Dopaminergic Interference for Treatment of Schizophrenia and Alcohol Use Disorder

Experimental studies in the rat

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Imagination, not intelligence, made us human

Terry Pratchett

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ABSTRACT

The neurotransmitter dopamine is involved in several different physiological functions as well as pathological conditions. Two conditions that have been suggested to be related to a low dopaminergic tone in the ventral striatum are substance use disorder, and negative symptoms in schizophrenia, both of which are difficult to treat. In this thesis, we aimed to investigate in the rat the possibility of elevating dopamine in the ventral striatum (nucleus accumbens, nAc) in order to treat these conditions. To this end, we utilized *in vivo* microdialysis to sample and analyse extracellular dopamine, *ex vivo* electrophysiological field potential recordings to analyse effects on primarily excitatory neurotransmission, as well as behavioural methods to study ethanol consumption and behavioural sensitisation. In paper I, we show that the combination of the smoking cessation agent varenicline and the anti-depressant bupropion has an additive effect on nAc dopamine, and eliminates the alcohol deprivation effect in an ethanol consumption study. In paper II, we showcased the effects of protracted amphetamine treatment on both ventral and dorsal striatal (dorsomedial striatum, DMS) dopaminergic signalling. Results show that the nAc appears more sensitive to both acute and repeated amphetamine challenge, and that repeated amphetamine results in both reduced basal dopamine release and a qualitatively different signalling via dopamine D2 receptors in this region. In paper III, we investigated the effects of psychosis-generating and non-psychosis-generating addictive substances with regards of their effect on nAc and DMS dopamine. Key findings showed a distinct difference between amphetamine and cocaine, both strongly pro-psychotic, and nicotine, which has low psychosis-generating potential.

Whereas amphetamine and cocaine both produced robust and similar elevations in dopamine in both the nAc and DMS, nicotine only had a noticeable effect in the nAc. In paper IV, findings from previous papers were combined in an effort to propose a way to selectively elevate dopamine in the nAc, without affecting DMS dopamine. We show that combining ethanol and nicotine produces an additive effect on nAc dopamine, with no marked interference on DMS dopamine, findings that we could then reproduce using varenicline and the glycine transport inhibitor Org24598.

The combined findings presented in this thesis lend support to the possibility of raising nAc dopamine for treatment of alcohol use disorder and selectively raising nAc dopamine, for treatment of negative symptoms of schizophrenia.

Keywords: Dopamine, striatum, addiction, schizophrenia

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SAMMANFATTNING PÅ SVENSKA

Dopaminerg manipulation för behandling av schizofreni och alkoholberoende – experimentella studier i råtta

Signalsubstansen dopamin är involverad i flera olika kroppsliga funktioner samt sjukdomstillstånd. Två tillstånd som kan kopplas till en låg dopaminerg ton i ventrala striatum (nucleus accumbens, nAc) är substansbrukssyndrom (t ex alkoholberoende) och negativa symtom (initiativlöshet, nedstämdhet, social isolering) vid schizofreni, vilka båda är svårbehandlade.

I denna avhandling använde vi råttmodeller för att undersöka 1) om en farmakologisk behandling som höjer dopamin i nAc, och troligen även i andra delar av hjärnan, kan sänka alkoholkonsumtion, och 2) om en annan farmakologisk behandling skulle kunna öka dopaminnivåerna selektivt i nAc. I detta syfte använde vi *in vivo* mikrodialys för att provta och analysera dopamin i hjärnan, *ex vivo* elektrofysiologiska fältpotentialsmätningar för att analysera effekter på neurotransmission, samt beteendemetoder för att studera alkoholkonsumtion och beteendesensibilisering.

I artikel I visar vi att kombinationen av rökavvänjningsmedlet vareniklin och det antidepressiva medlet bupropion har en tilläggseffekt på dopamin i nAc och en synergistisk effekt vad gäller sänkning av den så kallade alkoholdeprivationseffekten, ett svar som anses förutsäga effekt på människa. I artikel II studerade vi effekterna av upprepad amfetaminbehandling på både ventral och dorsal striatal (dorsomediala striatum, DMS) dopaminsignalering. Vi visar att nAc verkar mer känsligt för både akut och upprepad amfetaminbehandling och att vi får både en sänkt basal dopaminfrisättning och en kvalitativt förändrad signalering via dopamin D2 receptorer efter upprepad amfetaminexponering. I artikel III undersökte vi effekterna av psykosgenererande och icke-psykosgenererande beroendeframkallande substanser med avseende på deras effekt på dopamin i nAc och DMS. Vi såg en distinkt skillnad mellan amfetamin och kokain, båda starkt pro-psykotiska, och nikotin, som har låg psykosgenererande potential. Medan amfetamin och kokain båda ger stora och snarlika höjningar av dopamin i både nAc och DMS, hade nikotin bara en märkbar effekt i nAc. I artikel IV kombinerades resultat från tidigare artiklar i ett försök att föreslå ett sätt att selektivt höja dopamin i nAc, utan att påverka

dopamin i DMS. Vi visar att kombinationen av etanol och nikotin ger en additiv effekt på dopamin i nAc, utan markant störning på DMS-dopamin, fynd som vi sedan kunde reproducera med vareniklin och glycintransporthämmaren Org24598.

