2012

Cognitive enhancers: a pharmacological intervention for the treatment of substance dependence

https://hdl.handle.net/2144/31622

BOSTON UNIVERSITY

SCHOOL OF MEDICINE

Thesis

COGNITIVE ENHANCERS:

A PHARMACOLOGICAL INTERVENTION FOR THE TREATMENT OF SUBSTANCE DEPENDENCE

by

PHILIP SIVA VITTOZZI WONG

B.A., University of Southern California, 2010

Submitted in partial fulfillment of the

requirements for the degree of

Master of Arts

2012

Approved By

First Reader [redacted]

Terrell T. Gibbs, Ph.D.
Associate Professor of Pharmacology

Second Reader [redacted]

Alexander Y. Walley, M.D., M.Sc.
Assistant Professor of Medicine

COGNITIVE ENHANCERS:

A PHARMACOLOGICAL INTERVENTION FOR THE TREATMENT OF SUBSTANCE DEPENDENCE

PHILIP SIVA VITTOZZI WONG

Boston University School of Medicine, 2012

Terrell T. Gibbs, Ph.D., Associate Professor of Pharmacology

ABSTRACT

Dependence from addictive substances is a serious public health concern in the United States. Alcohol appears to be the most popular abused substance, while cigarette smoking has the highest rates of mortality. Though not as popular, illicit drugs such as cocaine and opioids are able to cause incredible damage to the lives of addicted individuals and to the people around them. The toxic injuries produced in the brain and the presence of withdrawal symptoms often result in cognitive deficits. Individuals that are able to terminate the consumption of drugs often have a hard time regaining their previous cognitive abilities. This partially contributes to the high incidence of relapse, which represents a major problem faced by the medical community. So far treatment has relied on cognitive behavioral therapy and a number of pharmacological agents. Even when combined, these approaches have not yielded satisfying results. For some types of addictions, such as the one for cocaine, there are no approved medications. Therefore research has made tremendous efforts to understand how the brain responds to addictive substances with the hope that such knowledge will lead to new pharmacological treatments. Cognitive enhancers are a promising class of drugs that is under investigation for the treatment of substance dependence. Most of them have been tested for their ability to decrease drug craving and consumption. Some of them are also being examined for their ability to reverse the cognitive deficits produced by previous drug exposure. The present thesis will examine the current literature on four cognitive enhancers: atomoxetine, reboxetine, selegiline and modafinil. Even if still in the preliminary stages, the clinical trials on reboxetine have obtained the highest rate of success. On the other hand, modafinil is the only cognitive enhancer that has been tested for reversing cognitive deficits. Compelling results in a clinical trial make modafinil one of the most exciting projects in this field of research. Atomoxetine and selegiline have mostly failed the clinical stage, but more studies are needed to determine their usefulness. In general, the potential ability to reverse cognitive deficits is not supported by the current literature and more research should be focused in this direction.

TABLE OF CONTENTS

LIST OF TABLES

ABBREVIATIONS

ACh	acetylcholine
ADHD	Attention-deficit hyperactivity disorder
cAMP	cyclic adenosine monophosphate
CYP2A6	cytochrome P450 2A6
CBT	cognitive based therapy
CPP	conditioned place preference
CRF	corticotropin releasing factor
DA	dopamine
DAT	dopamine Transporter
Δ^9-THC	(-)Δ^9-6a, 10a-trans-tetrahydrocannabinol
DSP-4	N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride
EM-1	endomorphine-1
EPSP	excitatory postsynaptic potential
FTS	forced swim test
GABA	γ-amino-butyric-acid
ICPSD	internal capsule stimulus induced poststimulus spike discharge
IPSP	inhibitory postsynaptic potential
LC	locus ceruleus
MA	methamphetamine
MAO	monoamine oxidase
mGluR	metabotropic glutamate receptor

ME	(Met)5enkephalin acetate
MSN	medium spiny neurons
NAc	nucleus accumbens
nAChR	nicotinic acetylcholine receptor
NE	norepinephrine
NMDA	*N*-Methyl-D-aspartic acid
OFC	orbitofrontal cortex
PFC	prefrontal cortex
SNc	substantia nigra pars compacta
VMAT	vesicular monoamine transporter 2
VTA	ventral tegmental area

INTRODUCTION

Definition of addiction

The word addiction derives from the Latin verb *addicere*, to give up, to surrender. However, the term does not have a universal definition in the scientific field. Some experts argue that substance dependence refers to the tolerance and/or withdrawal symptoms associated with substance abuse, while the term addiction entails a broader variety of behavioral and physical manifestations (1). Other experts in the field believe that the terms addiction and dependence can be used interchangeably (2). This latter approach will be the one used in the present thesis.

One of most authoritative definition is found in the Diagnostic and Statistical Manual of Mental Disorders (3). Substance dependence is described as a number of symptoms that prevent an individual from terminating the intake of a substance despite numerous negative consequences. It is diagnosed when a patient fulfills three or more of the following criteria within a 12-month period (Table 1).

Table 1. Criteria for substance dependence. In order to be diagnosed for substance dependence, a patient must meet three or more of the following criteria within a 12-month period of time.

1	Tolerance, the intake of an increasingly higher quantity of a substance in order to achieve the desired effect.
2	Withdrawal, a series of unpleasant feelings arising from a lack of substance intake.
3	Impaired control, manifested as the unintentional use of a substance in larger quantities and for longer periods of time.
4	Impaired control, manifested as the unsuccessful termination of a substance intake despite a conscious desire to do so.
5	Longer time spent in trying to find a substance, using it and recuperating from its effects.

6	The loss of interest in relationships, work and hobbies. Attention is mostly directed to substance related activities.
7	The inability to cease the use of a substance even in the clear presence of serious psychological and physical repercussions.

The presence of these physical and behavioral conditions are consistent across different types of addictions. However, there is considerable variety in the severity of each category. For example, withdrawal symptoms are evident in a heroin addict, while almost undetectable in a marijuana user.

An important feature of addiction that is not stressed enough in these 7 categories is relapse. In fact addiction is considered a chronic disorder, capable of affecting an individual even after a long period of abstinence. Relapse is by far the greatest problem that is being tackled by researchers and doctors in the field (1). The definitions presented above clearly suggest that addiction is a serious disorder. The most alarming facts about addiction will be examined by the following section.

Societal impact of addiction

Addiction is an enormous burden to society, having negative effects on health care, economy and legal system of a country. The following chart summarizes the prevalence of the most popular abused substances and the economic costs they have on the American society.

Table 2. Quantitative perspective on the societal impact of addiction. * (billions of dollars per year)

	Economic cost*	Millions of users (2)	Deaths per year
Nicotine	193 (1)	69.6	443,000 (4)
Alcohol	184.6 (2)	131.3	24,263 (5)
Illicit drugs	180.9 (3)	22.6	37,485 (6)

Although these statistics are of great value in assessing the problem of addiction, they do so only on a quantitative level. To have a better sense of the real burden, one must consider the economic costs on a qualitative level also. For example, in the 185 billion dollars spent due to alcohol addiction, statisticians have included damages such as to drunk driving. This already represents a huge portion of deaths in the US. Similarly, in the 181 billion dollars spent on illicit drug abuse, statisticians have included losses in productivity, such as the unyielding future of children whose parents became addicts.

Furthermore, one must also consider the moral damages caused by addiction. Numbers will never be able to express this kind of burden. Yet, people that are affected by addiction directly or indirectly must be confronted with enormous psychological adversities.

Therefore, finding a solution to this problem becomes an immediate priority for the American society. With such purpose in mind, scientists are studying new ways to treat addiction, since the current therapies are not doing enough to fight the problem. However, coming up with new treatments is only possible with a profound understanding of the neurobiology of addiction. There is now a great deal of knowledge on the way that the brain responds to substances of abuse, which will be the topic of the next section.

NEUROBIOLOGY OF ADDICTION

Opioids

The nucleus accumbens (NAc) is a brain region located in the ventral striatum and it has been associated with reward, motivation and drug addiction. Anatomically it is divided into the core and the shell subregions, which are believed to be distinct in function and receptors expressed. The main type of afferent neurons originating in the NAc are the medium spiny neurons (MSNs), which use γ-amino-butyric-acid (GABA) as their neurotransmitter. Firing of MSNs is regulated by presynaptic glutamate, acetylcholine (ACh) and GABA inputs. In turn, these presynaptic terminals are themselves highly modulated, for example by binding and activation of opioid receptors (7).

Brundege and Williams (7) have carried out electrophysiological studies to better assess the function of opioids in the NAc. [Met]5enkephalin acetate (ME), an endogenous peptide selective for the μ and δ opioid receptor, was able to decrease AMPA receptor-mediated excitatory postsynaptic potentials (EPSPs) by approximately 30% in MSNs of the NAc shell. In a second experiment ME inhibited glutamate mediated EPSPs and GABA mediated inhibitory postsynaptic potentials (IPSPs) in both the core and shell of the NAc. The inhibition of the IPSPs was greater in magnitude (47% - 68%) than the inhibition of EPSPs (29% - 39%). Both experiments were repeated with an endogenous opioid peptide selective for the δ receptor. However, in this condition the inhibition was not comparable to the one of ME.

The results from these experiments shine some light on the possible ways in which opioids act on the brain. Increased excitation in the NAc is believed to represent a common effect exerted by addictive drugs. ME was able to inhibit IPSPs more than EPSPs, indicating that opioids might ultimately cause excitation of MSNs by removing GABAergic inhibition. A finding worthy of attention from this publication is that the μ opioid receptor seems to be the main type responsible for affecting the NAc, given that stimulation of the δ opioid receptor did not have any effect. This was also confirmed by earlier studies (8).

Aside from modulation produced by glutamate and GABA, neurons in the NAc are significantly affected by dopaminergic projections coming from the ventral tegmental area (VTA). Addictive drugs tend to increase the release of DA from these projections and increase excitation of MSNs. Britt and McGehee (9) have recently tried to elucidate how opioids affect this dopaminergic synapse. In their experiments endomorphine-1 (EM) was used, a peptide selective for the μ opioid receptor. Upon a single electrical stimulus, 1μM of EM decreased the DA overflow by approximately 41% in the NAc shell. On the other hand, upon burst stimulation at of 25 Hz, the membrane potential profile of dopaminergic neurons did not change before and after the exposure to EM.

Previous evidence has suggested that dopaminergic neurons in the striatum do not possess opioid receptors (10). To explain their findings, Britt and McGehee hypothesized that opioids can affect the release of DA in the NAc by binding to receptors located on cholinergic neurons. Activation of μ opioid receptors on cholinergic interneurons decreases the release of ACh, resulting in decreased activation of nAChRs on dopaminergic terminals and consequently a decrease in DA release. This mechanism of action is strikingly similar to that of nicotine, which is believed to desensitize the nAChRs. Britt and McGehee were able to demonstrate that EM-1 decreases the firing rate of cholinergic interneurons of the NAc.

Dopaminergic neurons in the NAc are usually found in either of two states. During tonic activity, DA release is low and can be paralleled to 'background noise.' During phasic activity, DA release is high and it is often referred to as burst firing. A large difference in DA levels between these two states is particularly influential in the reinstatement of an addiction. Therefore addictive drugs can increase this difference by either enhancing burst firing or by lowering tonic activity. The results obtained by Britt and McGehee indicate that opioids operate through the second mechanism. In fact, EM-1 attenuated DA overflow upon a single stimulus, but did not affect the DA release upon burst firing.

To summarize, opioids are likely to exert their function in the NAc by: 1) inhibiting GABAergic IPSPs on MSNs and 2) inhibiting cholinergic modulation of DA terminals. These findings, while certainly insightful, do not seem to agree with other sources suggesting that opioids increase the extracellular levels of DA in the NAc. The authors address this concern by suggesting that an inhibition of tonic activity ultimately leads to a higher frequency of burst firing and therefore higher levels of DA. This can be explained by hypothesizing that the removal of tonic activity removes the concerted activation of D1 and D2 receptors on GABAergic MSNs that project to the VTA. The removal of this activation could thus represent a removal of inhibition and an increase in burst firing activity. The results gathered by Britt and McGehee are compelling, but their claims remain hypothetical and still need further validation.

Alcohol

While most addictive substances are believed to excite the VTA and the NAc through indirect means, ethanol seems to have a direct action on dopaminergic neurons. In these type of cells, hyperpolarization causes the opening of Ih cation channels, resulting in a spontaneous low frequency depolarization similar to the one found in pacemaker cells. Okamoto et al. (11) have carried out electrophysiological experiments on mouse brain slices containing VTA and substantia nigra (SNc) neurons to assess the effects of ethanol on Ih channels. Superfusion of brain slices with ethanol causing a concentration of 50 mM increased the firing rate by 10.2 % and augmented the cation current amplitude by 11.7%. On the other hand 20 mM produced only a 5% increase in the firing rate and did not affect the amplitude of the cation current. ZD7288 (30 μM), an inhibitor of Ih channels, was able to suppress the firing frequency of DA neurons, indicating that the Ih channels were responsible for the pacemaker activity. Furthermore, to assess the effects of repeated alcohol use, mice were injected a dose of ethanol for five consecutive days, resulting in a plasma concentration of 50 mM. The density of Ih channels decreased from 11.4 to 8.8 pA/pF. The half activation potential and activation kinetics did not change, suggesting that the properties of the channel itself were not affected.

