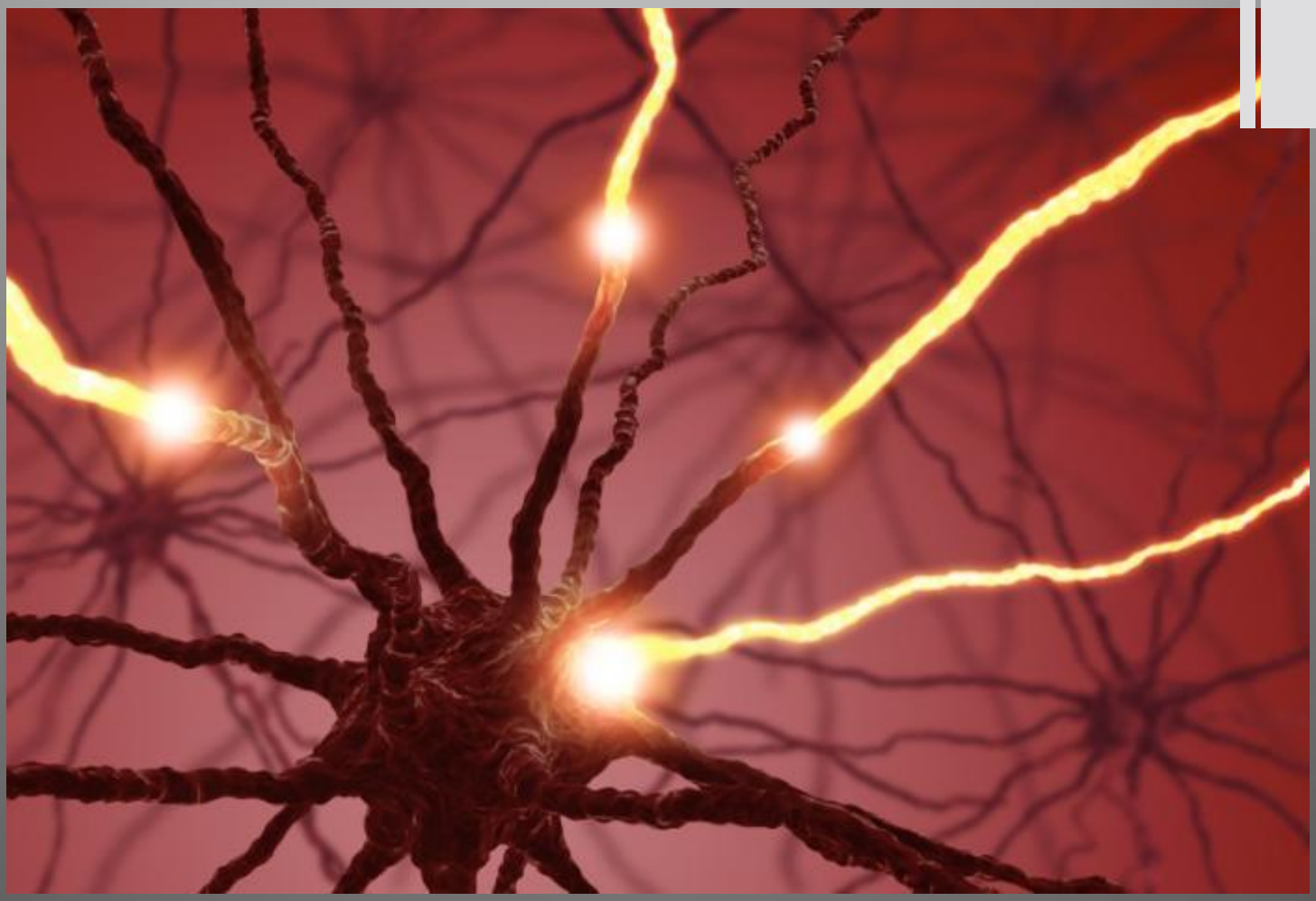

Increased extracellular serotonin due to SSRIs and its role in substance-dependence related behaviours

Bachelor Thesis
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Picture cover: (Iliades, 2014)

Abstract

An increasing number of studies support a role for serotonin (5-HT) and its transporter (SERT) in substance dependence related behaviours. Moreover, selective serotonin reuptake inhibitors (SSRIs), increase extracellular 5-HT levels by blocking SERT. Contradicting effects on substance dependence related behaviours are reported in SERT knockout rodents and SSRI treated rodents, although both show increased synaptic 5-HT. Several case studies describe development of alcohol dependence following a treatment with SSRIs. However, in multiple studies on SERT knockout rats, an increase of substance dependence related behaviours was observed. Therefore this thesis focussed on the modulating effects of increased synaptic 5-HT (due to SSRIs) on substance dependence related behaviours.

Concluding, increased synaptic 5-HT seems to have an inhibiting or satiating effect on substance related behaviours. Increased synaptic 5-HT due to SSRI use even shows a potential therapeutic role in substance dependence. However, SERT knockout rats and humans with polymorphisms in the gene encoding for SERT are associated with enhanced substance dependence and related behaviours. Possibly prenatal exposure to SSRIs or low expression of SERT may increase substance dependence related behaviour. However, more research is needed on prenatal and long-term exposure to SSRIs in models for depression and addiction to understand its effect on substance dependence related behaviours.

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Introduction

The neurotransmitter serotonin or 5-hydroxytryptamine (5-HT) is known for its regulating effect on various behavioural and physiological functions including impulsivity, aggression and feeding behaviour.[1,2] Dysregulation of the serotonergic system is often associated with several disease states including depression and consequently 5-HT is a main target in the treatment of affective disorders. [3,4]

Moreover, 5-HT also seems to play an important role in the reward-system. Although the majority of research on reward has focused on the dopamine and opioid systems, an increasing number of studies support the idea that 5-HT receptors are important mediators in the reward system. [5] 5-HT seems to be playing an important role in representing the value of the rewards and regulation of dopamine neurotransmission. [6,7]
For this reason, increasingly more studies are done on the effects of 5-HT receptors and their effect on addiction. [8] Furthermore, research has focussed on the 5-HT reuptake transporter (SERT) to which antidepressants like selective serotonin reuptake inhibitors (SSRIs) bind. SSRIs are one of the most prescribed antidepressants and they modulate extracellular 5-HT levels by blocking SERT, thereby increasing extracellular 5-HT levels. [9,10]

Illustrative is a case description of Miss X, who developed alcohol dependence following a treatment with SSRIs for her depression. When she switched to an 5-HT(3) receptor antagonist, her alcohol dependence disappeared. Ninety-three cases have been reported in which SSRIs are linked to addiction. [11] Considering that antidepressants, such as SSRIs, affect the extracellular 5-HT levels drastically and that associations between the 5-HT and the reward system are present, one could argue that increased 5-HT levels due to SSRI treatment increases the dependence of a substance and related behaviour.
Data from Homberg et al. (2008) support an increase in dependence when extracellular 5-HT is increased. The SERT knockout rat, a strain lacking the 5-HT transporter and therefore has increased extracellular 5-HT, showed to be more sensitive to the rewarding effects of cocaine. [12]
In contrast, acute and chronic fluoxetine (a SSRI) treatment, which blocks SERT, has shown to decrease the sensitivity of rats to rewarding brain stimulation [13].