Resultaten som presenteras i denna avhandling ger stöd för möjligheterna att 1) öka dopamin i nAc för att behandla alkohol-brukssyndrom, och 2) att selektivt öka dopamin i nAc för att behandla negativa symtom vid schizofreni utan att samtidigt förvärra de så kallade positiva symtomen (hallucinationer, vanföreställningar), som antas utövas i DMS.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Söderpalm, B, **Danielsson, K**, de Bejczy, A, Adermark, L, Ericson, M. *Combined administration of varenicline and bupropion produces additive effects on accumbal dopamine and abolishes the alcohol deprivation effect in rats*
Addiction Biology 2020; doi: 10.1111/adb.12807

II. **Danielsson, K**, Lagström, O, Ericson, M, Söderpalm, B, Adermark, L. *Subregion-specific effects on striatal neurotransmission and dopamine-signalling by acute and repeated amphetamine exposure*
Neuropharmacology 2021;
doi: 10.1016/j.neuropharm.2021.108638

III. **Danielsson, K**, Stomberg, R, Adermark, L, Ericson, M, Söderpalm, B. *Differential dopamine release by psychosis-generating and non-psychosis-generating addictive substances in the nucleus accumbens and dorsomedial striatum*
Translational Psychiatry 2021;
doi: 10.1038/s41398-021-01589-z

IV. **Danielsson, K**, Stomberg, R, Adermark, L, Ericson, M, Söderpalm, B. Sub-region-specific modulation of striatal dopamine in Wistar rats. Manuscript

CONTENT

LIST OF ABBREVIATIONS

5-HT - 5-hydroxytryptamine
aCSF - Artificial cerebrospinal fluid
ADE - Alcohol deprivation effect
ADHD - Attention Deficit Hyperactivity Disorder
AMPA-R - α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ANOVA - Analysis of variance
ARRIVE - Animal Research: Reporting of In Vivo Experiments
AUD - Alcohol use disorder
CNS - Central nervous system
D1 - Dopamine receptor D1
D2 - Dopamine receptor D2
DA - Dopamine
DAT - Dopamine transporter
DMS - Dorsomedial striatum
DSM V - Diagnostic and statistical manual for mental disorders
GABA - γ-aminobutyric acid
HPLC - High performance liquid chromatography
HSD - Honestly significant difference test
MSNs - Medium spiny neurons
nAc - Nucleus accumbens
nAChRs - Nicotinic acetylcholine receptors
NMDA - N-Methyl-D-aspartic acid
PFC - Prefrontal cortex
PPR - Paired pulse ratio
PREPARE - Planning Research and Experimental Procedures on Animals: Recommendations for Excellence
PS - Population spike
SN - Substantia nigra
THC - Tetrahydrocannabinol
VTA - Ventral tegmental area

1 INTRODUCTION

1.1 Dopamine

Dopamine is a catecholamine neurotransmitter that is crucial to several different processes in the brain, such as reward, motor control, motivation and executive function. It was first identified in the human brain in 1957 (Montagu, 1957), and only a few years later Arvid Carlsson discovered that the concentration of dopamine varied across the brain (Carlsson, 1959). This suggested that dopamine, which had previously been assumed to only function as an intermediate in the synthesis of noradrenaline and adrenaline, could have a more specific role in the central nervous system. Carlsson and colleagues found that the concentration of dopamine was highest in the corpus striatum (Bertler and Rosengren, 1959; Carlsson, 1959), which contains areas such as the nucleus accumbens, the caudate nucleus and caudate putamen. Reserpine, a substance that was known to cause Parkinsonism (a motor function syndrome characterised by tremor, muscular rigidity, bradykinesia and impaired postural balance) was found to deplete dopamine (along with noradrenaline and serotonin) in the brain. They also showed that rabbits pre-treated with reserpine regained normal motor control if administered L-DOPA, a catecholamine precursor (Carlsson et al., 1957), but not when given L-5-HTP (a serotonin precursor). It was thus concluded that catecholamines, and especially dopamine, which accumulated markedly in the corpus striatum after L-DOPA, play an important role in the control of motor function (Carlsson, 1959).

1.2 Pathological conditions involving dopaminergic dysfunction

Since those ground-breaking findings in the late 1950's, the role of dopamine has continued to evolve. It has been implicated as a key player in many different pathological conditions, perhaps most notably Parkinson's disease (Cotzias et al., 1967; Evans and Lees, 2004), but also attention deficit hyperactivity disorder (ADHD) (Thapar et al., 2013), depression (Belujon and Grace, 2017) and more. In this thesis, the focus will be on substance use disorder, or addiction, and schizophrenia, conditions where dysfunctional dopaminergic signalling has been implicated.

Substance use disorder

Addiction is a chronic neuropsychiatric condition with a high relapse rate (McLellan et al., 2000). In order to receive a diagnosis, the patient has to experience at least two of several symptoms listed in the DSM 5 guidelines in one year. These symptoms include lack of control over the intake of a substance, inability to stop using the substance, cravings, and a continued intake despite negative consequences (table 1).