Following the rationale of the authors, this data indicated that alcohol increased basal activity of dopaminergic neurons in the VTA and SNc by acting on Ih channels. In addition, alcohol developed tolerance by decreasing the density of these channels after repeated use. This could explain the decreased DA levels found in the midbrain during withdrawal from drinking. However, all of these claims remain of uncertain validity due to the fact that the ethanol concentration used in the study were relatively high. With such elevated levels of alcohol, specificity is lost, a scenario that is not a good representation of what happens in recreational drinking.

Another molecular target of ethanol that has received a lot of attention is the GABAergic neuron of the VTA. This type of cell is known to inhibit DA neurons that project to the NAc. The GABA neurons of the VTA are in turn excited by NMDA and inhibited by $GABA_A$ receptor activation. Stobbs et al. (12) have carried out in vivo experiments to elucidate the function of ethanol on GABA neurons. 0.25, 1 and 2 mg/kg of ethanol (comparable to plasma levels lower than 50mM) dose dependently decreased internal capsule stimulus induced poststimulus spike discharge (ICPSD) of VTA GABA neurons. Substituting the $GABA_A$ receptor agonist muscimol for ethanol did not cause comparable decreases in ICPSD, suggesting that ethanol does not bind to the $GABA_A$ receptor. However, the $GABA_A$ receptor could still have an indirect role in the modulation of neuronal activity induced by ethanol. On the other hand, NMDA was able to moderately but significantly increase ICPSD. Furthermore, the authors of the above study observed that either doubling the concentration of the NMDA antagonist dizocilpine or adding an equivalent dose of ethanol caused very a similar increase in ICPSD inhibition. Together the results imply the possibility that ethanol can cause disinhibition of VTA DA neurons by inhibiting NMDA receptor activation on the GABA neurons of the VTA.

While the above study shows no evidence of ethanol acting on the $GABA_A$ receptors of the VTA, in the NAc this receptor seems to have an important role. Supporting this idea is an experiment carried out by Rewal et al. (14) demonstrating that $GABA_A$ receptors containing the α4 subunit in the NAc shell play a significant role in the development of ethanol dependence. Rats infected with viral RNA interference reducing the expression of the α4 subunit decreased alcohol intake and preference. While these findings contribute more information on the ways in which ethanol produces its effects, the experiments carried out by Rewal et al. also raise problematic questions. The finding that α4 containing $GABA_A$ receptors mediate the addictive effects of ethanol contradicts previous established dogmas suggesting that excitation instead of inhibition in the NAc is responsible for the development of alcoholism. The literature reviewed above shows how intricate the effects of ethanol are and that contradictions prevent its mechanism of action from being fully elucidated.

Psychostimulants

a. *Amphetamines*

Amphetamines are one of the most diverse class of drugs, due not only to the wide variety of possible derivatives, but also to their numerous molecular targets. After the discovery that amphetamine can increase the levels of DA in the striatum (14) by inhibiting the DA transporter (DAT) (15), an impressive amount of literature has been devoted to elucidating its mechanism of action. Pifl and colleagues (16) examined the DA efflux induced by amphetamine on COS-7 cells expressing the human DAT and the human vesicular monoamine transporter 2 (VMAT2). In these cells amphetamine was able to dose-dependently release more than twice the amount of DA compared to cells transfected with only the DAT. On a qualitative level, the pattern of DA efflux over time was also influenced by the expression of these transporters. In DAT cells the release of DA induced by amphetamine reached a peak and then returned to baseline. On the other hand, the release of DA in cells with DAT and VMAT2 was sustained after reaching its peak. The authors also noticed that amphetamine had a higher potency with DAT cells. Together these findings imply a number of possible mechanisms of action for amphetamine. The ability to release DA from intracellular storage vesicles can explain the additional DA released when cells possess VMAT2. The increased potency of amphetamine in cells expressing only the DAT is due to the fact that DA is limited to the cytoplasm. Releasing neurotransmitter in this case is more rapid compared to mobilizing DA stored in the vesicles. This confirms once again that amphetamine can release DA from intracellular vesicles. Finally, after observing that cocaine, a blocker of the DAT, did not cause any efflux of neurotransmitter in DAT cells, the authors hypothesized that amphetamine mediates the release of DA by reversing the DAT.

Other studies on amphetamine have identified three additional molecular targets. One of them, tyrosine hydroxylase, is an enzyme that converts tyrosine into L-DOPA, a precursor of DA. Larsen et al. (17) demonstrated a three fold increase in the amount of L-DOPA produced with a methamphetamine infusion compared to control. Another enzyme targeted by amphetamines is monoamine oxidase A, which is responsible for the breakdown of DA. Ramsay et al. (18) have shown that d-amphetamine maintains the reduced state of MAO-A by stabilizing the semiquinone form of FAD. Lastly, amphetamine has also been shown to bind the D2 autoreceptor. Schmitz and colleagues (19) have hypothesized that the increase in extracellular DA caused by amphetamine activates the D2 autoreceptor, which inhibits the exocytosis of DA in a negative feedback manner. Upon a single electrical stimulation, infusion of amphetamine decreased the amplitude of DA efflux by 82%, concordant with an inhibition of DA exocytosis.

This finding is still in agreement with previous evidence showing that amphetamine can relocate DA from the vesicles to the cytosol. By doing so, only a small quantity of DA gets released upon exocytosis. Schmitz et al. believe that D2 autoreceptor contributes to this decrease in DA exocytosis. To demonstrate this hypothesis, they repeated their experiment with the addition of sulpiride, a D2 receptor antagonist. The decrease in DA efflux only reached 47% compared to the previous 82%. To further confirm these findings, DA efflux was measured in D2 receptor knock-out mice. In these animals, DA released was decreased by only 23%. Therefore there is compelling evidence indicating that under the effects of amphetamine the D2 autoreceptor decreases exocytosis of DA.

To summarize, amphetamine most likely exerts its effects by: 1) blocking the reuptake of DA through inhibition of the DAT; 2) increasing the efflux of DA by reversing the transport of the DAT; 3) mobilizing DA from intracellular storage vesicles to the cytoplasm; 4) increasing the production of DA by stimulating TH activity; 5) decreasing DA breakdown by inhibiting MAO-A; 6) decreasing the exocytosis of DA by stimulating the D2 autoreceptor. While there seems to be an overall consensus on these molecular targets, debate continues to revolve around the exact biochemical mechanism that underlies the effects of amphetamine. For example, how amphetamine manages to release DA from storage vesicles remains unclear (41g). The answer to this question is now the focus of several recent projects.

b. *Cocaine*

Together with the amphetamines, cocaine is another psychostimulant that has been abused by millions of Americans for decades. After preliminary studies suggesting that the addictive properties of cocaine are mediated by a blockade of the DAT (20), Rocha et al. (21) investigated the ability of DAT knock out mice to self-administer cocaine. In their experiment, DAT-/- mice required significantly more time for establishing self-administration of cocaine compared to wild-type animals, suggesting that the DAT has an important function in the initiation of dependence. However, once established, self-administration in both groups was stable and dose dependent. The dorso/ventral striatum of DAT-/- mice presented with higher basal levels of DA compared to wild-type mice and a cocaine injection was not able to cause significant changes. This is in contrast with control animals in which cocaine causes DA levels to significantly increase from basal levels. Therefore, other molecular targets aside from the DA transporter contribute to the establishment of cocaine self-administration. To identify possible binding sites through autoradiography, the cocaine analog [125I]RTI-55 was used on striatal coronal slices. To show that [125I]RTI-55 was a good analog of cocaine, the authors demonstrated that pretreatment with cocaine eliminated any radioactive labeling. Significant binding of [125I]RTI-55 occurred in DAT knock out mice, while the serotonin transporter antagonist alaprocrate significantly decreased radioactive labeling. These results indicate that cocaine also binds to the serotonin transporter, which could therefore have a role in the initiation of self-administration.

The above study also attempted to demonstrate that cocaine can bind to the NET, but a pretreatment with a NET inhibitor did not change the pattern of [125I]RTI-55 binding. The involvement of this transporter in the addictive effects of cocaine has been controversial. Following an early study suggesting that cocaine is likely to possess an affinity for the NET (22), more recent publications have shown that noradrenergic transmission indeed has a significant role. In particular, Beveridge et al. (23) demonstrated that cocaine exposure alters the density of the NET in several brain regions. Using [3H]nisoxetine to localize the NET in brain slices of squirrel monkeys, the investigators observed that the hypothalamus presented with an increase in transporter density compared to the wild type animals. It is believed that cocaine could stimulate noradrenergic transmission to the hypothalamus, leading to an increase in the production of corticotropin releasing factor (CRF), a hormone associated with the initiation of drug intake. Higher levels of NET were also found in the basolateral amygdala, enthorhinal cortex and hippocampus. The interconnection between these brain regions is associated with cognitive functions such as memory and learning. The effects of cocaine on these areas might explain the presence of cognitive deficits after the repeated exposure to the drug.

From the above analysis it appears that cocaine produces its effects by interacting with all three monoamine transporters. Among them, the DAT remains the most influential one. Some studies have tried to gain a more in depth perspective on the alterations to the dopaminergic transmission. For example, Heien et al. (24) have analyzed the release of DA in the NAc after an infusion of cocaine in live rodents. Upon burst electrical stimuli applied to the SNc and VTA, cocaine increased the frequency and amplitude of DA concentration spikes. As mentioned in the review of opioids, burst firing has a central role in the reinstatement of substance dependence. However, the precise mechanisms that allow cocaine to promote burst firing remain elusive.

Nicotine

Cigarette smoking is one of the leading causes of death in the United States (25). The addictive substance contained in tobacco is nicotine. A clear description of how this compound acts on the brain has not been achieved yet. What makes this task difficult is the wide variety of receptor subunits, which have specific distribution in different parts of the brain. The subunit composition of nAChRs determines the binding affinity, desensitization and downstream effects of nicotine.

One of the most comprehensive studies on nicotine's mechanism of action has been carried out by Mansvelder and colleagues (26). Their focus has been analyzing GABAergic and glutamatergic projections that regulate the DA neurons of the VTA. In one of their experiments on rat brains, they inserted a stimulating electrode in GABAergic neurons of the VTA and NAc, which are both known to generate IPSPs in dopaminergic neurons. They moderated the intensity of stimulation such that dopaminergic cells experienced IPSPs 50% of the times. In these conditions, nicotine was able to increase the frequency of IPSP above 50%, suggesting that it can promote GABAergic transmission and therefore cause a downstream inhibition of DA neurons. IPSPs were also augmented in frequency and amplitude. However, in 7 out of 11 cells, the frequency dropped below baseline after reaching a brief peak, suggesting a possible desensitization of nAChRs. Methyllycaconitine, which is selective for the α7 subunit of the nAChR, did not change the IPSPs generated by nicotine. Mecamylamine, selective for non α7 subunits, completely abolished the nicotine induced increase in IPSP. The same effect was also produced by dihydro-β-erythroidine hydrobromide, an agent selective for β2 subunits. To further confirm the desensitization of nAChRs on GABA neurons, the authors infused the brain slices with 250 nM of nicotine. The frequency of IPSPs increased transiently and soon after returned to baseline. Immediately following this infusion, an additional 1 μM was applied, but no change was observed. They then repeated the experiment by measuring EPSPs, a measure of glutamate induced excitation from the PFC. A 250 nM infusion of nicotine increased EPSPs, which remained elevated and did not resume back to baseline levels. A subsequent 1 μM infusion of nicotine caused an even higher increase in EPSPs frequency.

The above results support the hypothesis that nicotine increases dopaminergic transmission in the VTA by simultaneously potentiating glutamatergic input and inhibiting GABAergic input. The first is probably achieved by activation of α7 containing nAChR on presynaptic glutamate terminals, which do not appear to desensitize. This increased glutamate input is thought to produce long term potentiation on the DA neurons of the VTA. On the other hand, GABA neurons are first activated, but then brought to a lower basal activity by desensitization of receptors most likely containing the β2 subunit. Together these putative mechanism of action could explain the addictive properties of nicotine.

In addition to glutamatergic and GABAergic neurons, nAChRs are also found on the DA neurons of the VTA. Activation of these receptors by cholinergic projections is believed to increase DA release. Zhang and Sulzer (27) have hypothesized that nicotine can directly affect the release of DA in the NAc by desensitizing nAChRs on DA terminals. For this purpose, stimulating electrodes were implanted in the NAc with the ability to generate activity similar to tonic or phasic activity. With a single stimulation, nicotine decreased the release of DA. However, the authors observed that the higher the frequency of stimulation, the lower the inhibition induced by nicotine. At the highest frequency, a condition which emulates phasic firing, nicotine had no effects. Therefore the results support the idea that nicotine increases the difference between the levels of DA released during tonic activity and burst firing. Because phasic activity has been associated with reinstatement of drug addiction, nicotine appears to generate dependence by potentiating the impact of burst firing in the NAc. Research trying to elucidate the precise mechanism that is responsible for these effects is still underway.

From biology to behavior

One of the challenges in addiction research has been to explain how the neurochemical changes elicited by drugs can influence so drastically the behavior of a substance user. A number of models have been developed to pinpoint what exactly causes the transition from an occasional drug use to a compulsive addiction.

The classical view suggests that the desire to experience euphoria is the reason for the repetitive consumption of substances. After prolonged use, tolerance starts to prevail and the euphoria previously experienced disappears almost completely. Instead, alleviating the anhedonic effects of withdrawal becomes the reason for the drug intake (1).

