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THE MONOAMINE STABILIZER (-)-OSU6162 - A POTENTIAL NOVEL TREATMENT FOR ALCOHOL USE DISORDERS

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THE MONOAMINE STABILIZER (-)-OSU6162 - A POTENTIAL NOVEL TREATMENT FOR ALCOHOL USE DISORDERS
THESIS FOR DOCTORAL DEGREE (Ph.D.)

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ABSTRACT

Alcohol use disorder (AUD) represent a major health problem worldwide. Despite the severe consequences of AUD, only four medications are approved in Sweden for this disease. In addition, the prescription rates are low, partly due to varying clinical efficacy of these medications. Consequently, new, more effective pharmacotherapies are needed. A main problem in the treatment of AUD is the long-lasting vulnerability to relapse. Relapse is typically triggered by: stress, acute exposure to the drug, or drug-associated cues or context. Impaired impulsive control, often seen in AUD individuals, might further contributes to relapse to alcohol drinking. The dopamine (DA) system is one possible treatment target for AUD. Dopamine D_2 receptors has been suggested to be involved in mediating alcohol's reinforcing properties and a decrease in DA release and a reduction in D_2 receptors have been found in detoxified AUD patients. This DA down-regulation is hypothesized to induce alcohol craving and contribute to relapse even after a long period of abstinence. In addition to the role of DA in AUD, DA has also been suggested to be involved in impulse behavior. The monoamine stabilizer (-)-OSU6162 (OSU6162) has the ability to stimulate, inhibit, or show no effect on DA-related behaviors depending upon the prevailing dopaminergic tone. In this thesis, we evaluated the potential of OSU6162 as a new treatment for AUD using validated preclinical models of behaviors related to AUD. We have, in this thesis, identified OSU6162 as a potential novel treatment for AUD. Using a battery of animal models, we showed that OSU6162 attenuated voluntary alcohol intake, alcohol withdrawal symptoms (tail stiffness and walking with broad gait), the motivation to seek alcohol, cue/priming-induced reinstatement (relapse) of alcohol seeking, and relapse-like drinking in in rats that had been drinking alcohol for a long period of time. Moreover, we showed that OSU6162 pre-treatment improved motor impulsivity in both alcohol and alcohol-naïve rats. Furthermore, OSU6162 did not induce conditioned place preference in either alcohol-naïve rats or rats that had been drinking alcohol before the experiment, indicating that OSU6162 does not possess any abuse liability on its own. Together these results highlights OSU6162's potential to prevent relapse triggered by alcohol craving, alcohol related cues, re-exposure to alcohol and or an urge to relieve abstinence symptoms. In addition, the improved impulse control following OSU6162 treatment might help AUD individuals to override a compulsive drug-taking behavior in response to craving and thereby possibly prevent relapse to alcohol drinking. In addition to the global health problems related to alcohol, an opioid addiction epidemic is ongoing in the United State. We therefore decided to examine the potential of OSU6162 to attenuate self-administration of the opioid oxycodone in rats. The results showed that OSU6162 attenuated operant oxycodone self-administration but had no effect on context-induced reinstatement, at least in the dose tested. These preliminary results indicate that OSU612 might have potential to decrease intake of oxycodone, however, further studies are needed to fully evaluate the potential of OSU6162 on oxycodone self-administration and reinstatement. In conclusion, the results in this thesis indicate that OSU6162 may serve as a novel treatment for AUD and provided the necessary rational for a clinical "proof-of-concept" study with OSU6162 in alcohol dependent patients. The clinical study was

successfully executed and supported our preclinical findings by showing that OSU6162 attenuated alcohol craving in alcohol dependent patients. Thus, the rapid and fruitful transition of OSU6162 from bench to clinic highlights the importance of the preclinical medication development program used in this thesis work.

LIST OF SCIENTIFIC PAPERS

I. Steensland P, **Fredriksson I**, Holst S, Feltmann K, Franck J, Schilström B, and Carlsson A (2012). The dopamine stabilizer (-)-OSU6162 attenuates voluntary ethanol intake and ethanol-induced dopamine output in the nucleus accumbens. *Biological Psychiatry, 72: 823-831.*

II. Feltmann K*, **Fredriksson I***, Wirf M, Schilström B and Steensland S (2015). The Monoamine Stabilizer (-)-OSU6162 Counteracts Down-Regulated Dopamine Output in the Nucleus Accumbens of Long-Term Drinking Wistar Rats. *Addiction Biology, 21: 1130-1140.*

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III. **Fredriksson I**, Wirf M, Carlsson A and Steensland P. The Monoamine Stabilizer (-)-OSU6162 Prevents the Alcohol Deprivation Effect and Improves Motor Impulsive Behavior in Rat. *Manuscript.*

IV. **Fredriksson I**, Bonci A and Steensland P. Evaluation of the Monoamine Stabilizer (-)-OSU6162 on Oxycodone Self-administration and Context-Induced Reinstatement in Rat. *Manuscript.*

CONTENTS

LIST OF ABBREVIATIONS

5CSRTT	5-Choice Serial Reaction Time Task
ADE	Alcohol deprivation effect
ANOVA	Analysis of variance
AUD	Alcohol use disorders
CPP	Conditioned place preference
DA	Dopamine
DSM	Diagnostic and Statistical Manual of Mental Disorders
FDA	US Food and Drug Administration
FR	Fixed-ratio
GABA	γ-aminobutyric acid
iv	Intravenous
NAc	Nucleus accumbens
NCT	Novel Cage Test
NIDA	National Institute on Drug Abuse
(-)-OSU6162	OSU6162
PFC	Prefrontal cortex
PR	Progressive-ratio
sc	Subcutaneously
VTA	Ventral tegmental area
WHO	World Health Organization

1 INTRODUCTION

1.1 THE BURDEN AND COST OF ALCOHOL USE

Alcohol is a psychoactive substance that is mainly consumed for its pleasurable effects. According to the World Health Organization (WHO), approximately 2 billion people worldwide consume alcohol beverages, and 76.3 million are suffering from alcohol use disorder (AUD) [1]. In 2012, 3.3 million deaths or 5.9% of all global deaths were associated with alcohol [2]. Moreover, alcohol is estimated to cause about 20–30% of vehicle accidents and homicides worldwide [1]. In addition, harmful alcohol use has been identified as a causal factor in numerous types of chronic diseases, such as AUD, cancers, cardiovascular diseases, diabetes, neuropsychiatric disorders, gastrointestinal diseases, and infectious diseases [1]. Globally, alcohol misuse has been listed by WHO as the fifth leading causes of morbidity and mortality and is responsible for 5.1% of the global disease burden [2].

In Sweden, approximately one million people drink excessive amounts of alcohol risking negative health effects and, approximately 446 000 individuals or 5.9 % of the population suffer from alcohol dependence or alcohol misuse (according the DMS-IV) [3]. Besides devastating medical and psychiatric consequences for the AUD individual, AUD is a heavy burden to family, friends and social services, as well as an enormous economic burden for society. In Sweden alone, alcohol misuse and addiction costs society around 49 million SEK annually [4]. Despite the major health problem, only four medications are approved as a treatment for AUD in Sweden. However, the clinical efficacies of these are varying and limited [5]. Given the seriousness of AUD, new, more effective, medications are truly needed.

1.2 ALCOHOL USE DISORDERS

1.2.1 Diagnostic Criteria for AUD

Alcohol use disorders is a chronic relapsing brain disease characterized by obsession of seeking and consuming alcohol despite negative consequences, loss of control in limiting the intake and emergence of a negative emotional state (e.g. dysphoria, anxiety, irritability) when alcohol is not on board [6]. Suffering from AUD is often seen by the society as a defect of character [7]. However, once established, AUD is a brain disorder that shares numerous characteristics with other chronical relapse medical conditions such as hypertension, diabetes and asthma [8]. Although, AUD cannot be treated without regards for its social and behavioral context, the same is also true for other chronic relapsing disorders. Thus, AUD should be viewed as a treatable disease, not as a character flaw. There is no biological marker for AUD. Instead clinicians use criterion-based diagnostic instruments. The International Classification of Diseases (ICD) system [9] and The Diagnostic and Statistical Manual of Mental Disorders (DSM) are both diagnostic instruments frequently used to diagnose AUD [6].

Table. 1. Diagnostic criteria for AUD according to DSM-5. A minimum of two symptoms are required to diagnose an AUD. Alcohol use disorders can be classified as mild (two to three symptoms), moderate (four to five) or severe (6 or more). Figure modified from [10].

Diagnostic Criteria for alcohol use disorders (AUD)
1. Alcohol are often taken in larger amounts or over a longer period of time than intended.
2. There is a persistent desire or unsuccessful efforts to cut down or control alcohol use.
3. A great deal of time is spent in activities necessary to obtain alcohol, use alcohol, or recover from its effects.
4. Craving, or a strong desire to use alcohol.
5. Recurrent alcohol use resulting in failure to fulfill major role obligations at work, school or home.
6. Continued alcohol use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of alcohol.
7. Important social, occupational or recreational activities are given up or reduced because of alcohol use.
8. Recurrent alcohol use in situations in which it is physically hazardous.
9. Continued use despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by alcohol.
10. Tolerance, as defined by either of the following:
(a) A need for markedly increased amounts of alcohol to achieve intoxication or desired effect.
(b) Markedly diminished effect with continued use of the same amount of an alcohol.
11.Withdrawal, as manifested by either of the following:
(a) The characteristic alcohol withdrawal syndrome.
(b) The same (or a closely related) substance are taken to relieve or avoid withdrawal symptoms.

The DSM was introduced by the American Psychiatric Association in 1952 and provides standardized criteria for mental disorders. In 2013, the fifth edition of the DSM (DSM-5) was published [10], which includes several important differences from the prior edition, DSM-IV [6]. To receive a diagnosis of AUD, at least two of the 11 criteria (displayed in Table 1) must be met during the same 12-month period. In DSM–IV, alcohol abuse and alcohol dependence were described as two distinct disorders. In contrast, in the DSM-5, alcohol abuse and alcohol dependence are integrated into one single disorder called “alcohol use disorders - AUD”. Alcohol use disorders is then subdivided into “mild”, “moderate” and “severe” AUD, based on the number of criteria fulfilled. The presence of two or three symptoms corresponds to mild AUD, four to five to moderate AUD, and six or more indicate severe AUD. Two other

changes made in DSM-5 are the addition of a new criterion involving craving and the criterion in DMS-IV regarding legal problems is eliminated [10].

In DSM-5, the diagnostic criteria for AUD could be grouped in four blocks: impaired control, social impairment, risky use, and pharmacological criteria (see Table. 1). Criteria 1-4 describe impaired control over alcohol use, such as having problems limiting the alcohol drinking, consuming more alcohol over time than was intended and craving for alcohol or a strong desire to drink. Moreover, criteria 5-7 focus on social impairments from consuming alcohol and involve negative outcomes of alcohol use affecting social and professional life, as well as important activities the individual is willing to give up in favor of alcohol. Furthermore, criteria 8-9 describe the risky use of alcohol such as, a recurrent alcohol use in situations in which it is physically hazardous and continued alcohol consumption despite knowledge of its physical or psychological problems. The last two criteria 10-11, describe tolerance and withdrawal, respectively [10]. Tolerance is a hallmark of addiction that could be defined as a decreased, reinforced efficacy of alcohol following repeated alcohol administration [11]. Thus, tolerance will increase the minimum dose alcohol needed to produce the same effect. Tolerance also includes a lesser sensitivity to the motor-impairing [12] and metabolic effects [13] of alcohol. Withdrawal occurs in absence of alcohol and is characterized as a great discomfort for the AUD individual. Some withdrawal symptoms are suggested to increase the motivation to seek and consume alcohol [14]. Alcohol withdrawal symptoms could be both physical (e.g. convulsion, sweating, and higher heart rate) and psychological (e.g. anxiety and dysphoria) [15, 16]. However, withdrawal is not necessary for a diagnosis but is highly associated with severe AUD [10].

1.2.2 Current Available Pharmacotherapies

Despite AUD's high prevalence and its multiple and serious consequences for patients and society, only four medications are approved for the treatment of AUD in Sweden: disulfiram, acamprosate, naltrexone and nalmefene. The same medications are approved in the United States, with the exception of nalmefene, which is not approved by the US Food and Drug Administration (FDA). Because all currently available medications have shown limited and varying clinical efficacy [5], new, more effective pharmacotherapies are truly needed.

1.2.2.1 Disulfiram

Disulfiram (Antabuse ®), FDA-approved for the treatment of AUD in 1949, was the first pharmacological treatment introduced on the market to treat AUD. Alcohol is metabolized in the liver, via the enzyme alcohol dehydrogenase, to acetaldehyde and then to acetate via the enzyme acetaldehyde dehydrogenase. Disulfiram works by blocking the metabolism of alcohol in the body. Specifically, disulfiram inhibits the degeneration of acetaldehyde to acetate by blocking the liver enzyme acetaldehyde dehydrogenase [17, 18]. This causes an accumulation of the toxic metabolite acetaldehyde which results in the development of a highly aversive state with symptoms such as sweating, flushing, headache, nausea and tachycardia [18, 19]. The accumulation of acetaldehyde following alcohol consumption may

also have toxic effects on several organ systems, including the liver [20]. The fear of provoking an unpleasant disulfiram-alcohol reaction [19] is believed to be the primary mechanism to facilitate abstinence from alcohol [7].

1.2.2.2 Naltrexone

Naltrexone (Revia®, Vivitrol®), is primarily an antagonist at the μ-opioid receptors, but also at the opioid κ- and δ-receptors. Although, naltrexone's mechanism of action is not completely understood, naltrexone most likely modulates the mesolimbic DA release induced by alcohol administration through interactions with the opioid system and thereby possibly decreasing the acute positive reinforcing properties of alcohol [7]. Indeed, a study has shown that mice lacking the μ-receptor do not self-administer alcohol [21].

Naltrexone was approved for the treatment of AUD by the FDA in 1994. The approval was based on initial clinical studies showing clinical evidence for efficacy for naltrexone in recently abstinent AUD individuals. Specifically, AUD individuals treated with naltrexone (50 mg/day) were less likely to relapse during the treatment period of 12 weeks compare to AUD individuals who received placebo [22, 23]. Nevertheless, 5 months after the treatment, the relapse rates for the naltrexone and placebo groups were similar. These early clinical studies were based on preclinical studies showing effect of naltrexone on alcohol consumption in rats [24]. Thus, preclinical studies have contributed to the approval of this agent, pointing to the beneficial effect of using preclinical studies in the development of new potential medications for AUD.

Generally, the efficacy and safety of naltrexone is well documented [25, 26], however, some mixed results do exist [27]. The different response to naltrexone might have a genetic explanation, where individuals carrying a specific variant of the gene encoding the μ-opioid receptor (OPRM1), might respond better to naltrexone [28]. Additionally, it has also been suggested that individuals with high levels of craving for alcohol or have a family history of AUD [28, 29] are more likely to benefit from naltrexone treatment [29].

More recently, in 2006, depot formulation of naltrexone was approved by the FDA and has showed evidence for clinical efficacy [30]. Depot formulations of naltrexone may offer some advantages over oral formulations such as increased compliance, a variable reported to be critical in determining the success of naltrexone treatment [31].