Table 1: DSM 5 guidelines for diagnosis of substance use disorder. In order to obtain a diagnosis, an individual has to have significant impairment or distress from their pattern of drug use, as well as at least two of the listed symptoms in one year (American Psychiatric Association, 2013).

Guidelines for diagnosis of substance use disorder	
1	Using more of a substance than planned, or using a substance for a longer interval than desired
2	Inability to cut down despite desire to do so
3	Spending substantial amount of the day obtaining, using, or recovering from substance use
4	Cravings or intense urges to use
5	Repeated usage causes or contributes to an inability to meet important social, or professional obligations
6	Persistent usage despite user's knowledge that it is causing frequent problems at work, school, or home
7	Giving up or cutting back on important social, professional, or leisure activities because of use
8	Using in physically hazardous situations
9	Persistent use despite the user's awareness that the substance is causing or at least worsening a physical or mental problem
10	Tolerance: needing to use increasing amounts of a substance to obtain its desired effects
11	Withdrawal: characteristic group of physical effects or symptoms that emerge as amount of substance in the body decreases

Substance use disorder, as many psychiatric disorders, is a complex condition with many different aspects influencing the progression and severity of the illness. Indeed, despite the fact that a large proportion of the human population uses some kind of addictive substance, only a fraction develops a true addiction, which further supports the idea of substance use disorder as a complex and heterogeneous disorder.

Even though many neurotransmitter systems are involved in the addiction process, the mesolimbic dopamine system has been especially highlighted in both the initial reinforcing effects and the addicted state (Koob and Le Moal, 1997). Most drugs of abuse are capable of elevating dopamine levels in the brain, and this is thought to be a key function in the addictive potential of a drug. It is also well established that dopamine signalling is altered in individuals with substance use disorders (Fowler et al., 1996; Volkow et al., 1990; Volkow et al., 1996). In fact, repeated administration of drugs of abuse, including psychostimulants or alcohol, impairs dopaminergic neurotransmission and increases, for example, self-stimulation reward thresholds (Der-Avakian and Markou, 2010; Schulteis et al., 1995), alterations which in turn may drive drug intake (Ahmed and Koob, 2004; Belujon et al., 2016; Kesby et al., 2018; Martinez et al., 2004; Martinez et al., 2005).

Treatment options include pharmacological and psychosocial interventions, targeting both the neurochemical as well as the social aspects of the disorder. The main goals of treatment are to obtain abstinence, when possible, to prevent relapse and/or to reduce drug intake to less harmful levels. The pharmacological treatment principles vary depending on the substance of abuse in question but can be roughly divided into three main categories, aversive, antagonist and substitution therapies.

An example of an aversive treatment is disulfiram, used in alcohol use disorder, which inhibits the enzymatic clearance of ethanol, leading to an accumulation of acetaldehyde. This, in turn, results in the manifestation of aversive effects, such as flushing, nausea, headaches, palpitations, increased blood pressure and chest pains (Barth and Malcolm, 2010), making ethanol intake less desirable for the individual. Antagonist treatments build on the idea that pharmacological blockade of the reinforcing effect of a drug will lead to extinction of the self-administrating behaviour, e.g. treatment with the opiate receptor antagonist naltrexone for opiate or alcohol use disorders (also the latter disorder is believed to involve opioid receptors). Substitution therapy,

on the other hand, works by replacing the drug of abuse with either a more controlled dosage or a less harmful and less potent substance. This can be exemplified by using nicotine gums, patches and similar to treat nicotine use disorder, or methadone or buprenorphine to treat opiate use disorder. The idea here is to lessen the impact of withdrawal symptoms, which can increase the risk of relapse in an abstinent individual, by still supplying nicotine, but replace the "ritual" of nicotine use with a less significant action (compare lighting a cigarette after dinner to taking a piece of gum). The long-term goal is to successively reduce the dose of nicotine and for the individual to ultimately no longer need the substitution. For opiate use disorder, however, substitution treatment most often becomes life-long. Substitution therapy also reduces craving, probably by stimulating the same receptors and producing the same down-stream effects, e.g. dopamine release, as the drug of abuse, albeit on a smaller scale and in a more controlled manner. The theory is that, by temporarily restoring e.g. a compromised dopaminergic signalling system, the desire to further increase dopamine levels, by using a drug, is lessened. The partial nicotine acetylcholine receptor agonist varenicline, which currently is the most efficient smoking-cessation aid, is thought to act in this manner but in addition works as a nicotine receptor antagonist. This drug thus acts both as a substitution and antagonist treatment. The same is the case with buprenorphine for opiate use disorder.

However, due to limited efficacy of available treatments, especially for alcohol use disorder (Fanshawe et al., 2017; Kranzler and Van Kirk, 2001), further research aimed at determining the neurobiological underpinnings of drug addiction and to use this knowledge for developing new pharmacological treatment principles is fundamental.

Schizophrenia

Schizophrenia is a neuropsychiatric disorder that affects approximately 1% of the global population, with a risk of debilitating disability and life-long illness for the affected individual (Jablensky, 2000; van Os and Kapur, 2009). Diagnosis generally occurs in late adolescence to early adulthood, and most commonly at the time of the first psychotic event the individual experiences.