Contradicting results have been found in the modulating role of increased synaptic 5-HT in substance dependence related behaviours and many questions remain unanswered. Therefore the aim of this paper is to shed a light on the modulating role of increased extracellular 5-HT (due to SSRIs) on substance dependence related behaviours. Subsequently the main question is as follows: Do alterations in the 5-HT system due to SSRI treatment affect the substance dependence and related behaviours? To answer this question, additionally the role of 5-HT in the reward system will be discussed.
The focus of this thesis lies on preclinical studies, but several clinical studies are also discussed. For the preclinical studies, SSRI treatment and SERT knockout models are discussed. Furthermore, human studies on SSRI treatment and its effect on substance dependence disorder is examined.

SSRIs

First line modern antidepressants can be divided into three classes: the 5-HT and norepinephrine reuptake inhibitors (SNRI), the norepinephrine‑dopamine reuptake inhibitor (NDRI) and the selective serotonin reuptake inhibitors (SSRIs). Because SSRIs relatively fewer side effects, lower pricing and the comparable effectiveness, this new line of antidepressants replaced the older standards such as tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs).[4] In a meta-analysis from Arroll et al. (2005) 56% to 60% responded well to active SSRI treatment compared with 42% to 47% for the placebo. [14] Concluding, apart from the discontinuations due to side effects, not all depressed patients patient benefit from SSRI treatment and the placebo effects seem to play a role.
The antidepressant effects of SSRIs are believed to lie on occupying the 5-HT transporters, which inhibit the reuptake of 5-HT from the synaptic cleft. This increases the 5-HT neurotransmission after prolonged treatment, by increasing the availability of 5-HT and desensitizing the presynaptic inhibitory 5-HT1A autoreceptors. [15,16] Approximately 80 percent occupancy of the 5-HT transporters has been measured after 4 weeks of treatment. [17]

Fluoxetine, fluvoxamine, sertraline, paroxetine, citalopram and escitalopram are currently the prescribed SSRIs for depression. [4].
SSRIs are chemically distinct and differentiate between each other in their affinity and selectivity to the 5-HT reuptake inhibitor. [18] Effects of SSRIs should balance levels of 5-HT in depressed patients. But imbalance in 5-HT and mechanisms behind the antidepressant effects of SSRIs are still in debate. [19,20] This follows from the debate around the monoamine hypothesis of depression. This hypothesis arose along with the discovery that monoamine depletion, which is caused by reserpine, increased depressive symptoms. [19] [21] Not long after that discovery, 5-HT was pointed out to be a key player in depression. [22] Though recently the low 5-HT hypothesis is being challenged by results which even support that depression follows from high levels of 5-HT and consequently high levels of glutamate. [23] Andrews et al. (2015) underline the energy regulating function of 5-HT that is disturbed in the first weeks of SSRI treatment. The symptom reduction from SSRIs, which only occur on the long term, can be considered to be an effect of compensatory responses of the brain, by tonically activated 5-HT(1A) receptors that eventually reduce the glutamate transmission . (see fig. 1) (Andrews et al. 2015)

