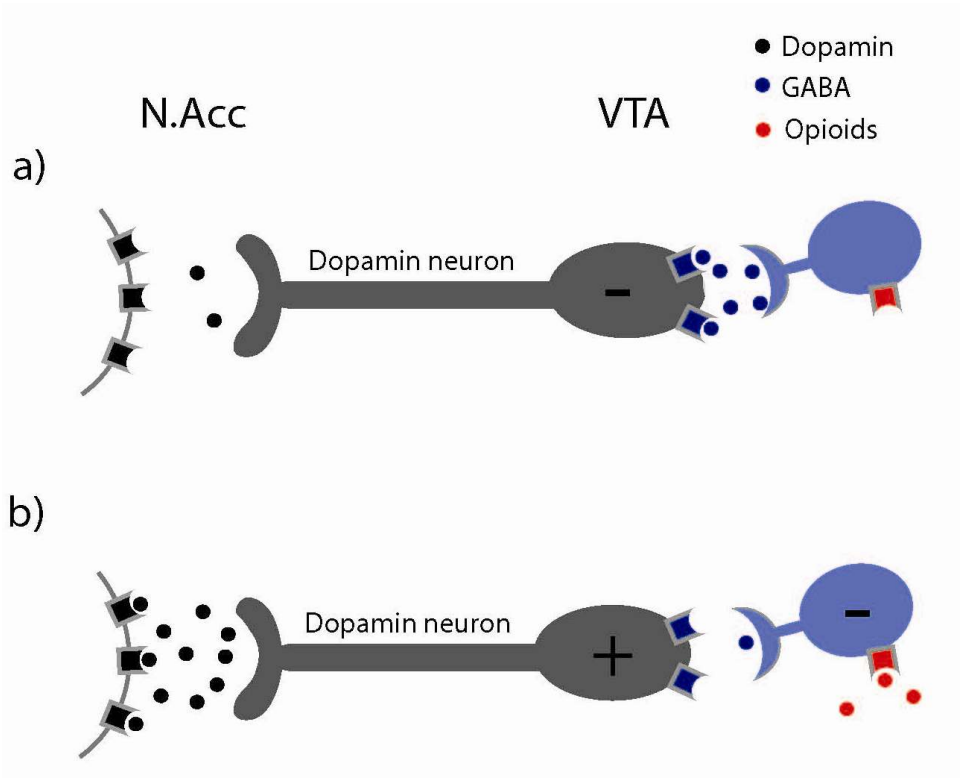


Figure 3. Interaction between the DA and opioid systems. The opioid and GABA systems exert a modulatory effect on the DA system. a) Normal physiological condition where the GABAergic neurons tonically inhibit release of DA in the VTA, and b) Activation of μ and δ receptors results in decreased GABA release and a subsequent disinhibition of the DA system, leading to a surge of DA release in the Nacc. The blockade of opioid receptors by naltrexone in turn results in the opposite action i.e., an increased release of GABA which in turn decreases the activity of the DA neurons.

1.6.4 Pharmacology of naltrexone

The pharmacology of a compound plays a central role in determining its efficacy as a treatment agent. An ideal pharmacotherapeutic agent for a difficult to treat population, such as substance dependent individuals would be one that is orally effective and has a long duration of action (Kreek, LaForge et al. 2002). Based on this, it is important to consider the pharmacokinetics of naltrexone to assess its suitability in amphetamine dependent individuals.

When taken orally, naltrexone is quickly absorbed and undergoes first-pass metabolism in the cytosol system in the liver. Naltrexone is then converted to several metabolites. The major metabolite is 6-β-naltrexol, which by itself is also known to reduce alcohol drinking in rats (Stromberg MF 2002) . Two other minor metabolites do not appear to contribute to naltrexone's pharmacological activity or toxicity, 2 hydroxy-3methoxy-6-β-naltrexol and 2-hydroxy-3-methyl-naltrexone. The mean

serum elimination half-life after chronic administration of a 50 mg daily dose is 9.7 hours for naltrexone and 11.4 hours for 6-β-naltrexol and its effects are evident upto 48 hours (Davidson, Emm et al. 1996; Ferrari, Bertolotti et al. 1998). In contrast, PET studies using C^{11} carfentanil show significant blocking of brain μ receptors for more than 72 hours after a single 50 mg dose (Lee, Wagner et al. 1988). This long duration of action of naltrexone might be desirable from a pharmacotherapeutic perspective of preventing acquisition or relapse (that is, not requiring daily treatment).

The evaluation of naltrexone´s pharmacology is also important to consider in the design of a trial, to investigate whether tolerance to naltrexone may develop after long-term intermittent administration. In human studies (O'Malley 1999; Heinala, Alho et al. 2001) there have been no reports of tolerance to the effects of naltrexone on alcohol drinking (with the exception of treatment of heroin dependent individuals). Importantly, there appeared to be an advantage of long-term naltrexone treatment (1 year) on days to relapse, compared to short term treatment.

1.6.5 Safety Profile

Nausea and vomiting are the most common side effects reported with naltrexone. Headache, anxiety and rashes are some of the less common side effects noted. These side effects resolve spontaneously after a few doses. There have been reports of dysphoria in patients with opiate addiction and also in a study with healthy volunteers (Mendelson, Ellingboe et al. 1980; Crowley, Wagner et al. 1985). This could be indicative of a mild opioid “withdrawal-like” reaction that occurs when naltrexone blocks opiate receptors that were in an activated condition, either due to stress or substance intake. Data on adverse events of naltrexone have been mixed. The discrepancy maybe due to a number of factors: 1) type of drug administered (e.g., opiates versus alcohol), 2) patient population (abstinent participants vs. active users), 3) design of the study (outpatient study vs. controlled laboratory study) and 3) time course of data collection (immediate vs. retrospective). Importantly, there have been no reports of hepatotoxicity at the recommended daily dosage of 50 mg. Lastly, DuPont Pharmaceuticals conducted a large safety study with naltrexone for alcohol dependence and concluded that naltrexone was safe under a variety of conditions (Croop, Faulkner et al. 1997).

1.6.6 Adherence Profile

Adherence to medication is a universal phenomenon in treating chronic medical illness. The most common stated reason for lack of adherence is side effects. However many people have a natural reluctance with regard to having to take a medication on a daily basis. In addition, unlike medications for other psychiatric problems, naltrexone does neither alleviate distressful psychiatric symptoms nor does it provide a sense of well-being. This could contribute to patient's resistance to taking medication as the effects are not clearly discernable in the absence of drug consumption**.** A strategy in addiction treatment studies to improve adherence to treatment, is the utilization of psychotherapy (more details in section 1.8).

1.7 HUMAN TESTING PARADIGMS IN ADDICTION RESEARCH

1.7.1 Human laboratory studies

Human laboratory studies in which stimulants are administered to healthy subjects have been a critical paradigm for testing potential pharmacotherapies for stimulant dependence (Fischman, Schuster et al. 1976; Fischman and Johanson 1998). Depending on the study, the design can vary from either presentation of cues or administration of a modest dose of the drug (referred to as priming dose) to assess subjective effects, craving and probability of relapse. The modulation craving (cue-induced and drug-induced), by a pharmacological agent, can provide insight into the mechanism of action of the agent and its role in reducing relapse (Jaffe, Cascella et al. 1989). The underlying hypothesis of such a study paradigm is that by blocking the positive effects of the drug, a "slip" (e g, a single occasion of use) would not lead to a full relapse. Another commonly used model in human laboratory studies has been the self-administration paradigm. In this model, subjects can self-administer drugs repeatedly, dictated by medical safety conditions. The subject is offered the alternative of getting the drug or a monetary value; thereby the behavior of drug taking can be clearly approximated and the effect of medication can be detected on a range of behaviors (e.g., when the break point occurs).

Two studies in the thesis (Jayaram-Lindstrom, Wennberg et al. 2004; Jayaram-Lindstrom, Konstenius et al. 2007) are based on the laboratory model, in which a priming dose is administered by the researcher, the former study using healthy subjects and the latter, amphetamine dependent individuals. By utilizing this paradigm in two different populations it has been possible to evaluate the potential

effect of naltrexone not only on surrogate efficacy variables but also to obtain medical safety data. Furthermore, the model provided valuable information on the probability of drug taking behavior (via assessments, self reports and urine toxicology) and also on the probable mechanism by which the medication may reduce amphetamine use, e.g., whether it blocks the euphoric effects or increases dysphoria. The human laboratory models also enable the gathering of a range of subjective data (e.g., drug effects, mood states and craving) which is important in assessing the interaction between the medication and the abused drug.

1.7.2 Outpatient Randomized Clinical Trials

Outpatient clinical trials remain the gold standard in assessing the efficacy of a medication. Clinical trials in addiction require some specific considerations with regard to outcome measures. Chief among them, is urine toxicology, which is the most informative outcome measure that can be analyzed both qualitatively and quantitatively. For studies in stimulant abusers, urine samples are typically collected 2-3 times a week for maximum sensitivity to repeated stimulant use. With respect to amphetamines, analyses are most frequently done with a cut off score of 500ng/ML with a level above this being considered, as indication of amphetamine use. Verification analyses are commonly performed using either gas chromatography-mass spectroscopy for quantitation, or immunoassay for semiquantitation. This data is usually combined with self-reported use of drugs to provide either confirmatory data or to estimate new drug use, as a heavy user may stop using for 2-3 days and yet have a positive urine test. Although abstinence is the goal in a majority of treatment studies, the self-reported use of drugs helps to differentiate between a “slip” and relapse to binge use. According to the new guidelines for treatment trials by National Institute of Drug Abuse (NIDA), a relapse is defined as a return to the baseline level of drug use. This is an important development in addiction treatment studies, as the primary outcome measure is commonly relapse to drug use and if not clearly defined, patients who experience a slip (single occasion of drug use) also get excluded from the analysis thereby increasing the risk of a negative finding.