The aetiology of schizophrenia is complex, and in large part still unknown, but twin studies indicate that genetic risk factors represent the largest overall contributor (Trifu et al., 2020) with mainly non-shared environmental factors explaining the remaining risk for developing the disorder. The symptoms of schizophrenia are categorised into the three main categories – positive, negative and cognitive symptoms. Positive symptomatology includes several of the hallmark diagnostic criteria of the condition, such as delusions and hallucinations (see table 2). The negative symptom category includes affective symptoms, such as anhedonia, lack of motivation, social withdrawal and an overall negative affective state. Finally, the cognitive symptoms include deficits in executive dysfunction, lack of attentiveness and dysfunctional working memory. Negative and cognitive symptoms usually manifest at a younger age than the positive symptoms but are often not identified as such until a diagnosis has been made. Of these categories, only the positive symptoms respond well to treatment with most antipsychotic compounds, with the exception of clozapine, an atypical antipsychotic that can also positively impact negative and cognitive symptoms, but which also, unfortunately, produces several adverse effects (obesity (metabolic syndrome), sedation, and hypersalivation) of which one may be directly life-threatening (agranulocytosis).

Diagnostic criteria

Table 2: DSM 5 guidelines for diagnosis of schizophrenia. The following criteria have to be fulfilled in order to diagnose an individual with schizophrenia.

	Guidelines for diagnosis of schizophrenia
A	At least two of the following (at least one of which must be 1, 2 or 3) for at least one month 1. Delusions 2. Hallucinations 3. Disorganised speech 4. Grossly disorganised or catatonic behaviour 5. Negative symptoms
B	Level of functioning has to be significantly and long term lowered compared to the previously achieved level
C	Continuous signs of the disturbance persist for at least 6 months, must include criterion A symptoms for at least one month
D	Schizoaffective disorder and depressive or bipolar disorder with psychotic symptoms ruled out
E	The disturbance is not caused by substance use or medical conditions
F	If a patient has a history of autism spectrum or communication disorders from childhood, schizophrenia diagnosis can be made in case of prominent delusions/hallucinations and other required symptoms of schizophrenia are present for at least 1 month

The earliest modern antipsychotic treatments, the so-called typical antipsychotics or first-generation antipsychotics, were developed in the 1950s. These drugs, such as chlorpromazine, the very first antipsychotic treatment cf. (Shen, 1999), were found to mimic the behavioural effects of reserpine but were hypothesized to produce these effects by blocking brain dopamine receptors instead of depleting dopamine stores. These findings in combination with other indices led to the so-called Dopamine Hypothesis, postulating that since dopamine receptor antagonism reduces psychotic (positive) symptoms, schizophrenia is a

condition defined by a hyperdopaminergic state in the brain (Carlsson and Lindqvist, 1963; McCutcheon et al., 2019; Stahl, 2018). Later it was confirmed that antipsychotics act primarily as dopamine-2 (D2) receptor antagonists (Johnstone et al., 1978; Peroutka and Synder, 1980). However, with the emergence of more detailed brain imaging studies, as well as findings that suggest that negative and cognitive symptoms can be worsened by D2-antagonism (Mueser and McGurk, 2004), the dopamine hypothesis has been modified. It has since been shown that while there is evidence of a hyperdopaminergic state in the associative striatum (McCutcheon et al., 2018), which is responsible for positive symptomatology, other studies indicate a hypodopaminergic state in other regions. These hypodopaminergic regions, primarily the prefrontal cortex and the ventral striatum, have tentatively been linked to the cognitive and negative symptoms (Juckel et al., 2006; Wolf et al., 2014).

The emergence of the atypical, or second generation, antipsychotics has also influenced the theories attempting to, at least in part, explain schizophrenia. Whereas the typical antipsychotics focus on dopamine antagonism, the atypical antipsychotics to a larger extent affect also other neurotransmitter systems, e.g. serotonin (Miyake et al., 2012). Compared to the typical antipsychotics, the atypical antipsychotics are less likely to cause the extrapyramidal motor control side effects seen with traditional treatment options (Leucht et al., 2013; Leucht et al., 2009). Today, the recommended first line of treatment for schizophrenia in Sweden are the second generation antipsychotic compounds aripiprazole, which is a partial agonist at dopamine D2 receptors, and risperidone (Läkemedelskommittén, 2022), both of which also have 5-HT2 blocking properties. The latter action appears to increase the antipsychotic effect and reduce the motor side effects, while simultaneously, unfortunately, increasing metabolic side effects.

More recently, other theories have gained attraction in the ongoing work to fully explain schizophrenia, beyond the dopamine hypothesis. The glutamate hypothesis of schizophrenia suggests that a deficit in glutamate signalling in the prefrontal cortex contributes to both

cognitive and negative symptomatology (Mei et al., 2018; Rolls, 2012; Uno and Coyle, 2019). Furthermore, besides more conventional neurotransmitters such as glutamate and dopamine, other neuroactive molecules have gained attention, such as kynurenic acid, a metabolite of the amino acid L-tryptophan which has been shown to be elevated in the cerebrospinal fluid from individuals with schizophrenia (Erhardt et al., 2003; Erhardt et al., 2007). Kynurenic acid has been shown to act directly as an antagonist of ionotropic AMPA and NMDA receptors (Elmslie and Yoshikami, 1985), as well as interacting with the glycine site on NMDA receptors (Kessler et al., 1989). NMDA receptor antagonists, e.g. Angel´s dust (PCP) and ketamine are known to produce schizophrenia-like symptomatology, including positive, negative and cognitive symptoms.