The incentive salience model argues that drug craving - rather than reward - constitutes the primary cause of an addiction. Therefore, a person can be drawn into repetitive substance use even without ever experiencing a pleasurable effect. A simple feeling of 'wanting' is sufficient for an occasional drug use to develop into a full-blown substance dependence (1).

The aberrant learning model is a compromise between these two theories. It states that after the perception of increased reward from a substance, the brain registers all of the environmental stimuli and conscious behaviors that best predict the procurement of that substance. This newly acquired information could be the drive for the repetitive drug taking. Proponents of this theory believe that this mechanism of action is analogous the one that allows humans to procure food and sex (1).

While a consensus has not been reached, it is possible that a combination of these theories might be the most valid model. Theoretically the hedonic effect, the drug 'wanting' and the learning of drug-associated stimuli might act at different stages of the addiction. Another possibility is that some models might be more applicable to some individuals rather than others depending on their genotype. In fact the topic of individual susceptibility has received a great deal of attention among experts. While single genes fully responsible for an addictive trait have not been identified, experts agree on the importance of the genetic makeup in determining the likelihood of substance dependence within an individual (1).

Currently approved medications

Opioids

Methadone was first approved in the US in 1947 (28). For the next fifty years it represented the only satisfactory treatment for opioid addiction. Methadone is a μ-opioid receptor agonist (29). Although it has been shown to decrease substance craving in most patients, many still fail their therapy and continue to have withdrawal symptoms (30). Other studies have reported an increase in craving for heroin after an additional administration of methadone (31). Additional evidence for the incomplete efficacy of methadone can be found in fMRI studies showing that drug cues increase the activity of brain regions that are associated with drug craving (32, 33).

The urge to find an alternative treatment has led to the introduction of buprenorphine, a partial μ-opioid receptor agonist. First approved in 1981 as an analgesic, it was used for the treatment of opioid addiction only after 2002 (34). Compared to methadone, some studies have shown that buprenorphine increases treatment retention (35), while other investigators obtained mixed results (36, 37).

The FDA has also approved Naltrexone, a μ-opioid receptor antagonist. However, because of its anhedonic effects, compliance has become a major problem. Sustained release formulations have been designed to avoid this issue, but their efficacy still needs to be proven (38).

Alcohol

There is evidence suggesting that ethanol might work by interfering with glutamatergic transmission. To address this problem, drug developers have created acamprosate, a partial agonist for the NMDA receptor (39). Acamprosate appears to reverse the changes in glutamate levels elicited by ethanol (29). Even though the mechanism of action is not completely elucidated, its efficacy has been well documented (40).

A completely different molecular strategy is used by disulfiram. By inhibiting alcohol dehydrogenase, this medication increases the levels of acetaldehyde, one of the metabolites of ethanol (29). Acetaldehyde causes flushing, vomiting and other adverse effects, thus making the consumption of an alcoholic beverage an unpleasant experience (41). Although the efficacy of disulfiram in treating alcoholism has been demonstrated, compliance is achievable only with the most motivated patients.

Naltrexone has also been approved for the treatment of alcoholism, but satisfying evidence on its efficacy is still warranted. Just like in the treatment of opioid addiction, the problem of compliance remains an important issue to be solved (29).

Nicotine

The nicotine gum was the first treatment approved for nicotine addiction. Since then, many other formulations have been marketed, with similar success rates. These medications, collectively known as nicotine replacement therapies, can increase the success rate of smoking cessation (42), but they fail to address other issues such as the long term prevention of relapse (43).

Consequently, different pharmacological approaches have been developed. For example, bupropion is an antidepressant that increases the levels of dopamine in the shell of the NAc and decreases the brain reward threshold (44). Some studies have also suggested that bupropion can block the effects of nicotine by direct binding to neuronal nAChRs (45). On the clinical side, compliance rates for bupropion are better than placebo, but nicotine craving is not completely alleviated. In addition, bupropion decreases only some of the symptoms of nicotine withdrawal (46).

Varenicline was the last approved drug for smoking cessation. It binds to all subtypes of nAChR with varying potencies. The highest affinity was found to be with the α4β2 receptor, which is thought to mediate the rewarding effects of nicotine. As a partial agonist, varenicline inhibits the binding of nicotine and partially increases dopamine in the NAc, ameliorating withdrawals and craving from nicotine (47). On the clinical side, varenicline is more effective than other currently approved medications, even in the long term abstinence. However, a number of patients in these clinical trials have failed to adhere to the therapy. Some adverse effects have also been documented (48).

Psychostimulants

There are no approved medications for the treatment of illicit psychostimulant addiction (cocaine, amphetamine, 3,4-Methylenedioxymethamphetamine (MDMA) and methamphetamine). This is an alarming situation, as their use is widespread. Cocaine users of age 12 and older amounted to 1.5 million last year, about 6.6% of total illicit drug users (1). Cognitive based therapy, which can be considered similar to behavioral medicine, has been the only therapy used so far. While its efficacy is significant, additional treatment resources are needed.

Comments

Out of all the approved drugs reviewed above, almost half of them have appeared on the market only in the last decade. Granted their considerable efficacy, the potential armamentarium available to a doctor has grown substantially. Physicians can now establish a personalized therapeutic plan based on the characteristics of the patient.

Adverse effects, motivation, length of abstinence and presence of psychiatric disorders are factors that help to determine which pharmacological intervention to use.

However, each of the drugs reviewed above has its own shortcomings. With some medications it is hard to achieve compliance, while others are limited by abuse potential. These difficulties are confirmed by the National Institute on Drug Abuse: last year 22.1 million individuals have been classified as having substance abuse or substance dependence. The statistics have not changed significantly since 2002 (1). This suggests that the current treatments are not doing enough. In fact, even with the vast progress made in the development of new pharmacological agents, relapse prevention remains the greatest problem faced by both patients and doctors. In addition, given the economic impact that addiction has on society, finding new therapeutics becomes a necessity for the scientific community. The next section will examine ways in which scientists are attacking the problem.

Pharmacological Strategies

A substantial amount of information has been unraveled on the way that drugs of abuse cause changes in the neurochemistry of the brain. This has allowed for the emergence of new pharmacological strategies, so far with promising results.

As previously discussed, increased dopamine release in the NAc is responsible for the rewarding and addictive effects of almost every substance of abuse. It follows that a successful strategy consists in using compounds that have similar molecular mechanisms on the NAc as the drug of abuse, with the exception of being safer, longer lasting and providing an inferior hedonic effect. Longer half lives could keep the patient away from repetitive consumption of substances. On the other hand, a small rewarding effect can alleviate the withdrawal symptoms. Known as agonists, these drugs were the first pharmacological strategy adopted in the fight against addiction. Despite being the oldest strategy, new drugs are still being developed under this regime with significant results (49).

A completely different approach is adopted by antagonists, agents that bind to the same receptors as the drugs of abuse, but elicit no molecular effect. Antagonists thus prevent other substances from binding the receptor and keeps them from causing any effect. Antagonists can also bind to the drug of abuse itself and prevent it from reaching its target receptor. This pharmacokinetic approach, consisting of antibodies to a specific drug, has shown some promise but substantial challenges still persist (49).

Partial agonists are agents that have properties of both agonists and antagonists. They bind to the same receptors targeted by drugs of abuse but cause only a moderate rewarding effect. This is sufficient to alleviate withdrawal symptoms but also prevents the binding of other drugs, keeping the patient from relapsing into the addiction (49).

The above strategies have relied on molecular mechanisms that target DA neurotransmission in the mesolimbic system. A different strategy is to focus on other neurotransmitters that indirectly affect dopaminergic neurons. For example, NE trafficking is implicated in the reinstatement of drug addiction caused by stress. Scientists have developed α2 agonists that reduce NE release from the locus ceruleus (LC) and decrease the reinstatement of heroin and alcohol addiction (50).

Another key player in the neurobiology of addiction is CRF. Animal studies suggest that CRF receptor antagonists can decrease the response to stress and therefore become a potential therapeutic strategy (51).

Glutamate is another important neurotransmitter involved in addiction. Increased release from the PFC to the NAc is responsible for the loss of control over drug seeking behavior, craving and relapse. Due to the importance of this neurotransmitter system, essentially every receptor involved in glutamate trafficking has been targeted. NMDA receptor antagonist have shown some efficacy in reducing alcohol craving. Agents that increase extracellular glutamate by acting on the cystine-glutamate antiporter are clinically effective in cocaine and nicotine studies. Agonist of the group II metabotropic glutamate receptors (mGluR) reduce drug seeking behavior for alcohol, nicotine and cocaine (49). Lastly, group I mGluR antagonists can decrease self administration of virtually all drugs in animal models (52).

Although the above pharmacological strategies hold great promise, this thesis will focus on a different class of drugs: cognitive enhancers. Even though they have common features with the strategies reviewed above, they can be considered a separate category because of their specific goal in the treatment of addiction.

The idea of using cognitive enhancers stems from the discovery that substance abusers have impaired cognitive functions such as learning, attention, memory, inhibitory control and decision-making (53). These mental functions are essential for a successful recovery, especially in cognitive based therapy (CBT). In fact, patients enrolled in CBT are required to learn new strategies to cope with their condition (49). A correlation study found that cocaine users who drop out of CBT tend to have significantly lower scores on tests that measure cognitive functioning (54). Similar findings emerged from a study with alcoholics (55). Cognitive enhancers aim at restoring these cognitive deficits, allowing patients to make better choices and undergo a more successful therapy. This thesis will now make a thorough review of four cognitive enhancers that are currently being investigated. Publications on the mechanisms of action, animal studies and clinical trials will be evaluated.

REVIEW OF COGNITIVE ENHANCERS FOR THE TREATMENT OF ADDICTION

Atomoxetine

Mechanism of action

Cognitive functioning is a process mediated by the prefrontal cortex (PFC) through the interconnection of multiple neurotransmitters systems. Among them, norepinephrine (NE) plays an important role in working memory, attention and responses to sensory stimuli. These functions are impaired in a number of disorders, such as attention-deficit hyperactivity disorder (ADHD) and substance dependence. Atomoxetine is a new pharmacological agent approved for the treatment of ADHD, but it is still under investigation for the treatment of substance dependence (56).

Atomoxetine exerts its effects by selectively blocking the presynaptic NE uptake transporter and thus increasing the synaptic levels of NE. In order to demonstrate this specific mechanism of action, Bymaster et al. (56) employed cell lines expressing the human NE, serotonin (5-HT) and dopamine (DA) transporters and infused them with ATO. The measured dissociation constant (Ki) were 5, 77 and 1451 respectively, demonstrating the selectivity of ATO for the NE transporter. To assess the ability of ATO to increase extracellular NE in the PFC, male Sprague-Dawley rats were administered the neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4), an agent known for depleting extracellular NE. ATO was able to bring cortical NE levels back to baseline levels even in the presence of DSP-4. In a subsequent experiment, researchers measured the extracellular levels of NE and DA in the PFC after ATO administration. Levels increased by 243% and 251% compared to baseline, respectively, while serotonin did not change significantly from controls. In the striatum and nucleus accumbens, DA and serotonin did not increase from baseline levels. These findings indicate that ATO selectively acts on brain regions and neurotransmitters that are involved in cognitive function, while leaving reward pathways unaffected. Therefore, as a potential therapeutic agent for addiction, ATO demonstrates minimal abuse liability.

A more recent publication has explored the effects of atomoxetine in other brain regions known to be affected by NE transmission. Sawnson and colleagues (57) have demonstrated that NE increases approximately by 200% of baseline levels in the PFC, occipital cortex, hypothalamus, hippocampus and cerebellum. This is in agreement with previous data suggesting that atomoxetine blocks the NET. In a second experiment, the

$\alpha 2$-adrenergic antagonist idazoxan was coadministered with atomoxetine and NE levels increased above 700% of baseline. The authors interpreted this outcome as evidence that the atomoxetine induced increase in NE is attenuated by $\alpha 2$ autoreceptor activation. This result contributes to better understand the mechanism of action of atomoxetine, but whether a combination of these two pharmacological agents is clinically useful remains unknown. Within the perspective of substance dependence, the authors also fail to discuss the implications of the increased extracellular NE in brain regions other than the PFC.

Despite a number of effects of uncertain significance, atomoxetine remains a promising candidate. In fact it also possesses antidepressant and anxiolytic properties. Desipramine is another NE reuptake inhibitor that also possesses these beneficial effects. This could be especially significant in the treatment of addiction since depression and anxiety are often experienced during withdrawal and represent the primary cause for relapse (56).

Animal Studies

Substance dependence is usually characterized by cognitive impairments, especially in the withdrawal phase. Attenuating these deficits can increase the effectiveness of cognitive behavioral therapy, which will ultimately allow the patient to remain abstinent for longer periods of time. Davis and Gould (58) carried out the first experiment showing that ATO can reverse cognitive deficits in a mouse model of nicotine withdrawal. The procedure, known as fear conditioning, consisted in presenting an auditory signal to the mice, followed by a footshock. When expecting a noxious stimulus, mice usually freeze in one position. By measuring the amount of time that the mice spent without moving, the investigators were able to determine if mice could learn to associate a painful event with the presentation of an environmental cue. Mice were subjected to chronic nicotine exposure, after which nicotine was eliminated and mice entered a phase of withdrawal. Mice receiving saline showed impaired fear conditioning, while the ones that received ATO showed a normal freezing behavior. This illustrates that ATO is capable of reversing fear conditioning deficits induced by nicotine withdrawal. Other animal studies using different models and experimental procedures have confirmed the potential therapeutic effects of ATO for nicotine dependence (59, 60). However, in the case of other types of addictions, it is not known if atomoxetine can be of therapeutic use. It has been reported that psychostimulant and opioid dependence also results in cognitive deficits (61), so the use of atomoxetine could be extended for other types of addictions.