Another important consideration in outpatient addiction treatment trials is, retention in treatment. To evaluate the goal of abstinence initiation or maintenance, retention to treatment is critical to be able to estimate the effects of the interventions made, during the course of treatment. One of the methods to increase retention in outpatient trials

has been the use of psychotherapy that often serves to enhance the changes initiated by the medical treatment. Two well documented psychotherapies (more details in section 1.8) in addiction treatment are contingency management and relapse prevention therapy (Ling, Rawson et al. 2006).

Compliance to medication is a common issue in all medication trials. This is usually related to either a) the side effect profile of the medication and b) the resistance by the patients to follow a regular medication regime. In addiction trials, compliance to medication can be enhanced by using newer formulations of the medication (e.g., slow release and depot injections). Lastly, in evaluating the efficacy of a medication it is important to consider the outcome measures sensitive to the medication's proposed mechanism of action. For example in a stimulant dependent population, one obvious treatment outcome measure would be abstinence, but the medication under investigation might have a better indication to reduce "heavy use". The use of the wrong outcome measure could result in a negative finding and clinically vital information would be lost. Two studies in this thesis are based on outpatient clinical trials (Jayaram-Lindstrom, Wennberg et al. 2005; Jayaram-Lindstrom 2007). The former was an open label trial while the latter a randomized double-blind placebo controlled trial (RCT). In the early 90's, open trials were considered the standard first step in the evaluation of new pharmacotherapy's in clinical medicine with the goal of providing important medical safety data (Meyer 1992). However, such a design has its inherent limitations and these are presented in more detail in the "Results" and "Discussion" section (paper III) of this thesis.

1.8 BEHAVIORAL TREATMENT FOR AMPHETAMINE DEPENDENCE

Because of the limited efficacy of existing pharmacotherapies, the success of psychotherapies is important to consider. Two major approaches have been commonly used in addiction treatment studies. 1) Cognitive behavior therapy (with a focus on relapse prevention) and 2) Contingency management. A brief description of each type is provided:

1) Cognitive behavior therapy (CBT)

   The main goal of this method is to assist the individual in gaining awareness of faulty and/or limiting behaviors and thought patterns and replacing them

with positive behaviors and thoughts. This form of therapy has been the most frequently evaluated approach for the treatment of substance abuse disorder and have a strong level of empirical support. The data suggest that CBT may hold particular promise in reducing the severity of relapses when they occur and in enhancing the durability of treatment effects. (Maude-Griffin, Hohenstein et al. 1998). Manual based CBT with a focus on relapse prevention therapy was utilized in the 2 chronic treatment studies of naltrexone (Jayaram-Lindstrom, Wennberg et al. 2005; Jayaram-Lindstrom 2007). Although the efficacy of psychotherapy cannot be evaluated in these studies due to lack of control condition (i.e. all patients received therapy), it could be speculated that psychotherapy could have contributed to the above average treatment retention and adherence rates observed in those studies.

2) Contingency Management procedures (CM)

The main goal of this method is to decrease behaviors maintained by drug reinforcers and increase behaviors maintained by nondrug reinforcers. Recent data from the NIDA clinical trials network (Roll, Petry et al. 2006) have reported that when a CM procedure was added to standard counseling approach for methamphetamine dependent individuals, it led to fewer positive urine samples to that group compared to the counseling-only group. A recent review of CM has reported that this approach also has a modest effect size in the substance dependent population (Griffith, Rowan-Szal et al. 2000).

2 GENERAL AIMS

Several lines of evidence point towards the involvement of the endogenous opioid system in the pathophysiology of stimulant addiction. The opioid antagonist, naltrexone has shown to modulate some of the behavioral and neurochemical effects of amphetamine in animal models. The aim of this thesis is to investigate the effects of acute and chronic dose of naltrexone, with the goal of evaluating its potential as pharmacotherapeutic agent in the treatment of amphetamine dependence.

Specific aims of the study

1. To investigate the interaction effects of an acute dose of naltrexone and amphetamine, in drug-naïve individuals, using a double blind placebo controlled trial design.

2. To investigate the interaction effects of an acute dose of naltrexone and amphetamine, in amphetamine dependent patients using double blind placebo controlled trial design.

3. To investigate the effects of chronic treatment with naltrexone in a 12-week open label clinical trial in amphetamine dependent patients, to obtain an estimate of compliance and medical safety data.

4. To investigate the effects of chronic treatment with naltrexone on the relapse to amphetamine use, in patients with amphetamine dependence, in a randomized placebo controlled trial.

3 MATERIALS AND METHODS

Table 4. Overview of four studies in the thesis

	Healthy volunteers	Amphetamine Dependent	No:of participants
Study I	X		12
Study II		X	20
Study III		X	20
Study IV		X	80

3.1 STUDY I

3.1.1 Subjects

Twelve males and seven female healthy volunteers between the ages of 20 and 45 years were recruited for the study from the Karolinska Institutet and surrounding community via advertisements. Subjects who had no history of substance abuse or dependence were selected for the study. Subjects were excluded from participation if they (1) had a diagnosis of major Axis-1 psychiatric disorder including any history of substance abuse or dependence in self and family, (2) had a serious medical condition such as history of cardiac or liver disease, (3) used nicotine more than once a week, (4) consumed more than 48 grams (women) or 60 grams (men) of pure alcohol per week, (5) had a positive result on alcohol breathalyzer on the test days, (6) showed traces of opiates, cannabis, amphetamines or benzodiazepines in the urine, (7) were pregnant or lactating, or (8) had a known allergy to naltrexone.

3.1.2 Procedure

The study was double blind and placebo controlled, comprising four possible combinations of naltrexone/placebo with dexamphetamine/placebo (medication details, section 3.5). There was an interval of 7 days between each session. The subjects received instruction to abstain from alcohol and nicotine for a 24-hour period and also from caffeine for 2 hours before testing. Subjects were allowed to proceed with the testing only if they recorded no measurable amounts of alcohol in their breath and illicit drugs could not be detected in the urine.

On the test days, the subjects arrived at 08:00 h having eaten a light breakfast approximately 2 hours prior to their arrival. Standardized batteries of tests were administered at scheduled time-intervals, post ingestion of study medication. These consisted of subjective, physiological and behavioral measures. All participants received debriefing at the end of each day to discuss any questions or experiences related to the session.

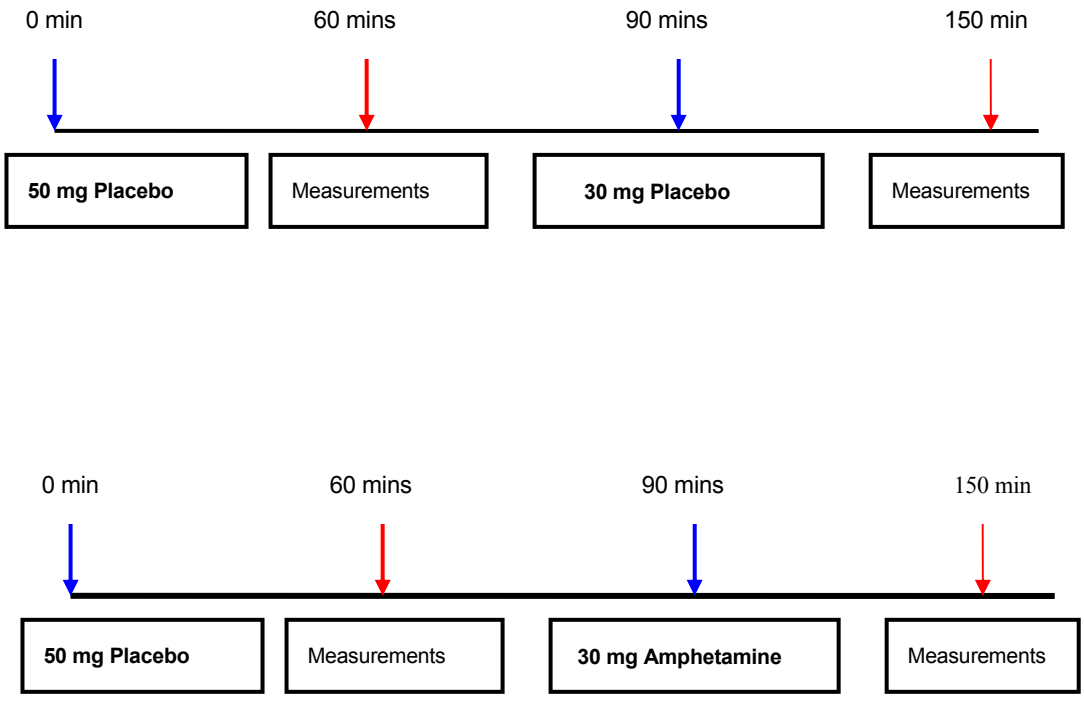


Figure 4. A schematic representation of the study procedure. Participants received a combination of naltrexone/placebo with dexamphetamine/placebo, on 4 test occasions. During each test day, a number of measurements were made at different time points

3.1.3 Measurements (also applicable to study II)

3.1.3.1 Subjective measures

The subjective effects were measured using Visual Analogue Scales (VAS). The VAS comprised of four questions measuring the subjective "high" produced by amphetamine (Johanson CE 1980). The subjects were asked to describe the current drug effects by marking on a 100-mm line. The subjects marked the VAS one hour after ingesting each of the study compounds (i.e. placebo, naltrexone or dexamphetamine) and continued to rate their experience over a period of 7 hours. The shortened version the Profile of Mood scale (POMS) was used to assess the general mood-state of the individuals (McNair DM, 1971). The subjects indicated the extent to which the various adjectives matched their current mood on a four-point scale, 60 min post ingestion of the study compounds.

3.1.3.2 Behavioural measures

Speech and the speed of reading were monitored and recorded by a stationary camera over a 3-minute period, 60 minutes after ingesting the study compounds.

3.1.3.2 Physiological measures

Heart and blood pressure were recorded manually. Sweat production was measured using the Galvanic Skin Response (GSR) via electrodes connected to the fingertips of the subjects. All physiological measures were also recorded 60 minutes after ingesting the study compounds.