Taken together, these various theories and hypotheses highlight the complexity of schizophrenia, as well as the need for further research into the mechanisms of the different neurotransmitters involved in the pathophysiology of schizophrenia. This knowledge is primarily needed to develop better treatments for the cognitive and negative symptoms in schizophrenia.

1.3 Dopaminergic pathways

Dopaminergic neurons are medium-sized neurons (around 15 µm in diameter) that communicate with long-range axonal projections. Following release, dopamine is taken up via the dopamine transporter (DAT) located on dopaminergic neuronal terminals (Ciliax et al., 1999), but possibly also on other cell types (Carboni et al., 1990), including astrocytes (Karakaya et al., 2007). Dopamine acts on dopamine D1-like (D1 and D3) receptors, which are G_q activated, and dopamine D2-like (D2, D4, D5) receptors, which are G_i-linked G-protein receptors (Neve et al., 2004). Dopamine D2 receptors also function as autoreceptors and are located on both dopaminergic cell bodies and axon terminals (Ford, 2014).

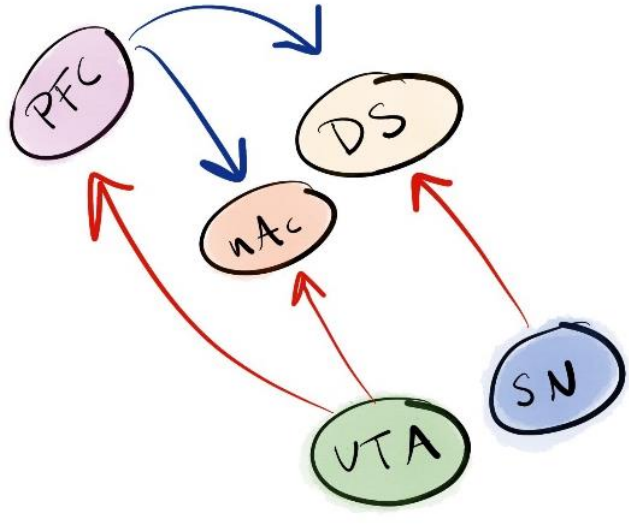


Figure 1: Schematic illustration of the two main dopaminergic pathways (red arrows). Dopaminergic afferents from the ventral tegmental area (VTA, in green) project to both the nucleus accumbens (nAc, in red) and the prefrontal cortex (PFC, in purple). The dorsal striatum (DS, in yellow) receives dopaminergic innervation from the substantia nigra (SN, in blue). Also illustrated are glutamatergic input from the PFC to the nAc and DS (blue arrows).

Dopaminergic neurons are tonically activated, firing at a low frequency, but also display burst activity (Grace and Bunney, 1984a, b). Dopaminergic neurons are relatively rare in the brain and are located in distinct and discrete cell groups, with projections to various other brain regions. There are three primary dopaminergic pathways. The cell bodies of the mesolimbic and mesocortical dopaminergic pathways are located in the ventral tegmental area (VTA) and the axons project to target areas including the nucleus accumbens (nAc) and prefrontal cortex (Ikemoto, 2007; Ikemoto, 2010; Pierce and Kumaresan, 2006) (Fig. 1). The cell bodies of the nigrostriatal pathway are located in the substantia nigra and the axons project to the nucleus striatum (caudate/putamen) (Smith and Bolam, 1990; Tritsch et al., 2012). The third major pathway is the tuberoinfundibular pathway, where dopaminergic cell bodies are located in the arcuate nucleus and project to the pituitary gland (Nestler et al., 2020). The work included in this thesis focuses on the mesolimbic and nigrostriatal pathways.

Striatum

One key brain region especially innervated by dopaminergic fibres is the nucleus striatum. The rodent striatal nucleus can be subdivided into the dorsal striatum, and nucleus accumbens. The dorsal striatum contains the dorsomedial striatum (DMS) roughly corresponding to the associate striatum, or nucleus caudate in humans, and the dorsolateral striatum, roughly corresponding to the motor striatum or the nucleus putamen in humans (Balleine and O'Doherty, 2010; McCutcheon et al., 2019). The nucleus accumbens can be roughly subdivided into nucleus accumbens shell and core (Calipari et al., 2016; Saddoris et al., 2015). The majority of neurons (95-97%) in the striatum are GABAergic medium spiny projection neurons (MSNs). The striatum also contains GABAergic interneurons (2-3%) and cholinergic interneurons (1-2%) (Tepper and Bolam, 2004). Even though the majority of neurons are GABAergic, the striatum is highly innervated by projections from cortical regions and the majority of synapses are glutamatergic (Omelchenko and Sesack, 2007; Russo and Nestler, 2013).