Instead of attempting to decrease cognitive deficits, studies with opioids and psychostimulants have employed ATO to attenuate drug craving. In a self-administration paradigm, Economidou et al. (62) trained rats to press a lever that delivered a dose of cocaine or heroin. Results demonstrated that ATO administered 20 minutes before a self-administration session, decreased significantly lever pressing for both substances. In another experiment, rats were trained to press the lever after the exposure to an environmental cue. Lever pressing caused an injection of cocaine. Following a period of abstinence, the environmental stimulus was presented without providing the lever. The time that the rats spent searching for the lever was a measure of drug craving and relapse.

Rats that were administered ATO 20 minutes before drug seeking sessions spent significantly less time looking for the cocaine lever after 1 week of abstinence. Other investigators have confirmed that ATO decreases craving for cocaine (63, 64, 65). The ability to extinguish addictive behaviors supports the idea that decreasing self-administration is a manifestation of intact learning processes. This is in agreement with evidence suggesting that atomoxetine has cognitive enhancing properties in the animal brain. Because of the extensive evidence gathered by preclinical studies, it can be concluded that ATO is effective in treating an animal model of nicotine and cocaine addiction. The support for the use of ATO is sufficient to legitimize studies on humans, the results of which are outlined below.

Clinical trials

In 2008 Stoop et al. (66) investigated the safety, tolerability and subject-rated effects of ATO in cocaine dependent individuals. After an ATO dose of 20 mg, the participants were asked to consume cocaine and give a subjective rating of their drug experience. Investigators also monitored heart rate, blood pressure, liver function and presence of side effects. ATO was able to decrease only the systolic blood pressure, while the subjective rating for cocaine effects did not change. ATO was therefore safe and tolerable in cocaine dependent individuals; however there was no evidence for an attenuation of drug liking. The authors argued that in order to see a statistically significant result, ATO doses should have been higher and maintained for longer periods of time. Successive studies employed larger doses of ATO. For example, Levin et al. (67)

carried out a 12-week open clinical trial with ATO doses of 80 mg per day in ADHD patients with a comorbid cocaine dependence. While ATO was able to reduce ADHD symptoms, cocaine use was not attenuated, as demonstrated by urine toxicology and self-reports. Possible circumstances that prevented significant results were the short duration of the clinical trial and a small sample size. More promising results have been achieved in amphetamine users. ATO attenuated blood pressure and heart rate increases induced by d-amphetamine. ATO also decreased the subjective rating score for drug experience but a change in drug consumption was not measured (68).

The only clinical trial investigating the effects of ATO on nicotine withdrawal has not produced satisfying results. The aim of the study was to establish whether ATO could decrease the craving for nicotine and attenuate attention deficits as a result of the nicotine withdrawal. Fifty participants were divided in two groups: those that smoked for the stimulatory effects of nicotine and those that smoked for relieving withdrawal symptoms. After being on an ATO regimen for seven days, the study subjects were kept from smoking overnight. Only the group that smoked for stimulation experienced decreased craving for nicotine, although ATO was not more efficacious than bupropion and varenicline. On tests that measured attention and vigilance, there was no difference between placebo and treatment with ATO. Based on these results, ATO can be a potential treatment only for a subset of smokers and does not compare to currently approved drugs for smoking cessation (69).

A similar scenario can be depicted for the effects of ATO on alcohol drinkers. Wilens et al. (70) carried out a double-blind, placebo controlled clinical trial with 80 participants affected by ADHD and a comorbid alcohol addiction. After at least 4 days of abstinence from alcohol, the study subjects were administered ATO for twelve weeks. The results indicated that ADHD symptoms were successfully diminished, but relapse to heavy drinking did not differ between ATO and placebo. However, those in the treatment group decreased their total heavy drinking days by 26% compared to controls. This was the only evidence of positive results and it was sufficient to consider ATO useful for the treatment of alcohol addiction. The authors argued that a better study design could have given more satisfying results. For example, the sample size was too small and the study subjects were mainly Caucasian (88%), making their experimental groups not representative of the real population.

A clinical trial in which ATO has almost completely failed is an open label study that assessed the safety and tolerability of ATO in marijuana users. Thirteen subjects seeking to terminate their addiction started the treatment regime but only eight completed the study. The percentage of abstinent days after treatment did not differ from baseline, although a trend towards reduction was observed. Urine samples could not confirm this trend, but the authors argue that the long half life of Δ9-THC might have been responsible for this effect. The most surprising finding was the incidence of mild to moderate gastro-intestinal adverse effects in 77% of the subjects. Withdrawal symptoms from an attempted decrease in consumption might have caused this unusual outcome. The authors argue that based on these results there is no evidence for any therapeutic effect of ATO in marijuana users that seek treatment (71).

Summary

After considering the results from both amphetamine and cocaine studies, it is still too early to reach a conclusion on the efficacy of ATO in treating psychostimulant dependence. In the future, larger clinical trials should take the next step and determine if ATO can decrease craving and cognitive deficits resulting from a cocaine or amphetamine addiction.

For other substances such as nicotine, ATO does not seem to be effective. Animal models of nicotine withdrawal provided strong evidence for the use of ATO, but results from the clinical trials did not match the expectations. ATO was effective only in a portion of the population and even in that subgroup, it was equivalent to already existing medications. However, given that only one nicotine study has been conducted, larger clinical trials should verify these results. Potential alterations to the study design might include more variety in the severity of withdrawal and other types of behavioral tests measuring nicotine craving.

Animal studies using ATO for alcohol dependence have not been produced yet. On the other hand a clinical trial has been conducted and results demonstrated that ATO was only partially effective in prolonging abstinence from drinking. The authors believed that the characteristics of the study participants and the low number of abstinent days were the reason for the unsatisfying nature of the results. While these concerns are certainly influential, it must be reminded that all study participants also had ADHD. Therefore future studies should try to enroll habitual drinkers that do not have other conditions. This patient population might find ATO more effective in decreasing consumption of alcohol compared to patients that have multiple disorders.

Finally, clinical trials on marijuana dependent individuals yielded inconclusive results after the occurrence of adverse events in the majority of the study participants. While this unusual event might have been a result of withdrawal symptoms, another possibility is a potential interaction between ATO and marijuana. Animal studies co-administering ATO and Δ9-THC might be able to verify if this interaction is real. If not, future studies should try to administer ATO through a different formula or route, with the intent of bypassing the digestive system and preventing GI side effects.

Reboxetine

Mechanism of action

Reboxetine is an antidepressant currently approved in Europe for the treatment of clinical depression and ADHD (72). It is a potent and selective inhibitor of the norepinephrine (NE) reuptake transporter, with almost no affinity for the dopamine (DA) and serotonin transporters. Even though reboxetine increases the levels of NE in the synapse, the ultimate effect is a decrease in firing and neurotransmitter releasing rate. This is due to the presence of α2 adrenergic autoreceptors on cell bodies and axon terminals, which get overactivated by high levels of extracellular NE and cause an inhibition of noradrenegic neurons. The locus ceruleus (LC) is the brain region mostly affected by reboxetine, where NE release is dose dependently decreased in both acute and long term administration (73).

The LC is especially susceptible to opioid drugs, where an acute exposure causes a decrease in the cAMP pathway followed by a decrease in firing rate. It is hypothesized that a compensatory mechanism is capable of overstimulating the cAMP pathway after a chronic exposure to opioids, ultimately resulting in an increased release of NE. The enhanced activity of these neurons after chronic exposure is believed to be the cause of withdrawal symptoms (74). Reboxetine might represent a potential therapeutic agent for its ability to decrease the release of NE from LC projections.

Reboxetine also possesses affinity for nAChRs, although binding is not competitive with acetylcholine. In particular reboxetine seems to target receptors that contain the α3-β4 subunits in hypothalamic synaptosomes. Non competitive binding to α4-β2 containing receptors was also documented in thalamic synaptosomes (75). In virtue of this properties reboxetine is being tested as a treatment of nicotine dependence.

Animal Studies

The only laboratory study examining the effects of reboxetine on an animal model of substance dependence was carried out by Rauhut et al. in 2002 (76). In their first experiment, rats were trained to self administer nicotine or sucrose. Upon an acute administration of reboxetine, self administration of nicotine decreased by 60% relative to baseline. On the other hand, the sucrose treatment group reduced their intake by 20%. These results indicate that reboxetine has specificity for nicotine and has little impact on the food-reward neurocircuitry.

The effects of long term treatment were also assessed. 14 days of reboxetine reduced both nicotine and sucrose self administration without eliciting tolerance in all of the animals. On the fifteenth day, the reboxetine treatment group was given a dose of saline and the nicotine intake increased to control levels. This confirmed that reboxetine was necessary to maintain a low nicotine self-administration.

In order to strengthen the validity of the results, the authors had to make sure that the decrease in nicotine self-administration was not due to a potential sedative effect of reboxetine. All rats were pretreated with reboxetine and successively divided in groups that received either nicotine or saline. Nicotine increased locomotion compared to saline, confirming that reboxetine does not have general sedative effects. A more direct evidence would have resulted from a measurement of sedation between reboxetine and saline treatments, which however was not carried out by the authors.

The results produced by Rauhut et al. suggest that reboxetine attenuates nicotine intake in animals. Based on their findings, it appears that reboxetine does not induce tolerance, is specific in maintaining a low self-administration of nicotine and does not produce general sedation. Therefore, both studies on the mechanism of action and studies on animal models support the idea that reboxetine might work in humans as a smoking cessation agent. On the other hand, unanswered questions remain as to whether reboxetine can attenuate the effects of other addictive drugs. Due to its ability to impact the noradrenergic transmission of the LC, future animal studies should begin with research involving opioid dependence.

Clinical studies

Even though preclinical studies provided support for the use of reboxetine in smoking cessation, clinical trials have explored its use in other types of substance dependence. Szerman et al. (77) have investigated the effects of reboxetine in 26 cocaine dependent patients seeking treatment. Urine toxicology, cocaine dependence, depression and anxiety were monitored during the 12 weeks of reboxetine administration. The data was taken from 20 participants and results showed a statistically significant effect of treatment in decreasing cocaine use compared to baseline. However, the authors pointed out that the participants able to complete the clinical trial were more likely from the start to remain abstinent. They suspected that the participants with more severe withdrawal symptoms dropped out of the study probably due to inefficacy of treatment. In fact individual susceptibility strongly influences the severity of a cocaine withdrawal syndrome. While the participants completing the trial found reboxetine to be beneficial, therapeutic efficacy on individuals with severe withdrawal symptoms is unknown.

Other promising results have been achieved by a recent clinical trial, which determined that reboxetine can attenuate the effects of MDMA. Cardiovascular related changes such as NE plasma levels, heart rate and blood pressure were all decreased in subjects pretreated with reboxetine. Drug high, stimulation and emotional excitation were also reduced in the treatment group. The authors ruled out a possible inhibitory pharmacokinetic interaction between reboxetine and MDMA, which could have explained the decrease in drug high. The treatment group actually had higher plasma levels of MDMA and its active metabolite 3,4-methylenedioxyamphetamine. Therefore the authors confirmed that reboxetine can pharmacologically attenuate the effects of MDMA (78).

The evidence emerged from these clinical trials provides support for the use of reboxetine in psychostimulant dependence. However larger clinical trials are needed to consolidate this hypothesis. In the case of cocaine dependence, more attention should be paid to those individuals affected by severe withdrawal symptoms. Even if reboxetine alone turns out to be ineffective in this patient population, it might serve as an effective supplement to other kinds of therapies. Successive studies should explore the use of reboxetine in conjunction with cognitive behavioral therapy or in conjunction with other pharmacological agents. After considering the above results, it appears that the reuptake of NE has a major role in the neurobiology of psychostimulants. While future clinical studies should continue in this direction, there is an urgent need for clinical trials on smoking cessation. The ability of reboxetine to antagonize nAChR has yet to be examined in humans despite the abundant evidence emerging from molecular studies.

Selegiline

Mechanism of action

In the central nervous system, neurotransmitters such as DA, NE and serotonin are broken down by two enzymes, catechol O-methyl transferase and monoamine oxidase. The latter is in turn is divided in the A (MAO-A) and B (MAO-B) types. While serotonin is mostly metabolized by the first type, DA and NE are equally broken down by both types. Selegiline is a potent irreversible MAO-B inhibitor and is currently used for the treatment of Parkinson's disease (79). It binds covalently to the flavin portion of MAO-B (80), in regions where this enzyme is highly concentrated, namely the thalamus, cortex, striatum and brainstem (81). At higher concentrations, selectivity is lost and both types of MAO are inhibited (82). Metabolism of DA by MAO-B causes the release of hydrogen peroxide, which contributes to the toxicity of addictive drugs that increase levels of DA. Thus, by inhibiting MAO-B selegiline has also a neuroprotective effect (79).

One of the active metabolites of selegiline is L-methamphetamine, which is the less active enantiomer of the common street drug. L-methamphetamine blocks the reuptake of DA by inhibiting the DA transporter. It has 40% of the potency of selegiline in terms of increasing the levels of DA. Other active metabolites include L-amphetamine and desmethylselegiline (80).