1.2.2.3 Acamprosate

Acamprosate (Campral®) was approved by the FDA in 2004, and is thereby the newest medication approved for treatment of AUD in US. Acamprosate has been shown to decrease craving and withdrawal distress in AUD individuals [32]. Acamprosate's exact mechanism of action is still not fully understood. Because the chemical structure of acamprosate is similar to γ-aminobutyric acid (GABA), acamprostate was initially believed to be a GABAergic acting drug. [33, 34]. However, later, acamprosate was demonstrated to rather act on NMDA receptor [35] and /or metabotropic-5 glutamate receptors [36] that may

modulate the hyperglutamatergic state associated with alcohol withdrawal. Evidence from a human magnetic resonance imaging study support acamprosate's ability to modulate glutamate neurotransmission as it suppressed central glutamate over 4 weeks of treatment [37]. However, recently, another mechanism of action was suggested, namely that calcium is the active moiety of acamprosate (calcium-bis(N-acetylhomotaurinate)) and that N-acetylhomotaurinate is a biologically inactive molecule [38], yet the mechanism of action is under debate.

Clinically, multiple trials have shown that acamprosate added to psychosocial interventions improved the duration and rate of abstinence [39, 40], whereas other trials have failed to find efficacy for acamprosate [41, 42]. However, recently, two meta-analyses showed a small overall positive treatment efficacy, particularly when abstinence was the treatment goal [43, 44].

1.2.2.4 Nalmefene

Nalmefene (Selincro®) was approved by the European Medicines Agency to treat AUD in 2013, but is not approved by the FDA. In contrast to disulfiram, naltrexone, and acamprosate which all are approved as an aid to maintain total abstinence, nalmefene is approved for the indication of reducing alcohol consumption. In addition, nalmefene is recommended to be used "as needed", meaning that the patient could take nalmefene in preparation for a situation with a perceived heightened risk of drinking.

Nalmefene is an opioid antagonist similar in both structure and activity to the opioid antagonist naltrexone. However, unlike naltrexone, nalmefene has shown no dose-dependent liver toxicity [45]. Moreover, nalmefene differ from naltrexone due to its partial agonist activity at the kappa-opioid receptor [46]. Multicenter clinical trials, in which nalmefene has been taken "as needed" in combination with psychosocial management, have reported efficacy of nalmefene in reducing alcohol consumption compared to placebo in alcohol dependent patients [47, 48].

1.2.3 Development of AUD

Chronic alcohol use might over time lead to AUD. The development of AUD might be viewed as a cycle of spiraling dysregulation of brain reward systems that progressively increases, resulting in compulsive alcohol use and a loss of control over alcohol drinking [49]. The brain is a highly reactive organ, which rapidly responds and adapts to its surroundings. Chronic intoxication of alcohol produces multiple neuroadaptions at the cellular, molecular and neurocircuitry levels that eventually may lead to the transition from controlled to compulsive alcohol use. Moreover, these neuroadaptations in the brain circuitry will also promote future alcohol consumption [50, 51].

Alcohol use is driven by both positive and negative reinforcement, however their relative contributions may change during the progression of AUD [52]. Positive reinforcement describes a situation in which a rewarding stimulus or experience (e.g., alcohol-induced

euphoria) increases the probability (and motivation for) for a response (e.g., alcohol-seeking behavior). Negative reinforcement refers to a process in which removal of an aversive stimulus or experience (e.g. removal of a negative emotional state) increases the probability of a response (e.g. relapse to alcohol drinking). Moreover, conditioned reinforcement is also implicated in the development and maintenance of AUD. In conditioned positive and negative reinforcement, stimuli that become associated with either alcohol or withdrawal might motivate subsequent alcohol-seeking behavior. The positive reinforcement of alcohol is suggested to play an important role in the beginning of alcohol use and abuse, whereas the negative reinforcing effects of alcohol become more critical for the motivation to drink alcohol during the transition to AUD [52, 53].

Alcohol use disorder, like addiction, has been conceptualized as a progression from impulsive to compulsive alcohol taking in a repetitive three-stage cycle: 1) binge/intoxication, 2) withdrawal/negative affect, and 3) preoccupation/anticipation, that feeds in to each other, become more intense and worsen over time (Fig. 1) [49]. These three stages of the cycle are associated with specific neurochemical and neurocircuitry changes that together lead to the loss of behavioral control over drug seeking and drug taking. Neuroadaptive changes in the brain reward, stress and executive function system are suggested to play a key role in the development of and the transition to AUD [50, 53].

Initially, in the binge/intoxication stage, alcohol use is primarily motivated by positive reinforcement [52]. The mesolimbic DA system plays an important role in mediating the positive reinforcing effect of alcohol [54]. Following repeated binge/intoxication of alcohol a downregulation of positive reward pathways occurs, leading to that an increased levels of alcohol is needed to trigger the brain reward system (tolerance development) [55]. Thus, tolerance to alcohol's positive reinforcing properties is an indication that the brain has started to change/adapt in response to repeated alcohol exposure.

During the binge/intoxication stage, environmental stimuli such as social context and places become associated with the pleasurable effects of alcohol [50]. This repeated association might trigger an associative learning process that causes the previously neutral stimuli to become a reinforcer in its own right (i.e. conditioned reinforcement). Conditioned reinforcement as a construct is proposed to precede and set the scene for incentive salience [50]. Incentive salience is mediated by the mesocorticolimbic DA system and defined "as a psychological process that transforms the perception of stimuli, imbuing them with salience, making them attractive, wanted, incentive stimuli" [56]. As a result of this, the "liking" (e.g. pleasure value) of alcohol becomes linked to "wanting" (i.e. desire or craving) the alcohol-associated stimuli. Following repeated alcohol exposure, this wanting might become pathologically amplified and provoke a strong desire or craving for alcohol which in turn might lead to resumption of addictive behaviors [55, 56]. Learned responses to conditioned drug-related stimuli might also elicit automatic responses, leading to drug-seeking and relapse in the absent of distinct craving [57]. Thus, both conditioned reinforcement and

incentive saline are suggested be implicated in cue-induced drug seeking, self-administration behavior and possibly also the transition to habit-like compulsive alcohol seeking [50].

In the withdrawal/negative affect stage, discontinued alcohol use produces a constellation of withdrawal symptoms. Withdrawal occurs in absence of alcohol and is characterized as a great discomfort for individuals with AUD [14]. Alcohol withdrawal symptoms could be both physical (e.g. convulsion, motor abnormalities, autonomic disturbances (e.g. sweating, higher heart rate)) and psychological (i.e. emergence of a negative-affective state characterized by anxiety, dysphoria, irritability) [15, 16]. Physiological aspects of withdrawal usually last up to 48 hours following termination of alcohol exposure, whereas the negative affecting state are more long lasting and may persist into protracted abstinence. This negative emotional state is proposed to be a major driving force in the maintenance of AUD [14]. Thus, during the withdrawal/negative affect stage, alcohol drinking is primarily motivated by the desire to avoid this negative emotional state (i.e., by negative reinforcement) [52, 58]. The transition from positively to negatively reinforced drug use, possibly induced by decreased function in reward system and recruitment of anti-reward systems, is sometimes referred to as the "the dark side of addiction" [59].

During the withdrawal/negative affect stage of the cycle, the dopamine (DA) component of the reward system (within-system neuroadaptions) is compromised and brain stress neurotransmitters (between-system neuroadaptations) such as corticotropin-releasing factor and dynorphin, in the neurocircuitry of the extended amygdala, are recruited [51]. These are example of counter-adaptations made by the central nervous system in an attempt to neutralize alcohol's effects. However, in the absent of alcohol, these opposing effects persist and form an "allostatic state". The allostatic state represents a new pathological reward set point outside the normal homostatic range [52]. These neuroadaptations are suggested to produce the withdrawal response [50, 51, 60].

A main problem in AUD is high rates of relapse, in which addicted individuals return to compulsive alcohol drinking long after acute withdrawal. Relapse correspond to the preoccupation/anticipation stage, which is characterized by exaggerated craving for alcohol use [53]. Conditioned reinforcement is suggested to contribute to the preoccupation/anticipation stage ("craving") stage [52]. The dysregulations that comprise the "dark side of addiction" are suggested to persist during protracted abstinence to set the tone for vulnerability to craving [59]. Craving might be driven by both environmental cues associated with alcohol availability and by internal states often linked to negative emotional states and stress. Conditioned craving is thought to have the ability to induce relapse to alcohol seeking and intake even after long periods of abstinence. During the preoccupation/anticipation stage, contextual cues via the hippocampus and stimuli cues via the basolateral amygdala converge with frontal cortex activity to drive drug seeking. In addition, other components in the frontal cortex are weakened, producing deficits in executive function [51]. Deficits in executive function might lead to impaired decision making and behavioral inhibition, which might further contribute to craving and relapse.

However, the reward deficits and the stress surfeit, carried over from the withdrawal stage, are hypothesized to be the primary driving force for relapse [61].

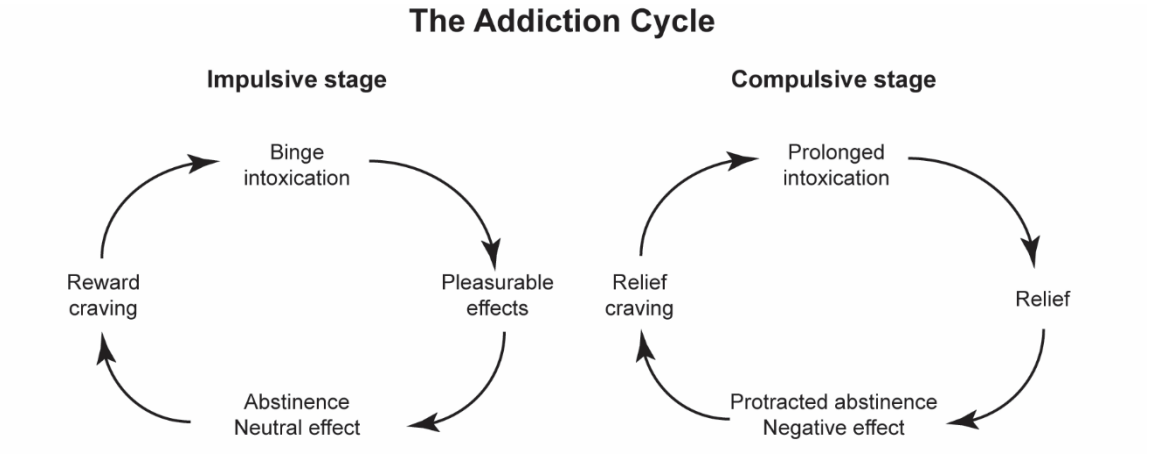

Fig. 1. Transition from alcohol use to AUD. A schematic picture of the addiction cycle. Initially alcohol is consumed for its positive reinforcing effects, driven by reward craving. However, over time, as the addictive process progresses a shift occurs. Alcohol use becomes compulsive and maintained by negative reinforcing effects (i.e. relief from a negative emotional state). Picture modified from [58].

Although, much of the early research focused on the acute rewarding effects of alcohol, the focus has been shifting towards identifying long-term neuroadaptive changes that may underlie relapse and excessive consumption of alcohol after periods of abstinence. The mesolimbic DA system is hypothesized to be implicated in the development of both positive and negative reinforcing effects of alcohol, and thereby contribute to the development and maintenance of AUD. Thus, the mesolimbic DA system represent one possibly target for medication development for AUD.

1.3 DOPAMINE

1.3.1 Brief History

In the late 1950s, Carlsson and colleagues discovered that DA was a neurotransmitter in its own right and not just a precursor of norepinephrine [62, 63]. Later, Carlsson and colleagues proposed that DA depletion in the striatum could be the cause of neurological symptoms in Parkinson's disease [64]. Today, we know that DA, in addition to motor control, is involved in the regulation of several important functions, such as cognition, emotions, motivation, reward, attention and endocrine activity. Furthermore, a dysregulation or dysfunction of the dopaminergic circuits has been implicated in several other brain disorders, including for example AUD, substance use disorder, schizophrenia, attention deficit hyperactivity disorder (ADHD) and Huntington's disease [65].

1.3.2 Dopamine Receptors

There are currently five known DA receptor subtypes, termed D_1-D_5. These receptors are divided into two families, the "D_1-like family" and the "D_2-like family", depending on their structural, pharmacological and signaling properties [66]. The D_1 and D_5 receptors are

included in the D_1-like family, whereas the D_2-like family consists of the D_2, D_3 and D_4 receptors. All of these receptors are G-protein coupled transmembrane receptors [66]. Generally, the D_1 and D_2 -like receptors exerts opposite effects by activation of different second messenger pathways [67, 68]. Pharmacological agents could typically distinguish between the two families, but possess usually less specificity within each family [66].

All DA receptors are localized postsynaptically, whereas the D_2 and D_3 receptor subtypes are expressed both pre- and postsynaptically [69]. In the seventies, it was discovered that the receptor-mediated feedback mechanism adjusting the activity of dopaminergic neurons is, at least partly, mediated by receptors located on the dopaminergic neurons themselves. Later, these receptors were shown to be almost exclusively of D_2 subtype and stimulation of these receptors result in inhibition of dopaminergic neuron with respect to firing rate, synthesis, release and metabolism of DA. These D_2 receptors are often referred to as autoreceptors and are largely located extrasynaptically [70]. Generally, activation of D_2 autoreceptors result in decreased locomotor activity, whereas activation of postsynaptic D_2 receptors result in stimulation of locomotor activity [69]. Dopamine autoreceptors are suggested to be approximately tenfold more sensitive to DA and DA agonists than postsynaptic receptors, [71], possibly due to the fact that autoreceptors, compared to postsynaptic receptors, are exposed to much lower DA concentrations.

DA receptors are expressed in regions that receive dopaminergic innervations with the D_1 receptor subtype displaying highest expression levels and being the most widely distributed receptor subtype [66]. In subcortical areas, the D_1 and D_2 receptor subtypes have roughly the same expression, however, in the cortex, the D_1 subtype has a higher level of expression than D_2 [66].

1.3.3 Dopamine Pathways

The dopaminergic system in the central nervous system can be divided into four distinct pathways: the nigrostriatal, the mesolimbic, the mesocortical, and the tuberoinfundibular pathway [72-74]. The nigrostriatal DA pathway originates in the substantia nigra and project predominantly to the dorsal striatum. The nigrostriatal pathway is involved in motor control. Symptoms of Parkinson's disease (e.g. inhibition of voluntary movements, muscular rigidity and tremor) is caused by degeneration of DA neurons in this system [75]. Moreover, extrapyramidal side effects such as parkinsonism, dystonia, akathisia, and tardive dyskinesia are believed to be caused by massive blockade of D_2 receptors (antipsychotic drugs) in the dorsal striatum [76]. The tuberoinfundibular pathway originates in the arcuate nucleus of the hypothalamus and projects to the pituitary stalk and is important in the inhibitory control of prolactin synthesis and secretion [77]. The prolactin regulation is mediated by the D_2 receptor. Thus, a common side effect from blockade of D_2 receptors are increased secretion of prolactin (i.e. hyperprolactemia). Both the mesolimbic and mesocortical pathways have their cell bodies in the ventral tegmental area (VTA) and these two pathways are sometime referred to as the mesocorticolimbic pathway [72]. The mesolimbic pathway comprises DA neurons that project from VTA via the medial forebrain bundle to limbic structures such as

ventral striatum (i.e. the nucleus accumbens (NAc)), hippocampus, and amygdala, whereas the mesocortical pathway projects to cortical regions (e.g. to the prefrontal cortex (PFC)). The mesolimbic and mesocortical systems are involved in regulation of emotional control, motivation, attention, reward, cognition and the reinforcing properties of most drugs of abuse. The mesocorticolimbic pathway is also known to be the common denominator of the brain reward systems, in which the dopaminergic neurons projecting from the VTA to the NAc are suggested to play a central role [78-80].