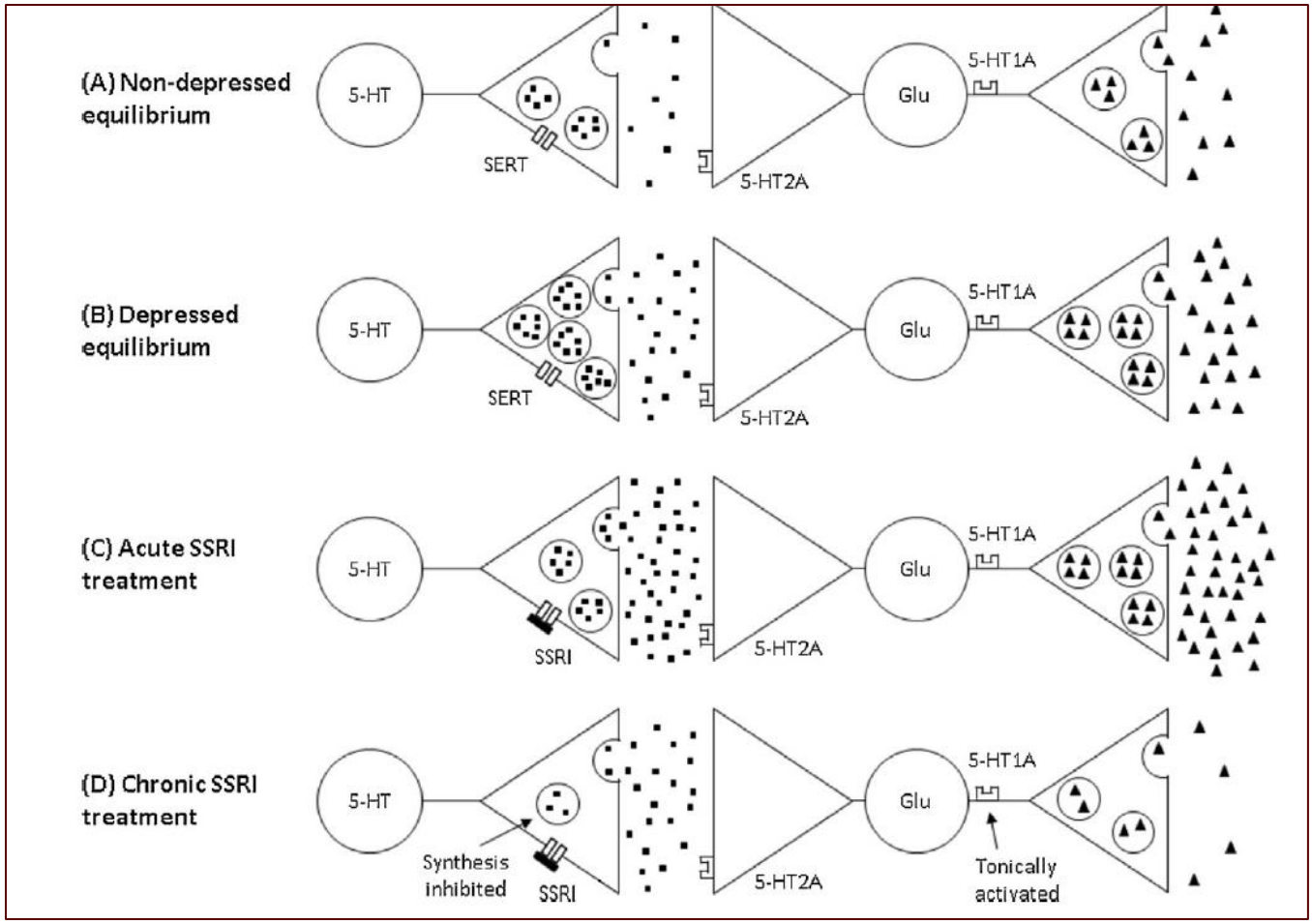

Figure 1 Hypothetical 5-HT and glutamate patterns in projection regions (e.g., the lateral PFC) over the course of depression and SSRI treatment. (A) Equilibrium 5-HT and glutamate transmission in the non-depressed state. (B) Equilibrium transmission of 5-HT and glutamate in the depressed state. (C) During acute SSRI treatment, blockade of the serotonin transporter (SERT) shifts the balance of 5-HT into the extracellular compartment. Extracellular 5-HT is therefore perturbed above the depressed equilibrium. Since SERT blockade mimics the effects of a sustained increase in 5-HT transmission, glutamatergic activity rises above the depressed equilibrium (D) Over prolonged (chronic) SSRI treatment, the brain's homeostatic mechanisms attempt to reverse the excess glutamatergic activity by inhibiting the synthesis of 5-HT, which eventually brings extracellular 5-HT back to the depressed equilibrium. These homeostatic responses reduce glutamatergic activity and produce the antidepressant response. Adapted from Andrews et al. (2015)

Substance dependence related behaviours and the role of 5-HT

One can refer to a substance use disorder in humans when a problematic pattern of use of a substance leads to clinically significant impairment or distress, and only when one is suffering from at least two of the eleven criteria documented in the DSM V, occurring within a 12 month period. In the DSM IV, substance abuse was seen as a mild or early phase of substance use disorder and dependence as the more severe manifestation. Now, in the DSM V the substance use disorder is a single diagnosis. (American Psychiatric Association, 2013) Briefly summarized, the eleven criteria are as follows: hazardous use, social/interpersonal problems related to use, neglected major roles to be able to use, withdrawal, tolerance, larger amounts used/prolonged use,

repeated attempts to quit/control use, much time spent using, physiological problems related to use and activities given up to use. [25]

First, the question arises whether SSRIs themselves cause dependence. Medical authorities do not consider SSRIs to cause dependence in humans. Despite the fact that SSRIs are widely used, the literature on substance dependence on SSRIs themselves is limited. The studies that do exist are case studies that describe mostly a previous history of substance abuse or polysubstance abuse. [26] Although the majority of the individuals using SSRIs do not abuse or depend on this antidepressant [26], this has been challenged by many for abrupt discontinuation of the treatment can cause many symptoms. The description in these symptoms are by some referred to as a discontinuation syndrome while others describe this as a withdrawal syndrome or dependence. [27]

The action of many addictive drugs result in increasing mesolimbic dopamine activity directly or indirectly. Dopamine is often pointed out as the key player in the mechanisms behind reward and addiction. [28] In more detail, the dopamine neuron activity encodes the difference between the expected reward and the actual reward. This is called reward prediction error and is considered to induce learning and modulate behavioural responses. [29] An important dopaminergic pathway in mediating drug-induced reward is the mesolimbic pathway. This pathway originates in the ventral tegmental area (VTA) and terminates in the Ventral striatum, including the Nucleus Accumbens (NAc) (see fig. 2). [30]

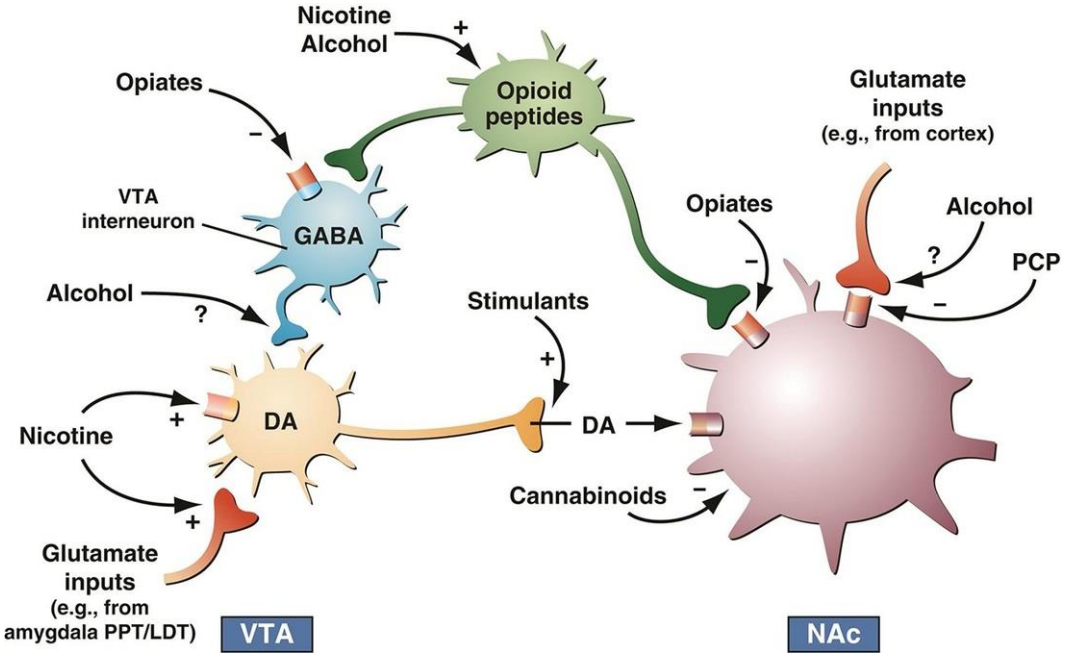

Figure 2. Simplified scheme of points of action of drugs of abuse on the VTA-NAc. Adapted from Nestler, 2005