The ability to inhibit MAO and the presence of L-methamphetamine as an active metabolite allows selegiline to increase the extracellular levels of DA in parts of the mesolimbic pathway. As such, selegiline can compensate for the lack of DA found in the midbrain after prolonged use of addictive drugs. Together with the above properties, the neuroprotective effects further supports the use of selegiline as a potential treatment for substance dependence.

Animal studies

Since selegiline produces its effects in multiple ways, He et al. (80) conducted an experiment that determined which of selegiline's mechanism of action was more significant in treating an animal model of opioid dependence. Answering this question would have contributed to the development of a more targeted treatment with less adverse effects. The investigators used clorgyline, an MAO-A inhibitor, rasagiline, an MAO-B inhibitor, and L-methamphetamine. After a training in morphine self-administration, rats were divided into control and treatment groups. Clorgyline and rasagiline were administered at two different doses. The lower dose (1.0 mg/kg) allowed selectivity for only one type of MAO, while the higher dose (10 mg/kg) was non selective. Both of the selective doses did not produce a significant effect compared to controls, whereas all other treatment groups experienced a decrease in opioid self-administration. Taken together these results demonstrate that all of rasagiline's mechanisms of action are important and that only non-selective doses are effective. In addition, selegiline shows promise as a treatment for opioid addiction.

Other studies confirmed the efficacy of selegiline in animal models of opioid dependence. Grasing and Ghosh (83) were able to attenuate behavioral deficits as a result of opioid withdrawal by pretreating rats with selegiline. After two or three weeks of morphine withdrawal, rats were subjected to a forced swim test (FTS), in which freezing behavior represents a measure of decreased central dopaminergic activity. Nucleus accumbens and striatal slices were obtained after the FTS. When they stimulated firing of neurons by perfusion with cocaine and amphetamine, DA efflux was decreased in animals that were withdrawn from morphine. Selegiline prevented both the decrease in DA efflux and immobility in the FTS. Results were significant for nucleus accumbens slices but not for striatal slices. The authors concluded that selegiline attenuates decreases in DA efflux induced by opioid withdrawal and that the nucleus accumbes is more affected than the striatum during opioid withdrawal.

Selegiline has also been used in alcohol self-administration studies. Cohen et al. (84) trained rats to distinguish between two levers, one that provided ethanol and one that provided water. All animals showed more than 90% preference for the ethanol lever. A series of MAO inhibitors were used in the study, including ones that were specific to only one isoform of MAO and others that lacked specificity. Selegiline was used at doses of 3 and 10 mg/kg, which according to Curet et al. (85), produced selectivity only for MAO-B in the rat brain. With other MAO inhibitors only nonselective doses were able to decrease self-administration. This led the investigators to confirm the evidence from previous studies suggesting the use of non-selective doses in order to obtain significant results. Selegiline was effective even at the selective dose because the metabolite l-methamphetamine might have contributed to the increase in available DA (84). Previous studies had confirmed the ability of selegiline in decreasing alcohol self-administration in a mouse model (86).

Similar animal studies with nicotine have not been carried out yet, but Siu et al. (87) discovered that selegiline strongly inhibits cytochrome P450 2A6 (CYP2A6), the major enzyme involved in the metabolism of nicotine. Individuals with a genetic deficiency in CYP2A6 are 1.75 times more likely to quit smoking because their body nicotine concentration takes longer time to fall and therefore these individuals are less likely to experience severe withdrawal. By inhibiting CYP2A6, selegiline slows down the metabolism of nicotine and therefore can work as a smoking cessation aid. By prolonging the levels of nicotine in the body, it can potentially function as an adjuvant to a nicotine replacement therapy.

Although promising results support the potential of selegiline in the treatment of multiple substance related disorders, animal studies that employed psychostimulants have not produced satisfying evidence for its efficacy. Yasar et al. (88) demonstrated that an injection of selegiline did not alter D-amphetamine self-administration. Therefore the authors concluded that selegiline was not suited for the treatment of psychostimulant addiction. Experiments using methamphetamine and cocaine models of dependence produced similar results (89).

Clinical Studies

Numerous clinical trials have been published on selegiline, especially in cocaine dependent individuals. Initial studies focused on its safety, tolerability, pharmacodynamics and pharmacokinetics. Aside from showing the absence of adverse effects and interactions between selegiline and cocaine, these preliminary studies also attempted to demonstrate the presence of therapeutic effects. An oral administration of selegiline was able to decrease the feeling of 'high' in cocaine dependent subjects (90). The transdermal patch was then introduced with the intent to: 1) avoiding first pass effect; 2) providing a more prolonged exposure; 3) reducing the levels of metabolites; 4) avoiding the inhibition of MOA in the intestine and liver - a known cause of adverse effects. The first study using this route of administration found that selegiline decreased only some of the physiological and subjective effects of cocaine (91).

These partially satisfying results were followed by a large clinical trial that specifically aimed at assessing the ability of selegiline to decrease cocaine intake. 300 cocaine dependent subjects were enrolled in a double-blind, placebo-controlled study in which transdermal patches of selegiline were administered daily for 8 weeks. Relative to baseline, the selegiline treatment group decreased the number of abstinent days by 11.4%, compared to a 14% in the placebo group. Urine toxicology and behavioral tests measuring the severity of substance dependence did not show a difference between the two treatment groups. Based on the results, transdermal patches of selegiline do not seem to work as a treatment for cocaine addiction (92).

The most recent publication evaluated the potential benefits of transdermal selegiline with higher doses of cocaine to best resemble the consumption usually found in cocaine addicts. Physiological effects of cocaine were unchanged with the administration of selegiline. Craving was reduced, but the reward following drug use was not decreased. In agreement with previous evidence, this clinical study confirmed the inadequacy of transdermal selegiline as a treatment for cocaine dependence (93).

Due to promising evidence from preclinical studies, selegiline has also been evaluated as a treatment for nicotine dependence. In a randomized double blind clinical trial, 109 habitual smokers were assigned to either a nicotine patch plus oral selegiline or just a nicotine patch. 25% of the first group remained abstinent for 52 weeks, compared to an 11% in the second group. However the results did not reach statistical significance. Tests for nicotine craving and the need for nicotine patches showed a significant effect of selegiline. The authors believed that selegiline could not be excluded as a viable option for smoking cessation and expressed the need for more clinical trials (94).

However successive studies could not validate the above findings. 51 subjects receiving oral selegiline hydrochloride for eight weeks did not achieve a greater smoking abstinence rate compared to placebo. While indices of nicotine withdrawal, dependence, craving and depression were decreased over time, these parameters did not differ between the two treatment arms. The reasons for these unsuccessful results were not clear. The authors indicated that the placebo group had an abnormally high percentage of abstinence compared to previous clinical trials. In addition, all of the study participants were mostly Caucasian with high educational achievements and a strong motivation to quit. Lastly, for the 6 months follow up data the participants in the study did not reach the required number for statistical power (95). Shortly after the publication of these results, another clinical trial produced negative results. A transdermal patch of selegiline for 8 weeks did not improve smoking abstinence relative to placebo (96).

Summary

Cocaine studies suggest that selegiline is not an effective treatment option. Three of the four publications reviewed above could not achieve a significant effect for selegiline. Despite the negative results, some authors have tried to justify the inefficacy of selegiline by indicating various experimental conditions that could have compromised the findings. For example, Elkashef et al. hypothesized that the transdermal route of administration decreased the concentration of metabolites such as L-methamphetamine and L-amphetamine, both of which could have had a crucial role in decreasing cocaine craving. Future clinical trials could return to the oral form of selegiline. In case the oral route turns out to be effective, it means that the active metabolites have a significant role in the pharmacology of selegiline in humans. Elkashef et al. also indicated that the study participants receiving selegiline in their clinical trial had been using cocaine for a longer period of time compared to cocaine users in the control group. This could have masked the effects of selegiline because of a more severe craving for cocaine. Even though the frequency of drug use during the 30 days prior to the clinical trial was the same across all individuals, this group difference might have influenced the behavioral aspect of their addiction. In other words, individuals that have been taking cocaine for longer periods of time probably have a more consolidated addictive behavior. Additional clinical trials could therefore adjust to this variable by creating the same average of past cocaine use across all treatment groups.

Nicotine studies have produced findings of similar nature. Both publications could not achieve a significant effect of selegiline in all their experiments. Again, the characteristics of the participants recruited might have played a significant role. For example, in Weinberger et al., the placebo group had an abnormally high percentage of abstinence compared to previous clinical trials. In addition, all of the study participants were mostly Caucasian with high educational achievements and a strong motivation to quit smoking. This might have hindered a potential difference between treatment and placebo. A common theme in both cocaine and nicotine studies has been the objective of decreasing substance intake. Instead, it would be interesting to see if selegiline has any use as a relapse prevention agent. Future clinical trials would have to enroll patients that have already started a period of abstinence, with or without the help of cognitive behavioral therapy. Lastly, the effects of selegiline in an opioid or alcohol dependent population have not been explored yet, despite the compelling evidence gathered from animal studies.

Modafinil

Mechanism of action

Modafinil is a wakefulness-promoting agent approved for the treatment of narcolepsy. Aside from its main usage, scientists are also testing this drug for its psychostimulant and neuroprotective properties. In fact modafinil is currently being employed in clinical trials for substance dependence, neurodegenerative diseases and a variety of psychiatric disorders. However, a clear mechanism of action has not been elucidated yet. Investigators have looked at numerous brain regions and molecular targets, but the results have been mostly inconclusive.

After the completion of preliminary studies showing that modafinil has mild psychostimulant effects, Mignot et al. carried out binding assays with neurotransmitter transporters that are usually bound by cocaine and amphetamine. With the dopamine (DA) transporter modafinil was found to have only weak binding (97). Contrary to these findings, Madras et al. (98) demonstrated significant binding to striatal DA transporters in a rhesus monkey model. The ability of modafinil to affect the release of DA has also been the subject of controversy. Simon et al. (99) found that both a tyrosine hydroxylase inhibitor and DA receptor antagonists were not able to alter the effects of modafinil on locomotion, suggesting that the release of DA is not affected. On the other hand, Winsor and Eriksson (100) made the opposite conclusion after showing that a DA autoreceptor antagonist blocked the effects of modafinil. Despite the diversity of results, most investigators agree on the fact that modafinil has a mechanism of action that is different than the ones of classic psychostimulant drugs (101). For example DA receptor antagonists and a tyrosine hydroxylase inhibitor blocked the effects of amphetamine but could not inhibit modafinil. L-DOPA co-administered with amphetamine caused a typical climbing behavior in mice, but not when co-administered with modafinil. Amphetamine was able to reverse reserpine-induced akinesia, while modafinil was not (99). Following this rationale, investigators have also agreed that the effects on DA transmission cannot be solely responsible for the increased locomotor activity seen in the animal models, suggesting the existence of multiple molecular targets.

Although a clear mechanism of action has not been described yet, the evidence so far reviewed still shows promise within the perspective of substance related disorders. If it is true that modafinil can increase the DA concentration within the striatum, then it can attenuate the decreases in striatal DA experienced during withdrawal from psychostimulants. At the same time, its marked difference with cocaine and amphetamine suggests that modafinil will unlikely cause dependence. In fact a number of studies have confirmed that modafinil does not have abuse liability (101).

Glutamate is another neurotransmitter that has been closely monitored as the main target of modafinil. Chronic exposure to substances of abuse causes a decrease in the basal levels of extracellular glutamate in the nucleus accumbens (NAc) (102). At high concentrations, modafinil increases the concentration of glutamate in the striatum (103). During abstinence, the initial decrease in extracellular glutamate in the NAc causes prefrontal cortex (PFC) projections to increase the release of glutamate. This process has been correlated with drug or cue induced reinstatement of drug seeking. It is hypothesized that the presynaptic stimulation of mGlut2/3 receptor can decrease the release of glutamate, which therefore could prevent reinstatement of drug seeking. Modafinil appears to increase the binding of glutamate to these receptors as demonstrated by an experiment showing that its effects were completely blocked by an mGlut2/3 receptor antagonist (103).

Modafinil also affects other neurotransmitters. For example, Ferraro et al. found that modafinil decreases GABA in the pallidum and striatum, another mechanism that could be responsible for increasing locomotor activity (103). Modafinil also shows binding to the NE transporter with an affinity that is comparable to that for the DA transporter. Since the adrenergic system is known for mediating the release of glutamate in the PFC, it is hypothesized that the effect of modafinil on glutamatergic transmission might be exerted indirectly through NE (101).

The mechanisms of action reviewed above have been limited to the ones most pertinent to the treatment of substance dependence. However, it is possible that the wake-promoting and neuroprotective properties acting on different parts of the brain can contribute to the broad effects of modafinil within the spectrum of substance-related disorders.

Animal studies

Most of the animal studies on modafinil have focused on opioid and cocaine dependence. Tahsili-Fahadan et al. (102) obtained compelling results with a rat model by investigating the ability of modafinil to block opioid seeking behavior. The experimental procedure, called conditioned place preference (CPP), involved the use of two communicating chambers each with distinguished features. In the first session of the experiment rats were injected morphine or modafinil and placed in one of two chambers. In the second session, the rats were injected with saline and then positioned in the other chamber. The rats were therefore trained to associate a specific substance with a specific environment. After the training period, the treatment doses were eliminated and the rats were given the opportunity to chose which chamber to go into. As predicted, the animal preferred the environment associated with morphine. In contrast, modafinil trained rats did not show CPP, which indicates that this drug does not have abuse potential and does not cause a reward. In the second experiment the rats underwent an extinction phase, where injections were terminated and the rats eventually lost preference for the morphine-associated chamber. An injection of morphine re-established CPP, but a pretreatment with modafinil blocked this effect. The authors concluded that modafinil has the ability to attenuate morphine induced reinstatement. To further investigate the effects of modafinil, a group of rats was pretreated with modafinil during the morphine training phase without undergoing extinction. Morphine was able to induce CPP, demonstrating that modafinil only affects relapse and does not attenuate opioid seeking behavior during a period of daily drug intake.