1.4 ALCOHOL AND DOPAMINE

Almost all drugs of abuse, including alcohol, activate the mesolimbic DA system, resulting in an increased DA release in the NAc [81]. However, the molecular mechanisms by which alcohol activates the mesolimbic DA system is still under debate. Alcohol does not interact with a specific receptor, instead alcohol interferes with several neurotransmitter systems in the brain reward and stress circuits. However, in this thesis the focus is on alcohol's acute and chronic effects on the mesolimbic DA system.

1.4.1 Brief History

In the 1950s Olds and Milner discovered that rats implanted with brain electrodes would work to self-administer electrical currents into some, but not all, brain areas [82]. In specific brain areas, electrical stimulation were found to be highly rewarding, leading to that rats preferred stimulation over food (even when they were hungry) and water (even when they were thirsty). Later, these brain structures were anatomically mapped in more detail and referred to as "the brain reward system". Normally, the brain reward circuitry leads to pleasurable effect through natural reinforces, such as food, water and sexual contact. When the brain is activated by drugs of abuse, the response is more powerful, for alcohol, as much as 3 to 5 times stronger, than activation triggered by natural reinforces [81, 83]. Because drugs of abuse are more powerful, they are hypothesized to hijack the reward systems [80], which might lead to the loss of interest for natural rewards often seen in AUD individuals.

1.4.2 Acute Effects of Alcohol

The mesolimbic DA system, which originates in the VTA and project to limbic regions such as the NAc, is a central component of the brain reward system [78-80, 84]. Dopamine is the neurotransmitter primarily involved in the mesolimbic system. Activation of this system has been suggested to be a key element in mediating the reinforcing and rewarding effects of alcohol [54]. This has been demonstrated by a numerous animal- and clinical studies [57, 78]. For example, like most drugs of abuse, systemic administration of alcohol elevates extracellular DA concentrations in the NAc [81, 85, 86]. Similarly, alcohol self-administration produces a dose-dependent rise in DA levels in the NAc in rats [87]. Moreover, previous studies have shown that alcohol increases the firing rate of VTA DA neurons in rats [88, 89]. In addition, in a human study, intoxicating doses of alcohol increased extracellular DA in the ventral striatum (including NAc) [90] and increased extracellular DA in the striatum has been shown to correlate with self-reported "high" in healthy controls [91].

Collectively, these studies indicate that activation of the mesolimbic DA system most likely play an important role for the positive reinforcing effects of alcohol. Supportively, reduced alcohol-reinforced responding have been demonstrated following microinjections, into the NAc or VTA, of pharmacological agents that interfere with DA transmission [92, 93]. Additionally, mice deficient in DA D_1 or D_2 receptors show attenuated operant self-administration of alcohol [94], alcohol intake and preference [95], as well as conditioned place preference for alcohol (CPP) [96]. However, in spite of extensive evidence, several early studies have challenged the involvement of accumbal DA in alcohol reinforcement by showing that lesions of the mesolimbic DA system do not completely abolish alcohol-reinforced behavior [97, 98]. These studies might indicate that DA is an important, but maybe not essential, component of alcohol reinforcement.

In addition to alcohol reward, activation of the mesocorticolimbic DA system has been hypothesized to play a key role in incentive salience. Specifically, the function of midbrain DA neurons might be to direct behavior toward salient rewarding stimuli [56]. Weiss and colleagues have demonstrated that even the anticipation of alcohol availability have the potential to increase DA levels in the NAc in rats conditioned to 10% alcohol [87]. Furthermore, in another study, Philpot and Kristein demonstrated that following repeated injections of alcohol, saline injections alone evoked DA increase in the NAc [99]. In addition, in abstinent alcohol dependent individuals, alcohol-associated stimuli activated the ventral striatum [100]. Together, these studies indicate that the mesocorticolimbic system might play a role for the initiation of alcohol seeking, which in turn might contribute to alcohol craving and relapse. Supportively, the DA receptor antagonist haloperidol have been shown to reverse conditioned reinforcing effects of a discrete stimulus previously paired with alcohol [101] and reinstatement of responding for alcohol induced by a discriminative stimulus was blocked by either DA D_1 or D_2 receptor antagonists [102]. Moreover, Hamlin and colleagues showed that systemic injections of the DA D_1 receptor antagonist SCH 23390 blocked context-induced reinstatement [103] and Chaudhri and colleagues found that SCH 23390 injections into either NAc shell or core attenuate this reinstatement [104].

A multitude of other neurotransmitters might contribute to the positive reinforcing properties of alcohol by direct or indirectly activation of the brain reward circuitry [55]. For instance, alcohol has been proposed to increases the release of endogenous opioid in the VTA, resulting in an inhibition of GABA-ergic interneurons. Normally, these GABA-ergic interneurons exert a tonic inhibition of dopaminergic VTA neurons. Therefore, an inhibition of the GABA-ergic interneurons will result in disinhibition of the dopaminergic neurons, which subsequently will release DA in NAc [54, 79, 105]. The subsequent release of DA in NAc is believed to contribute to the rewarding effects of alcohol, as well as opiates [54, 79, 106]. However, alcohol intake is also suggested to result in endogenous opioid release in terminal areas of mesolimbic and mesocortical projections [107]. Importantly, several other neurotransmitters, for example, GABA, glutamate, acetylcholine, serotonin, as well as hormones (e.g. ghrelin) have been suggested to play important roles for alcohol reward and intake [78].

1.4.3 Chronic Effetcs of Alcohol

Following chronic alcohol exposure, a dysregulated DA activity has been demonstrated, hypothesized to reflect adaptations in mesolimbic DA function to counter sustained stimulation by alcohol. In contrast to alcohol's acute effects on mesolimbic DA neurotransmission, withdrawal from chronic alcohol use is associated with mesolimbic DA hypofunction. This is supported by animal studies showing that chronic alcohol use induced a substantial reduction in extracellular NAc DA levels [108], downregulation of D_2-receptors [109], and a decreased activity in VTA DA neuron [110]. Additionally, human imaging studies have shown decreased DA release [111], paralleled by a reduction in DA D_2 receptors [112] in detoxified alcohol dependent individuals. Moreover, following 3 weeks of alcohol drinking, changes in NAc DA turnover and synthesis was shown 2 months after alcohol withdrawal in mice [113], indicating that decreased DA release might be a long-lasting phenomenon. Thus, the persistence of DA deficient during protracted abstinence might have implications for vulnerability to relapse [114], as well as the adverse symptoms associated with protracted alcohol withdrawal. Interestingly, in a clinical study, a slow rate of recovery of DA receptor function predicted relapse and poor treatment outcome in alcohol dependent individuals [115]. In summary, this hypofunction of the mesolimbic DA system following chronic alcohol exposure is suggested to play an important role in the maintenance of AUD by motivating resumption of alcohol drinking during withdrawal to reverse DA deficits. Supportively to this hypothesis is a study showing that rats undergoing alcohol withdrawal will self-administer just enough alcohol to return DA levels to normal [116].

The transition from controlled alcohol use to AUD not only involve a compromised brain reward system, but also the recruitment of the brain stress systems such as the corticotropin-releasing factor, norepinephrine and dynorphin in the extended amygdala [14, 55]. These dysregulations in the stress systems are producing aversive or stress-like states and are suggested to contribute to the negative emotional states in withdrawal and protracted abstinence [61]. Thus, the combination of decreased reward system function and recruitment of brain stress systems provides a powerful motivation for relapse during both acute and protracted abstinence. A compound with DA stabilizing properties might have potential to counteract both positive and the negative reinforcing effects of alcohol, and thereby provide one potential drug candidate.

Following chronic alcohol use, components in the PFC might be compromised, resulting in deficits in executive function [51]. Generally, the function of the PFC is to engage executive function and disruption in this system might lead to impaired decision making and behavioral inhibition. Deficits in executive function, particularly in the preoccupation/anticipation, are often seen in AUD individuals [117, 118], which might increase the vulnerability to relapse. In fact, clinical studies have found detoxified AUD individuals to demonstrate poor inhibitory control in several behavior tasks, such as stop signal serial reaction [119], continuous performance task [120] and Go/No-Go task [121]. In addition to be involved in AUD, the DA system and D_2 receptors are suggested to be involved in the complex regulation of impulsive behavior [122]. Indeed, long-term alcohol vapor exposure, profoundly disrupted D_2/D_4

modulation of PFC neural activity in rats medial PFC [123]. Moreover, a decreased DA transmission in PFC [124] and an association between a reduction in striatal D_2/D_3 receptors and a decreased metabolic activity in prefrontal regions necessary for executive control (e.g. inhibitory control) [125] have been observed in AUD individuals. In addition, low striatal DA D_2 receptor availability have in turn been linked to increased impulsivity in rodents [126], as well as in social drinkers and individuals with AUD [127], Thus, enhancement of cognitive functions (e.g. improve impulse control), possibly through modulation of DA transmission, might provide an additional approach to prevent relapse.

1.5 DOPAMINE AS A POTENTIAL THERAPEUTIC TARGET FOR AUD

Given the involvement of DA in the development and maintenance of AUD, the DA system has previously been evaluated as a potential treatment target for AUD [128]. Activation of the mesolimbic DA pathway is believed to contributing to the acute reinforcing properties of alcohol [129] and traditional DA receptor antagonists (neuroleptics) reduce alcohol craving and alcohol reinforcement [128]. However, the use of DA antagonists are limited by severe side effects (including extrapyramidal effects) resulting from excessive DA inhibition. In addition, the DA antagonist flupenthixol induced relapse in recently detoxified AUD patients [130]. Dopamine agonists have also been evaluated as potential therapeutics based on the hypothesis that chronic alcohol consumption might create a dysphoric state as a result of decreased DA release [111], paralleled by a reduction in D_2 receptors [112]. Although medications that increase DA activity might theoretical have potential in the treatment of AUD, reported results have been conflicted [128]. For example, the DA D_2 agonist bromocriptine, was suggested to reduced drinking in alcohol dependent individuals [131], but a subsequent, randomized, double-blind, placebo-controlled study failed to show any difference in relapse between bromocriptine and placebo [132]. Recently, the partial D_2-agonist, aripiprazole was shown to decrease alcohol consumption in alcohol preferring AA-rats [133], as well as alcohol intake in AUD patients [134-136]. However, in a 12-week, double-blind, placebo-controlled treatment study with 295 AUD individuals, Anton and colleagues did not find any effect of aripiprazole in heavy drinking days compared to placebo [137]. Nevertheless, these results indicate that normalization, rather than antagonism or agonism, of the DA system might be a promising strategy for AUD treatment. Interestingly, aripiprazole has been shown to have DA stabilizing properties, which also has been demonstrated for (-)-OSU6162 (OSU6162) [70], the compound evaluated in this thesis. However, it should be noted that aripiprazole's and OSU6162's mechanism of action are not the same.

1.6 THE MONOAMINE STABILIZER (-)-OSU6162

1.6.1 Brief History

The monoamine stabilizer (-)-OSU6162 (OSU6162) [138] belongs to a pharmacological class of compounds originally named "dopamine stabilizers" based on the ability of these compounds to act normalizing on dopaminergic signaling depending upon the prevailing

dopaminergic tone [70]. Dopamine has an essential role in the expression of spontaneous and stimulant-induced activity [139]. Therefore, locomotor activity has been used as a behavioral model to describe DA stabilizers. The first DA stabilizers were synthesized by Dr. Arvid Carlsson and colleagues as part of a project aiming at developing a novel treatment for schizophrenia with better pharmacokinetic properties and less severe side effects [70].This project lead to the discovery of compounds with intriguing effects on the DA system; One of them being the partial D_2 agonist, (-)-3-PPP [140]. A partial D_2 agonist will bind to DA receptors located both pre- and postsynaptic and produce agonist activities, but lower than that of DA. The level of receptor activation will correspond to the intrinsic activity of the partial D_2 agonist. (-)-3-PPP has undergone trials in schizophrenic patients [141, 142] and was found to possess antipsychotic activity without inducing any serious side effects. However, the antipsychotic action was not sustained for more than 1 week, possibly due to desensitization of the receptor by the agonist [141]. Thus, it was suggested that a partial D_2 receptor with lower intrinsic activities may possess a more long-lasting antipsychotic activity. The partial D_2 agonist, aripiprazole, a further development of (-)-3-PPP [143], is one such compound and has been approved by the FDA for the treatment of schizophrenia [144, 145]. Thereby, aripiprazole, was the first claimed "dopamine stabilizer" to be commercially available.

OSU6162 is a further development from (-)-3-PPP and has the ability to stimulate, inhibit, or show no effect on DA-related behaviors depending upon the prevailing dopaminergic tone [138, 146, 147]. For example, OSU6162's ability to suppress DA activity is illustrated by inhibition of amphetamine-induced hyperlocomotor activity in rats [146] and sub-human primates [148]. In contrast, stimulation of DA activity is shown through increased locomotor activity in rats habituated to their environment [138, 146, 147]. Importantly, these buffering effects on DA transmission were observed without the presence of motor impairments or catalepsy [138, 146]. The normalizing and stabilizing profile of OSU6162 is further supported by a previous positron emission tomography study in anaesthetized rhesus monkeys, showing a reduction in the striatal L-[11C]DOPA influx rate in monkeys with high baseline values and an increased striatal L-[11C]DOPA influx rate in monkeys with low baseline values [149]. Together, these studies indicate that OSU6162's DA stabilizing properties appears to depend on the current baseline in DA activity.

1.6.2 OSU6162's Mechanism of Action

The exact mechanisms behind OSU6162's ability to modify DA-related behaviors in a bidirectional manner are not fully understood. Although, OSU6162, like (-)-3-PPP and aripiprazole, has been shown to display partial agonist effects in vitro [150, 151], and is considered a "dopamine stabilizer", the observed effects of OSU6162 cannot be explained by partial D_2 receptor agonism [70]. In fact, OSU6162 have failed to demonstrate any intrinsic activity in vivo [138, 146]. In a behavior study, evaluating the effects of OSU6162, (-)-3-PPP and aripiprazole on locomotor activity, all three compounds caused inhibition of locomotor activity in rats introduced to a novel stimulating environment (highly active rats). However,

only OSU6162 was able to induce any activation in rats habituated to their environment (inactive rats) [147]. However, following reserpine-induced DA-depletion (i.e. in the absence of DA) a minor stimulation of locomotor activity was observed in rats treated with (-)-3-PPP [140], an effect indicating D_2 receptor stimulation. In contrast, OSU6162 did not induce any behavioral activation in rats treated with reserpine [138], and failed to induce rotational behavior in 6-hydroxy-DA-lesioned rats [152], suggesting lack of D_2 agonist-like effects. Instead, these studies indicate that the behavioral activating effects of OSU6162 is due to an indirect effect mediated by DA.

Based on early in vitro studies, OSU6162 was suggested to be a D_2/D_3 receptor antagonist with very low affinity, and with no detectable affinity for a selection of other receptor types [138]. Indeed, the ability of OSU6162 to blunt amphetamine-induced hyperactivity was lost in DA D_2 knockout mice [153], giving support for the involvement of D_2 receptor blockade in the behavioral effects of OSU6162.