Besides dopamine other neurochemicals, like 5-HT, are associated with regulating dependence related behaviours. Dopaminergic neurons in the VTA have been observed to receive input from 5-HT containing axon terminals. After staining the VTA of rats, direct synaptic contacts between 5-HT terminals and dopaminergic cells have been detected. [32] Electrophysical studies in primates, comparing firing patterns in the dorsal raphe nucleus (DRN) and the substantia nigra pars compacta (SN), were the first to also measure activity in serotonergic

neurons related to value of rewards. Using single-unit recording to measure firing patterns they found that the SN neurons showed phasic responses to reward prediction errors and that the DRN neurons showed tonic firing patterns related to expected and received reward.[6] Most interestingly, mice with genetic deletion of the dopamine transporter show dependence related behaviours in response to cocaine, measured by self-administration and conditioned place preference models[33,34], supporting that non-dopaminergic mechanisms add to the rewarding effects of psychostimulants. Further, double dopamine/5-HT transporter knockouts do not show cocaine place-preference[35], implying a role for 5-HT in cocaine reward. Together these results suggest a role for 5-HT in motivational and hedonistic behaviours.

The 5-HT system consists of 14 receptors. The number of studies on the different ways in which the 5-HT receptor subtypes affect Reward-related behaviours is increasing. However not all 5-HT receptors use the same mechanisms and therefore affect the reward related behaviour in different directions. [8] Several 5-HT subtypes, including the 5-HT(1B), 5-HT(1A), 5-HT(2C) and 5-HT(3), did already show to have modulating effects on the mesolimbic dopamine activity and on the behavioural effects of addictive drugs.[7] Because increased extracellular 5-HT in SERT knockout animals and SSRI treated patients may alter the behavioural effects of addictive drugs, studies on 5-HT agonists can tell more about the effects of SSRIs.

5-HT(1B) receptor agonists have been shown to potentiate both the rewarding effects of cocaine and the cocaine self-administration.[36] More recently, stimulation of the 5-HT1B receptor, using the selective 5-HT(1B) receptor agonist CP 93129, has shown to increase the mesolimbic dopamine in a microdialysis study.[37] Furthermore this study showed that the 5-HT(1B) receptor potentiated the alcohol induced dopamine increase in the nucleus accumbens.[38]
In contrast to stimulation of the 5-HT1B receptor, administration of the 5HT(2C) receptor agonist showed a reduced cocaine induced locomotion and self-administration.[39]
Furthermore, the 5-HT(3) receptor agonist 1-(m-chlorophenyl)-biguanide (CPBG) enhanced the alcohol induced dopamine release in the VTA. Also the 5-HT(3) receptor antagonist 3-tropanyl-indole-3-carboxylate (ICS 205-930) reversed this stimulating effect.[40] More recently it has been shown that combined administration of a 5-HT(3) antagonist (either zacopride or ICS 205-930) and ethanol prevented rats from self-infusing ethanol into the posterior VTA. This suggests an important modulating effect of 5-HT(3) receptors in ethanol's rewarding effects.[41]

The 5-HT(1A) receptors have often shown to modulate dopamine mediated behaviour and dopamine release in the NAc.[7] However, activation of either mainly presynaptic autoreceptors or mainly the postsynaptic receptors appear to affect the cocaine induced behaviour in opposing directions. Stimulation of mainly the postsynaptic receptors increased cocaine induced locomotion using the 5-HT(1A) agonist 8-OHDPAT. Although small doses of this agonist, which appears to preferentially stimulate the autoreceptors, decreased locomotion and basal 5-HT release. This effect was reversed by co-administration of WAY 100635, a selective 5-HT(1A) receptor antagonist.[42] It seems autoreceptors and postsynaptic receptors of these kind have opposing modulating effects on reward related behaviours. A recent review of Müller et al. (2007) concluded that stimulation of the autoreceptors will most likely facilitate addiction-related behaviours by limiting the 5-HT response in terminal areas, while stimulation of postsynaptic receptors will possibly inhibit the expression of various addiction-related behaviours directly. [43]

More research is needed to comprehend how 5-HT affects the dopamine reward system more precisely. Nevertheless, these data underline the complexity of the serotonergic system, but show the important modulating role of 5-HT on substance dependence related behaviours. Understanding of the inhibiting or stimulating effects of each 5-HT receptor subtype on the reward system would clarify more on the modulating effects of SSRIs on these pathways. SSRIs increase the extracellular 5-HT and this 5-HT presumably binds to all 5-HT receptor subtypes, thereby affecting the substance related behaviour in diverse and complex ways.[7] When 5-HT binds to inhibiting and stimulating receptor subtypes at equal amounts, the overall effect on substance dependence related behaviours may possibly even be negligible. Moreover, only 5-HT receptors in the mesolimbic pathway are described in this paragraph but receptors localised in different brain regions may also affect substance dependence related behaviours.

Animal studies

5-HT transporter knockout rodents

In animal studies, SERT knockout rodent strains can serve as a model to study lifelong SSRI use. SERT knockout strains in rodents lack functional SERT, which decreases the 5-HT reuptake and the 5-HT turnover. This increases the extracellular 5-HT and thereby affects the different 5-HT receptors that are free to bind.[44] They have shown higher extracellular 5-HT and no changes in the dopamine levels, because of their reduction in SERT.[44–46]