Nucleus accumbens

The nAc is innervated by dopaminergic projections originating in the VTA (Ikemoto, 2007), and can be functionally and anatomically divided into the core and shell regions. The core is physiologically more similar to the dorsal striatum, whereas the shell is part of the extended amygdala (Alheid and Heimer, 1988), and this physiological subdivision also reflects certain functional differences. The shell region appears to be important in hippocampal-dependent spatial information processing, and the core plays a role in the control of amygdala-dependent learning (Corbit et al., 2001; Ito and Hayen, 2011).

Nucleus accumbens in substance use disorder

Pathology of the nAc has especially been linked to substance use disorder. In fact, preclinical studies have repeatedly shown that dopaminergic projections from the VTA to the nAc are crucial for the rewarding and reinforcing effects by drugs of abuse. Local microinjections of different drugs of abuse into the nAc and/or VTA have repeatedly shown to induce condition place preference (CPP) (Campos-Jurado et al., 2020; Liao et al., 2000; Watson et al., 2010), a method used to evaluate the rewarding (or aversive) properties of a drug.

Even though the role of the nAc has especially been implicated in the acute stages of reward and drug reinforcement, there is also support for a sustained control by the nAc over drug taking behaviour. In fact, deep brain stimulation of the nAc may lead to alleviation of alcohol use disorder, supporting a role for the nAc in addiction therapy (Kuhn et al., 2009). Drugs of abuse have repeatedly been shown to increase extracellular dopamine in the nucleus accumbens (ventral striatum); both when given passively and when self-administered, in rodents and in man, but the underlying mechanisms of action vary across drugs. In fact, the signalling pathways and the neurotransmitters involved are not fully established. Psychostimulants, such as amphetamine and cocaine, act directly on dopamine terminals, and increase extracellular dopamine levels by inhibiting or reversing the dopamine uptake mechanism

(Fleckenstein et al., 2007; Venton et al., 2006). Nicotine activates nicotinic acetylcholine receptors, especially in the VTA (Yan et al., 2018); while morphine increases accumbal dopamine output by acting on opioid receptors located on inhibitory GABAergic interneurons in the VTA (Johnson and North, 1992) causing disinhibition. Alcohol-induced dopamine release may involve acetaldehyde (Foddai et al., 2004; Melis et al., 2009), the first metabolite of ethanol which also is produced in the brain (Hipolito et al., 2007), and/or ethanol itself, and indirect or direct effects in the VTA and/or in the nAc. Glycine receptors in the nucleus accumbens have been shown to play a key role in ethanol induced dopamine release, where inhibition of glycine receptors prevents the dopamine elevation induced by both local and systemic administration of ethanol (Lido et al., 2011; Molander and Soderpalm, 2005). Notably, also nicotinic acetylcholine receptors (nAChRs) in the VTA are involved both in ethanol-induced and ethanol cue-induced dopamine release in the nAc and this action appears to involve endogenous acetylcholine release in the VTA in response to glycine receptor activation in the nAc (Ericson et al., 2008). These nicotinic receptors subtypes are different from those responsible for the dopamine release produced by nicotine itself cf. (Ericson et al., 2003).

Nucleus accumbens in schizophrenia

Neurotransmission in the nAc is tightly regulated by glutamatergic inputs from the prefrontal cortex and dopaminergic inputs, making the nAc a vulnerable brain region in schizophrenic patients. In fact, smaller nAc have been reported in patients diagnosed with schizophrenia (Schaub et al., 2021), and reduced activity in this brain region correlates with the severity of negative symptoms in medication-free schizophrenics (Juckel et al., 2006; Wolf et al., 2014). The nAc may thus be especially linked to the negative symptomatology in schizophrenia. Interestingly, the rate of substance abuse among individuals with schizophrenia is significantly higher (50%) than among the general population (Chiang et al., 2019; Hunt et al., 2018; Voce et al., 2019; Voce et al., 2018). As elevation of dopamine levels in the nAc is a

hallmark of drugs of abuse it is possible to speculate that the use of drugs within the schizophrenic population can be a way of self-medication for negative symptoms, a line of reasoning that is supported by some clinical studies wherein patients with schizophrenia and substance use disorder report fewer negative symptoms than those with schizophrenia only (Potvin et al., 2006).

Dorsomedial striatum

Dorsomedial striatum (DMS) is innervated by dopaminergic projections arising from the substantia nigra pars compacta but there is also some innervation arising from the VTA (Ikemoto et al., 2015; Lu et al., 2021). The DMS is a sub-region of the brain that has been implicated in motor control, but also goal-directed behaviour and decision-making (Gremel and Costa, 2013; Yin et al., 2005). In fact, mild dopamine lesions of the DMS have been shown to impair behavioural flexibility, and inhibitory control in mice (Lhost et al., 2021).

DMS in substance use disorder

While the DMS has retrieved less attention in regulating drug reward, control of reward related actions has repeatedly been shown to be regulated by the DMS (Allen et al., 2022). In fact, recent studies suggest that dopamine signalling in the DMS is a key controller of the development of compulsive reward seeking (Seiler et al., 2022), suggesting that the DMS could be involved in more advanced stages of drug addiction. Indeed, repeated exposure to either alcohol or cocaine has been suggested to impair cognitive flexibility by inflicting on DMS function (Ma et al., 2022; Zhao and Chen, 2020).