3.1.4 Statistical analysis

The primary goal of the study was to investigate if naltrexone would attenuate the subjective effects of amphetamine as measured by the VAS. The primary measure of the study was defined as the mean score of the four VAS items. Further, in order to calculate a change from baseline, the mean score of subjective high during four time-points minus the baseline score was calculated. This score was compared between the four conditions with a repeated measure ANOVA with LSD post hoc test. The secondary measures (speed of reading, eye-blinks, POMS, GSR, heart rate and pulse) were also analysed by comparing the change scores (from baseline to one hour after the last medication) over the four conditions with repeated measure ANOVA and LSD post hoc tests.

3.2 STUDY II

3.2.1 Subjects

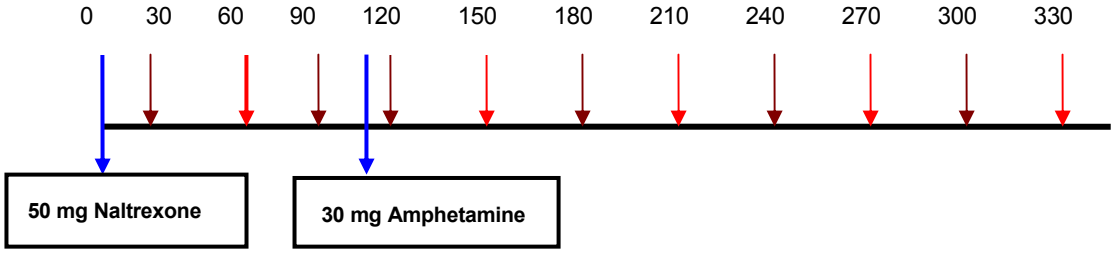
Twenty abstinent amphetamine dependent patients between the ages of 20-45years were recruited for the study from the outpatient substance dependence clinic (The Magnus Huss Policlinic) at the Karolinska University Hospital, Stockholm. As this was an outpatient study, for ethical reasons the patients recruited were from a larger pool of currently drug-free amphetamine dependent patients awaiting psychostimulant treatment for their attention deficit hyperactivity disorder (ADHD) diagnosis. The inclusion criteria for the study were 1) Males; 2) DSM IV criteria for amphetamine dependence; 3) DSM IV criteria for ADHD; 4) drug-free from amphetamine for a minimum of 30 days; 5) residence in Stockholm county. The exclusion criteria were 1) dependence on any substance other than amphetamine and nicotine; 2) any other major psychiatric diagnosis (other than ADHD and amphetamine dependence); 3) testing positive on urine toxicology on the morning of testing and between the test days.

3.2.2 Procedure

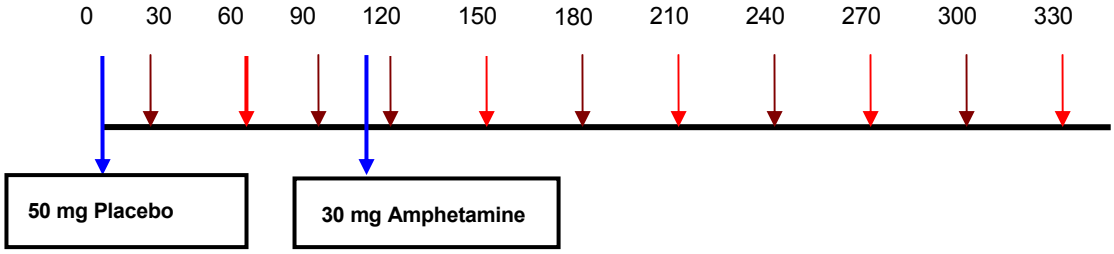
The study was a double-blind placebo-controlled cross-over design. Prior to starting the session, breath alcohol levels were assessed and supervised urine samples were collected to verify abstinence from commonly abused drugs. In event of relapse the patients were considered drop-outs (as measured by self- reports and urine toxicology) and referred back to the clinic for treatment. The study protocol allowed for drop-outs to be replaced, to meet the total sample size of 20.

On arrival to the clinic, patients received a standardized breakfast following which a venous catheter was inserted in their left arm to draw blood at regular time intervals during the day. Patients were also provided with a standardized lunch. Adverse events (AE) were monitored systematically during the test days and also on the visits between the test days, by the study physician. All patients underwent 2-3 urine toxicology tests between the two test sessions to monitor any drug use (i.e., in a span of one week). Patients received debriefing at the end of each test day to discuss questions and experiences related to the testing.

Day 1: The scale represents the time points of administration of test batteries and study medication



Day 2: Scale represents the time points of administration of test batteries and study medication



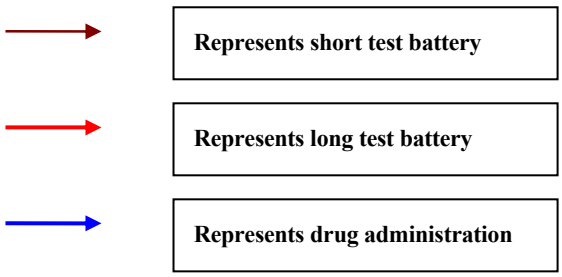


Figure 5. Schematic representation of the study procedure.

3.2.3 Measurements

3.2.3.1 Subjective and physiological measurements (similar to Study I, in addition testing were done at more time points and the subjective tests batteries were divided into short (VAS scale and blood pressure only) and long battery(all tests) in Study II.

3.2.3.2 Biological samples

Measurements of plasma cortisol were obtained at baseline (prior to ingestion of placebo/naltrexone) and then at scheduled intervals (-120, -60, 0, +15+ 30, +45, +60, +90, +120, +150, +180, +210).The samples were collected in heparin tubes and stored on ice immediately. They were then centrifuged at 4 °C and the serum was transferred to a microtube and stored at -20 °C until assayed. Plasma cortisol concentrations were measured by standard radioimmunoassay at the clinical chemistry laboratory, Karolinska University Hospital. Plasma samples (2mL) were separated and collected to perform the pharmacokinetic analysis. Dexamphetamine was quantified in plasma using electrospray liquid chromatography-mass spectrometry (Agilent 1100 LC-MS) using amphetamine-D5 as an internal standard. A QC sample containing 25 ng/ml of amphetamine in blank plasma was analysed together with the study samples.

3.2.3.3 Pharmacokinetic evaluation

The pharmacokinetics of dexamphetamine was evaluated by compartment analysis. Initial estimates were obtained from the JANA stripping program (Dunne 1985). The final estimates of the pharmacokinetic parameters were obtained from the PC-NONLIN program (The American Statistican, 1986). The reciprocal of measured plasma concentrations was used as weights in the iterative procedure. The data was fitted to a one-compartment model with a zero or first order absorption phase (10 and 14 cases, respectively).The optimal pharmacokinetic models were established by visual inspection of the fitted plasma concentration time curves and from the weighted squared residuals using the F-ratio test (Boxenbaum HG 1974).

3.2.4 Statistical analysis

The primary hypothesis of the study was that naltrexone attenuates the subjective effect of amphetamine in patients diagnosed with amphetamine dependence. The primary

outcome measure was the difference in subjective measures of amphetamine effects. This was operationally defined as the composite score of the four VAS scales for the various time points, during each test day, comparing naltrexone versus placebo. The primary outcome measure was analyzed using repeated measures of ANOVA.
The score of the Craving for Amphetamine Scale and POMS were analyzed in a similar manner, where the aggregate scores of the scales were calculated for the various time points, compared between the two treatment groups, using repeated measures of ANOVA.The secondary outcome measure (heart rate, pulse, GSR) was the difference in physiological measures of amphetamine effects. This was computed by calculating composite scores, for the various time points during the test dates, comparing naltrexone versus placebo condition. The secondary outcome measures were analyzed using repeated measure of ANOVA. All values are expressed as the mean ± SD.

3.3 STUDY III

3.3.1 Subjects (also applicable to study IV)

A total of 100 treatment-seeking amphetamine dependent individuals (study III & IV) between 20-65 years of age were recruited from the Stockholm metropolitan area via advertisements in local newspapers and written information to social workers.

All eligible patients had to fulfill the DSM IV criteria for amphetamine dependence and have used amphetamine for a minimum of 12 days in the last 12 weeks. Exclusion criteria included the following: 1) current DSM IV diagnosis of any other substance dependence syndromes except nicotine, 2) history of major psychiatric disorder (e.g., schizophrenia or psychosis) or current psychiatric condition requiring medication,
3) use of any opioid medication or illicit opiates in the last month, 4) current use of benzodiazepines, 5) traces of any illicit substance in the urine (amphetamine, benzodiazepines, cannabis, cocaine, dextropropoxyphen, opiates), 6) serious somatic disease (e.g., seizure disorder, glaucoma, arteriosclerosis, hyperthyroidism),
7) aspartate aminotrasferase (AST) or alanine aminotransferase (ALT) activity greater than three times the upper reference range, or serum bilirubin concentration greater than twice the upper reference range, and 8) pregnant or lactating women.

3.3.2 Procedure

Twenty amphetamine dependent patients were recruited for a 12-week open label trial of naltrexone. The research nurse dispensed medication on a weekly basis, in blister containing 7 tablets of naltrexone 50mg. In addition, patients received a thirty minute session of relapse prevention therapy. Details of the study procedure were as follows:

Table 4. Study design over 12 weeks of treatment. (Schematic representation of study procedure also applicable to study IV)

Measurements	**Screening**	**Inclusion Week 0**	**Treatment Week 1-12**
ASI interview		X	X (week 12)
DSM-IV interview	X		
Time-LineFollow-Back (TLFB)	X	X	X
Physical examination	X		X
Blood samples (w 4, 8 & 12)	X		X
Urine toxicology test (2 times a week for w1-12 and once a week for w13-24)	X	X	X
Medication (naltrexone 50mg (once weekly in blister of 7 tablets)			X
Relapse prevention therapy (once a week)			X
Assessments:			
a) Craving scale		X	X
b) Adverse events		X	X

3.3.3 Measurements

The amphetamine consumption pattern was assessed by using the Time Line Follow-Back interview (TLFB) at baseline and on a weekly basis during treatment, to determine both quantity and frequency of use (Sobell L. C. & Sobell 1992). Weekly assessments included urine toxicology and craving for amphetamine (using the VAS scale). In addition tolerability of treatment was assessed by 1) monitoring adverse events on a weekly basis and 2) analysing laboratory tests (AST, ALT, GT and serum bilirubin).