These SERT knockout rodent strains can also be used to study the effect of chronically increased extracellular 5-HT on substance dependence related behaviours. In a study of Homberg et al. (2008), SERT knockout rats, heterogeneous rats and wildtype rats could self-administer different doses of cocaine (0.3 mg/kg, 0.6 mg/kg or 0.9 mg/kg). Results showed that SERT knockout rats self-administered higher amounts of cocaine than the wildtype strain and the heterogeneous strain at doses of 0.3 mg/kg and 0.9 mg/kg, this has not been observed with 0.6 mg/kg. The conditioned place preference test showed that the SERT knockout rats were more sensitive to the rewarding effects of cocaine. Moreover, the SERT knockout rats also showed increased cocaine induced locomotion compared to the wildtype rats. This suggests that chronic increased extracellular 5-HT may also increase cocaine dependence. Additionally the sensitivity of 5-HT(1A) receptors was determined by measuring the hypothermic response to the 5-HT(1A) receptor agonist 8-OHDPAT. 8-OHDPAT- induced hypothermia was decreased in SERT knockout rats. Furthermore, S15535, a selective somatodendritic 5-HT(1A) receptor agonist, potentiated the stress-induced hyperthermia (SIH) in SERT knockout rats. These thermal responses may be explained by desensitized 5-HT(1A) receptors, while 5- HT(1A) receptor binding was reduced in selective brain regions. The authors point out that either occupation of the 5-HT(1A) receptors by the high 5-HT tonus or the desensitization of these receptors contribute to the cocaine supersensitivity.[12]

This theory is further supported by the results from Verheij et al. (2014). In this study basal 5-HT levels measured in the NAc and hippocampus of SERT knock out rats were significantly higher compared to the wildtype rats (see figure 1a). However they found a significant lower increase of extracellular 5-HT in the NAc and the hippocampus in the SERT knockout rats (compared to the wildtype) after administration of cocaine (see figure 1b). Verheij et al. speculated that the significant lower increase in cocaine induced 5-HT release in SERT knock out rats contributes to the enhanced cocaine self-administration. This lower increase is ascribed to the enhanced release of 5-HT from storage vesicles caused by cocaine. Further, this study supports that differences

between the genotypes in cocaine induced 5-HT response are most likely not due to dopamine and noradrenaline, since no significant differences between the two genotypes were found in dopamine and noradrenaline levels after administration of cocaine. [46]
The lower phasic 5-HT release in SERT knockout rats found after cocaine administration, causing an increase in self-administration, supports the inhibiting effect of 5-HT on the dopamine pathway. Additionally it underlines that a decrease in functional 5-HT reuptake inhibitors exhausts releasable 5-HT stored in intracellular vesicles, which causes the 5-HT peaks in reaction to cocaine to be very low.

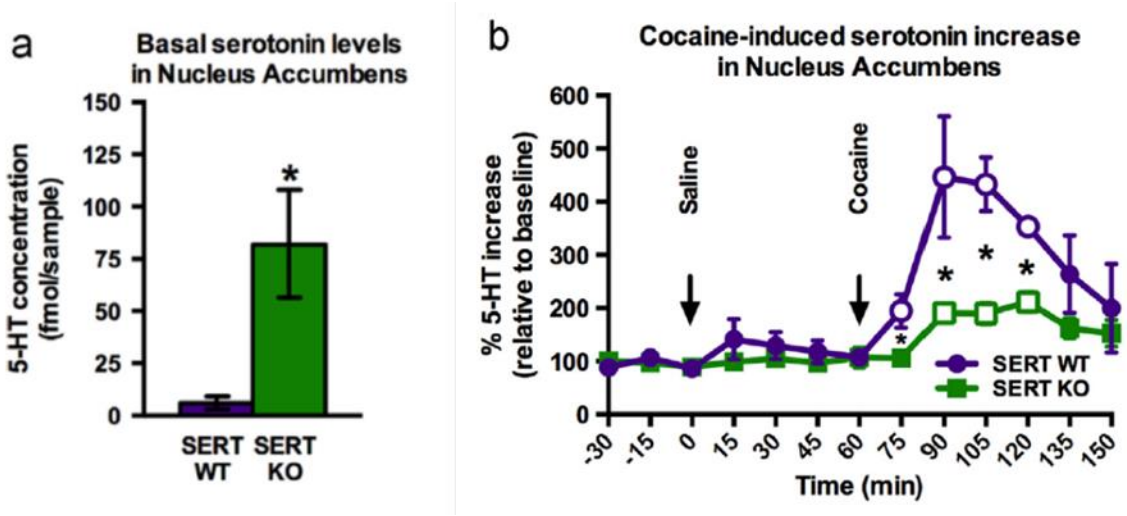

Figure 3a: Increased baseline levels of extracellular serotonin (5-HT) in the nucleus accumbens shell of SERT KO rats. Figure 3b: Reduction in the COC-induced increase of extracellular accumbal serotonin (5-HT) in SERT KO rats. Adapted from Verheij et al., 2014

Similar results in terms of self-administration have been found in SERT knockouts, when administered MDMA. The SERT knockout rats showed to be more sensitive to the initial and following reinforcing properties of MDMA, increasing their intake over time. The authors even suggest that their results imply that humans with lower SERT activity will also be more sensitive to MDMA.[47]

In summary, chronic elevated extracellular 5-HT levels in the SERT knockout rodents have proven the enhancing effects on self-administration of MDMA and cocaine. In terms of mechanisms, desensitization or occupation of the 5-HT(1A) receptors by the high 5-HT tonus and the minimal 5-HT peak seen after cocaine and MDMA administration may explain the sensitivity to cocaine and MDMA in SERT knockout rats. These previous results indicate that it is the inability of cocaine and MDMA to induce phasic 5-HT release in the SERT knockout rodents, which enhances the reinforcing effects of these substances. This evidence supports the primary inhibiting or satiating effects of 5-HT on dependence related behaviours. However, when extracellular 5-HT is chronically increased this seems to increase dependence related behaviours.

Interestingly, an inhibiting function of 5-HT on natural reward behaviour has also been found in a study from Sanders et al. (2007), although the authors consider the opposing roles of 5-HT and dopamine to be oversimplified. They investigated the effect of chronic fluoxetine treatment (daily injections (10 mg/kg) for three weeks) or genetic deletion of SERT in mice on natural food reward using multiple behavioural tests. They

consistently found that chronic pharmacological blockade or genetic deletion of SERT resulted in a reduction of operant responding for natural reward and a general decrease in locomotion. Opposed to increased dopaminergic activity, which enhances operant responding for natural reward and increases locomotion. Though this study did not find an effect of the SERT deletion or blockade in the Pavlovian-to-Instrumental transfer task (PIT), which measures the ability of Pavlovian conditioned cues that predict reward to enhance operant responding. These results suggest that the effect of increased extracellular 5-HT does not only decrease operant responding to natural reward but can be generalised to overall decrease of goal-directed behaviours. [48]

This implies that the chronically increased synaptic 5-HT in SERT knockout rats and SSRI treated rats does not affect natural reward behaviours and substance reward behaviours in the same manner. Though increased synaptic 5-HT seems to inhibit natural reward behaviours in SERT knockout rats and possibly even overall goal-directed behaviours, cocaine and MDMA does not inhibit them from increasing their self-administration and locomotion.