In two different studies, OSU612 was shown to induced dose-dependent increase in prolactin levels [146, 154], an effect associated with D_2 receptor blockade. In addition, functional experiments have reported that OSU6162, like traditional D_2 antagonists, elevate DA synthesis, DA release, and DA metabolism in untreated rats [138]. However, animal behavior experiments have demonstrated a clear distinction between OSU6162 and traditional D_2 receptor antagonists [70]. For example, OSU6162 (3-60 mg/kg), over a D_2 occupancy range of 37 to 87%, showed a tone-dependent mixture of stimulatory and depressant effects on locomotor activity in rats without inducing any catalepsy [146]. In contrast, in the same study, over a similar occupancy range, haloperidol (a traditional D_2 antagonist) inhibited psychostimulant activity but induced catalepsy and failed to activate rats habituated to their environment [146].

There is no obvious mechanism by which OSU6162 could stabilize dopaminergic transmission. However, a proposed mechanism of OSU6162 is a relative preference for extrasynaptic versus synaptic D_2 receptors [70]. This hypothesis postulates that, in case of low to normal dopaminergic tone, OSU6162 may stimulate dopaminergic signaling by blockade of D_2 autoreceptors. Instead, in case of an elevated dopaminergic tone, OSU6162 may dampen transmission mediated by extrasynaptic D_2 heteroreceptors [70]. Another suggested mechanism is that OSU6162 might have a dual action on D_2 receptors by interaction with both an allosteric and an orthosteric site on the receptor in order to stimulate or inhibit dopaminergic neurotransmission [150]. When the DA level is low, OSU6162 acts on the allosteric site on D_2 receptors causing an enhanced effect of DA, presumably due to a conformation change. This change might in turn lead to an increased activation of the D_2 receptor or an increased affinity to DA [150]. On the other hand, if DA levels are high, OSU6162 will bind to the orthosteric site and antagonize the receptor, causing DA inhibition [150]. However, additionally, mutually counterbalancing effects, involving both agonism and antagonism in different receptor location or sites, probably exist. Indeed, recently, OSU6162 was shown to have stabilizing properties on the serotonergic neural circuits, acting as a partial

agonist, notably on the 5-HT_{2A} receptors [155]. Thus, the wider classification "monoamine stabilizer" is a more appropriate name for this class of compounds.

1.6.3 Clinical Applications of OSU6162

OSU6162, with its unique ability to stabilize DA transmission, might constitute a potential treatment for a variety of conditions involving dysregulation or dysfunction in the dopaminergic circuits of the brain. Indeed, OSU6162 has been tested as potential therapeutics for Huntington's disease [156, 157], schizophrenia [158, 159], and more recently for mental fatigue following stoke or traumatic brain injury [160]. All these clinical studies have demonstrated that OSU6162 appears to be clinically safe with side effects of mild severity. Thus, a great advantage of OSU6162 compared to traditional D_2 antagonists, might be the lack of extrapyramidal reactions [161]. Moreover, stabilizing of dopaminergic transmission would be particularly useful in the treatment of conditions involving both increased and decreased DA signaling, as suggested in the case of AUD. OSU6162's potential to target the DA system in brain regions relevant for AUD is supported by a recent human PET study showing that OSU6162 preferentially binds to D_2/D_3-receptors in the striatum [162]. In addition, Natesan and colleagues, demonstrated that OSU6162 induced expression of Fos, a marker of neuronal activity [163], preferentially in NAc compare to dorsolateral striatum in rats [146].

1.7 ANIMAL MODELS OF AUD - FOCUS ON VOLUNTARY ALCOHOL INTAKE

Animal models are important in promoting knowledge of neurobiological mechanisms and neurocircuitries involved in drug-seeking behavior and new potential targets for medication development [164]. In addition, in animal experiments, experimental conditions can be controlled and therefore give essential insight into casual relationships that would be impossible, or unethical, to do in humans. Rats are a common and suitable species for determining the biological basis of drug self-administration and relapse because their reward pathways display a high degree of similarity to humans [165]. Alcohol use disorder is a complex disorder with a major heterogenecity, where genetic predisposition, age of onset, incidence of withdrawal, environment factors and pattern of drinking differs a lot between AUD individuals [166]. Thus, no single animal model can capture the complexity of or manifest all symptoms of AUD. Instead, different animal models are used to reflect various aspect of AUD, such as excessive alcohol drinking, increased motivation to take alcohol, and relapse to alcohol seeking [167]. When developing and utilizing animal models, the model's face, construct, and predictive validity are important to consider. The face validity refers to the similarity in symptoms between the animal and the human condition. The construct validity refers to whether there is a similarity in the mechanisms underlying the behavior in the model and the behavior in the human condition. The predictive validity refers to whether the results obtained predict the response in human, such as a treatment response [168].

The studies in this thesis combine a battery of different animal models, used to model different aspects of AUD, to evaluate the potential of OSU6162 as a novel treatment for

AUD. Moreover, we use outbred (standard) laboratory rats and focus on voluntary alcohol intake models to, in the best way, represent the heterogeneous human population and resemble human alcohol consumption, respectively. Therefore, forced alcohol intake models will not be described here.

1.7.1 Alcohol Intake Models

In humans, alcohol is taken voluntarily and ingested orally. Thus, voluntary alcohol consumption becomes an important aspect in animal models of alcohol intake. Operant self-administration models and home-cage alcohol drinking models are both voluntary alcohol intake models [167]. Voluntary alcohol intake models can from a behavioral perspective be classified as operant and non-operant procedures, which are distinguished from each other on the basis of the behavior required to obtain alcohol. In home-cage alcohol drinking models, i.e. non-operant models, rats are presented with an alcohol bottle in the home-cage, whereas in the operant self-administration models, rats are required to produce an operant response (e.g. press a lever) to get access to alcohol [167].

Historically, it has been a challenge to obtain high alcohol consumption in standard laboratory rats because rats do not generally prefer alcohol over water. Consequently, several different initiation procedures have been developed in order to increase the alcohol consumption, such as water/food deprivation [169] or taste masking with sucrose fading procedures [170]. Despite these efforts, rats rarely maintain high alcohol consumption when the initiation procedures are removed. In addition, results from models using food or fluid deprivation might be difficult to interpret because alcohol consumption could be motivated by thirst or the need for the calories, and not by the pharmacological effects of alcohol. Another attempt to increase alcohol consumption in rats is the use of rat strains selectively bred for high preference for alcohol [171, 172]. However, in drug development, interpretation of data using genetically modified animals might be difficult, as inbred rats are not easily translated into the general human population. Nevertheless, selectively bred rat lines might be a good model to evaluate genetic components underlying susceptibility or resistance to AUD.

1.7.1.1 Intermittent Access 20% Ethanol Model

The Intermittent Access 20% Ethanol (IA20E) model was first introduced in the early 1970s and showed that standard laboratory rats voluntary consume high levels of alcohol without any initiation procedure, if an intermittent schedule to 20% alcohol in a 2-bottle-choice paradigm was applied [173]. This early study showed that repeated cycles of free-choice alcohol intake led to considerably higher alcohol intake compared to rats given continuous access to alcohol [173]. More recently, the model was revived [174] and have since then gain popularity in the alcohol field [175].

In the IA20E model, rats get access to alcohol in the home-cage during specific days of the week (typically Monday, Wednesday, and Friday). Water is available *ad libitum*. At early stages of this procedure, rats consume relatively low levels of alcohol but will gradually escalate over time [173, 174] reaching an alcohol intake between 3-6 g/kg/day [175]. This

drinking pattern might model the transition from social-like drinking to excessive alcohol drinking in humans. Although, this model has several important advantages, a limitation with this model might be that a variability in alcohol intake exists and all rats due not escalate or drink excessive amounts of alcohol [175]. However, this individual variability in alcohol intake could also be seen as an advantage of the model, because in a way this represents the heterogeneous human population.

Previous studies have suggested that the IA20E model in addition to study initiation, and maintenance of excessive alcohol intake, also might be used to study binge-like alcohol drinking [175]. In fact, Carnicella and colleagues showed that the IA20E model induced pharmacologically relevant blood ethanol concentrations (BECs) reaching a mean BEC value of > 80 mg% [176], which meets the criteria of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) for binge drinking in humans (National Institute on Alcohol Abuse and Alcoholism, 2004). Although rats reach high levels of alcohol consumption in the IA20E, the method might not be sufficient to induce alcohol dependence. Models that are suggested to induce alcohol dependence in rats are using forced alcohol exposure, e.g. the alcohol vapor model [177]. However, an obvious limitations using alcohol vapor procedures are the lack of face validity. In addition, this excessive, experimenter-imposed alcohol exposure might act as a non-specific stressor in the rats, which might lead to confounding results.

1.7.1.2 Operant Self-Administration Model

Alcohol's positive and negative motivational effects are believed to be important factors in motivating drinkers to increase or decrease their alcohol intake [178]. Therefore, a variety of animal behavioral models aim to measure alcohol's motivational effects have been developed. One such model is the operant self-administration model, which is based on procedures originally developed by B.F. Skinner in 1938 using food as reward [179].

The operant self-administration model is a commonly used model in which animals are trained to self-administer alcohol typically by pressing a lever in operant conditioning chambers [180]. The operant chamber is usually equipped with two levers defined as active and inactive. The active lever is linked to the alcohol delivery, whereas the inactive lever is linked to vehicle or has no programmed consequence. The inactive lever is used as a measure of nonspecific behavioral activity. The self-administration model is used to explore the motivational aspects of alcohol seeking (craving) in rodents [178]. Using this model, different schedules of alcohol reinforcement can be applied, which will determine how hard the animal will have to work to obtain one alcohol delivery. Operant training is typically performed on a fixed-ratio (FR) 1 schedule of reinforcement, in which every operant response (e.g. lever press) is reinforced with the delivery of alcohol. Another commonly used schedule is the progressive-ratio schedule (PR) of reinforcement. In the PR schedule of reinforcement, the requirement for obtaining alcohol (the number of lever presses) are progressively increased within the session [167]. The PR schedule of reinforcement is used to determine an animal's willingness to work to obtain alcohol and the point at which responding ceases is called the

"breakpoint" [181]. The breakpoint is thought to provide an index of the reinforcing efficacy of the drug [182].

1.7.1.3 Pros and Cons with Operant vs Non-Operant Models

The IA20E model present good face validity for human alcohol consumption, because the alcohol is ingested orally and voluntary in repeated cycles. Moreover, in a study using in vivo microdialysis, a decreased DA overflow in NAc was observed following 7 weeks of drinking in the IA20E model [183]. Furthermore, we have demonstrated that the IA20E model might induce a hyperglutamatergic state in rats, typified by an increased NMDA- and AMPA-induced currents in pyramidal cells in the medial PFC, following 3 months of high alcohol consumption. These two studies, showing neuroadaptations in the mesolimbic DA system and on glutamatergic transmission, are examples of studies that provide the IA20E model with construct validity. Moreover, naltrexone and acamprosate, two medications approved for treatment of AUD, have been demonstrated to decrease alcohol intake in this model [174, 184]. In addition, varenicline has been shown to decrease alcohol intake in the IA20E model [185], as well as percent heavy drinking days and drinks per drinking day in alcohol dependent patients, compared with placebo, in a multicenter, double-blind, placebo-controlled clinical trial [186]. Together these translational studies, give the IA20E model predictive validity and support the potential of this model as a good preclinical tool in drug development.

An advantages with the use of an operant self-administration model, compared with the IA20E model, is that the operant self-administration model provides more flexibility in the experimental design which thereby could provide more information. For example, the temporal distribution of the responses can be analyzed [167]. On the other hand, the operant self-administration model requires a relatively long training period, and not all rats will successfully acquire operant alcohol self-administration [175]. However, once stable responding levels are achieved, operant self-administration is very versatile model in terms of behavioral assessment [167]. The operant self-administration model has had an important role in the preclinical validation and characterization of naltrexone [187, 188] and acamprosate [189, 190], indicating some predictive validity. Although, humans do not have to press a lever to gain access to alcohol, the operant self-administration model still present some face validity, because humans ingest alcohol orally and drink alcohol voluntary where they control the amount consumed and the pattern of consumption.

In summary, the IA20E model focus on consummatory aspects, whereas the operant self-administration model captures both the consummatory and the motivational aspects of alcohol consumption [178]. Therefore, in this thesis (Paper I), we use both the IA20E model and the operant self-administration model (a PR schedule of reinforcement) to evaluate OSU6162's potential to decrease alcohol consumption and the motivation to seek alcohol.

1.7.2 Relapse-like Drinking Models

A main problem in the treatment of AUD is the long-lasting vulnerability to relapse [191]. Relapse to alcohol could be modelled by the alcohol deprivation model [192] and by reinstatement models [193].

1.7.2.1 The Alcohol Deprivation Model

The alcohol deprivation model is based on the observation of a temporary rise in alcohol intake following a period of forced abstinence, a so-called alcohol deprivation effect (ADE) [167]. The ADE was first described in rats by Sinclear and Senter [194] and has in recent years become a widely used paradigm in examining relapse-like drinking behavior [195]. The exact mechanism behind the ADE is not clear, but alcohol itself might act as a cue (i.e. taste, smell) or/and a priming stimuli triggering the ADE [167]. Alternatively, the ADE might by caused by an increased reinforcing value of alcohol [192].

1.7.2.2 The Reinstatement Model

The reinstatement model was introduced to the drug addiction field in the early 1970s [196] and has over the years become a well-established model to study drug-seeking behavior [193]. The reinstatement model refer to the resumption of extinguished lever-pressing behavior after non-contingent exposure to drug or non-drug stimuli [197]. In the reinstatement model, rats are trained to self-administer a drug of abuse, such as alcohol, by typically pressing a lever in an operant conditioning chambers. The alcohol seeking behavior (lever pressing) is then extinguished in the absence of alcohol. During the reinstatement test, reinstatement of drug seeking behavior is defined as significantly higher responding on the lever previously paired with drug following exposure to the experimental manipulations as compared with the control manipulations [197]. Re-exposure to alcohol [24, 198], exposure to environmental contexts [199] or alcohol cues[200] previously paired with alcohol, and exposure to a stressful stimuli [198] are different manipulations typically used to reinstate alcohol seeking in laboratory animals.

1.7.2.3 Pros and Cons with Relapse-like Drinking Models

The concept of ADE has been observed in individuals with AUD [201] and the model has therefore been suggested to have face and construct validity [167]. Regarding predictive validity, naltrexone and acamprosate, have shown to prevent the ADE in rats [192, 202], as well as craving-induced relapse in humans [44].

For the reinstatement model, it should be noted that in contrast to relapse in AUD individuals, this model measure relapse-like behavior in a drug-free state. Thus, this model might be better referred to as a model measuring alcohol-seeking behavior [167]. However, the reinstatement model is suggested to have face validity for relapse due to the fact that factors reported to reinstate alcohol seeking in laboratory animals (alcohol-priming, cue, and stress) also provoke relapse and craving in humans [201]. In addition, naltrexone and acamprosate, which has been shown to decreases relapse tendencies in humans, has been shown to

decrease alcohol-priming or cue-induced reinstatement of alcohol seeking in rats [188, 203, 204], supporting the predictive validity of the reinstatement model.

A limitation with both the reinstatement and the ADE model, is the use of a forced abstinence period (i.e. extinction training or removal of the alcohol from the cage). In AUD individuals, abstinence from alcohol, unless treated in an inpatient facility, is typically initiated when alcohol is available due to the negative consequences associated with excessive drinking [205, 206]. However, recently, new models have been developed in an attempt to more closely mimic the human condition. For example, Marchant and colleagues have developed a model of context-induced relapse where active alcohol-taking behavior is suppressed by negative consequences using a mild footshook [207].