Because this was the first report of modafinil on opioid dependence, it is still early to make a conclusion on its potential benefits. Based solely on the results from the above study, modafinil shows promise in prolonging abstinence from opioids. The only negative outcome in this study was the increase in morphine induced CPP with a smaller dose of modafinil, the cause of which is still not clear. Future experiments should keep assessing the effects of modafinil at low doses to shine more light on this unexpected outcome. Another insightful project would be assessing relapse prevention through other types of protocols, such as drug or cue induced reinstatement of self-administration after an extinction phase. Studies on relapse prevention are more likely to succeed as experiments carried out during daily drug intake have not yielded positive results.

While only one report has explored the effects of modafinil on opioid dependence, more studies have been conducted on cocaine. Deroche et al. (104) found that modafinil did not potentiate or weaken cocaine self-administration. After extinction, rats pretreated with modafinil did not decrease the reinstatement of cocaine self-administration upon a cocaine injection. Other negative results have been produced by Bernardi et al. (105) who demonstrated that modafinil increased cocaine CPP. Because of the unsatisfactory data, both authors believe that modafinil should not be recommended for the treatment of cocaine dependence.

Contrary to the above findings, other investigators believe that modafinil might have a therapeutic potential. Newman et al. (106) used a discrimination study, where animals were trained to differentiate cocaine from saline. Monkeys receiving an injection of cocaine had to press a lever in order to obtain food. A different lever was associated with a saline injection. In the experimental session, after an injection of modafinil, monkeys pressed the lever previously associated with cocaine, suggesting a similar effect for both drugs. From a second experiment, the investigators found that modafinil at 32 mg/kg/day can decrease self administration of cocaine given at 0.0032 mg/kg and at 0.01 mg/kg. This was the main outcome of the study, which was used by the authors to justify their recommendation of modafinil for addiction treatment. In the last experiment, after completion of a cocaine self-administration regimen, saline was substituted in the injections. Self-administration slowly decreased, until it reached negligible values. Upon an injection of modafinil, self-administration of saline returned back to elevated levels. This was evidence that modafinil can elicit past cocaine seeking behavior.

The results demonstrate a strong link between modafinil and cocaine. The similarity between the two drugs is considered by the authors as an advantage for treatment. In fact, as evident from their discussion, the authors seem to recommend modafinil as a replacement therapy. At the same time, they dismissed the potential for abuse liability after observing that modafinil shows a slow rate of onset. This line of thought is flawed since modafinil cannot possibly be a replacement therapy without being reinforcing to some extent. The major flaw might reside in the results showing the effects of modafinil and cocaine are similar. In fact these findings are in contrast with previously described studies, which highlight the marked difference between psychostimulants and modafinil. A possible reason for obtaining different results might be due to the use of low doses. As previously illustrated in Tahsili-Fahadan et al., low doses of modafinil might have opposite effects compared to higher doses.

Aside from a flawed reasoning, the results from the third experiment further questions the usefulness of modafinil. The fact that the monkeys relapsed to a cocaine seeking behavior is certainly a negative outcome. Finally, a total of eleven animals were used for the research, a number that would be appropriate only for a preliminary study. While it appears that modafinil does not have the ability to treat cocaine dependence in animal models, only larger studies will be able to confirm it. For future research, it would be interesting to see whether modafinil is capable of preventing relapse, a factor that plays a major role in the treatment of addiction.

Clinical studies

A preliminary clinical study provided evidence that modafinil can increase cocaine abstinence compared to placebo (107). However, more robust studies were needed to confirm these results. In a study published in 2009, Anderson et al. (108) showed that modafinil is only partially effective in the treatment of cocaine dependence. 210 participants were randomly divided in groups receiving either placebo, modafinil at 200mg or 400mg. The treatment was administered once daily for 12 weeks in conjunction with cognitive behavioral therapy once a week. The main outcome measure was percent of cocaine non-use days as reported by self-assessment and confirmed by urine tests. After completion of the study, the modafinil groups did not differ from controls. No difference was also found in urine tests. However, the maximum number of consecutive non-use days was increased in the participants taking modafinil. Positive results were also found in a number of behavioral tests measuring addiction severity and cocaine craving, where 200mg of modafinil was shown to be partially effective. However, because the main outcome gave results contrary to the hypothesis, the authors decided to carry some post hoc tests. They noticed that in Dackis et al. (107), alcohol dependence represented one of the exclusion criteria during the recruitment of patients. For this reason, Anderson and colleagues decided to re-analyze the data without the participants that had a drinking addiction. Both percent of cocaine non-use days and maximum days of abstinence were significantly higher in the modafinil group compared to placebo. The authors therefore concluded that modafinil can be effective only in a portion of the cocaine dependent population due to a suspected interaction between alcohol, cocaine and modafinil.

While the potential of modafinil in the treatment of cocaine addiction remains unknown, more satisfying results emerged from a study conducted by Ghahremani et al. (109). The effects of modafinil between 16 abstinent methamphetamine (MA) dependent patients were compared to the ones of 19 healthy individuals. The main outcome was the percentage of correct responses in a behavioral test, where participants were shown a picture and asked to chose one of two possible answer keys. Upon a correct response a blue light turned on, while a red light indicated an incorrect choice. Some pictures inverted their correct answers without any warning during the course of the test. Therefore the participants were not only being tested in their ability to learn a task, but also whether they were mentally flexible to switch the correct answer. Both MA dependent and healthy individuals were tested at baseline, showing a superior performance by the healthy individuals. In the second part of the study, 200 mg of modafinil or placebo were administered two hours before the test to both groups. Modafinil increased the accuracy for the MA dependent group up to control levels, while healthy individuals did not experience a change in their performance. During each test, the brains of the study subjects were subjected to fMRI scans, which showed an increased activation of the anterior cingulate complex (ACC) in MA dependent subjects taking modafinil. None of these changes were encountered when placebo was administered and never emerged in the fMRI scans of healthy subjects. Other studies showed similar results to the ones examined above (110).

Summary

From the evidence collected so far it is not clear whether modafinil can be used to treat cocaine dependence. Ideally a treatment needs to be effective for the whole patient population, something that modafinil fails to do. Yet it seems like modafinil still has some beneficial effects, which prevents from excluding it as a possible candidate for substance dependence treatment. More studies are needed to uncover the various unknowns regarding modafinil. The fact that it is effective for sleeping disorders suggests that the oral route of administration is capable of providing the right concentration in the brain. Therefore future studies using different routes of administration are unlikely to succeed. The 200 mg dose is also something that should not be altered in future research, since the 400 mg was not superior in any of the experiments conducted. Because of the suspected interaction between modafinil, cocaine and alcohol, animal studies would be required to verify whether this interaction is real. Finally, since strong results were not obtained on the ability of modafinil to decrease cocaine use, additional studies could direct their attention to relapse prevention. This is a very plausible option given that modafinil has mild psychoactive properties, something that a drug for the treatment of cocaine dependence should have.

From the evidence gathered so far on methamphetamine studies, it appears that modafinil can improve learning in MA dependent individuals. However, more clinical trials are needed, especially ones that can recruit a higher number of patients. All studies produced so far have had a small number of participants. The next question to be answered is whether modafinil can increase abstinence in MA dependent subjects undergoing cognitive behavioral therapy. The need for these additional studies is also supported by the results coming from the fMRI scans. It is known from earlier publications that MA dependent patients experience a decreased activation of the ACC during cognitive tests of control and vigilance. The ACC has also been correlated with improved performance in the behavioral tests like the one used by Ghahremani et al. Thus, the fact that the MA dependent patients experienced an increased activation of the ACC during the study is a significant achievement. Modafinil thus shows promise as a supplement to cognitive behavioral therapy in MA dependent subjects. Another possible route to be investigated is the potential benefits of modafinil in decreasing MA consumption or relapse. However the incentive to pursue this direction is hindered by inconclusive results from cocaine studies.

DISCUSSION

Cognitive enhancers represent a new approach to the treatment of substance dependence. They often have mechanisms of action similar to addictive drugs, which allows them to either decrease drug consumption or increase the rate of abstinence. Some cognitive enhancers target neurotransmitter systems more specifically involved with cognitive function, compensating for the deficits resulting from long term exposure to addictive substances.

Among all the cognitive enhancers reviewed so far, selegiline has the most interesting mechanism of action due to the variety of changes that it causes in the brain. The function that is most relevant to substance dependence is the decrease in DA breakdown by inhibition of MAO. There is reason to believe that targeting MAO would be beneficial in a brain that has decreased levels of DA in the mesolimbic system. Another interesting function is the decrease in hydrogen peroxide as a result of the MAO inhibition. This allows selegiline to have neuroprotective properties, especially because MAO is found in the mitochondria, where oxygen radicals are especially harmful to the survival of a cell. Investigators have also found other neuroprotective effects that are not related to the inhibition of MAO, which contribute to its use in Parkinson's disease and as a potential treatment for addiction (79).

The extensive literature on the putative function of selegiline has encouraged researchers to conduct a significant amount of animal studies. Most of them have shown promising results. However, clinical trials did not test selegiline on the same addictive drugs used in the animal models. For example selegiline shows efficacy in treating animal models of opioid and alcohol dependence, but not for cocaine and nicotine. Clinical studies have focused on cocaine and nicotine, without achieving successful results. Investigators have not yet conducted clinical research on opioid and ethanol addiction. To summarize, even though selegiline appears to be ineffective in cocaine and nicotine dependent humans, it might prove to be therapeutic in other kinds of addiction. The potential of this pharmacological agent remains to be further investigated.

Research on modafinil also needs to be further delved into. The available information on this drug makes it one of the most exciting cognitive enhancers to investigate. Its mechanism of action is still not fully elucidated, due mostly to the multiplicity of neurotransmitter system that it affects. Contrary to the case of selegiline, animal studies have been influential in directing the clinical trials. Cocaine studies in rats and monkeys have been followed by similar studies in humans. Results from the latter show that modafinil decreases consumption only in the absence of a comorbid alcohol addiction. Therefore the efficacy of modafinil in cocaine dependence remains uncertain and more research is needed for a final consensus. The wake promoting and cognitive enhancing properties of modafinil led to the idea of using it to reverse cognitive deficits found in withdrawal from addictive drugs. Animal studies that can prove this idea still need to be conducted. In clinical trials however, modafinil has been the only cognitive enhancer tested for modifying cognitive function in humans during a period of abstinence. The results indicate that modafinil is effective in enhancing cognitive functions, making it the most exciting project in substance dependence research.

Reboxetine is the cognitive enhancer that has the highest success rate in clinical trials, even though this might be due to the fact that only preliminary studies have been conducted. Substances that cause dependence, such as cocaine and amphetamine, increase NE transmission. On the other hand, reboxetine dampens the levels of synaptic NE by indirectly stimulating α2 adrenergic autoreceptors. Therefore, by causing changes in the brain that are opposite to ones caused by addictive drugs, reboxetine is unlikely to cause dependence and it is a good candidate for treating substance related disorders.

Another molecular target of reboxetine is the nAChR. Investigators conducted an experiment aimed at decreasing nicotine self-administration in a rat model. Even though results from this study were encouraging, clinical trials have not verified if reboxetine works as a smoking cessation agent.

One clinical trial in patients with cocaine addiction and another one in patients with MDMA addiction have been conducted, both of which show that reboxetine is effective. Although still in the preliminary stage, these studies make reboxetine a promising cognitive enhancer for attenuating the effects of psychostimulants. In fact none of the literature on the other drugs reviewed so far have achieved results of comparable success.

Research with Atomoxetine has made the least progress compared to other cognitive enhancers. The major difficulty has been trying to translate its efficacy from the laboratory to the clinic. Aside from acting on brain regions responsible for cognitive function, atomoxetine also has anti-depressant effects. These qualities led to animal studies that tried to improve cognitive function and decrease consumption of cocaine. In both cases investigators generated the most convincing evidence out of all the animal studies conducted on cognitive enhancers. This success was surprisingly not reflected in the human condition. Atomoxetine could not maintain abstinence from alcohol drinking and it was not superior to already existing medications for smoking cessation. In marijuana users it was not effective and it caused adverse side effects. Lastly, preliminary studies seemed encouraging for amphetamine users but not for cocaine users.

A review of the available literature on cognitive enhancers has pointed out that numerous clinical trials have been conducted with minimal preclinical evidence. This discrepancy probably originated from the fact that many of the cognitive enhancers under investigation have already been approved for other pathological disorders. Because these medications are safe and tolerable, going straight to the clinical trial might be a more direct way to assess the efficacy of a drug. The caveat is that the therapeutic potential of some cognitive enhancers is unknown before being tested on humans. Therefore a classical dilemma in medical research arises. On one hand, the translational aspect of medicine has been recognized as the standard approach that best predicts the success of a clinical trial. According to this approach, animal studies should precede clinical studies. On the other hand, there are numerous examples of animal studies that are not good predictors of success in clinical trials - as in the case of atomoxetine. Therefore one could also argue for a more 'trial and error' approach.