SSRI treatment in animals

To further explore the role of 5-HT in dependence related behaviours this paragraph will provide more details about SSRI treated animals. Increased synaptic 5-HT due to fluoxetine treatment (5 or 10 mg/kg for 5 days) has been shown to decrease the intake of cocaine in rats [49]. More studies, which will be discussed next, support a decrease in dependence related behaviours after fluoxetine treatment.

As mentioned in the introduction, acute and chronic fluoxetine treatment have shown to decrease the sensitivity of rats to rewarding brain stimulation. In this study of Lee et al., (1998), eight Wistar rats were implanted with electrodes either into the ventral tegmental area (VTA) or medial forebrain bundle (MFB). The rats had to perform an operant response to receive electrical stimuli in these areas. This method is called an discrete-trial current-threshold self-stimulation procedure. The results showed that reward thresholds were elevated when administered acute intraperitoneally fluoxetine (2.5, 5.0, 10.0, and 20.0 mg/kg) treatment, but also after chronic fluoxetine (5.0 mg/kg) treatment for 21 days. [13] Increased reward thresholds are correlated with decreased abuse liability. [50] Considering that no evidence was found for tolerance, these results support that fluoxetine itself may not be addictive and most importantly that it may inhibit abuse. It should be mentioned that the sample size in this study is small, interpretations should therefore be drawn with caution.

Moreover, a discrete-trial current-threshold self-stimulation procedure was also used in a more recent study to rewards in rats. Here, the rats were administered a 5-HT(1A) receptor agonist (8-OH-DPAT) or fluoxetine. A low dose of the 5-HT(1A) agonist lowered the reward thresholds, which is correlated with increased abuse liability.[50,51] Whereas a high dose elevated these thresholds. Furthermore the 5-HT(1A) antagonist *p*-MPPI did not change the stimulation behaviour, but when given before the agonist it reversed the lowering and elevating effects on the thresholds. These data support that the 5-HT(1A) receptor is responsible for mediating both increases and decreases in reward behaviour. Most importantly, a high dose of fluoxetine (10 mg/kg) elevated reward thresholds and responses latencies. Supporting that Fluoxetine may inhibit abuse . However lower doses (2.5 and 5.0mg/kg) increased response latencies and did not affect thresholds. [51] These data also support a possible therapeutic role for Fluoxetine in drug abuse and dependence, when given in high doses. Furthermore it underlines the important modulating role of the 5-HT(1A) receptor, which has been discussed before.

Referring back to the study of Sanders et al. [48], an overall decrease in goal directed behaviour would also explain the decrease in self-stimulation as well and would not be favourable in SSRI treatment for depression as well as substance dependence.

Human studies

Few studies have been done on the modulating effect of SSRIs on substance dependent related behaviours in humans. These will be discussed in the next paragraph. However, several studies exist on the modulating effects of the 5-HT transporter and the 5-H(3) receptor on dependence related behaviours.

As described before, 5-HT(3) receptors play a role in the rewarding effects of alcohol [40,41] It should be noted that this receptor is, opposed to the other 5-HT receptors, a ligand-gated ion channel. First evidence supports a modulating role of the 5-HT(3) receptor function in alcohol sensitivity [52] Recently, Enoch et al. (2011) showed that gene variations in humans encoding for the 5-HT(3) receptor affect the risk on alcohol dependence. [53] Preceding to this study, Walstab et al. (2008) discovered that a polymorphism (Tyr129Ser, rs1176744) in the 5HTR3B gene, encoding for a subunit of the 5-HT receptor, increased the mean receptor open time up to seven times. [54]

This led to the study from Enoch et al. (2011), which looked further into the relation between this polymorphism and alcohol dependence by genotyping 360 alcohol dependent African American men. They showed that the polymorphism in the 5HTR3B gene, which increases receptor sensitivity, predicted alcohol dependence. Furthermore, low activity of the promoter region in the human 5-HT transporter gene (5-HTTLPR) predicted cocaine/heroin dependence. The authors concluded that the increased risk for dependence was an effect of increased 5-HT(3) receptor sensitivity plus increased synaptic 5-HT as an result of these polymorphisms. [53] Most interestingly previous studies support that polymorphisms in the same promotor region (5-HTTLPR) decreases the expression of the 5-HT transporter gene and increases the risk for alcohol dependence and substance use disorders. [55,56] A meta-analysis of 145 studies was performed to investigate how strong the link between the 5-HT transporter gene and alcohol dependence is. A significant association has been found between at least 1 short allele in 5-HTTLPR and alcohol dependence. This points towards an modulating role of the 5-HT transporter in alcohol dependence. Nevertheless the authors also stress that publication bias could affect the results that were found. [57]
Although these studies seem to corroborate an increased risk of dependence due to decreased functional 5-HT transporters, results on SSRI treatment in non-depressed humans are conflicting. This will be discussed in the next paragraph.

SSRI treatment in humans

Few studies exist on dependence in humans following SSRI treatment. An illustrative example is the case description of Miss X, who developed alcohol dependence following a treatment with SSRIs, as discussed in the introduction. Although more studies of these kind exist, they limit themselves mostly to case studies. [11] Because clinical studies can provide more reliable data, this paragraph will focus on the clinical studies.