In summary, the ADE model appears to be suitable to study the impact of alcohol re-exposure on relapse, whereas the reinstatement model can address the impact of environmental factors and stress on alcohol seeking prior to drug use [195]. Thus, the ADE model and the reinstatement model might be a good complement to each other in the screening for new anti-relapse agents. We therefore, in this thesis, used both the ADE model (Paper III) and a cue/priming-induced reinstatement model (Paper I) to evaluate the potential of OSU6162 to attenuate relapse-like behavior. In addition, we used a context-induced reinstatement model [208] to examine the effect of OSU6162 on oxycodone seeking induced by re-exposure to the drug seeking environment (Paper IV). This model have previously been used to reinstate drug seeking behavior for several drugs of abuse including heroin [209] and oxycodone (Bossert et al., unpublished data).

2 AIM OF THE STUDY

The overall aim of this thesis was to evaluate the potential of OSU6162 as a novel treatment for AUD using validated preclinical models of behaviors related to AUD.

Specific Aims:

Paper I: To evaluate OSU6162's potential to decrease alcohol intake, alcohol seeking, alcohol withdrawal symptoms, and cue/priming-induced reinstatement of alcohol seeking in long-term alcohol drinking rats.

Paper II: To evaluate the rewarding properties of OSU6162 in alcohol-naïve and long-term alcohol drinking rats using CPP.

Paper III: To evaluate the effects of OSU6162 on relapse-like alcohol drinking and motor impulsivity in alcohol-naïve and long-term alcohol drinking rats.

Paper IV: To examine the effects of OSU6162 on oxycodone self-administration and context-induced reinstatement and thereby evaluate the potential of OSU6162 on another drug of abuse.

3 MATERIAL AND METHODS

This section provides a brief description of the animals, the drugs, the different animal models, and statistics used in this thesis. Detail descriptions of the animals, drugs and methods could be found in the papers/manuscripts. It should be noted that the microdialysis experiments (Paper I: Conducted and analyzed by Björn Schilström; Paper II: Conducted and analyzed by Kristin Feltmann) and the novel object recognition experiment (Paper II: Conducted and analyzed by Kristin Feltmann) were not included in this thesis project and the methodological aspects will therefore not be described here. Information about these studies could be found in Paper I and Paper II.

3.1 ETHICAL CONSIDERATIONS

Despite the severe consequences of AUD, only four medications have been approved in Sweden for this disease. The varying and limited clinical efficacy of these pharmacotherapies justify the search for more effective medications to help AUD individuals reduce their drinking and combat their disease. Thus, this thesis provides valuable information for a potential novel medication for AUD which might benefit a huge patient group that today lack adequate treatment.

The work presented in this thesis consist of animal experiments, all designed to minimize the number of animals and their suffering. The experiments in Paper I-III were carried out in strict accordance with the recommendations in the Swedish Animal Welfare Act and approved by the Swedish Ethical Committee on Animal Research in Stockholm (diary numbers: N427/11, N475/12, and N163/14). The experiments in Paper IV were performed in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (8th edition), under protocols approved by the Animal Care and Use Committee (# 14-BNRB-25).

3.2 ANIMALS

Male Rcc Han Wistar rats were purchased from Harlan (Netherlands) and used in Paper II and III and for all behavior experiments in Paper I. In Paper IV, male Sprague Dawley rats were used and obtained from Charles River Laboratories (Wilmington, MA, USA). All rats were housed under standard laboratory conditions with controlled temperature and humidity. Food and water were freely available in all experiments except for the 5-Choice Serial Reaction Time Task (5CSRTT) experiment in Paper III and the food self-administration experiment in Paper IV, in which food restriction was applied (20 g/day). Rats were housed individually in most of the experiments (for details see papers/manuscripts).

3.3 DRUGS

The monoamine stabilizer (-)-OSU6162 [(S)-(-)-3-(3-methanesulfonyl-phenyl)-1-propyl-piperi- dine] (or PNU-96391) was generously donated by Dr. Arvid Carlsson (Sahlgrenska Academy, University of Gothenburg) and dissolved in saline (B. Braun Malsungen AG,

Germany). OSU6162 was injected subcutaneously (sc), at a volume of 5 ml/kg, 30 or 60 min prior to the start of the test sessions. The OSU6162 doses used in this thesis were based on a previous study showing that this dose range induces high striatal D_2 receptor occupancy without inducing catalepsy in rats [146]. Saline was used as vehicle.

Alcohol drinking solutions were prepared in tap water from 95% (v/v) ethanol (Apoteket AB, Stockholm, Sweden) in Paper I and 96% (v/v) ethanol (Solveco AB, Sweden) in Paper II and III. The other drinking solutions, used in Paper I, were prepared in tap water from NaCl (Sigma Aldrich, Stockholm, Sweden) or sucrose (Sigma Aldrich, Stockholm, Sweden). Oxycodone, used for the self-administration experiments in Paper IV, was received from the National Institute on Drug Abuse (NIDA) pharmacy and dissolved in sterile saline. The oxycodone doses were based on unpublished data from Dr. Yavin Shaham's laboratory (NIDA).

3.4 ANIMAL MODELS

3.4.1 Intermittent Access 20% Ethanol (IA20E) Model

The IA20E method induces voluntary intake of high amounts of alcohol without the use of an initiation procedure [173, 174]. In the IA20E method, rats had access to 20% alcohol during three 24-hour drinking-sessions per week (Monday, Wednesday and Friday) and water was always available. On Alcohol days, each rats was given access to one bottle of 20% alcohol and one bottle of water. After 24 h, the alcohol bottle was replaced with a second water bottle for the subsequent 24 h. The following day, the second water bottle was replaced with the 20 % alcohol bottle. The location of the alcohol bottle was alternated from the previous session to control for side preferences. During the weekend, rats receive free access to water. Alcohol intake per kilogram of body weight (g/kg), the preference for alcohol over water (the ratio of alcohol to total fluid intake), water intake and total fluid intake were calculated by weighing the rats and the bottles. Bottles were typically weighed both 4 and 24 hours after the fluids were presented with some exceptions where only the 24 h measurements were taken. The procedures for the salty- and sweet-solution experiments (Paper I) were identical with the IA20E model, with the exception that the alcohol solution was replaced with a bottle of 0.175% sodium chloride (NaCl) or 5% sucrose, respectively.

In Paper I, the effects of acute and repeated OSU6162 treatment on alcohol intake were evaluated. The acute OSU6162 treatment data were analyzed using a repeated measures analysis of variance (ANOVA) with the within-subjects factor of OSU6162 treatment (0, 15, and 30 mg/kg). Moreover, the repeated OSU6162 treatment data were analyzed using a repeated measures ANOVA with the within-subjects factors of OSU6162 dose (0 and 30 mg/kg), and day (5 alcohol sessions during treatment and 5 drinking sessions post treatment).

3.4.2 Modified Novel Cage Test (NCT)

The Novel Cage Test (NCT) [210] was developed to evaluate emotional reactivity in mice by quantifying exploration and risk assessment behaviors. In this thesis, a modified NCT was

used which was adjusted to rats and used to examine the level of alcohol withdrawal symptoms (tail stiffness and walking with abnormal/broad gait [211]), as well as locomotor (waking), exploration (investigating and rearing), risk assessment (stretch attend posture and stretch approach), displacement (grooming) and anxiety-like behaviors (freezing and motionless) during 5 minutes of spontaneous behavior. The rats were placed in the center of the open field arena (40 x 40 cm) and their behaviors were video-recorded for 5 min using a digital camera placed above the cage. The NCT was conducted during the dark phase of the light/dark cycle and performed under dim light. The frequency (how often the behavior occurs) and duration (total time of an occurrence) of the behaviors were recorded with EthoLog® [212].

In paper I, the effects of OSU6162 treatment on alcohol withdrawal symptoms were examined. The data from the NCT did not pass the tests for normality and were therefore analyzed using non-parametric analyses. The overall main effect was determined using Kruskal-Wallis test and followed by post hoc analyses with Mann Whitney-U test.

3.4.3 Operant Alcohol Self-administration

The operant self-administration model is commonly used to measure alcohol intake and the motivation to seek alcohol [180]. The operant self-administration procedure was based on previous studies showing that rats readily self-administer 20% alcohol without the use of sucrose fading or any other initiation procedure [175, 213]. The operant self-administration training was conducted in standard Med Associates self-administration chambers. Each chamber was equipped with two levers. Lever presses on the active lever activate the pump, whereas lever presses on the inactive lever had no programmed consequences. During training, rats earned alcohol at a volume of 0.1 ml paired with a discrete tone-light cue. In addition, an olfactory cue (orange scent) predictive of alcohol was also used. In the end of the training, rats earn alcohol during 60 min sessions (FR 3 schedule of reinforcement) 3 days a week (Monday, Wednesday and Friday). During testing, a PR schedule of reinforcement was used to determine the rats' willingness to work for alcohol [181]. The PR method was adapted from [214] and the number of lever presses required for one alcohol delivery increased within the session according to a pre-defined exponential function [215]. Total number of lever presses established in the last successfully completed ratio during the session was defined as the breakpoint [181].

In Paper I, the effects of OSU6162 on self-administration using a PR schedule of reinforcement were evaluated. The PR test data were analyzed using repeated measures ANOVA with the within-subjects factor of OSU6162 treatment (0, 15, and 30 mg/kg).

3.4.4 Cue-induced Reinstatement

The reinstatement model [196] is a commonly used model to study reinstatement (relapse) of drug-seeking behavior [193]. In the cue-induced reinstatement experiment, rats are trained to self-administer 20% alcohol as described in 3.4.3. Following the alcohol self-administration training, rats were exposed to extinction sessions. During extinction sessions, responses on

the previously active lever were not reinforced with alcohol or the activation of the discrete tone-light cue. After extinction of lever-pressing, rats were tested for cue/priming-induced reinstatement of alcohol seeking. During testing, responding on the active lever led to contingent presentations of the tone-light cue previously paired with alcohol delivery but not alcohol [216]. A small amount of alcohol was presented in the liquid dispenser as an additional cue, or priming at the start of the session; however, no additional alcohol was delivered.

In paper I, the effects of OSU6162 treatment on cue/priming-induced reinstatement of alcohol seeking were examined. The reinstatement data were analyzed using an ANOVA with the between-subjects factor of treatment (0 and 30 mg/kg).

3.4.5 The Alcohol Deprivation Model

The alcohol deprivation model is a relapse-like drinking model and is based on the observation of a temporary rise in alcohol intake following a period of forced abstinence [194], a so-called ADE. Rats were drinking alcohol in the home-cage using the IA20E model for approximately 10 weeks and were thereafter subjected to an abstinence period of 18 days. Following the abstinence period, the alcohol bottles were reintroduced and measurements of alcohol intake, preference for alcohol over water, water intake and total fluid intake were taken. Bottles were weighed both 4 and 24 h after the fluids were presented.

In Paper III: The effects of OSU6162 to attenuate the ADE were evaluated. The data from the ADE test were analyzed using paired Student's t-test. Prior to the trial it was planned to compare difference before and after abstinence period within each treatment group.

3.4.6 Conditioned Place Preference (CPP)

The CPP model [217] is a classical model of drug reward, in which laboratory animals are trained to associate one distinct compartment (context) with drug injections and a second compartment with injections of vehicle [218].

The CPP experiments was carried out as described previously [219]. The CPP-boxes consisted of two compartments separated by a guillotine door, and with distinct visual and tactile cues. The CPP experiments consisted of three phases: pre-conditioning (day 1; 15 min), conditioning (day 2–5; 60 min/session), and post-conditioning test (day 6; 15min). The initial side preference (i.e. the side where the rat spends more than 50% of the time) was determined during the preconditioning phase. During the conditioning phase (guillotine door closed), each rats received two injections per day with six hours in between, using a biased procedure (i.e. drugs were paired with the least preferred compartment and vehicle with the preferred compartment). Four conditioning sessions were chosen as three to four sessions are generally used to obtain robust CPP to rewarding substances in rodents [220, 221]. Control rats were paired with vehicle on both sides. During the post-conditioning test, rats were again given free access to both compartments and time spent in each compartment was measured during 15 min. Expression of CPP was evaluated by comparing time spent in the drug-paired

compartment during post-conditioning, with time spent in the same compartment during pre-conditioning.

In paper II, the effects of OSU6162 with regards to abuse liability were evaluated. The CPP data were analyzed using paired Student's t-test within each treatment group as determined a priori.

3.4.7 5-Choice Serial Reaction Time Task (5CSRTT)

The 5CSRTT paradigm measures motor impulsivity and attention [222]. Rats are trained in standard Med Associate chambers. Each chamber had a pellet dispenser on the right wall and five nose-poke holes on the left wall. The 5CSRTT procedure was performed as described previously [223] except that a modified training protocol was used. This training protocol is described in detail in Paper III (Table 1). Briefly, rats are trained to respond to a brief visual stimulus presented randomly in one of five nose-poke holes. Correct response (i.e. response in an illuminated hole) is rewarded with a food pellet. No reward is given upon incorrect response (i.e. response in a non-illuminated hole), omission (no response when starting a trial) or premature response (responding before presentation of the visual stimulus). Each session is terminated after 100 trials or 40 min, whichever occurred first. The number of premature responses is used as a measurement of impulsive behavior [222]. The number of premature responses, trials, correct responses, omissions, latency to respond (i.e. time between stimulus onset and nose poke) and latency to collect (i.e. time to collect the pellet followed a correct response) were recorded.

In Paper III, the effects of OSU6162 on motor impulse behavior were examined. The 5CSRTT data were analyzed using repeated-measures ANOVA with the within-subjects factors of treatment (baseline, 0, 15 and 30 mg/kg) and condition (ITI-5s and ITI-7s session). Baseline was defined as mean of responding during the week before the first OSU6162-testing.

3.4.8 Intravenous Self-administration of Oxycodone

To enable intravenous (iv) self-administration, a catheter was inserted into the right jugular vein as described elsewhere [224, 225] and in Paper IV. Rats were trained and tested in standard Med Associates self-administration chambers. Each chamber had two levers on opposing walls. Lever presses on the retractable active lever activated the infusion pump, whereas lever presses on the non-retractable inactive lever had no programmed consequences. Rats were trained to self-administer oxycodone (6 h/day) at a dose of 0.1 mg/kg for 5 days. During training and testing, rats earned oxycodone infusions at a volume of 65 µl paired with a discrete tone-light cue under a FR 1 schedule of reinforcement.

In Paper IV, the effects of OSU6162 on oxycodone self-administration were tested. The self-administration data were analyzed using repeated measures ANOVA with the within-subjects factor of OSU6162 dose (0, 7.5, 15, 30 mg/kg).

3.4.9 Context-induced Reinstatement

In humans, relapse is often triggered by exposure to environments previously associated with the drug [226]. This phenomenon is modeled in rats using the ABA renewal model [227]. In this model, rats are first trained to self-administer a drug in one context (A) and then lever pressing is extinguished by placing the rat in a different environment (B). During testing, re-exposure to the initial environment (A) leads to context induced-reinstatement of drug seeking [208].