In animal studies, repeated exposure to psychostimulants results in an increased locomotor response to the drug, referred to as behavioural sensitization. Behavioural sensitization has been proposed to reflect many of the neurochemical changes that are characteristic for drug addiction and is an established model for investigating drug-induced effects on the function of the nervous system (Robinson and Berridge,

1993; Steketee and Kalivas, 2011). Ablation of D2-expressing medium spiny neurons in the DMS has been shown to prevent behavioural sensitization to amphetamine (Durieux et al., 2012), suggesting that the DMS may play a role following extended drug exposure.

DMS in schizophrenia

While a hypodopaminergic state is presumed to exist in the nAc of individuals diagnosed with schizophrenia, dopamine hyperfunction in the associative striatum is one of the most robust pathophysiological observations in patients with schizophrenia (Abi-Dargham et al., 1998; Conn et al., 2020; Katthagen et al., 2020). In fact, there is evidence for upregulated dopamine synthesis (Katthagen et al., 2020), dopamine release (Shen et al., 2012) as well as lowered expressions of DAT (Sekiguchi et al., 2019), in the associative striatum of patients with schizophrenia.

1.4 Research questions

Paper I

Can robust but controlled elevation of dopamine levels in the nAc reduce ethanol intake in a rat model predictive of clinical effect in man?

Even though accumbal dopamine levels have been linked both to the positive and negative reinforcing effects of ethanol in animal models, real effective treatments manipulating dopaminergic mechanisms are still lacking. Naltrexone is an established drug for treating alcohol use disorder and is supposed to produce its effect by antagonizing the dopamine releasing effect of ethanol both in rats (Gonzales and Weiss, 1998) and in man (Heilig and Egli, 2006; Spagnolo et al., 2014), thereby at least partly blocking the rewarding effect of ethanol (Valenta et al., 2013). However, the effect size of this treatment is low (Cohen`s d = 0.2) (Jonas et al., 2014; Soyka and Chick, 2003; Srisurapanont and Jarusuraisin, 2002) and the number needed to treat is 8-12. Further, direct manipulations of the dopamine system, such as stimulation (Gorelick and Wilkins, 2006; Malcolm et al., 2001; Malcolm et al., 2000) of dopamine receptors, nor partial activation/blockade of these receptors with aripiprazole (Coffin et al., 2013; Newton et al., 2008) have yielded any impressive results. Indeed, the most illustrative study in this regard may be the study by Wiesbeck et al. (Wiesbeck et al., 2001) comparing a depot formula of the dopamine D2 receptor antagonist flupenthixol to placebo during one year after an initial detox. This study showed that relapses to heavy drinking came earlier, were longer and more severe in the active treatment group. At the initiation of this trial, the brain imaging studies showing reduced baseline DA activity in sober alcoholics were not available. In light of the latter findings, the outcome of the flupenthixol study may be explained by a further reduction of an already low dopamine activity, which probably enhances the craving for ethanol.

With respect to enhancement of dopamine activity most human studies have been performed with the dopamine D2 receptor agonist bromocriptine and these have also been largely negative (Gorelick and Wilkins, 2006). An obvious drawback with this type of treatment is that only one of five postsynaptic dopamine receptors are engaged and that this D2 subtype also acts as a somatodendritic and terminal autoreceptor. Activation of such inhibitory D2 autoreceptors reduces both neuronal firing and dopamine release.

Varenicline is an interesting substance in this regard. This partial agonist at α4β2 nicotinic receptors produces a limited but sustained dopamine release in nAc and simultaneously prevents further dopamine activation by nicotine (Ericson et al., 2009). This dual effect probably explains the compound´s established effect as a smoking cessation aid (Cohen´s d = 0.45) (O'Malley et al., 2018). This substance also prevents the pronounced dopamine elevation produced by ethanol and nicotine in combination, while it was less clear whether it antagonized the dopamine elevation produced by ethanol itself (Ericson et al., 2009). More recent studies indicates that this is not the case (Feduccia et al., 2014; Goldstein et al., 2022), which probably is explained by the fact that the nicotinic receptors involved in ethanol-induced dopamine release are different from those engaged by varenicline (see above). Therefore, the reduced ethanol intake observed in animal models after varenicline is most likely not explained by blockade of ethanol-induced dopamine release but probably by its dopamine activating effect.

To date four varenicline trials for the treatment of alcohol use disorders have been performed. In two of these studies, evidence was obtained for a reduction of self-reported alcohol intake with an effect size of 0.35-0.4 (Cohen´s d) (Litten et al., 2013; O'Malley et al., 2018). In another study, there was a profound reduction of self-reported ethanol intake, but this did not significantly separate from placebo. On the other hand, ethanol intake measured as a reduction of the 100% specific ethanol metabolite phosphatidyl-ethanol in blood was reduced in the varenicline

but not in the placebo group (de Bejczy et al., 2015). The effect size was approximately 0.35 (Cohen´s d).

It is reasonable to assume that the emerging effect of varenicline in the treatment of alcohol use disorder also is explained by its slight elevation of low dopamine levels in an individual with alcohol use disorder rather than by a tentative blockade of the dopamine release induced by ethanol. In that case, it should be possible to enhance this effect by combining varenicline with another dopamine-elevating agent with another mechanism of action. For this purpose, we choose the combined dopamine/noradrenaline reuptake inhibitor bupropion, which presently is registered for treatment of depression and smoking cessation.