In a study of Curry et al. (2012), in which 192 adolescents diagnosed with major depressive disorder, without pre-existing alcohol or substance abuse disorder, were observed during and after treatment. The short-term depression treatment consisted of cognitive behaviour therapy, fluoxetine alone, the combination of both, or clinical management with pill placebo. 25.5 Percent of the adolescents developed an alcohol dependence or substance use disorder during the 60 months following treatment. Although not significantly, fluoxetine treatment or the combination of fluoxetine and cognitive behaviour therapy showed a lower probability of developing alcohol or substance dependence respectively, compared to the cognitive therapy alone or placebo. However results confirmed a significant reduction in the probability of subsequent substance use disorder (to substances such as marijuana, cocaine, opiates and hallucinogens) when patients reacted well to treatment. Although this was not found for alcohol use disorder. [58]

Alcohol dependence

A decrease in alcohol consumption after treatment with fluoxetine has been observed in a study of Naranjo et al. (1990). Fluoxetine (60 mg/day but not for 40 mg/day) was found to significantly decrease alcohol consumption by 17.3 percent. Interestingly the amount of cigarettes smoked in 29 male pre-alcoholics was increased. This shows that the effects of an SSRI on substance consuming behaviours are significant but can be diverse between different addictive substances. Also here it should be noted that 60 mg/day is a substantially higher dose than needed for antidepressive effects. [59]

Additional double-blind studies support the possible therapeutic effect of SSRIs in lowering alcohol intake. A study on 16 alcohol-dependent subjects showed that 40 mg/day citalopram significantly decreased daily alcoholic intake. Data suggested that citalopram decreasing the urge to drink and the reinforcing effects of alcohol. (Naranjo et al. 1992) Furthermore, a study on 10 subjects treated with fluoxetine (up to 80 mg daily) or placebo for 28 days, showed that fluoxetine reduced alcohol intake by 14 percent. [61]

In contrast, other studies do not support the possible therapeutic effects of fluoxetine in alcohol dependence. In a study on 28 male subject with severe alcohol dependence, fluoxetine treatment (60 mg/day) for 12 weeks did not reduce clinically significant relapse rates. Although not significant, subjects with comorbid cocaine dependence relapsed more than twice as often compared to subject with alcohol dependence alone [62] Furthermore a study on 101 alcohol-dependent subjects, fluoxetine treatment (up to 60 mg/day) for 12 weeks in combination with weekly psychotherapy did not show any significant differences in alcohol intake than the placebo treated subjects. However, both groups showed an reduction in alcohol intake during treatment.[63]

Most studies on SSRI treatment for alcohol dependence selected currently non-depressed patients, however past depressions may influence the results. A recent study on sertraline (200 mg/day) treatment or placebo for 14 weeks in non-depressed alcohol dependent patients showed that this group was very heterogeneous and conflicting results may be explained by presence or absence of lifetime depressive disorder. Absence of lifetime depressive disorder increased the therapeutic potential of sertraline in alcohol dependence. In contrast, no apparent therapeutic potential was found for sertraline in patients with lifetime depression. Authors do mention that study's results require replication. [64]

Treatment with SSRIs in cases of alcohol dependence remains controversial. Most studies support an therapeutic role of SSRIs in alcohol dependence. However some studies claim that SSRIs increase alcohol

consumption.[11] Nonetheless these results limit themselves mostly to case studies and may be influenced by the current or lifetime depressive disorder as well.

Substance dependence

Furthermore, fluoxetine treatment (60mg/day) for 33 weeks has been shown to reduce cocaine use in patients that co-depend on opioids, [65] and several studies on SSRIs as an potential treatment for cocaine dependence support possible therapeutic effects of SSRIs.
Treatment of 5 adult males with histories of cocaine abuse with fluoxetine (40 mg/kg) for 4 weeks did not have any negative interactions with cocaine and decreased the subjective ratings of cocaine's positive mood effects. [66] Additionally, citalopram (20 mg per day) use for 12 weeks combined with cognitive behavioural therapy (CBT) and contingency management (CM) reduced cocaine-positive urine tests during treatment compared to placebo treated subjects. [67] And Sertraline (200 mg/day) treatment for 12 weeks delayed the relapse in recently abstinent cocaine-dependent patients with depressive symptoms. [68] Together these results also imply a potential therapeutic role of SSRIs in cocaine abuse/dependence.

In summary, SSRI treatment in patients depending on alcohol, cocaine and MDMA seems to have therapeutic potential. In contrast, genetic deletion of SERT enhances dependence related behaviours. While both SSRI treatment and SERT deletion enhances synaptic 5-HT, this seems to contradict each other. However SERT knockout rats lack SERT from the beginning of development, while SSRI treatment blocks SERT only during treatment. Lack of SERT during development possibly causes neurophysiological adaptions that explain the behavioural differences. Interestingly, SSRI treatment in pregnant woman has already shown to effect neurotrophic growth factor (NGF) signalling in placental cells. [69] Furthermore, prenatal SSRI use disrupted a wide range of neurobehavioural outcomes in infants [70], and in rats neonatal SSRI exposure showed to have lasting effects on behaviour and 5-HT circuitry. [71] These results support acute and long-term effects of exposure to SSRIs during development.
Additionally, SSRI treatment in adult rats showed to have an enhancing effect on the proliferation of hippocampal cells. [72] More recently, chronic fluoxetine treatment (28 days) in adult male mice has shown to increase dendritic branching and maturation of adult-born hippocampal granule cells. [73] These results support neurophysiological changes due to SSRI treatment during adulthood as well.

Conclusion

In this thesis the effect of increased synaptic 5-HT (due to SSRI treatment) on substance dependence related behaviours was reviewed. 5-HT seems to play an important role in modulating substance dependence related behaviours. Several studies have supported a role for 5-HT in reward behaviours besides dopamine and stimulation of different 5-HT subtypes in the mesolimbic pathway showed both increases and decreases in reward behaviours. The mechanisms behind this modulating role remain uncertain. Therefore, more research is needed to understand which receptor subtypes in which brain regions modulate substance related behaviours.

SERT knockout strains serve as a good model for chronically increased synaptic 5-HT, because they lack functional SERT, which decreases the 5-HT reuptake and the 5-HT turnover. This is similar to the effect of SSRIs which block functional SERT and thereby increase synaptic 5-HT. Interestingly, studies on SERT knockout rats show enhanced self-administration of MDMA and cocaine. Moreover, it seems that the inability of cocaine and MDMA to induce phasic 5-HT release in the SERT knockout rats and desensitization/occupation of the 5-HT(1A)receptor enhances the reinforcing effects of these substances.
Likewise, multiple studies in humans associated polymorphisms in the 5-HT transporter gene with alcohol and substance dependence. Studies suggested decreased expression of SERT increased the risk for substance and alcohol dependence.

Overall these studies support an inhibiting or satiating effect of synaptic 5-HT on substance dependence related behaviours. However decreased expression of the 5-HT transporter is associated with increased substance dependence and related behaviours. Nonetheless, natural reward behaviour is not increased in SERT knockout rats or SSRI treated rats, suggesting different underlying mechanisms for natural reward.