Compliance to treatment was assessed by 1) patients self-report of medication use, 2) presence of 6-β-naltrexol, a metabolite of naltrexone, in the urine (9 µg/ml was set as a limit for detection of 6-β-naltrexol in the urine), and 3) total number of treatment days attended (9/12 weeks).

3.3.4 Statistical analysis:

Initially the sample was described with regards to basic demographics and drug consumption patterns prior to treatment. Treatment outcome was expressed in terms of amphetamine use during the 12-week treatment period as compared to 12-week period prior to treatment. To compare study completers to noncompleters, the variables were analysed by Students *t*-test and analysis of variance (ANOVA). Compliance was defined by attending 9/12 of treatment. Tolerability was described by self-reports and physician's ratings of adverse events and from liver markers such as ALT, AST and GGT.

3.4 STUDY IV

3.4.1 Procedure

This was a randomized, double-blind, single-site placebo-controlled trial of naltrexone for amphetamine dependence. All eligible patients had a 2 week lead-in period to assist with and confirm their current drug free state. Post the lead-in period, 80 patients were randomized to either placebo or naltrexone treatment for 12 weeks. The randomization process was conducted by the Karolinska University Hospital pharmacy.

3.4.2 Measurements

At intake each patient underwent a physical examination, urine and blood analysis along with the Structured Clinical Interview for DSM IV diagnosis. Current symptoms of ADHD were assessed at baseline using a check list of DSM IV criteria. The

Addiction Severity Index (ASI) was administered pre (week 0) and AT post treatment (McLellan, Kushner et al. 1992).

Weekly assessments included, self-reports of drug consumption using TLFB and craving for amphetamine (instruments used were similar to study III). Adverse effects monitoring was carried out by both the physician (interview) and by the patient (self-rating of intensity and duration) using a standardized form. At weeks 4, 8 and 12, liver enzymes, bilirubin and hematological markers were measured.

Urine samples were screened for amphetamines by an immunoassay method with a cutoff level of 500 ng/ML. Urine samples were screened for amphetamines by an immunoassay method with a cutoff level of 500 ng/ML. The confirmation analyses of the positive samples comprised amphetamine, MDA and MDMA (reporting limit 300 ng/mL) and were performed using a liquid chromatography-tandem mass spectrometry (M. Andersson 2007).

3.4.3 Statistical analysis

The primary outcome measure of the study was abstinence from amphetamine use, as measured by negative amphetamine urine samples during 12 weeks of treatment (max 24 samples). All missing urine samples were imputed as positive in the analysis. The primary analysis was carried out according to the Intention-To-Treat (ITT) approach. Treatment efficacy was analyzed by repeated measures of ANOVA, comparing naltrexone and placebo treated patients over 12 weeks. A secondary completer analysis on the primary outcome measure was conducted using the same statistical method (a completer was defined, as per protocol as a patient who provided at least 16 of the total of 24 urine samples). Rates of continuous abstinence from amphetamine were computed by a Kaplan-Meier analysis, where the time-dependent survival (non-relapse as measured by negative amphetamine urine samples) probabilities for both treatment groups were received both according to ITT and completer principles. All secondary measures were analyzed by repeated measures of ANOVA, comparing naltrexone and placebo patients over 12 weeks of treatment. Patients were compared on baseline characteristics using x^2 tests for categorical characteristics and t-tests for continuous characteristics in order to assess efficiency of randomization procedure to ensure homogeneity between the two treatment groups

3.5 STUDY MEDICATION

3.5.1 Naltrexone and matching placebo for studies I –IV

All studies utilized a naltrexone dose of 50mg (ReVia, DuPont). The placebo and naltrexone capsules (or tablets) were obtained through the Karolinska University Hospital pharmacy. Depending on the design of the study, the medications were either packaged in boxes containing blisters of 7 tablets (Study III) or 7 capsules (Study IV) each, for a week or in single boxes containing dosage for the day (for the acute dose studies I and II). Boxes were labeled with the patient number, study identification number, the investigators name and packed by the pharmacy.

3.5.2 Dexamphetamine and matching placebo for studies I & II

For study I & II, dexamphetamine 30mg (Metamina^{R}, the dextrorotatory isomer of amphetamine; Recip AB) and matching placebo capsules were obtained from the Karolinska University Hospital Pharmacy and packaged for each patient in a similar manner as the naltrexone/placebo capsules.

3.6 STUDIES CONDUCTED WITH HUMAN SUBJECTS

Participants in the studies provided their written consent for participation in the study. All the studies and consent forms were approved by the regional ethical review board in Stockholm, the Swedish Medical Products Agency and conducted in accordance with Good Clinical Practice (ICHGCP, 1996) and the Declaration of Helsinki.

4 RESULTS AND DISCUSSION

4.1 THE EFFECT OF NALTREXONE ON THE SUBJECTIVE RESPONSE TO AMPHETAMINE IN HEALTHY SUBJECTS (PAPER I)

The effects of an acute dose of naltrexone on the subjective, physiological and behavioural effects of amphetamine were investigated, in drug-naïve individuals.

The main outcome of the study, subjective "high" was measured using the VAS, compared over the four experimental conditions (Fig. 6). There was a statistically significant effect between the treatment conditions ($F_{3,\ 33}$ = 15.9; $p<0.001$). As expected, amphetamine produced its expected effects of increasing subjective "high" when compared to placebo ($p<0.001$). Post-hoc comparisons showed that the subjective effects during the placebo-amphetamine condition were significantly greater than the naltrexone-amphetamine condition ($p<0.05$).

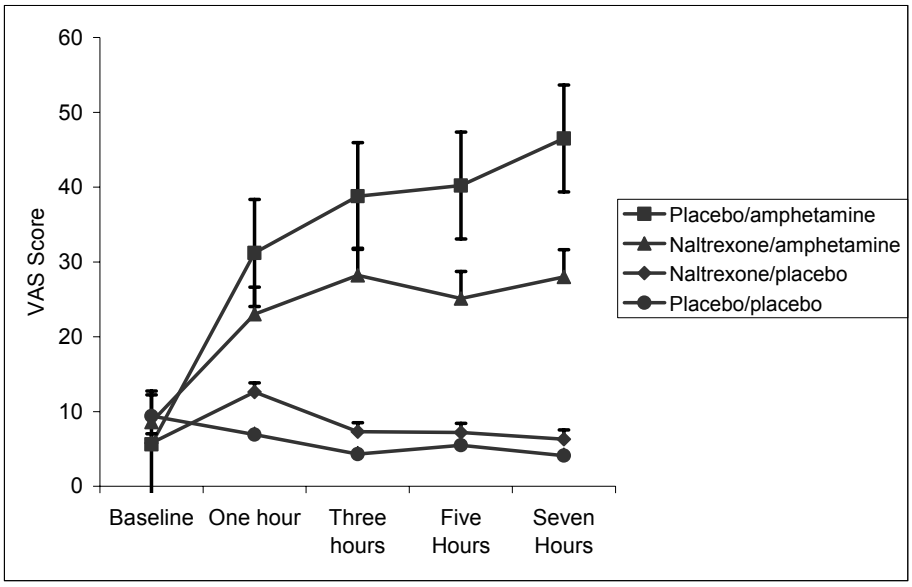


Figure 6. VAS mean scores (±SD) of subjective "high" over the four treatment conditions and at different time points: baseline and after 1, 3, 5 and 7 hours. Naltrexone (50 mg) significantly ($p<0.05$) attenuated the subjective effects of dexamphetamine (30 mg) when compared to placebo. Data points show mean of all twelve volunteers over the 4 conditions.

As a secondary analysis of subjective high, the specific items of the composite VAS were analyzed (Fig. 7). The results of the separate VAS items were consistent with the overall results of the VAS. In all of the four items the placebo-amphetamine condition produced the most robust scores and the naltrexone-amphetamine condition produced a significant reduction subjective effects produced by amphetamine. Of the items, "like

the drug" showed the least difference in the placebo-amphetamine condition compared to the naltrexone-amphetamine conditions.