What remains certain is that the potential of cognitive enhancers has yet to be revealed. This is especially true regarding the ability to ameliorate cognitive deficits experienced during abstinence. Future clinical studies will hopefully learn from the mistakes of the past and uncover new therapeutic effects for this class of drugs. The present thesis was able to examine only four pharmacological agents, but others cognitive enhancers such as memantine and galantimine are also under investigation. Research conducted in the upcoming years will continue to feed excitement and progress in the discovery of a treatment for substance dependence.

LIST OF JOURNAL ABBREVIATIONS

Addict Biol.	Addiction Biology
Alcohol. Clin. Exp. Res.	Alcoholism, Clinical and Experimental Research
Am. J. Drug Alcohol Abuse	American Journal of Drug and Alcohol Abuse
Am. J. Psychiatry	American Journal of Psychiatry
Behav. Brain Res.	Behavioural Brain Research
Biochem. Pharmacol.	Biochemical pharmacology
Biochim. Biophys. Acta.	Biochimica et Biophysica Acta
Biol. Psychiatry.	Biological Psychiatry
BMC Clin Pharmacol.	BMC Clinical Pharmacology [electronic resource]
Clin. Pharmacol. Ther.	Clinical Pharmacology and Therapeutics
Cochrane Database Syst. Rev.	Cochrane Database of Systematic Reviews
Curr. Top. Behav. Neurosci	Current Topics in Behavioral Neuroscience
Drug Alcohol Depend.	Drug and Alcohol Dependence
Eur. J. Neurosci.	The European Journal of Neuroscience
Eur. J. Pharmacol.	European Journal of Pharmacology
Eur Neuropsychopharmacol.	European Neuropsychopharmacology: the Journal of the European College of Neuropsychopharmacology
Exp Clin Psychopharmacol.	Experimental and Clinical Psychopharmacology
Hum Psychopharmacol.	Human Psychopharmacology
Int. Rev. Neurobiol.	International Review of Neurobiology

J Affect Disord.	Journal of Affective Disorders
JAMA	Journal of the American Medical Association
J. Clin. Psychiatry	Journal of Clinical Psychiatry
J. Med. Chem	Journal of Medicinal Chemistry
J. Nerv. Ment. Dis.	Journal of Nervous and Mental Disease
J. Neurochem.	Journal of Neurochemistry
J. Neurophysiol.	Journal of Neurophysiology
J. Neurosci.	Journal of Neuroscience
J. Pharmacol. Exp. Ther.	The Journal of Pharmacology and Experimental Therapeutics
J. Psychopharmacol (Oxford)	Journal of psychopharmacology / British Association for Psychopharmacology
Mol. Pharmacol.	Molecular Pharmacology
Nat. Neurosci.	Nature Neuroscience
N. Engl. J. Med.	New England Journal of Medicine
Neuropsychol Rev.	Neuropsychology Review
Neurosci. Lett.	Neuroscience Letters
Pharmacol. Biochem. Behav.	Pharmacology, Biochemistry, and Behavior
Proc. Natl. Acad. Sci.	Proceedings of the National Academy of Sciences of the United States of America
Psychol Addict Behav.	Psychology of Addictive Behaviors: Journal of the Society of Psychologists in Addictive Behaviors
Trends Pharmacol. Sci.	Trends in Pharmacological Sciences

REFERENCES

1. P. Condon T, W. Balmer C. 71 - Neurobiology of drug addiction. In: *Neurobiology of Disease*. Burlington: Academic Press; 2007:771-779. Available at: http://www.sciencedirect.com/science/article/pii/B9780120885923500736. Accessed September 18, 2011.

2. Koob GF, Moal ML. *Neurobiology of addiction*. Academic Press; 2006.

3. First MB, ed. *Diagnostic and Statistical Manual of Mental Disorders.* 4th ed. Washington DC: American Psychiatric Association; 2000.

4. Harwood, H. Updating Estimates of the Economic Costs of Alcohol Abuse in the United States: Estimates, Update Methods, and Data. Report prepared by The Lewin Group for the National Institute on Alcohol Abuse and Alcoholism, 2000.

5. CDC - Chronic Disease - Tobacco - At A Glance. Available at: http://www.cdc.gov/chronicdisease/resources/publications/AAG/osh.htm. Accessed September 18, 2011.

6. P. Condon T, W. Balmer C. 71 - Neurobiology of drug addiction. In: *Neurobiology of Disease*. Burlington: Academic Press; 2007:771-779. Available at: http://www.sciencedirect.com/science/article/pii/B9780120885923500736. Accessed September 18, 2011.

7. Brundege JM, Williams JT. Differential modulation of nucleus accumbens synapses. *J. Neurophysiol.* 2002;88(1):142-151.

8. Johnson SW, North RA. Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J. Neurosci.* 1992;12(2):483-488.

9. Britt JP, McGehee DS. Presynaptic opioid and nicotinic receptor modulation of dopamine overflow in the nucleus accumbens. *J. Neurosci.* 2008;28(7):1672-1681.

10. Trovero F, Herve D, Desban M, Glowinski J, Tassin JP. Striatal opiate mu-receptors are not located on dopamine nerve endings in the rat. *Neuroscience*. 1990;39(2):313-321.

11. Okamoto T, Harnett MT, Morikawa H. Hyperpolarization-activated cation current (Ih) is an ethanol target in midbrain dopamine neurons of mice. *J. Neurophysiol.* 2006;95(2):619-626.

12. Stobbs SH, Ohran AJ, Lassen MB, et al. Ethanol suppression of ventral tegmental area GABA neuron electrical transmission involves N-methyl-D-aspartate receptors. *J. Pharmacol. Exp. Ther.* 2004;311(1):282-289.

13. Rewal M, Jurd R, Gill TM, et al. Alpha4-containing GABAA receptors in the nucleus accumbens mediate moderate intake of alcohol. *J. Neurosci.* 2009;29(2):543-549.

14. Heikkila RE, Orlansky H, Mytilineou C, Cohen G. Amphetamine: evaluation of d- and l-isomers as releasing agents and uptake inhibitors for 3H-dopamine and 3H-norepinephrine in slices of rat neostriatum and cerebral cortex. *J. Pharmacol. Exp. Ther.* 1975;194(1):47-56.

15. Jones SR, Joseph JD, Barak LS, Caron MG, Wightman RM. Dopamine neuronal transport kinetics and effects of amphetamine. *J. Neurochem.* 1999;73(6):2406-2414.

16. Pifl C, Drobny H, Reither H, Hornykiewicz O, Singer EA. Mechanism of the dopamine-releasing actions of amphetamine and cocaine: plasmalemmal dopamine transporter versus vesicular monoamine transporter. *Mol. Pharmacol.* 1995;47(2):368-373.

17. Larsen KE, Fon EA, Hastings TG, Edwards RH, Sulzer D. Methamphetamine-induced degeneration of dopaminergic neurons involves autophagy and upregulation of dopamine synthesis. *J. Neurosci.* 2002;22(20):8951-8960.

18. Ramsay RR, Hunter DJB. Inhibitors alter the spectrum and redox properties of monoamine oxidase A. *Biochim. Biophys. Acta.* 2002;1601(2):178-184.

19. Schmitz Y, Lee CJ, Schmauss C, Gonon F, Sulzer D. Amphetamine distorts stimulation-dependent dopamine overflow: effects on D2 autoreceptors, transporters, and synaptic vesicle stores. *J. Neurosci.* 2001;21(16):5916-5924.

20. Giros B, Jaber M, Jones SR, Wightman RM, Caron MG. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature.* 1996;379(6566):606-612.

21. Rocha BA, Fumagalli F, Gainetdinov RR, et al. Cocaine self-administration in dopamine-transporter knockout mice. *Nat. Neurosci.* 1998;1(2):132-137.

22. Bennett BA, Wichems CH, Hollingsworth CK, et al. Novel 2-substituted cocaine analogs: uptake and ligand binding studies at dopamine, serotonin and

norepinephrine transport sites in the rat brain. *J. Pharmacol. Exp. Ther.* 1995;272(3):1176-1186.

23. Beveridge TJR, Smith HR, Nader MA, Porrino LJ. Effects of chronic cocaine self-administration on norepinephrine transporters in the nonhuman primate brain. *Psychopharmacology (Berl.)*. 2005;180(4):781-788.

24. Heien MLAV, Khan AS, Ariansen JL, et al. Real-time measurement of dopamine fluctuations after cocaine in the brain of behaving rats. *Proc. Natl. Acad. Sci. U.S.A.* 2005;102(29):10023-10028.

25. Mokdad AH, Marks JS, Stroup DF, Gerberding JL. Actual causes of death in the United States, 2000. *JAMA*. 2004;291(10):1238-1245.

26. Mansvelder HD, Keath JR, McGehee DS. Synaptic mechanisms underlie nicotine-induced excitability of brain reward areas. *Neuron*. 2002;33(6):905-919.

27. Zhang H, Sulzer D. Frequency-dependent modulation of dopamine release by nicotine. *Nat. Neurosci.* 2004;7(6):581-582.

28. Drugs@FDA. Available at: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.SearchAction&SearchType=BasicSearch&SearchTerm=methadone. Accessed September 19, 2011.

29. Edens E, Massa A, Petrakis I. Novel pharmacological approaches to drug abuse treatment. *Curr Top Behav Neurosci.* 2010;3:343-386.

30. Dyer KR, White JM. Patterns of symptom complaints in methadone maintenance patients. *Addiction*. 1997;92(11):1445-1455.

31. Curran HV, Bolton J, Wanigaratne S, Smyth C. Additional methadone increases craving for heroin: a double-blind, placebo-controlled study of chronic opiate users receiving methadone substitution treatment. *Addiction*. 1999;94(5):665-674.

32. Langleben DD, Ruparel K, Elman I, et al. Acute effect of methadone maintenance dose on brain FMRI response to heroin-related cues. *Am J Psychiatry*. 2008;165(3):390-394.

33. Wang W, Li Q, Wang Y, et al. Brain fMRI and craving response to heroin-related cues in patients on methadone maintenance treatment. *Am J Drug Alcohol Abuse*. 2011;37(2):123-130.

34. Drugs@FDA. Available at: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.SearchAction&SearchType=BasicSearch&SearchTerm=BUPRENORPHINE. Accessed September 18, 2011.

35. Johnson RE, Jaffe JH, Fudala PJ. A controlled trial of buprenorphine treatment for opioid dependence. *JAMA*. 1992;267(20):2750-2755.

36. Kosten TR, Schottenfeld R, Ziedonis D, Falcioni J. Buprenorphine versus methadone maintenance for opioid dependence. *J. Nerv. Ment. Dis.* 1993;181(6):358-364.

37. Strain, E.C., Stitzer, M.L., Liebson, I.A., Bigelow, G.E., 1994a. Buprenorphine versus methadone in the treatment of opioiddependent cocaine users. Psychopharmacology (Berlin) 116, 401.

38. Lobmaier P, Kornor H, Kunoe N, Bjorndal A (2008) Sustained-release naltrexone for opioid dependence. Cochrane Database Syst Rev: CD006140.

39. Naassila M, Hammoumi S, Legrand E, Durbin P, Daoust M. Mechanism of action of acamprosate. Part I. Characterization of spermidine-sensitive acamprosate binding site in rat brain. *Alcohol. Clin. Exp. Res.* 1998;22(4):802-809.

40. Mason BJ. Treatment of alcohol-dependent outpatients with acamprosate: a clinical review. *J Clin Psychiatry*. 2001;62 Suppl 20:42-48.

41. Fuller RK, Branchey L, Brightwell DR, et al. Disulfiram treatment of alcoholism. A Veterans Administration cooperative study. *JAMA*. 1986;256(11):1449-1455.

42. Stead LF, Perera R, Bullen C, Mant D, Lancaster T. Nicotine replacement therapy for smoking cessation. *Cochrane Database Syst Rev*. 2008;(1):CD000146.

43. Buchhalter AR, Fant RV, Henningfield JE. Novel pharmacological approaches for treating tobacco dependence and withdrawal: current status. *Drugs*. 2008;68(8):1067-1088.

44. Paterson NE, Balfour DJ, Markou A. Chronic bupropion attenuated the anhedonic component of nicotine withdrawal in rats via inhibition of dopamine reuptake in the nucleus accumbens shell. *Eur. J. Neurosci.* 2007;25(10):3099-3108.

45. Slemmer JE, Martin BR, Damaj MI. Bupropion is a nicotinic antagonist. *J. Pharmacol. Exp. Ther.* 2000;295(1):321-327.

46. Shiffman S, Johnston JA, Khayrallah M, et al. The effect of bupropion on nicotine craving and withdrawal. *Psychopharmacology (Berl.)*. 2000;148(1):33-40.

47. Coe JW, Brooks PR, Vetelino MG, et al. Varenicline: an alpha4beta2 nicotinic receptor partial agonist for smoking cessation. *J. Med. Chem.* 2005;48(10):3474-3477.

48. Ebbert JO, Wyatt KD, Hays JT, Klee EW, Hurt RD. Varenicline for smoking cessation: efficacy, safety, and treatment recommendations. *Patient Prefer Adherence*. 2010;4:355-362.

49. Potenza MN, Sofuoglu M, Carroll KM, Rounsaville BJ. Neuroscience of behavioral and pharmacological treatments for addictions. *Neuron.* 2011;69(4):695-712.

50. Lê AD, Harding S, Juzytsch W, Funk D, Shaham Y. Role of alpha-2 adrenoceptors in stress-induced reinstatement of alcohol seeking and alcohol self-administration in rats. *Psychopharmacology*. 2004;179:366-373.