The self-administration chambers were modified to two contexts that differed from each other in terms of their auditory (fan on/off), visual (houselight white/red light) and tactile (narrow/wide grid) cues [209, 228]. The contexts are referred to as A and B, where A is the oxycodone self-administration (training) and reinstatement (testing) context and B is the extinction context. Both contexts had two levers on opposing walls as described in 3.4.8. Rats were trained to self-administer oxycodone in context A for 6 h/day for 14 days. During training, rats earned oxycodone infusions at a volume of 65 µl paired with a discrete tone-light cue under a FR 1 reinforcement schedule. Oxycodone was infused at a dose of 0.1 mg/kg/infusion for the first seven sessions and at the dose of 0.05 mg/kg/infusion for the last seven sessions. Following the oxycodone self-administration training, rats were exposed to extinction sessions (6 h/day). During these sessions, responses on the previously active lever led to contingent presentations of the discrete tone-light cue, but were not reinforced with oxycodone. After extinction of lever-pressing in context B, rats were tested for context-induced reinstatement under extinction conditions (lever presses led to the presentation of the tone-light cue but not oxycodone).

In paper IV, the effects of OSU6162 on context-induced reinstatement of oxycodone seeking were examined. The reinstatement data were analyzed using a repeated measures ANOVA with the within-subjects factors of OSU6162 dose (0 and 15 mg/kg), and Context (A and B).

3.5 STATISTICS

The statistics used for the different experiments in this thesis are described under each method above. IBM SPSS statistics (version 20.0, SPSS Inc., Chicago, Illinois) or GraphPad Prism (GraphPad Software, La Jolla, CA, USA) were used to perform all statistical analyses. For all analyses a P-value of <0.05 was considered significant.

4 RESULTS

4.1 PAPER I: THE DOPAMINE STABILIZER (-)-OSU6162 ATTENUATES VOLUNTARY ETHANOL INTAKE AND ETHANOL-INDUCED DOPAMINE OUTPUT IN THE NUCLEUS ACCUMBENS

The purpose of this study was to evaluate OSU6162's potential as a novel treatment for AUD using several well-established animal models. We hypothesized that OSU6162 might have the ability to stabilize DA activity during acute alcohol drinking and abstinence, which potentially may decrease alcohol intake, attenuate alcohol craving, dampen withdrawal symptoms, and prevent relapse to alcohol drinking.

4.1.1 Effects of Acute OSU6162 Treatment on Voluntary Alcohol Intake

The effects of OSU6162 on alcohol consumption was first evaluated in a group of rats voluntary consuming moderate amounts of alcohol (2.9 ± 0.3 g/kg/24 h; n = 9) and subsequently in a second group of rats that voluntary emerged in to high (4.6 ± 0.3 g/kg/24 h; n = 7) and low (1.9 ± 0.2 g/kg/24 h; n = 6) alcohol consumers. After at least 3 months of home-cage alcohol consumption, rats were given an injection of vehicle or OSU6162 (0, 15, and 30 mg/kg) once a week in a counterbalanced order, using a within-subjects design. The results showed that both OSU6162 doses significantly decreased alcohol intake compared with vehicle in rats that had voluntary consumed high levels of alcohol (Fig. 2C). In the moderate alcohol consuming rats (Fig. 2B), only the higher OSU6162 dose significantly decreased voluntary alcohol intake. In contrast, OSU6162 treatment had no effect in low alcohol consuming rats (Fig. 2A).

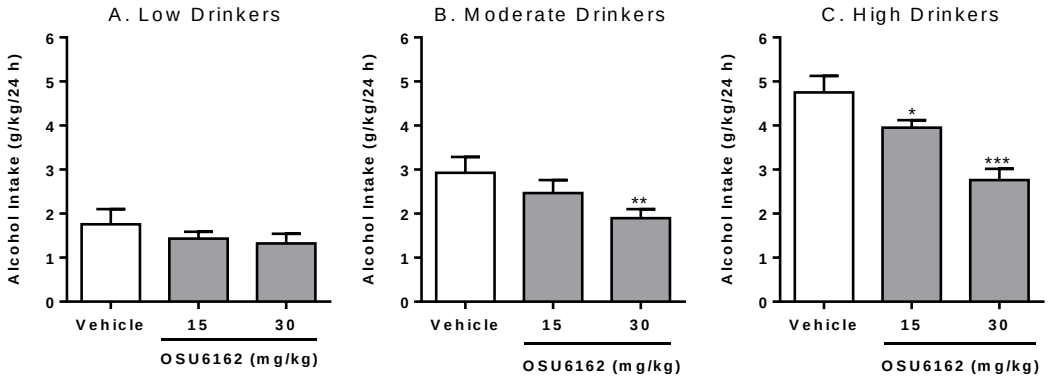

Fig. 2. Acute OSU6162 treatment decreased alcohol intake in moderate and high alcohol consuming rats. All rats had voluntarily consumed 20% alcohol in the home-cage for at least 3 months before being subjected to acute OSU6162 treatment (0, 15, 30 mg/kg, sc). Each rat received all doses in a counterbalanced order. Both OSU6162 doses significantly decreased alcohol intake in the rats voluntarily consuming high amounts of alcohol (**C**; n = 7). A significant reduction in alcohol intake was found only after treatment with the higher OSU6162 dose in rats consuming moderate amounts of alcohol (**B**; n = 9). However, OSU6162 treatment had no significant effect in the rats voluntary consuming low levels alcohol (**A**; n = 6). All values are expressed as mean ± SEM, *p < 0.05; **p <0.01; ***p <0.001 compared with corresponding vehicle at the 24 h time-point.

4.1.2 Effects of Repeated OSU6162 Treatment on Alcohol Intake

Next, the high alcohol-consuming rats were after the last acute OSU6162-administration given 3 additional weeks of voluntary alcohol consumption (4.6 ± 0.3 g/kg/24; n = 7) Thereafter, half of the rats were given a daily injection of OSU6162 (30 mg/kg) during 8 days (Mon-Fri plus Mon-Wed) and the remaining half received vehicle. Following 2 weeks of voluntary alcohol consumption, the experiment was repeated with the assigned dose reversed. The results showed that repeated OSU6162 treatment significantly decreased alcohol intake for all five alcohol-drinking sessions without inducing any tolerance or rebound increase in alcohol intake after the treatment was ended in high alcohol consuming rats (Fig 3A). Moreover, repeated OSU6162 treatment significantly increased the water intake (Fig. 3B) for all five alcohol-drinking sessions. In addition, repeated OSU6162 treatment showed a significantly decreased preference for alcohol over water (Fig. 3C) as a result from the OSU6162-induced decrease in alcohol intake and increase in water intake. Finally, there was no significant effect on the total fluid intake or on the water intake on the 3 days when alcohol was not present (data not shown).

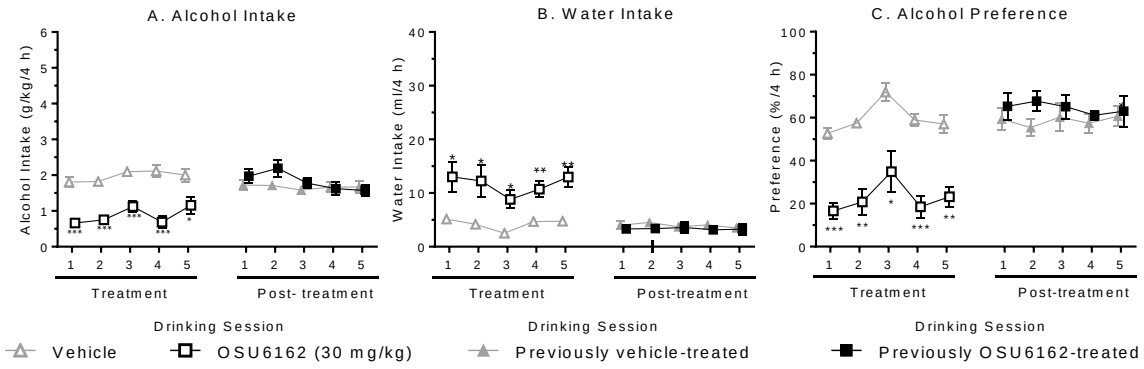

Fig. 3. Repeated OSU6162 treatment selectively reduced voluntary alcohol intake and preference. The rats (n = 7) had been consuming high amounts of alcohol for approximately 5 months before the repeated OSU6162 treatment was initiated. Each rat received both OSU6162 (30 mg/kg, sc) and vehicle treatment in a counterbalanced order. The repeated OSU6162 treatment significantly decreased alcohol intake **(A)** and increased the water intake **(B)** for all five alcohol sessions. Consequently, the preference for alcohol over water was decreased **(C)**. When the repeated OSU6162 treatment was terminated, the alcohol intake **(A)**, the preference for alcohol over water **(C)** and the water intake **(B)** immediately went back to baseline. All values are expressed as mean ± SEM, *p < 0.05; **p <0.01; ***p <0.001 compared with vehicle at the corresponding treatment day (4 h time-point).

4.1.3 Effects of OSU6162 Treatment on Alcohol Withdrawal Symptoms

Rats (n=20) voluntary consumed high amounts of alcohol in the home-cage using the IA20E model for approximately 5 months and were thereafter divided into two groups with equal baseline alcohol consumption. After 23 hours of abstinence (acute abstinence), the rats got an injection of OSU6162 (30 mg/kg) or vehicle, before being subjected to an open field arena (40 x 40 cm) and video-recorded for 5 min. The effect of OSU6162 on alcohol withdrawal symptoms (tail stiffness and walking with broad gait [211]) was evaluated. The test was

repeated after additional 2 weeks of abstinence (protracted abstinence). As a control for the alcohol-induced withdrawal symptoms, one group of alcohol-naïve rats (n = 16) were subjected to the NCT but did not receive any pharmacological treatment. The result showed that OSU6162 treatment significantly attenuated acute alcohol withdrawal symptoms (Fig. 4) but had no significant effect on alcohol withdrawal symptoms after protracted abstinence (data not shown).

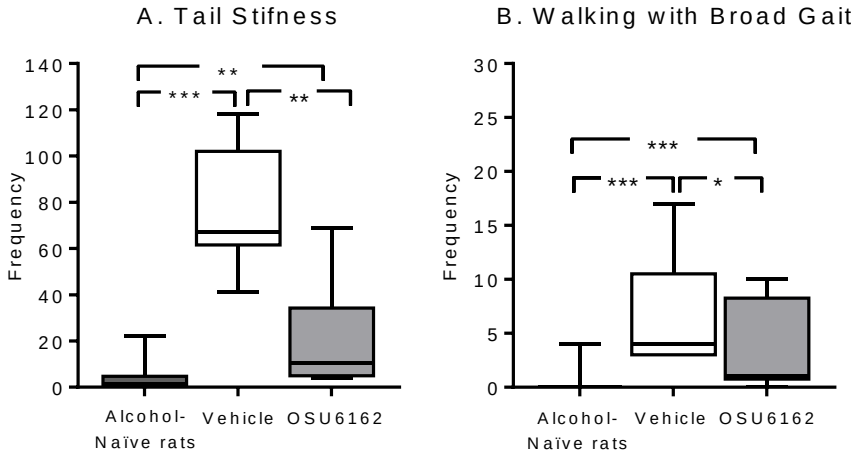

Fig. 4. OSU6162 treatment attenuated acute alcohol withdrawal symptoms. The effects of OSU6162 on alcohol withdrawal symptoms (tail stiffness and walking with abnormal broad gait) were evaluated in rats that had voluntarily consumed high amounts of alcohol for approximately five months before the test. The frequency of both tail stiffness **(A)** and walking with broad gait posture **(B)** was significantly increased in both OSU6162 and vehicle treated animals compared to alcohol-naïve rats. During acute alcohol withdrawal OSU6162 treatment significantly attenuated the alcohol withdrawal symptoms compared to vehicle. All values are expressed as median and range, *p < 0.05; **p < 0.01; ***p < 0.001 compared to corresponding vehicle or as indicated. Alcohol-naïve rats (n = 16), vehicle (n = 10) and OSU6162 (n = 10).

4.1.4 Effects of OSU6162 on Cue/Priming-induced Reinstatement

Following 6 months of operant alcohol self-administration training, the alcohol seeking behavior was extinguished without the presence of the cues. After extinction of lever-pressing, half of the rats received OSU6162 (30 mg/kg; n=10) and the other half vehicle (n=9), 60 minutes before the start of the cue/priming-induced reinstatement test. During the test session, the cues were reintroduced in the chambers and as an additional cue, or priming, a small amount of alcohol was presented in the liquid dispenser at the start of the session; however, no additional reward was delivered. The numbers of active lever presses were compared following OSU6162 and vehicle pre-treatment. The results showed that OSU6162 has the ability to attenuate cue/priming-induced reinstatement of alcohol seeking (Fig. 5A) in rats that had been exposed to alcohol for a long period of time.

4.1.5 Effects of OSU6162 on Alcohol Self-administration

After the cue/priming-induced reinstatement test the rats went back on regular self-administration training (FR 3 schedule of reinforcement) for a month. Next, the effect of

OSU6162 on alcohol-seeking was evaluated using a PR schedule of reinforcement, where the delivery of 20% alcohol was contingent on a visual and auditory cue. The PR test was performed once a week during 3 weeks and each rat received both OSU6162 doses (15 and 30 mg/kg) and vehicle in a counterbalanced order. Active lever presses and breakpoint were compared between OSU6162 and vehicle. The results revealed that OSU6162 attenuated operant alcohol self-administration under a PR schedule of reinforcement (Fig. 5B) in rats that had voluntarily consumed alcohol for seven months before treatment.

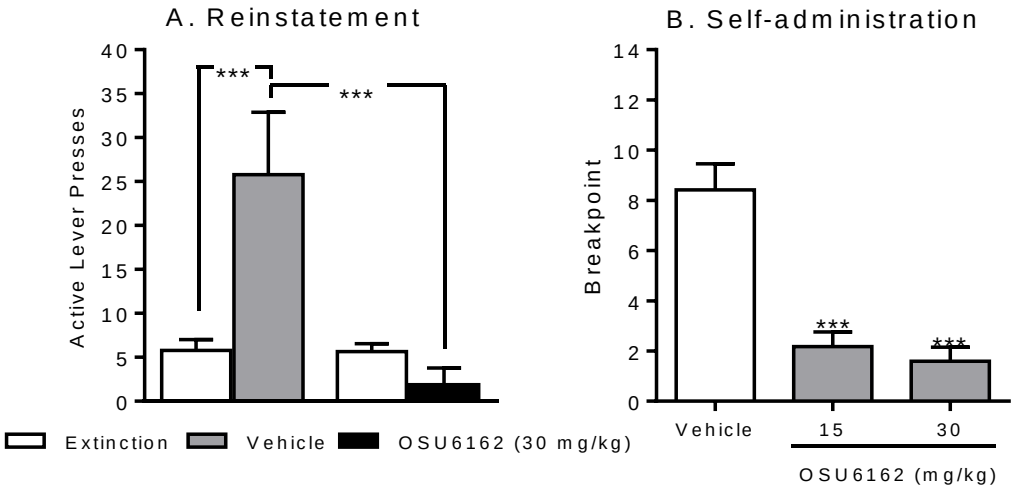

Fig. 5. OSU6162 treatment attenuated cue/priming-induced reinstatement and breakpoint. The effects of OSU6162 on cue/priming-induced reinstatement of alcohol seeking and operant self-administration of alcohol were examined in rats trained to self-administer 20% alcohol. In the reinstatement test, the cues previously associated with the delivery of alcohol were reintroduced. The vehicle-treated rats (n = 10) reinstated their alcohol-seeking behavior as shown by a significant increase in the number of active lever press compared with the extinction level (**A**, left panel). The cue/priming-induced reinstatement was significantly attenuated by OSU6162 (30 mg/kg; n = 9) treatment (**A**, right panel). In the progressive ratio test (n = 17), both OSU6162 (15 and 30 mg/kg) doses significantly decreased the breakpoint **(B)** compared to vehicle. All values are expressed as mean ± SEM, ***p <0.001

4.2 PAPER II: THE MONOAMINE STABILIZER (-)-OSU6162 COUNTERACTS DOWN-REGULATED DOPAMINE OUTPUT IN THE NUCLEUS ACCUMBENS OF LONG-TERM DRINKING WISTAR RATS

In Paper I, we also showed using microdialysis (conducted and analyzed by co-author Björn Schilström and the method is described in more details in Paper I) in NAc of alcohol-naïve rats, that OSU6162 increased the DA output when given alone. This could possibly indicate that OSU6162 has an abuse liability. However, since the OSU6162-induced DA increase was rather slow and long lasting in contrast to the rapid peak seen after use of traditional drugs of abuse [81, 229], this possibility seems unlikely. Nevertheless, the abuse liability of OSU6162 has to be further evaluated.