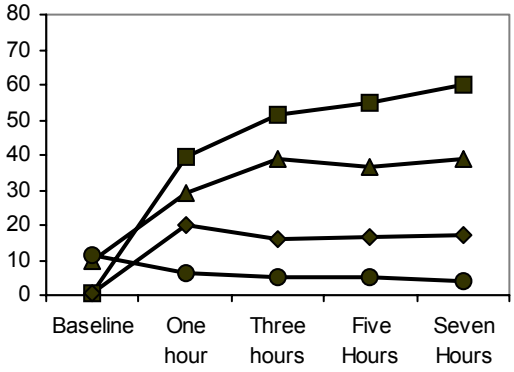


Fig 7a. "**Feel the drug** "

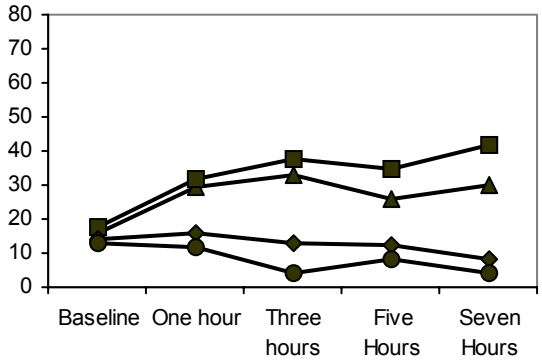


Fig 7b. "**Like the drug** "

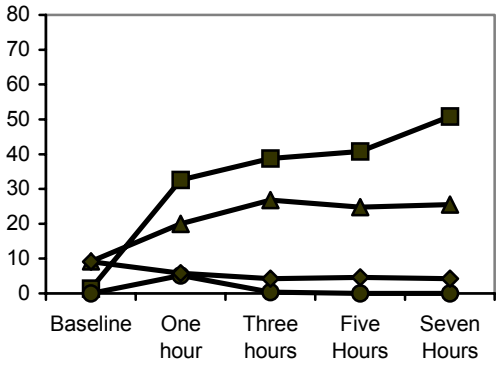


Fig 7c. "**Feel aroused** "

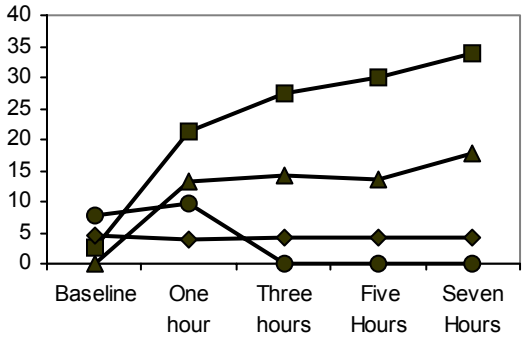


Fig 7d. "**Want more** "

Figure 7. VAS mean scores of the individual items of the VAS scale over the four condition and over time(placebo/amphetamine!; naltrexone/amphetamine4; naltrexone/placeboɜ; placebo/placebo)). X-axis: mean score of the VAS scale; Y-axis: time points of subjective ratings. Data points show mean score of twelve subjects, $p<0.05$.

In addition to the VAS, the POMS was utilised to evaluate any further effects on mood. Ratings on the fatigue dimension of the scale were significantly increased by pre-treatment with naltrexone ($p<0.05$).

As expected, treatment with amphetamine produced an elevation of blood pressure ($p<0.05$). However, pre-treatment with naltrexone did not influence any of the physiological or behavioural measures.

With respect to tolerability of naltrexone in combination with amphetamine, the main adverse events reported in this study were mild nausea and fatigue (n=4) that abated within 2-3 hours. Similar effects have previously been reported in other studies using acute doses of naltrexone in healthy volunteers (Hollister, Johnson et al. 1981; Davidson, Swift et al. 1996) demonstrating the relative safety of naltrexone.

The results of the study demonstrated that, in this population of drug-naïve individuals, a 30 mg dose of amphetamine produced it's the prototypical effects of increased subjective arousal and increased peripheral response. The subjective "high" produced by amphetamine was blunted by the pre-treatment with 50 mg dose of naltrexone. A probable explanation for this effect is that naltrexone attenuated the direct subjective or mood altering effects of the drug (e.g., euphoria, high or liking). Previous treatment studies (with alcohol and cocaine dependence) have reported that naltrexone not only reduces the subjective effects of the stimulant but may also affect the likelihood of future drug use by decreasing both the craving and liking of the drug (McCaul, Wand et al. 2000; Schmitz, Stotts et al. 2001). The finding that naltrexone blunts the reinforcing effects of amphetamine in healthy individuals, forms the basis of evaluating naltrexone also in dependent patients.

4.2 NALTREXONE ATTENUATES THE SUBJECTIVE EFFECTS OF AMPHETAMINE IN PATIENTS WITH AMPHETAMINE DEPENDENCE (PAPER II)

The primary hypothesis of the study was that pre-treatment with naltrexone would attenuate the subjective effect of amphetamine also in patients diagnosed with amphetamine dependence**.** Psychostimulants influence neuroendocrine function that is tightly linked to regulation of the opioid system, thus a secondary aim was to investigate if naltrexone blunts the effects of amphetamine via modulation of the HPA axis.

Figure 8, displays the primary outcome measure of the study, i.e. the subjective effects of the dexamphetamine challenge for the two treatment groups over time. First, there was a main effect for time-point of measurement ($F = 419.6$; $p<0.001$), showing that the amphetamine challenge invoked a subjective drug effect over time. Furthermore there was also a main effect for treatment condition ($F=482.1$; $p<0.001$) showing that the placebo condition produced a higher subjective drug effect compared to the naltrexone condition, i.e. naltrexone significantly reduced the subjective effects invoked by dexamphetamine. The difference between the two treatment conditions emerged from the time-point 150 of measurement [$t(19) = -5.17$, $p<0.001$].

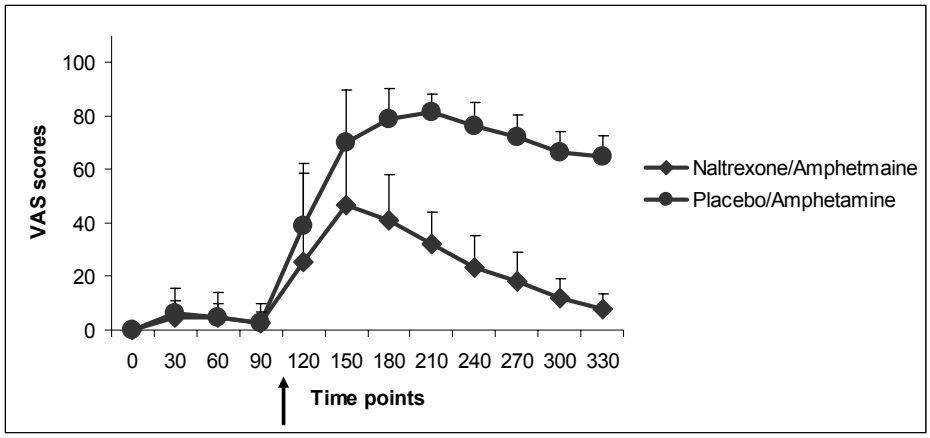
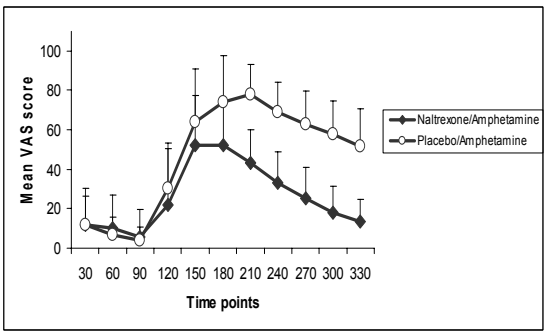
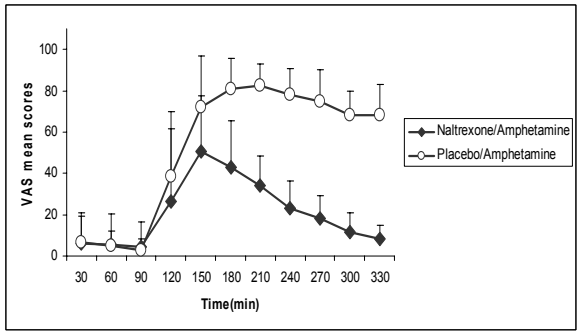


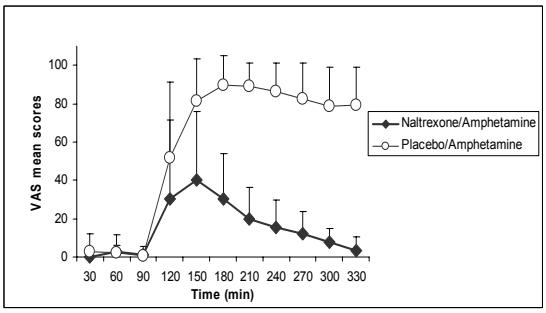
Figure 8. VAS mean scores (± SD) of subjective high over the two treatment conditions and at different time points. Pre-treatment with naltrexone (50 mg) significantly ($p<0.01$) attenuated the subjective effects of dexamphetamine (30mg) when compared to placebo. A single dose of naltrexone/placebo was administered at time point 0. The arrow indicates the administration of the 30 mg dose of dexamphetamine. Data points shows mean scores for all twenty patients over the 2 conditions

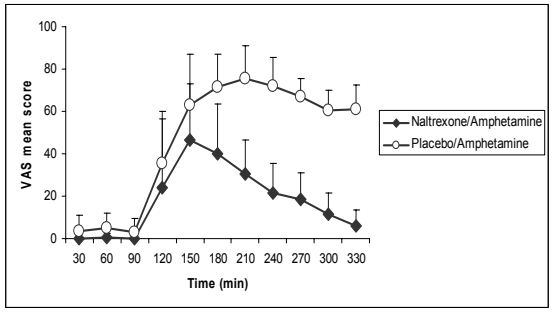
As a secondary analysis of subjective high, the specific items of the composite VAS were analysed (Fig. 9). After Bonferroni correction of multiple comparisons, the results of each of the separate VAS items ("feel the drug", "like the effect", "feel aroused" and "want more") were consistent with the overall results of the VAS. With regards to the POMS scale (subscales of vigor and fatigue) there was no difference between the two treatment conditions.





Feel the drug **Like the effect**





Want more **Feel aroused**

Figure 9. Mean scores of the individual items of the VAS scale, over the two treatment conditions (naltrexone/amphetamine ♦;placebo/amphetamine ○). Data points show mean score (± SD) for twenty patients.

The effect of interaction between naltrexone and amphetamine was evaluated also on measures of craving in dependent patients.The Naltrexone treatment condition produced a significantly lower mean craving score when compared to the placebo condition using the craving for amphetamine scale ($F= 44.8$, $p<0.001$).