51. Lê AD, Harding S, Juzytsch W, et al. The role of corticotrophin-releasing factor in stress-induced relapse to alcohol-seeking behavior in rats. *Psychopharmacology (Berl.)*. 2000;150(3):317-324.

52. Olive MF. Cognitive effects of Group I metabotropic glutamate receptor ligands in the context of drug addiction. *Eur. J. Pharmacol.* 2010;639(1-3):47-58.

53. Ersche KD, Fletcher PC, Roiser JP, et al. Differences in orbitofrontal activation during decision-making between methadone-maintained opiate users, heroin users and healthy volunteers. *Psychopharmacology (Berl.)*. 2006;188(3):364-373.

54. Aharonovich E, Hasin DS, Brooks AC, et al. Cognitive deficits predict low treatment retention in cocaine dependent patients. *Drug Alcohol Depend.* 2006;81(3):313-322.

55. Bates ME, Pawlak AP, Tonigan JS, Buckman JF. Cognitive impairment influences drinking outcome by altering therapeutic mechanisms of change. *Psychol Addict Behav*. 2006;20(3):241-253.

56. Bymaster FP, Katner JS, Nelson DL, et al. Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: a potential mechanism for efficacy in attention deficit/hyperactivity disorder. *Neuropsychopharmacology*. 2002;27(5):699-711.

57. Swanson CJ, Perry KW, Koch-Krueger S, et al. Effect of the attention deficit/hyperactivity disorder drug atomoxetine on extracellular concentrations of norepinephrine and dopamine in several brain regions of the rat. *Neuropharmacology*. 2006;50(6):755-760.

58. Davis JA, Gould TJ. Atomoxetine reverses nicotine withdrawal-associated deficits in contextual fear conditioning. *Neuropsychopharmacology*. 2007;32(9):2011-2019.

59. Reichel CM, Linkugel JD, Bevins RA. Nicotine as a conditioned stimulus: impact of attention-deficit/hyperactivity disorder medications. *Exp Clin Psychopharmacol*. 2007;15(5):501-509.

60. Gould TJ, Rukstalis M, Lewis MC. Atomoxetine and nicotine enhance prepulse inhibition of acoustic startle in C57BL/6 mice. *Neurosci. Lett*. 2005;377(2):85-90.

61. Ersche KD, Sahakian BJ. The neuropsychology of amphetamine and opiate dependence: implications for treatment. *Neuropsychol Rev*. 2007;17(3):317-336.

62. Economidou D, Dalley JW, Everitt BJ. Selective norepinephrine reuptake inhibition by atomoxetine prevents cue-induced heroin and cocaine seeking. *Biol. Psychiatry*. 2011;69(3):266-274.

63. Economidou D, Pelloux Y, Robbins TW, Dalley JW, Everitt BJ. High impulsivity predicts relapse to cocaine-seeking after punishment-induced abstinence. *Biol. Psychiatry*. 2009;65(10):851-856.

64. Brenhouse HC, Dumais K, Andersen SL. Enhancing the salience of dullness: behavioral and pharmacological strategies to facilitate extinction of drug-cue associations in adolescent rats. *Neuroscience*. 2010;169(2):628-636.

65. Sofuoglu M, Hill K, Kosten T, Poling J. Atomoxetine Effects on Stress and Dextroamphetamine Responses in Humans. 2008a(in submission).

66. Stoops WW, Blackburn JW, Hudson DA, Hays LR, Rush CR. Safety, tolerability and subject-rated effects of acute intranasal cocaine administration during atomoxetine maintenance. *Drug Alcohol Depend*. 2008;92(1-3):282-285.

67. Levin FR, Mariani JJ, Secora A, et al. Atomoxetine Treatment for Cocaine Abuse and Adult Attention-Deficit Hyperactivity Disorder (ADHD): A Preliminary Open Trial. *Journal of Dual Diagnosis*. 2009;5(1):41-56.

68. Sofuoglu M, Sewell RA. Norepinephrine and stimulant addiction. *Addict Biol*. 2009;14(2):119-129.

69. Ray R, Rukstalis M, Jepson C, et al. Effects of atomoxetine on subjective and neurocognitive symptoms of nicotine abstinence. *J. Psychopharmacol. (Oxford).* 2009;23(2):168-176.

70. Wilens TE, Adler LA, Weiss MD, et al. Atomoxetine treatment of adults with ADHD and comorbid alcohol use disorders. *Drug Alcohol Depend.* 2008;96(1-2):145-154.

71. Tirado CF, Goldman M, Lynch K, Kampman KM, Obrien CP. Atomoxetine for treatment of marijuana dependence: A report on the efficacy and high incidence of gastrointestinal adverse events in a pilot study. *Drug and Alcohol Dependence.* 2008;94(1-3):254-257.

72. Kobayashi T, Washiyama K, Ikeda K. Inhibition of G-protein-activated inwardly rectifying K+ channels by the selective norepinephrine reuptake inhibitors atomoxetine and reboxetine. *Neuropsychopharmacology.* 2010;35(7):1560-1569.

73. Szabo ST, Blier P. Effect of the selective noradrenergic reuptake inhibitor reboxetine on the firing activity of noradrenaline and serotonin neurons. *Eur. J. Neurosci.* 2001;13(11):2077-2087.

74. Nestler EJ. Historical review: Molecular and cellular mechanisms of opiate and cocaine addiction. *Trends Pharmacol. Sci.* 2004;25(4):210-218.

75. Miller DK, Wong EHF, Chesnut MD, Dwoskin LP. Reboxetine: functional inhibition of monoamine transporters and nicotinic acetylcholine receptors. *J. Pharmacol. Exp. Ther.* 2002;302(2):687-695.

76. Rauhut AS, Mullins SN, Dwoskin LP, Bardo MT. Reboxetine: attenuation of intravenous nicotine self-administration in rats. *J. Pharmacol. Exp. Ther.* 2002;303(2):664-672.

77. Szerman N, Peris L, Mesías B, et al. Reboxetine for the treatment of patients with Cocaine Dependence Disorder. *Hum Psychopharmacol.* 2005;20(3):189-192.

78. Hysek CM, Simmler LD, Ineichen M, et al. The norepinephrine transporter inhibitor reboxetine reduces stimulant effects of MDMA ("ecstasy") in humans. *Clin. Pharmacol. Ther.* 2011;90(2):246-255.

79. Magyar K. The pharmacology of selegiline. *Int. Rev. Neurobiol.* 2011;100:65-84.

80. He S, Grasing K. L-methamphetamine and selective MAO inhibitors decrease morphine-reinforced and non-reinforced behavior in rats; Insights towards

selegiline's mechanism of action. *Pharmacol. Biochem. Behav.* 2006;85(4):675-688.

81. Gerlach M, Youdim MB, Riederer P. Pharmacology of selegiline. *Neurology.* 1996;47(6 Suppl 3):S137-145.

82. Waldmeier PC, Felner AE. Deprenil: loss of selectivity for inhibition of B-type MAO after repeated treatment. *Biochem. Pharmacol.* 1978;27(5):801-802.

83. Grasing K, Ghosh S. Selegiline prevents long-term changes in dopamine efflux and stress immobility during the second and third weeks of abstinence following opiate withdrawal. *Neuropharmacology.* 1998;37(8):1007-1017.

84. Cohen C, Curet O, Perrault G, Sanger DJ. Reduction of oral ethanol self-administration in rats by monoamine oxidase inhibitors. *Pharmacol. Biochem. Behav.* 1999;64(3):535-539.

85. Curet O, Damoiseau-Ovens G, Sauvage C, et al. Preclinical profile of befloxatone, a new reversible MAO-A inhibitor. *J Affect Disord.* 1998;51(3):287-303.

86. George SR, Fan T, Ng GY, et al. Low endogenous dopamine function in brain predisposes to high alcohol preference and consumption: reversal by increasing synaptic dopamine. *J. Pharmacol. Exp. Ther.* 1995;273(1):373-379.

87. Siu ECK, Tyndale RF. Selegiline is a mechanism-based inactivator of CYP2A6 inhibiting nicotine metabolism in humans and mice. *J. Pharmacol. Exp. Ther.* 2008;324(3):992-999.

88. Yasar S, Gaál J, Panlilio LV, et al. A comparison of drug-seeking behavior maintained by D-amphetamine, L-deprenyl (selegiline), and D-deprenyl under a second-order schedule in squirrel monkeys. *Psychopharmacology (Berl.).* 2006;183(4):413-421.

89. Winger GD, Yasar S, Negus SS, Goldberg SR. Intravenous self-administration studies with l-deprenyl (selegiline) in monkeys. *Clin. Pharmacol. Ther.* 1994;56(6 Pt 2):774-780.

90. Bartzokis G, Beckson M, Newton T, et al. Selegiline effects on cocaine-induced changes in medial temporal lobe metabolism and subjective ratings of euphoria. *Neuropsychopharmacology.* 1999;20(6):582-590.

91. Houtsmuller EJ, Notes LD, Newton T, et al. Transdermal selegiline and intravenous cocaine: safety and interactions. *Psychopharmacology (Berl.)*. 2004;172(1):31-40.

92. Elkashef A, Fudala PJ, Gorgon L, et al. Double-blind, placebo-controlled trial of selegiline transdermal system (STS) for the treatment of cocaine dependence. *Drug Alcohol Depend.* 2006;85(3):191-197.

93. Harris DS, Everhart T, Jacob P 3rd, et al. A phase 1 trial of pharmacologic interactions between transdermal selegiline and a 4-hour cocaine infusion. *BMC Clin Pharmacol.* 2009;9:13.

94. Biberman R, Neumann R, Katzir I, Gerber Y. A randomized controlled trial of oral selegiline plus nicotine skin patch compared with placebo plus nicotine skin patch for smoking cessation. *Addiction.* 2003;98(10):1403-1407.

95. Weinberger AH, Reutenauer EL, Jatlow PI, et al. A double-blind, placebo-controlled, randomized clinical trial of oral selegiline hydrochloride for smoking cessation in nicotine-dependent cigarette smokers. *Drug Alcohol Depend.* 2010;107(2-3):188-195.

96. Killen JD, Fortmann SP, Murphy GM Jr, et al. Failure to improve cigarette smoking abstinence with transdermal selegiline + cognitive behavior therapy. *Addiction.* 2010;105(9):1660-1668.

97. Mignot E, Nishino S, Guilleminault C, Dement WC. Modafinil binds to the dopamine uptake carrier site with low affinity. *Sleep.* 1994;17(5):436-437.

98. Madras BK, Xie Z, Lin Z, et al. Modafinil occupies dopamine and norepinephrine transporters in vivo and modulates the transporters and trace amine activity in vitro. *J. Pharmacol. Exp. Ther.* 2006;319(2):561-569.

99. Simon P, Hémet C, Ramassamy C, Costentin J. Non-amphetaminic mechanism of stimulant locomotor effect of modafinil in mice. *Eur Neuropsychopharmacol.* 1995;5(4):509-514.

100. Wisor JP, Eriksson KS. Dopaminergic-adrenergic interactions in the wake promoting mechanism of modafinil. *Neuroscience.* 2005;132(4):1027-1034.

101. Minzenberg MJ, Carter CS. Modafinil: a review of neurochemical actions and effects on cognition. *Neuropsychopharmacology.* 2008;33(7):1477-1502.

102. Tahsili-Fahadan P, Carr GV, Harris GC, Aston-Jones G. Modafinil blocks reinstatement of extinguished opiate-seeking in rats: mediation by a glutamate mechanism. *Neuropsychopharmacology.* 2010;35(11):2203-2210.

103. Ferraro L, Antonelli T, O'Connor WT, et al. The effects of modafinil on striatal, pallidal and nigral GABA and glutamate release in the conscious rat: evidence for a preferential inhibition of striato-pallidal GABA transmission. *Neurosci. Lett.* 1998;253(2):135-138.

104. Deroche-Gamonet V, Darnaudéry M, Bruins-Slot L, et al. Study of the addictive potential of modafinil in naive and cocaine-experienced rats. *Psychopharmacology (Berl.).* 2002;161(4):387-395.

105. Bernardi RE, Lewis JR, Lattal KM, Berger SP. Modafinil reinstates a cocaine conditioned place preference following extinction in rats. *Behav. Brain Res.* 2009;204(1):250-253.

106. Newman JL, Negus SS, Lozama A, Prisinzano TE, Mello NK. Behavioral evaluation of modafinil and the abuse-related effects of cocaine in rhesus monkeys. *Exp Clin Psychopharmacol.* 2010;18(5):395-408.

107. Dackis CA, Kampman KM, Lynch KG, Pettinati HM, O'Brien CP. A double-blind, placebo-controlled trial of modafinil for cocaine dependence. *Neuropsychopharmacology.* 2005;30(1):205-211.

108. Anderson AL, Reid MS, Li S-H, et al. Modafinil for the treatment of cocaine dependence. *Drug Alcohol Depend.* 2009;104(1-2):133-139.

109. Ghahremani DG, Tabibnia G, Monterosso J, et al. Effect of modafinil on learning and task-related brain activity in methamphetamine-dependent and healthy individuals. *Neuropsychopharmacology.* 2011;36(5):950-959.

110. Hester R, Lee N, Pennay A, Nielsen S, Ferris J. The effects of modafinil treatment on neuropsychological and attentional bias performance during 7-day inpatient withdrawal from methamphetamine dependence. *Exp Clin Psychopharmacol.* 2010;18(6):489-497.

VITA

[redacted]

[redacted]