Increased 5-HT due to SSRI (Fluoxetine Citalopram and Sertraline) treatment in clinical and preclinical studies show mostly a potential therapeutic role in dependence on cocaine and alcohol, supporting the inhibiting effect of SSRIs on substance dependence. Although, several case studies described development of alcohol dependence after SRRI use, these studies limit themselves mostly to case studies. Further, one should consider that current or lifetime depression could affect the effect SSRIs have on substance/alcohol dependence. An overview of the studies described on substance dependence related behaviours in SERT knockout rats and SSRI treated humans and rats, can be found in table 1 (pg. 16-18).

Studies on increased synaptic 5-HT due to decreased SERT expression or due to SSRI treatment seem conflicting. A possible explanation between the contradicting results found in SERT knock out rats/humans with polymorphisms in SERT and SSRI treated subjects on substance related behaviours, could lie in the different developmental conditions. SERT knockout strains and low SERT expressing humans lack SERT from the beginning of development, possibly causing neurophysiological adaptions that explain the behavioural differences. Interestingly, studies on SSRI exposure during prenatal and neonatal stages of rats and humans already showed to affect behaviour and 5-HT pathways. Possibly prenatal exposure to SSRIs or low expression of SERT may affect substance dependence related behaviour. This would hold implications for SSRI use during pregnancy and identifying patients with an increased risk for substance dependence. More research is needed to investigate this relationship between prenatally increased synaptic 5-HT and subsequent substance dependence.

Additionally, one could consider that long-term exposure to SSRIs in adults, which has shown to affect neuronal plasticity in rodents, modulates substance dependence related behaviours. Case studies on SSRI use in non-dependent patients who subsequently develop substance dependence are limited in amount and significance. However, fluoxetine treatment for 231 days has shown to reduce cocaine intake in patients with dependence disorder as well as in shorter treatments with SSRIs (see table 1). This contradicts that changes in dependence behaviour and neurophysiology appear after long term exposure to increased synaptic 5-HT in humans with an addiction. Nonetheless, more research is needed on long-term SSRI use (e.g. months) in models for addiction and depression with regard to its effect on substance dependence related behaviours.

Concluding, 5-HT and its transporter play an important role in modulating dependence related behaviours and several studies showed they may affect these behaviours . However, little is known about the mechanisms and more research is needed to understand the therapeutic effects and potential hazardous effects of SSRI use.

Table 1 overview of studies discussed on increased synaptic 5-HT and its effect on substance dependence related behaviours

Study subjects	**Treatment**	**Length treatment**	**Results**	**Reference**
	Chronic changes in SERT			
Rats	SERT knockout (lifelong increased synaptic 5-HT)	-	Increased intake cocaine and desensitized or occupied 5-HT(1A) receptors	(Homberg et al. 2008)
Rats	SERT knockout (lifelong increased synaptic 5-HT)	-	Lower increase of extracellular 5-HT in the NAc and the hippocampus after administration of cocaine	(Verheij et al. 2014)
Rats	SERT knockout (lifelong increased synaptic 5-HT)	-	Increased intake MDMA and more sensitive to reinforcing properties.	(Oakly et al. 2014)
Humans (alcohol dependent)	-	-	low activity of the promoter region in the human 5-HT transporter gene (5-HTTLPR) predicted cocaine/heroin dependence.	Enoch et al. (2011) Walstab et al. (2008) Gerra et al. (2004)
Rats	SERT or SSRI treatment	-	Reduction of operant responding for natural reward and a general decrease in locomotion	(Sanders et al. 2007)

Study subjects	Treatment	Length treatment	Results	Reference
Acute and long-term blockade of SERT with SSRIs				
Rats	fluoxetine treatment (10 mg/kg)	21 days	Reduction of operant responding for natural reward and a general decrease in locomotion	(Sanders et al. 2007)
Rats	Fluoxetine (5 or 10 mg/kg)	5 days	Decrease cocaine intake	(Carroll et al. 1990)
Rats	Fluoxetine (2.5, 5.0, 10.0, and 20.0 mg/kg)	21 days	Increased reward tresholds	(Lee 1998)
Rats	Fluoxetine (10 mg/kg)	-	Increased reward tresholds	(Harrison et al. 2001)
Humans	-	-	Polymorphism in the 5HTR3B gene or in the promoter region in the 5-HTTLPR gene predicted alcohol dependence or cocaine/heroin dependence.	(Enoch et al. 2011) (Gerra et al. 2004) (Feinn et al. 2005) (McHugh et al. 2010)
Humans (adolescents diagnosed with major depressive disorder)	Fluoxetine		Lower probability of developing alcohol or substance dependence (n.s.)	(Curry et al. 2012)
Humans (male pre-alcoholics)	Fluoxetine (60 mg/day but not for 40 mg/day)	28 days	Decreased alcohol intake by 17.3% increased amount of cigarettes smoked.	(Naranjo et al. 1990)
Humans (alcohol-dependent)	Citalopram (40 mg/day)	7 days	Decreased daily alcoholic intake	(Naranjo et al. 1992)

Humans (alcohol-dependent)	fluoxetine (up to 80 mg/day)	28 days	Decreases alcohol intake by 14 %.	(Gorelick & Paredes 1992)
Humans (alcohol-dependent)	fluoxetine (60 mg/day)	84 days	Did not reduce clinically significant relapse rates	(Kabel & Petty 1996)
Humans (alcohol-dependent)	fluoxetine treatment (up to 60 mg/day)	84 days	No significant differences in alcohol intake than the placebo	(Kranzler et al. 1995)
Humans (alcohol-dependent)	sertraline (200 mg/day)	98 days	In absence of lifetime depressive disorder, the therapeutic potential of sertraline in alcohol dependence increased	(Pettinati et al. 2001)
Humans (cocaine and opioid dependent)	fluoxetine treatment (60mg/day)	231 days	Reduced cocaine use	(Winstanley et al. 2011)
Humans (history of cocaine abuse)	fluoxetine (40 mg/kg)	28 days	Decreased the subjective ratings of cocaine's positive mood effects	(Walsh et al. 1994)
Humans (cocaine dependent)	citalopram (20 mg per day) combined with CBT and CM	84 days	Reduced cocaine-positive urines	(Moeller et al. 2007)
Humans (recently abstinent cocaine-dependent)	Sertraline (200 mg/day)	84 days	Delayed the relapse	(Oliveto et al. 2012)

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