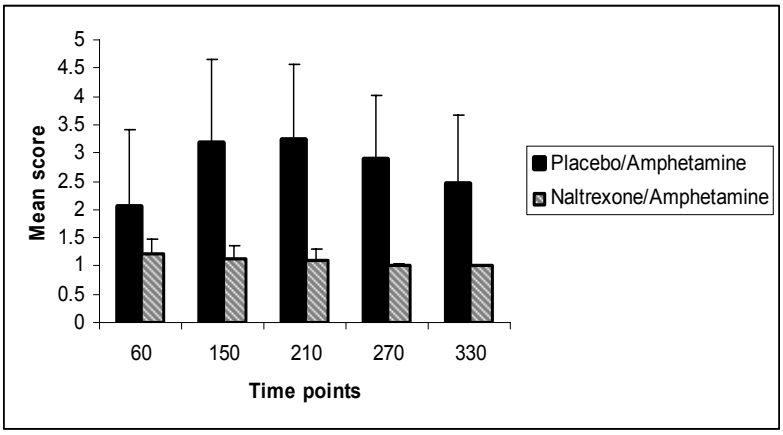


Figure 10. Mean craving scores (n=20) measured by the Tiffany craving scale for the naltrexone versus placebo condition. Pre-treatment with naltrexone significantly reduced the craving levels when compared to placebo ($p<0.001$).

Figure 11, displays interaction effect between treatment and time for the cortisol levels between the two groups. The mean baseline plasma cortisol concentration (predrug baseline) was 349mmol/l, with no difference between the two groups. Patients in the naltrexone/amphetamine condition had significantly higher levels of cortisol than the placebo/amphetamine condition ($F=12.2$; $p<0.05$).

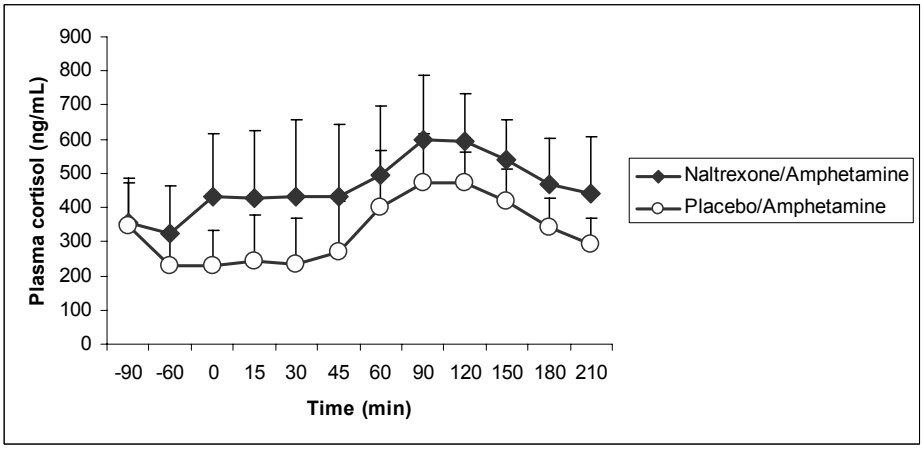


Figure 11. Plasma cortisol concentration (n=12) for the two treatment conditions naltrexone/amphetamine (♦) and placebo/amphetamine (○). A significant difference was observed between the two treatment conditions ($p<0.05$). A single dose of naltrexone/placebo was administered at time point -60 followed by a 30mg dose of dexamphetamine at time point 0.

Lastly, to our knowledge there is no study investigating the pharmacokinetic interaction of naltrexone and amphetamine. Figures 12a and 12b show the dose normalized drug exposure, i.e. AUC/mg/kg, and the elimination half-life, respectively, as a function of age of the patients**.** There were no differences in the pharmacokinetics of oral dexamphetamine between week 1 and week 2 (i.e. between the placebo/dexamphetamine and naltrexone/dexamphetamine condition).

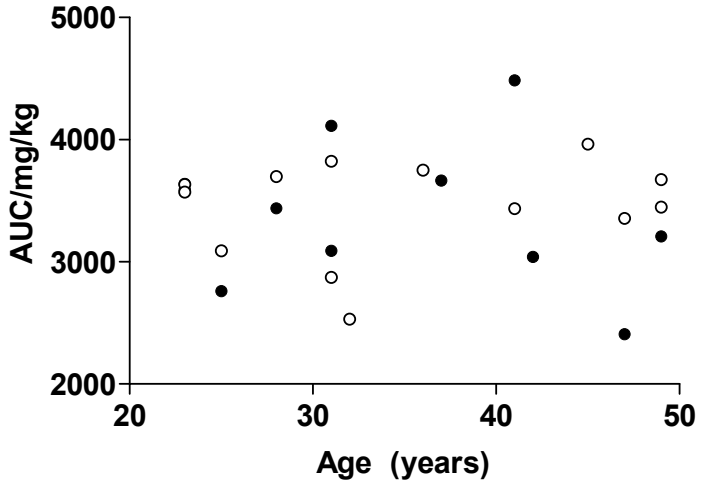


Figure 12a. The relationship between systemic dexamphetamine exposure and age of the patients. AUC/mg/kg (dose-normalized area under the plasma concentration time curve) of oral dexamphetamine in week 1 (○) and week 2 (●), for the two conditions; placebo/dexamphetamine and naltrexone/ dexamphetamine. There was no statistical difference in AUC/mg/kg between weeks 1 and 2, p=0.649 (Mann-Whitney U-test).

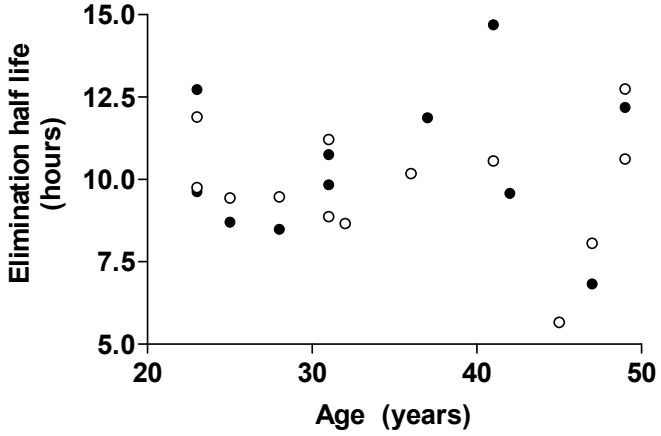


Figure 12b. The relationship between terminal half-life of oral dexamphetamine and age of the patients. There was no statistical difference in elimination half-life of dexamphetamine between week 1 (○) and week 2 (●), p=0.531 (Mann-Whitney U-test)

The results demonstrated that pre-treatment with naltrexone significantly blunted the subjective effects of a challenge dose of amphetamine, also in dependent individuals. The findings provide evidence of naltrexone's modulatory effect in patients who may have developed tolerance to certain effects of amphetamine. In addition, the data provides a proof-of-concept that naltrexone not only dampens the subjective effect of amphetamine in the event of drug use but also decreases the likelihood of additional drug consumption (as evidenced by the reduction in "want more" and craving).

The combination of naltrexone and amphetamine produced a greater elevation of cortisol when compared to placebo and amphetamine. This is in line with an earlier study assessing the effect of the opioid antagonist, naloxone, on the response of the HPA axis to the stimulant drug methylphenidate (Joyce and Donald 1987). Thus far, the pharmacological effects of naltrexone have been discussed in terms of its ability to blunt the subjective effects of the drug. From the current results, it could be hypothesized that pre-treatment with a single dose of naltrexone attenuated craving through its ability to transiently increase cortisol levels(O'Malley, Krishnan-Sarin et al. 2002) and in turn reduce the rewarding effects of amphetamine. These findings are preliminary and it remains to be determined whether the acute elevations may also persist during intermediate or long- term treatment with naltrexone.

The pharmacokinetic data revealed that treatment with naltrexone did not affect the uptake or elimination of amphetamine, irrespective of the body weights of the patients. By ruling out such an interaction, the results suggest that the mechanism of naltrexone (i.e. blunting of some of the subjective effects of dexamphetamine) is related to its pharmacodynamic properties. The findings that a single dose of naltrexone reduces the subjective arousal of amphetamine, motivates examining the effect of naltrexone in a chronic dosing model, in amphetamine dependent individuals.

4.3 AN OPEN LABEL TRIAL OF NALTREXONE FOR AMPHETAMINE DEPENDENCE: COMPLIANCE AND TOLERABILITY (PAPER III)

The effect of chronic treatment with naltrexone in amphetamine dependent individuals was investigated using an open label study design. Eleven out of 20 patients completed the 12 weeks of treatment. Five patients relapsed to amphetamine use and were lost to follow-up while 3 patients dropped out after reporting adverse events. Abstinence from amphetamine was achieved by two patients during the 12 weeks of treatment (according both to subjects self-reports and urine drug screening).The total proportion of urine samples testing positive for the presence of naltrexone's metabolite (6-β-naltrexol) was 69% (112 out of 163). The patients who completed the study had a significantly higher proportion of tests with urinary concentration of the metabolite, compared to the patients who did not (77% compared to 22%, $t_{18} = 6.0$, $p < 0.001$).

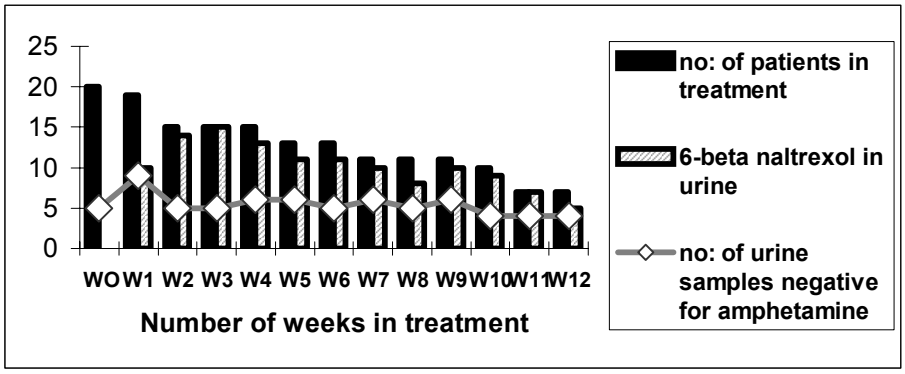


Figure 13. Compliance to treatment, expressed as the number of patients attending each week, the presence of 6-β-naltrexol in the urine samples (medication dispensing begins from week 0 and thus the first detection 6-β-naltrexol starts week 1) and the number of urine samples negative for the presence of amphetamine.