4.2.1 Effects of OSU6162 on CPP

To evaluate the abuse liability of OSU6162, alcohol-naïve rats (n=16) and rats that had been drinking alcohol for 3 months (n=15), using the IA20E model, were subjected to the CPP model. During conditioning (4 days), rats were injected twice a day with OSU6162 (30 mg/kg, sc) or vehicle (morning and afternoon), using a biased procedure (i.e. OSU6162 was paired with the least preferred compartment and vehicle with the preferred compartment). Expression of CPP was evaluated by comparing time spent in the drug-paired compartment during post-conditioning, with time spent in the same compartment during pre-conditioning. The results showed that OSU6162 did not induce CPP in either the alcohol-naïve rats (Fig. 6A) or rats that had been drinking alcohol (Fig. 6B) for 3 months before the CPP experiment.

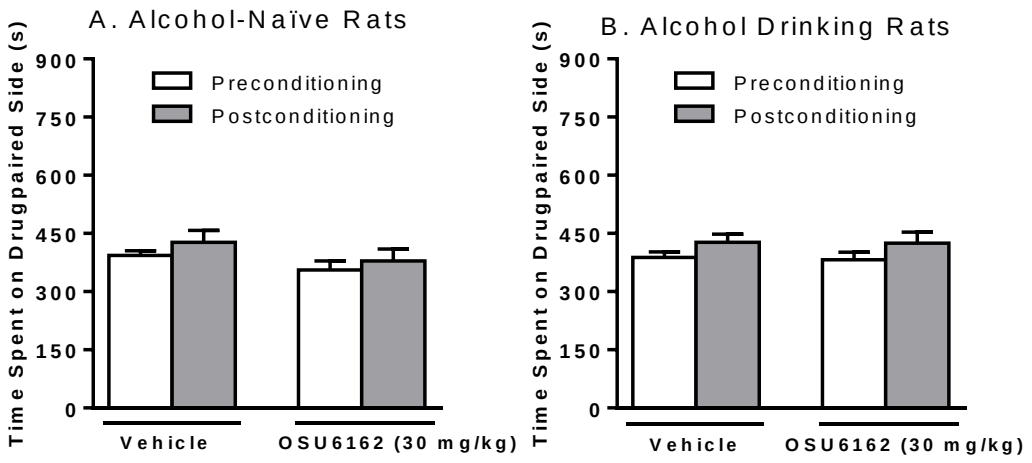

Fig. 6. OSU6162 treatment did not induce CPP. The effects of OSU6162 (30 mg/kg) or vehicle (saline) on CPP were evaluated in alcohol-naïve rats (n=16) and rats that had been drinking alcohol for 3 months prior to the experiment (n=15). OSU6162 did not induce any significant CPP compared to vehicle in either the alcohol-naïve rats **(A)** or the long-term alcohol drinking rats **(B)**. All values are expressed as mean ± SEM.

4.3 PAPER III: THE MONOAMINE STABILIZER (-)-OSU6162 PREVENTS THE ALCOHOL DEPRIVATION EFFECT AND IMPROVE MOTOR IMPULSIVE BEHAVIOR IN RATS

The purpose of this study was to evaluate the effects of OSU6162 on relapse-like alcohol drinking and motor impulsivity. A main problem in the treatment of AUD is the long-lasting vulnerability to relapse [191]. Impaired impulsive control, often seen in AUD individuals [230], might be one factor contributing to relapse to alcohol drinking [231]. The DA system is suggested to be involved in the complex regulation of impulsive behavior [122]. Rodent data show that high DA activity in the NAc enhances impulsive behavior, and can be attenuated by DA antagonism [232]. In contrast, disrupted activity, presumably DA hypoactivity, in prefrontal regions might increase impulsive behavior [233]. Thus, neuroanatomically distinct brain regions are linked to different aspects of impulsive behavior, and can be modulated by both increase and decrease in DA activity. We therefore hypothesized that OSU6162 might have the ability to regulate impulsive behavior, which potentially may improve impulse control.

4.3.1 Effects of OSU6162 on the ADE

Following approximately 10 weeks of alcohol drinking in the IA20E model and 18 days of forced abstinence, rats were divided in two groups with equal alcohol consumption (OSU6162 =3.3±0.3 g/kg/24 h; vehicle=3.2±0.2 g/kg/24 h) based on the last drinking day before the abstinence period. On the test day, rats were injected with either OSU6162 (30 mg/kg) or vehicle (saline), 60 min before the reintroduction of the alcohol. The results showed that an ADE was observed in the vehicle treated rats, as shown by a significantly increased alcohol intake compared to baseline alcohol drinking. In contrast, OSU6162 treatment significantly attenuated the ADE as shown by a decreased alcohol intake at the 4 h time-point (data not shown) and a non-significant trend towards a reduction in alcohol intake at the 24 h time-point (Fig. 7A). Moreover, OSU6162 treatment significantly decreased the preference for alcohol over water at both the 4 h (data not shown) and the 24 h time-point (Fig. 7B).

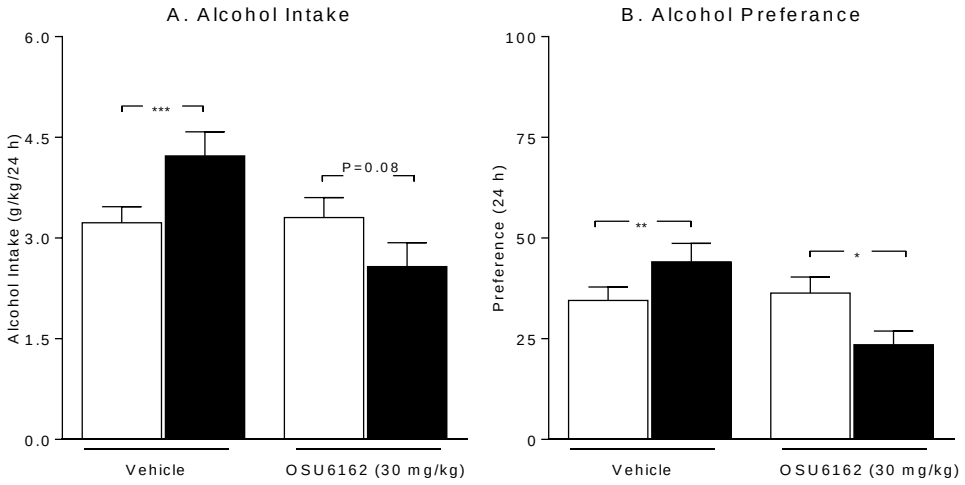

Fig. 7. OSU6162 treatment attenuated the ADE. Rats got voluntary intermittent access to alcohol in their home-cage during approximately 10 weeks and were thereafter subjected to 18 days of forced alcohol abstinence. Prior to the reintroduction of the alcohol, the rats were divided in to two groups with equal baseline alcohol consumption and given an injection of vehicle (n=10) or OSU6162 (30 mg/kg; n=6). An ADE was observed in vehicle treated rats (**A**: left panel) but not in rats treated with OSU6162 (**A**: right panel). Moreover, in rats treated with vehicle, a significant increased preference for alcohol was seen (**B**: left panel), whereas the preference for alcohol was significantly reduced in rats treated with OSU6162 (**B**: right panel). All values are expressed as mean ± SEM, *P<0.05; **p <0.01 ***P<0.01; compare to corresponding baseline at the 24 h time-point.

4.3.2 Effects of OSU6162 on Motor Impulse Behavior

Rats were trained in the 5CSRTT for 3-4 months before given access to water (n = 13) or alcohol (n = 14) for 7 weeks. Following drinking, an injection of OSU6162 (15 or 30 mg/kg, sc) or vehicle (saline) was given to all rats in a counterbalanced order. OSU6162 tests were conducted twice a week (Tuesdays and Fridays) every other week, with baseline 5CSRTT training sessions on the intervening days. On Tuesdays the rats were tested under an ITI of 5 s, the same ITI-length used on baseline training. In contrast, on Fridays, the rats were

challenged with a prolonged waiting period before presentation of the visual stimulus (ITI were changed from 5 to 7 s). This manipulation has been shown to robustly provoke premature responses [126]. The results showed that OSU6162 (30 mg/kg) significantly improved motor impulsivity in both alcohol-naïve and alcohol drinking rats, as shown by a decreased premature responding in the 5CSRTT (Fig. 8).

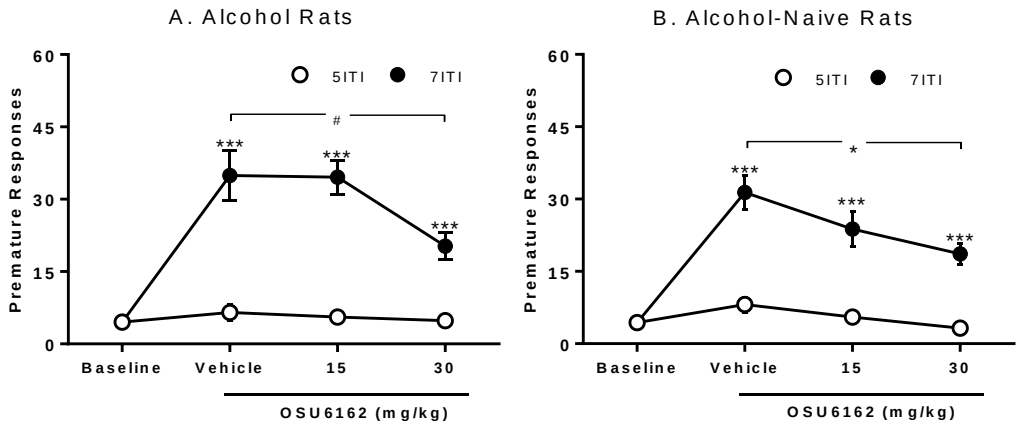

Fig. 8. OSU6162 treatment attenuated motor impulse control. Following 3-4 months of 5CSRTT-training and 7 weeks of home-cage alcohol or water drinking, both alcohol **(A)** and alcohol-naïve rats **(B)** significantly increased their premature responses compare to baseline when the ITI was prolonged from 5 to 7 seconds in the 5CSRTT. However, the highest OSU6162 (30 mg/kg) dose significantly reduced the number of premature responses compare to vehicle in both alcohol **(A)** and water **(B)** pre-exposed rats. All values are presented as mean ± SEM, n=13-14 per group; ***p <0.001 compared to corresponding baseline and #p <0.05; compared to corresponding vehicle within the ITI-7s- session.

4.4 PAPER IV: EVALUATION OF THE MONOAMINE STABILIZER (-)-OSU6162 ON OXYCODONE SELF-ADMINISTRATION AND CONTEXT-INDUCED REINSTATEMENT IN RAT

Finally, we decided to evaluate the effects of OSU6162 on another drug of abuse using iv self-administration. The opioid addiction epidemic is ongoing in the US [234]. Drug overdoses is the leading cause of accidental death in the US and these overdoses are mostly related to prescription pain relievers (such as oxycodone and fentanyl) and heroin [234]. We therefore decided to evaluate the potential of OSU6162 as a novel treatment for opioid addiction by evaluating the effect of OSU6162 on oxycodone self-administration and context-induced reinstatement of oxycodone seeking in rats. The mesolimbic DA system has been suggested to contribute to the rewarding effects of opiates [60, 106]. Moreover, a decreased activity in NAc have been shown after chronic administration of opiates in rats [108]. Despite the large body of evidence of an involvement of the mesolimbic DA system, behavioral pharmacological experiments with DA receptor antagonists have shown minimal effect on self-administration of opiate agonists, at least in doses that does not cause sedation [235, 236]. We hypothesized that OSU6162 might have the ability to stabilize DA activity in both the acute and abstinence phase of oxycodone self-administration, which potentially may attenuate craving and reinstatement to oxycodone seeking.

4.4.1 Effects of OSU6162 on Oxycodone Self-administration

The effects of OSU6162 on oxycodone self-administration (0.1 mg/kg/infusion) were examined in rats trained to self-administer oxycodone. Following oxycodone self-administration training, rats got 3 days of baseline oxycodone self-administration for 2 h/day before the start of the test. Rats were tested (n=9) every other day (within-subjects design) for oxycodone self-administration (2 h/day) after pre-treatment with OSU6162 (7.5, 15, and, 30 mg/kg) or vehicle in a counterbalanced order. The results showed that the two higher doses of OSU6162 (15 and 30 mg/kg) significantly decreased oxycodone self-administration compared to vehicle (Fig. 9A).

4.4.2 Effects of OSU6162 on Context-induced Reinstatement

After extinction of the operant response in context B, the effect of OSU6162 on context-induced reinstatement of oxycodone seeking in context A were examined. Rats (n = 10) received vehicle or OSU6162 (15 mg/kg) in a counterbalanced order using a within subjects design. The results showed that exposure to the oxycodone context reinstated active lever responding after extinction, but there was no difference between vehicle or OSU6162 treatment (Fig. 9B).

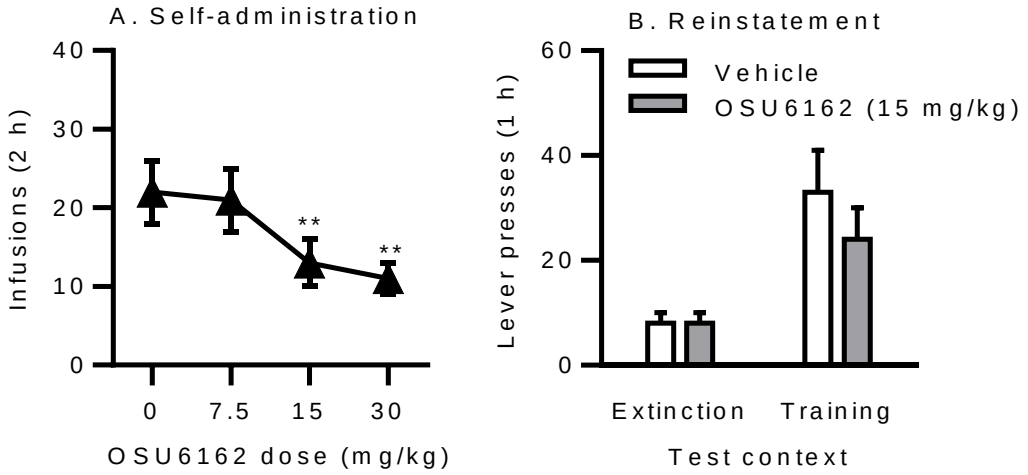

Fig. 9. OSU6162 treatment attenuated oxycodone self-administration but had no effect on context-induced reinstatement. Self-administration test **(A)**: Number of oxycodone infusions and active and inactive lever responses during the self-administration test sessions with OSU6162. Rats (n = 9) received an injection with vehicle or OSU6162 (7.5, 15, and 30 mg/kg, sc) 60 min before the test sessions every other day using a within subjects design. Reinstatement test **(B):** Number of active lever presses in rats (n = 10) tested in the extinction and oxycodone contexts after injections of vehicle or OSU6162 (15 mg/kg, sc) before exposure to the oxycodone context and the extinction context using a within subjects design. Data are presented as mean±SEM ** $p<0.01$ compared to vehicle.