Chronic treatment with naltrexone led to a significant reduction in amphetamine consumption during treatment when compared to baseline among the study completers ($p<0.01$), as assessed by TLFB. The level of consumption of amphetamine (Table 5) during treatment was significantly lower than prior to treatment (on average 0.79 grams/day before treatment and 0.17 grams/day during treatment; $t_{10}=3.2$; $p<0.01$). The frequency of consumption during treatment was also significantly lower than prior to treatment (60% of the days prior to treatment vs. 22% of the days during treatment; $t_{10}= 4.5$; $p<0.01$). Among the completers, there was a significant reduction in amphetamine-positive urine toxicology test ($p<0.01$).There was also a significant correlation (r_{xy} =

0.60; $p<0.05$) between the daily drug use as reported in TLFB and the positive urine tests.

Table 5. Consumption patterns of amphetamine and alcohol at the start of treatment, during week 1-6, and during week 7-12. There was a significant reduction in the level of amphetamine consumption at the end of treatment when compared to baseline levels ($p<0.01$).

	Amphetamine **Average (g/day)**	**Alcohol** **Average (g/day)**
Prior to treatment (Average of 12 weeks)	0,8	26
Week 1-6	0,2	9
Week 7-12	0,1	7

In open label trials (compared to double-blinded studies), compliance to treatment may have more to do with e.g., other factors such as the characteristics of the patients, and thus a number of measures need to be included to evaluate the efficacy of the pharmacotherapy that maybe unrelated to compliance. Hence supplementary assessments were made on a weekly basis e.g., craving scale. The results demonstrated that among the patients who completed the study (defined as attending 9/12 weekly visits) there was a pronounced decrease in craving for amphetamine during the course of treatment as compared to baseline (Figure 14).

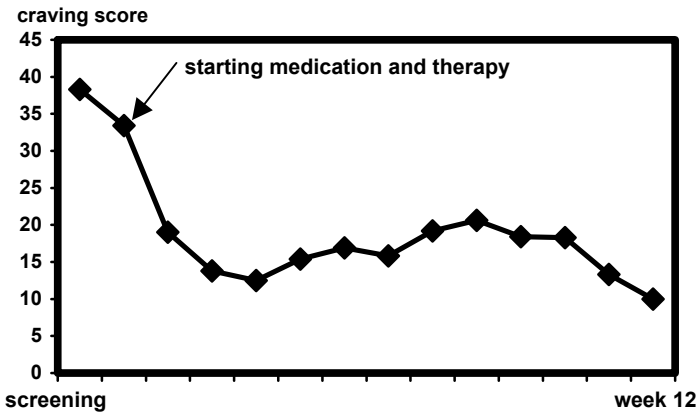


Figure 14. The pattern of subjective craving for amphetamine, over the course of 12 weeks of treatment (completer analysis; n=11)

In conclusion, the results demonstrate that chronic naltrexone pharmacotherapy was well tolerated in abstinent and currently using patients. The absence of any elevation in liver enzymes level during treatment further add to the safety and tolerability data of naltrexone, also in amphetamine dependent individuals. Since this was an open label study, it was difficult to draw conclusions on the efficacy of the treatment, however the data from the patients who complied with treatment highlights the probable role by which naltrexone mediates its effect i.e., in the event of drug consumption. It is probable that naltrexone may prevent a "slip" from becoming a binge episode by reducing the reinforcing effects and craving for amphetamine. This could imply that naltrexone reduced the direct subjective "rewarding" effects of amphetamine and in turn reduced consumption. Overall, the data demonstrates that naltrexone was well tolerated with moderate rates of compliance, supporting the feasibility of investigating this compound in a larger placebo-controlled trial as a potential pharmacotherapy for amphetamine dependence.

4.4 NALTREXONE FOR AMPHETAMINE DEPENDENCE: A RANDOMISED PLACEBO CONTROLLED TRIAL (PAPER IV)

In paper IV, the effect of chronic treatment with naltrexone was evaluated in amphetamine dependent individuals, in a randomised placebo controlled trial.

Among the 80 patients enrolled, 55 (68.7%) completed the study, with no difference in retention between the two treatment groups (naltrexone: 72.5% vs. placebo: 65%).
On the primary outcome measure, the ITT analysis showed that during the treatment, the naltrexone group had a significantly higher mean number of amphetamine negative urine samples, compared to the placebo group, $F(1,78)=5,02$, $p<0.05$ (Figure 15).
There was also an effect of time in treatment, $F(23, 56)=8.11$, $p<0.05$, showing that the mean number of negative urine samples became lower over time for both treatment groups. The mean percentage of negative urine samples during the twelve week trial for naltrexone treated patients was 65.21 (SD=36.12) and for placebo treated patients 47.71 (SD=33.67).

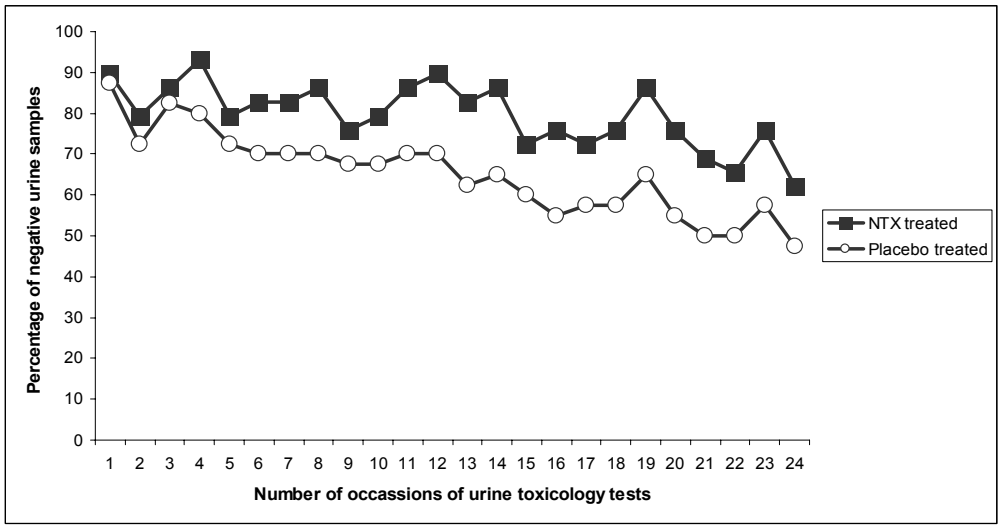


Figure 15. Percentage of negative urine samples for the two treatment groups over 12 weeks (2 urine tests/per week) of treatment (n=80; ITT analysis).

A similar pattern in results was found in the completer analysis, which showed that the naltrexone group had a significantly higher mean number of amphetamine negative urine samples compared to the placebo group, $F(1, 53)=4, 15$, $p<0.05$ (Figure 16). There was a decrease in the mean number of negative urine samples for both groups as

an effect of time in treatment, F (23, 31) =3.36, 00 p<0.05. The mean percentage of negative urine samples during the twelve week trial for naltrexone treated patients was 79.74 (SD=28.79) and for placebo treated patients 64.10 (SD=27.97).

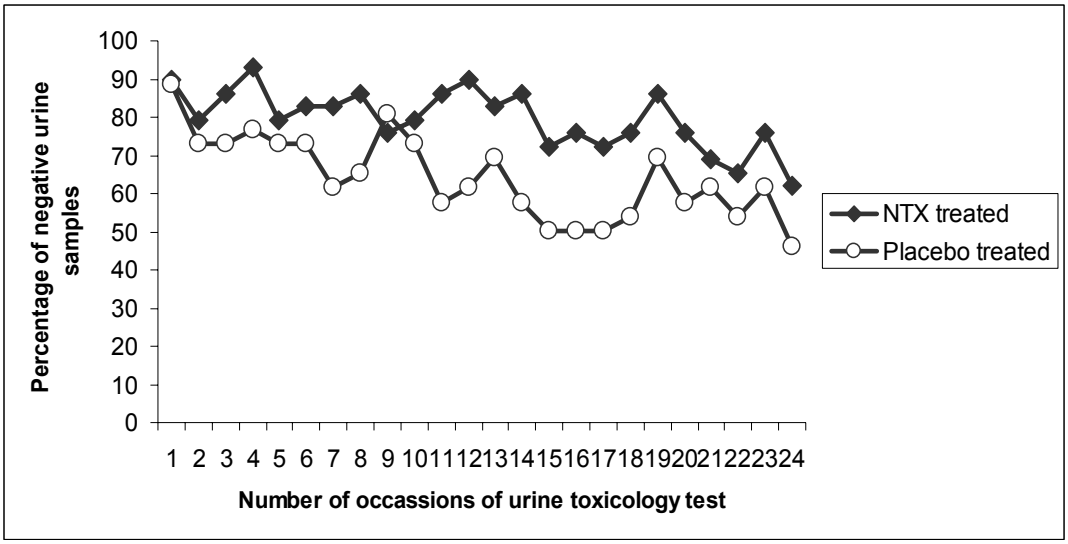


Figure 16. Percentage of negative urine samples for the two treatment groups over 12 weeks of treatment (n=55; completer analysis).

Survival analyses were used to examine the rate of continuous of abstinence from amphetamine. The results showed that the treatment groups differed in rate of continuous abstinence, both in the ITT (Breslow test, t=6.36, p<0.05) and in the completer analysis (Breslow test, t=5.34, p<0.05), in favor of naltrexone treatment (Figure 17 displays results for the ITT analysis).

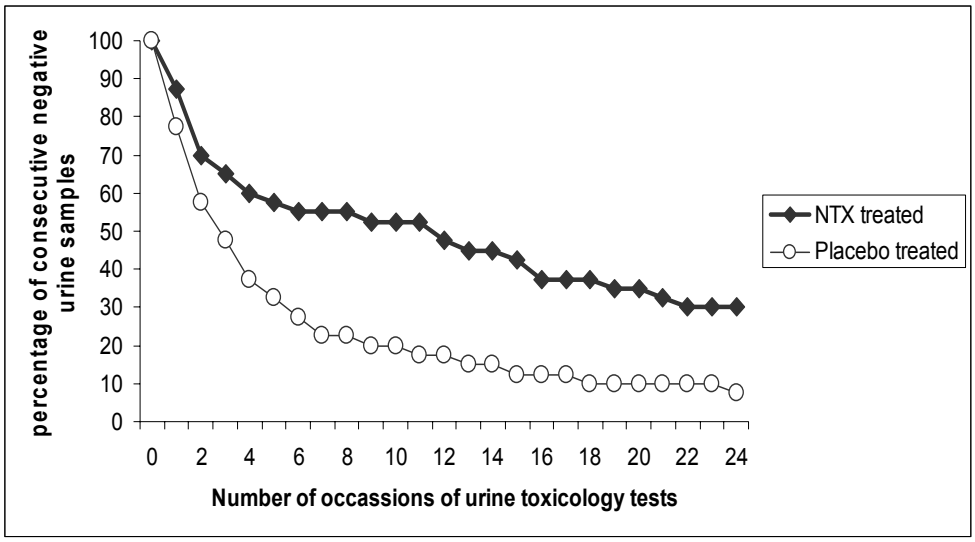


Figure 17. Survival curves representing rate of continuous abstinence for the two treatment groups across the 12 weeks (displayed as the number of occasions of urine toxicology; 2 urine tests/week) of treatment (n=80; ITT analysis).

Analysis of urinary concentration of 6-β-naltrexol revealed that more than half of the patients (25/40) in the naltrexone group displayed compliance to medication (defined as the presence of metabolite concentration in >8 of 12 weekly tests). In addition, there was a positive correlation between medication compliance and number of amphetamine-negative urine samples ($P=.69$, $p<0.05$), i.e. the higher the compliance to medication, the higher the abstinence rates for the naltrexone group.

Results of weekly measures of craving demonstrated that the two treatment groups were similar in the mean craving scores at week 0 (3.5, ± 1.4). There was an interaction effect between treatment and time, ($F(3)=5.0$, $p<0.05$) indicating that the naltrexone treated group had a greater reduction in craving scores over 12 weeks as compared to placebo.

The results provides strong support for the involvement of the endogenous opioid system in amphetamine dependence, and shows for the first time a significant effect of medication on the probability of relapse to amphetamine abuse. Treatment with naltrexone reduced the percentage of amphetamine-positive urine samples in patients with chronic amphetamine dependence.

Among the individuals randomized to naltrexone more than half displayed successful compliance to medication. There was a strong positive correlation between compliance to medication and the percentage of amphetamine-negative urine samples. This indicates that the ability of naltrexone to promote a clinically significant reduction in drug consumption is highly dependent on medication compliance.

Continued treatment with naltrexone also led to a reduction in craving scores as compared to placebo over 12 weeks of treatment and this in line with previous treatment studies on alcohol and cocaine dependence (McCaul, Wand et al. 2000; Schmitz, Stotts et al. 2001). Taken together, the efficacy of naltrexone as an anticraving medication could be promising also in the amphetamine dependent population**.**

The potential neurobiological mechanism of naltrexone's effects on amphetamine can be inferred from pre-clinical findings. Naltrexone is a non-selective opioid antagonist and binds to opioid receptors (high affinity to μ and δ receptors) which are localized on inhibitory interneurons that regulate DA neurons. Blockade of these receptors subsequently leads to a reduction of DA release (Hitzemann, Curell et al. 1982; Hurd and Ungerstedt 1989) Naltrexone may reduce amphetamine reward by blocking the opiate receptors that influence the mesolimbic DA neurons and thereby interfere with amphetamine-stimulated release of DA. It can thus be speculated that the reduction in rewarding effects of amphetamine observed in the studies, might be linked to naltrexone's attenuation of amphetamine-induced DA release.

In conclusion, naltrexone attenuates the subjective rewarding effects of amphetamine in both acute and chronic dosing models. The results of the clinical trial further consolidate the finding that chronic treatment with naltrexone leads to a sustained effect on the behavioral and subjective correlates of reward, i.e. sustained reduction in amphetamine consumption and craving.

5 SUMMARY AND CONCLUSIONS

5.1 SUMMARY

Amphetamine is the second of the most commonly abused illicit drugs in Sweden, after cannabis. The abuse of, and dependence on amphetamine is known to cause severe mental and physical health problems. From the existing literature, it is evident that till date no pharmacological treatment exists for amphetamine dependence**.** Based on the recently discovered relationship between amphetamine and the endogenous opioid system, the present thesis aimed at evaluating naltrexone as a potential pharmacological agent in the treatment of amphetamine dependence.

1) An acute dose of naltrexone blunted the subjective effects (euphoria, aurosal and liking) of amphetamine in healthy individuals The combination of naltrexone and amphetamine was well tolerated with no serious adverse events.

2) An acute dose of naltrexone modulated the subjective effects (euphoria, arousal and liking) and craving for amphetamine, in amphetamine-dependent individuals. The results from the subjective effects together with the pharmacokinetic data suggest that naltrexone mediates its effect by blunting the reinforcing effects of amphetamine.

3) Chronic treatment with naltrexone in an open label-design, demonstrated that naltrexone was tolerated in both actively abusing and abstinent amphetamine-dependent individuals. A decrease in amphetamine consumption and craving was observed in patients who complied with treatment.

4) Chronic treatment with naltrexone in a randomised placebo-controlled trial reduced consumption and craving for amphetamine, in dependent persons. The results further confirm the medical safety data concerning using naltrexone as a pharmacotherapy for amphetamine dependence.

5.2 CONCLUSIONS

The effects of naltrexone in an acute and chronic dosage model, was examined in drug naïve and amphetamine dependent individuals, with the aim of evaluating naltrexone as a potential pharmacotherapy for amphetamine dependence. The data from the four resulting papers are presented in this thesis.

The amphetamine dependent patients recruited in the studies differ from the amphetamine users in other parts of the world in one main respect. The patients recruited were mono-dependent i.e., their primary drug of choice was amphetamine and they did not fulfill the DSM IV criteria for any other substance dependence excepting nicotine. At present this is still a unique situation observed only in Sweden, as amphetamine abusers in other parts of world are most often diagnosed with dual dependence. This could be either due to availability and pricing of other drugs or a different profile of users. The current situation in Sweden could be considered an advantage in relation to the studies conducted, for a number of reasons; 1) the ability to perform proof-of-concept studies with a pharmacological compound in a homogeneous sample, 2) the patients were relatively less sick and hence able to adhere to treatment, 3) recruitment and compliance rates were better in comparison other countries, although marginally.

The effects of an acute dose of naltrexone on the reinforcing effects of amphetamine (euphoria, arousal, and liking) were evaluated in both drug naive and drug dependent individuals. The result that naltrexone attenuated the subjective effects in both populations, in the absence of any significant side effects, demonstrates that its effects are related to modulation of the reinforcing effects of amphetamine.

Naltrexone has previously been investigated also in cocaine dependence. Results from clinical trials examining the effect of chronic naltrexone treatment in cocaine dependence have been mixed (Schmitz et al, 2001, Schmitz et al, 2004). An explanation for this effect could be related to the demographics of the patients recruited in these studies. The latter study by Schmitz et al. (2004) was conducted in a sample of cocaine-alcohol dependent individuals and populations with concurrent dependence and may benefit from a higher (100m) dose of naltrexone(Kiyatkin and Brown 2003). This line of argument is further supported by a recent study (Pettinati,

Kampman et al. 2007) demonstrating an effect of a higher dose of naltrexone (150mg) in reducing cocaine and alcohol use in men.

In the two landmark clinical studies of naltrexone with alcohol dependence (O'Malley, Jaffe et al. 1992; Volpicelli, Alterman et al. 1992) naltrexone was shown to be effective in decreasing alcohol consumption and craving. The most striking effects observed in these studies was that the treatment with naltrexone resulted in a reduction of full-blown relapse (<5 drinks) among those patients who resumed drinking, with a risk of relapse among placebo-treated patients more than twice that of naltrexone-treated. Overall the results of the studies in this thesis are in line with the earlier findings, suggesting role of naltrexone also in amphetamine dependence, i.e., the effectiveness of naltrexone in reducing amphetamine relapse.

Craving is a central component in addition that often precipitates relapse, thus this was systematically measured in the three studies comprising patient populations. The patients who participated in each of these studies differed in one main respect, i.e., the use pattern; at baseline and during the trial. In paper III, abstinence was not a requirement but encouraged. In paper II, only abstinent patients were recruited and any patient who relapsed was replaced by a new one (hence the final analysis only included abstinent patients), and finally in paper IV all patients started the study by attaining abstinence, with the study aiming at relapse prevention. Despite the differences in use patterns, naltrexone had a marked effect on the craving for amphetamine. In other words, in patients with current use of amphetamine, naltrexone prevented a slip from becoming a binge and in abstinent patients it probably enabled the maintenance of a drug-free status. It could therefore be suggested that the reduction in drug consumption by naltrexone most likely results from its blunting of both pleasurable drug effects and craving. Furthermore, there were no cases of overdose with amphetamine in any of the studies. This suggests that in the absence of the pleasurable effects along with a greater control over impulses, there is a reduction in likelihood of patient increasing his/her dose to achieve a larger effect. In conclusion, naltrexone attenuates the subjective rewarding effects of amphetamine in both acute and chronic dosing models. The results of the clinical trial further consolidates the finding that chronic treatment with naltrexone leads to a sustained effect on the behavioral and subjective correlates of reward, i.e. sustained reduction in amphetamine consumption and craving.

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