5 DISCUSSION

The main findings in this thesis were that OSU6162 attenuated several alcohol-mediated behaviors, including voluntary alcohol consumption, operant alcohol self-administration under PR schedule of reinforcement, alcohol withdrawal symptoms, ADE and cue-induced reinstatement of alcohol seeking, in rats that had voluntarily consumed alcohol for an extended period of time. In addition, OSU6162 decreased motor impulsivity, as measured by the 5CSRTT, in both alcohol-naïve and alcohol drinking rats.

The results in paper I and III highlight four characteristics desirable for an AUD medication. First, repeated OSU6162 treatment significantly decreased alcohol intake compared with vehicle on each treatment day, indicating that no tolerance to the ability of OSU6162 to decrease alcohol intake in rats developed over time. This finding is in agreement with a previous study showing maintained anti-dyskinetic effects of OSU6162 in a primate model of Parkinson's disease [237]. Second, there was no marked rebound increase in drinking when the OSU6162 treatment was terminated. However, the rapid return to baseline drinking after the treatment indicates that continuous OSU6162 treatment might be needed to control drinking in a clinical situation. Third, OSU6162 attenuated cue-induced reinstatement, relapse-like alcohol drinking (ADE) and blunted acute alcohol withdrawal symptoms, which indicates that OSU6162 might have potential to prevent relapse triggered by alcohol craving, re-exposure to alcohol, alcohol related cues, and/or an urge to relieve abstinence symptoms. Fourth, OSU6162 improved motor impulsivity, which indicate that OSU6162 might have beneficial effects on cognitive functions including impulse control. A characteristics desirable for a potential AUD medication because AUD individuals often suffer from cognitive impairments [117, 118].

The results in Paper I showed that OSU6162 significantly decreased voluntary alcohol intake in rats consuming moderate and high amounts of alcohol, whereas there was no significant effect in rats consuming low amounts of alcohol. The reason for the diverse results of acute OSU6162 treatment between the three groups is not fully understood, but we hypothesize that the long-term consumption of high and moderate amounts of alcohol might have induced changes in the DA system, rendering these rats more sensitive to the effects of OSU6162. This hypothesis is supported by early animal studies showing a downregulation of D_2-receptors [109] and a marked reduction in extraneural DA output [238] in the mesolimbic DA system after chronic alcohol consumption. Furthermore, a recent study, using the IA20E model, showed a significantly reduced DA output in the NAc in rats that had been drinking high amounts of alcohol for 7 weeks compared with alcohol-naïve rats [183]. In addition, in our microdialysis study, in Paper II, we showed that long-term voluntary alcohol drinking for 10 months significantly reduced DA output in the NAc compared to alcohol-naïve rats. Moreover, the alcohol-induced DA-peak was blunted and there was a subsequent shift in DA levels below baseline in the long-term drinking compare to the alcohol-naïve rats. Thereby, it is possible that OSU6162 treatment presumably decrease alcohol intake in high and moderate consuming rats by normalize their possible hypodopaminergic state and leave the low alcohol

consuming rats unaffected due to their assumed already "normal" or less affected DA system. This idea is supported by the microdialysis experiment in Paper II, where OSU6162-pretreatment normalized the alcohol-induced DA-peak and prevented the DA levels to dip below baseline in alcohol drinking rats. Finally, the diverse results, following acute OSU6162 treatment in low, moderate, and high drinkers, give further support for OSU6162 ability to stimulate, suppress, or show no effect on DA-related behaviors depending on the prevailing dopaminergic tone [138, 146, 147].

The mechanism behind the findings of attenuated alcohol self-administration, alcohol withdrawal symptoms, cue-priming induced reinstatement and ADE following OSU6162 treatment is not fully understood. The mesolimbic DA system is impaired upon cessation of chronic alcohol use and this decreased function are hypothesized to contribute to alcohol withdrawal symptoms and the switch from positive to negative reinforcement [51, 52, 60]. Indeed, a downregulated DA system has been demonstrated in both humans and rodents during alcohol withdrawal [109-112, 238]. As maintained above, in our microdialysis study, we showed that long-term alcohol drinking in the IA20E model, induced DA deficits in NAc compare to alcohol-naïve rats. Moreover, OSU6162 treatment normalized the alcohol-induced DA-peak and counteracted this hypodopaminergic state. Thus, OSU6162 treatment might attenuate alcohol withdrawal symptoms by normalizing alcohol-induced DA deficits. Moreover, previous studies have demonstrated that alcohol self-administration and even the anticipation of alcohol availability produces a rise in DA levels in the NAc in rats [87]. Therefore, the mechanism of OSU6162 to attenuate alcohol self-administration, cue/priming-induced reinstatement and ADE might be to act as an antagonist and decrease the possible increased level of DA back to baseline. Thus, OSU6162 might have decreased the positive reinforcing effects of alcohol during the PR test, as well as decreased the alcohol seeking induced by environmental stimuli previously associated with alcohol.

In paper I, we also conducted a battery of control experiments to evaluate the specificity of the effects observed after OSU6162 administration on alcohol-mediated behavior. These control experiment showed that OSU6162 i) was more efficacious than the currently available FDA-approved AUD medication naltrexone in decreasing voluntary alcohol intake, ii) had no significant effect on voluntary intake of a salty solution, iii) significantly decreased the intake of a sucrose solution, iv) and had no effect on general motor activity. The unaffected general motor activity and intake of a salty solution indicate that rats following OSU6162 treatment have an intact general motor activity. This idea is in agreement with previous findings showing no general motor impairments of OSU612 using the same dose-range [138, 146] as in this thesis. The lack of motor impairments is further supported by the increased water intake in IA20E model following acute and repeated OSU6162 treatment (Paper I). Moreover, in paper I, we also revealed a lack of significant effect on inactive lever pressing in the PR-experiment and an increased response on the inactive lever during cue-induced reinstatement. In addition, using the 5CSRTT, we found that the latency to respond and to collect the reward following OU6162 treatment was unaffected (Paper III). Together these results provide support for an intact general motor activity and confirm the capacity of

that rats to perform the operate task as well as drink the alcohol solution. The finding that OSU6162 decreased intake of a sucrose solution, had no effect on intake of a salty solution or water when alcohol was not present, indicating that the effects of OSU6162 might be selective for intake of substances with highly reinforcing properties. In fact, sucrose has been shown to be a powerful reinforcer in rats [239]. This idea is further supported by the control experiment in paper IV, showing that OSU6162 decreased self-administration of palatable food pellets, which have previously been shown to be highly rewarding in rats and strongly preferred over both methamphetamine [224, 225] and heroin [240]. The control experiment with naltrexone, showed that OSU6162 might be superior to naltrexone from some aspects. Specifically, naltrexone-treated rats decreased both their alcohol intake and total fluid intake. In contrast, the OSU6162-treated rats compensated the loss in fluid intake from the decreased alcohol intake with an increased water intake, resulting in a maintained total fluid intake.

In Paper III, we found that OSU6162 improved motor impulsivity in both alcohol-naïve and alcohol drinking rats as measured with the 5CSRTT. These results indicate that improvement of motor impulse control might be one mechanism behind OSU6162's ability to blunt relapse-like behavior such as the ADE and cue/priming-induced reinstatement of alcohol seeking in long-term drinking rats. In the 5CSRTT experiment, OSU6162 treatment also induced a trend towards an increased omission rate, decreased correct responding and number of trials, possibly indicating a sedative effect. However, the unaffected latency to respond and to collect the reward following treatment even with the highest OSU6162 (30 mg/kg) dose indicates an intact motor activity and suggests that sedation is not the reason for the decreased premature responding. The lack of sedative effect is also supported by the control experiments in Paper I (discussed above), showing that OSU6162 (30 mg/kg) had no effect on general motor activity or intake of a salty solution. We therefore hypothesize that the increased omission rate, decreased correct responding and number of trials following the OSU6162 treatment in the 5CSRTT might indicate a decreased motivation to seek the reward (i.e. palatable food pellets) which is expressed as a decreased performance during the 5CSRTT. This suggestion is supported by our control experiment in Paper IV, in which OSU6162 significantly attenuated intake of this food pellets in an operant self-administration procedure. In addition, as mentioned above, these food pellets are strongly preferred over both methamphetamine [224, 225] and heroin [240] in rats.

In regards to OSU6162's potential therapeutic applicability in a patient population with AUD, it should be noted that we also showed in Paper I and II, using microdialysis, that OSU6162 increased the DA output in NAc in both alcohol-naïve and long-term alcohol drinking rats. This could possibly indicate that OSU6162 has an abuse liability. However, since the OSU6162-induced DA increase was rather slow and long lasting in contrast to the rapid peak seen after use of traditional drugs of abuse [81, 229], this possibility seems unlikely. This suggestion is supported by the results in paper II showing that OSU6162 did not induce CPP in either the alcohol-naïve rats or rats that had been drinking alcohol for three months before the start of the experiment. These results indicate that OSU6162 is not rewarding on its own and most likely does not possess any abuse liability.

In Paper IV, our preliminary results showed that OSU6162 attenuated self-administration of oxycodone but had no effect on context-induced reinstatement of oxycodone seeking. Previous, behavioral pharmacological experiments with DA receptor antagonists have shown minimal effect on self-administration of opiate agonists, at least in doses that does not cause sedation [235, 236]. However, the decreased self-administration of oxycodone following OSU6162 treatment in the present study is in line with a previous study showing that tentagrun, a partial DA D_2 agonist, attenuated heroin self-administration under both FR- and-PR schedule of reinforcement [84]. This is also in agreement with the results in Paper I, showing that OSU6162 attenuated alcohol self-administration under a PR schedule of reinforcement. Together, the present study and the study by Zhang and colleagues, indicate that compounds that are able to buffer the DA activity rather than fully block DA transmission might represent novel candidates for pharmacological interventions of drug abuse, at least for alcohol and opioids. In Paper IV, we found that rats reinstated to oxycodone seeking when placed in their initial (training) environment, but had no ability to attenuate this reinstatement. However, we only included one dose of OSU6162 (15 mg/kg) in this experiment and it is possible that a higher dose would be needed to attenuate context-induced reinstatement. Indeed, a higher dose of OSU6162 (30 mg/kg) was used in the positive alcohol study on cue/priming-induced reinstatement. Previous studies have shown that DA D_1 antagonists have the potential to attenuate context-induced reinstatement of heroin seeking [241, 242]. However, studies evaluating the role of D_2 receptors in context-induced reinstatement of opiate seeking are limited. Future studies are needed, preferably doing a full dose-response curve with OSU6162, include more doses of oxycodone, and a PR schedule of reinforcement, to fully evaluate the effects of OSU6162 on oxycodone self-administration and context-induced reinstatement of oxycodone seeking.

In conclusion, this thesis show that the monoamine stabilizer OSU6162 has the ability to attenuate voluntary alcohol consumption, operant alcohol self-administration, alcohol withdrawal symptoms, relapse-like alcohol drinking and cue-induced reinstatement of alcohol seeking, in rats that had voluntarily consumed alcohol for an extended period of time. In addition, we showed in this thesis that OSU6162 improve motor impulse control, which might help AUD individuals to override a compulsive drug-taking behavior in response to craving and thereby possibly prevent relapse to alcohol drinking. Based on these findings and the favorable side effect profile of OSU6162 [160], a "proof-of-concept" double-blind placebo-controlled clinical study in 56 alcohol dependent patients was recently conducted [243]. The clinical study was successfully executed and the results support the predictive validity of this preclinical evaluation. Specifically, OSU6162, compared to placebo, significantly attenuated craving after intake of alcohol and induced significantly lower subjective "liking" of the consumed alcohol, effects driven by individuals with high levels of baseline impulsivity. Furthermore, none of the patients felt "high" or wanted more of the OSU6162-medication when the study was ended [243].

6 SUMMARY AND CONCLUDING REMARKS

Alcohol use disorder is a common chronic relapse disorder, causing serious medical, economic and social consequences. Globally, AUD is among the top five leading causes of morbidity and mortality. Despite the major health problem, only four medications are approved for treatment of AUD. However, the clinical efficacies of these are limited and new, more effective, medications are truly needed.

As mentioned before, the DA system is one possible treatment target for AUD. Dopamine D_2 receptors has been suggested to be involved in mediating alcohol's reinforcing properties and a decrease in DA release and a reduction in D_2 receptors have been found in detoxified AUD patients. This DA down-regulation is hypothesized to induce alcohol craving and contribute to relapse even after a long period of abstinence. The fact that OSU6162 might have the ability to modulate DA transmission without inducing any severe side effects makes OSU6162 unique as a compound with D_2 receptor blockage properties. Thus, a great advantage of OSU6162 compared to traditional D_2 antagonists, might be the lack of extrapyramidal reactions. This unique mechanism of action highlights the potential of OSU6162 for treatment of a variety of conditions, including AUD, involving dysregulation or dysfunction in the DA system.

In this thesis, we have identified OSU6162 as a potential medication for AUD using validated animal models. For example, we showed that OSU6162 attenuated voluntary alcohol consumption, operant alcohol self-administration under PR schedule, alcohol withdrawal symptoms, cue-induced reinstatement of alcohol seeking and the ADE in rats that have been drinking alcohol for an extended period of time. In addition, we found that OSU6162 treatment improved motor impulse control in both alcohol-naïve rats and alcohol drinking rats. Based on these preclinical findings and the favorable side effect profile of OSU6162, a "proof-of-concept" double-blind placebo-controlled clinical study in 56 alcohol dependent patients was recently conducted. The clinical results confirmed the predictive validity of our preclinical evaluation. Specifically, OSU6162 significantly attenuated craving after intake of alcohol and induced significantly lower subjective "liking" of the consumed alcohol compared to placebo. Interestingly, these effects were driven by individuals with high levels of baseline impulsivity. In addition, none of the patients felt "high" or wanted more of the OSU6162-medication when the study was ended, giving support for our results in paper II showing that OSU6162 did not induce CPP in either the alcohol-naïve rats or rats that had been drinking alcohol for a long time. Collectively, these preclinical and clinical results give further support for OSU6162 as a novel treatment for AUD and merit for a full-scale randomized clinical efficacy trial in AUD patients. A positive outcome of such a study would benefit a large patient group that today lack adequate treatment.

The opioid epidemic is ongoing in the United State and new medications for opioid addiction are crucial. We therefore, in paper IV, evaluate the potential of OSU6162 on oxycodone self-administration and context-induced reinstatement of oxycodone seeking. We found that

OSU6162 treatment attenuated operant oxycodone self-administration but had no effect, at least in the dose tested, on context-induced reinstatement. The present study is still promising and give support for a full preclinical evaluation.

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