We hypothesize that the combined administration of varenicline and bupropion, as compared to administration of either drug by itself, will show a larger effect on dopamine levels and on the alcohol deprivation effect, a rat model predictive of clinical effect in man (Spanagel and Holter, 2000).

Papers II and III

Can the pharmacology of drugs of abuse give clues to the treatment of negative and positive symptoms of schizophrenia, respectively?

As mentioned, drug abuse is more common in schizophrenic patients than in healthy controls, and the use of some drugs, e.g. nicotine, is extremely common in schizophrenic patients (Hughes et al., 1986). A hallmark for drugs of abuse is their dopamine elevating effects in the brain. However, even though the positive symptoms of schizophrenia have been linked to dopamine hyperactivity not all drugs of abuse exacerbate the positive symptoms. For example, psychotic symptoms following nicotine, ethanol or heroin intake are rare or even non-existing (nicotine). This in stark contrast to amphetamine, cocaine and THC which all are well-known pro-psychotic agents, especially in individuals with or with heredity for schizophrenia (Moore et al., 2007). Nevertheless, and surprisingly, even these latter drugs are sometimes used by schizophrenic patients. The reason might be that drugs of may

reduce negative symptomatology – they increase motivation and initiative, and produce hedonic and pro-social effects, and even though these effects are very likely to come with increased psychotic symptoms in some cases (e.g. following amphetamine), this might be considered worthwhile by some individuals.

We hypothesize that the reason why drugs of abuse that do not produce psychosis but still may alleviate negative symptoms is that they selectively elevate extracellular dopamine levels in the nAc, as compared to the DMS. Psychosis generating drugs, on the other hand, elevate dopamine similarly in both these brain regions. If this is true, the mechanisms of action of drugs of abuse not elevating dopamine in DMS might be exploited in the search for new treatments of negative symptomatology.

An interesting phenomenon observed in patients using central stimulants is that while tolerance to the hedonic effects may develop, the opposite, i.e. sensitization, often develops to the pro-psychotic effects. This observation indicates that these two pharmacological effects, reward and psychosis, are produced in different neuronal circuitries. If the mechanisms underlying the enhanced sensitivity to the psychotic effects could be understood this could indicate targets for new treatments of positive symptomatology.

We hypothesize that sub-chronic administration of amphetamine in a manner producing a sensitization phenomenon differentially affects neurotransmission in the nAc and DMS.

Paper IV

Can selective, powerful pharmacological elevation of dopamine levels in the nAc but not in the DMS be achieved?

The results obtained in paper III showed that both nicotine and ethanol have dopamine elevating properties that might explain their pro-social effects and their simultaneous low propensities to induce psychosis. There is extensive evidence showing that these two drugs often are used in combination (Husky et al., 2007; McKee et al., 2007) and despite this

extensive co-use worldwide, psychosis induced by the combination of nicotine and ethanol does not appear to be a clinical problem. Still, these two drugs produce additive dopamine elevating effects in the nAc (Ericson et al., 2009; Tizabi et al., 2007).

We hypothesize that the combined administration of nicotine and ethanol produces an additive and pronounced dopamine elevating effect in the nAc but essentially no effect in the DMS.

Nicotinic acetylcholine receptors of the α4β2 subtype are involved in the dopamine elevating effects of nicotine and glycine receptors are involved in those of ethanol (for references, see above). Hence, co-administration of agents other than ethanol and nicotine manipulating these two receptor populations should produce a similar selective dopamine elevation in the nAc but not in the DMS.

We hypothesize that the combined administration of varenicline, a partial agonist at α4β2 nicotinic receptors, and the glycine uptake 1 inhibitor Org 24598 produces an additive dopamine elevating effect in the nAc but essentially no effect in the DMS.

2 AIM

Aim 1: to investigate if the combination of varenicline and bupropion has an additive effect on nAc DA and if the combination can have a beneficial effect on ethanol intake in a voluntary ethanol consumption model.

Aim 2: to investigate how the baseline function of the DA systems in nAc and DMS are altered and how they respond to the respective drug after chronic intermittent administration of amphetamine.

Aim 3: to investigate the DA activating effects of nicotine, ethanol and morphine in the nAc and in the DMS and compare them to those of amphetamine, cocaine and THC.

Aim 4: to investigate if nicotine and ethanol, or drugs releasing DA by similar mechanisms as these drugs, will show additive effects on DA release in nAc, but not in DMS.

3 METHODS, MATERIALS AND METHODOLOGICAL CONSIDERATIONS

The materials and methods are described in-depth in the individual papers. The following chapter will cover a brief description of the methods and a more detailed discussion about the pros and cons of each method, and of how the method has been applied in the work included in this thesis.

3.1 ANIMALS

The animals used in this thesis are all male Wistar Han outbred rats, of the RccHan strain, obtained from the vendor Envigo, Netherlands. It has been observed that animals from different vendors, even if they are of the same stock or strain, can differ from one-another in certain behaviours (for example in response to ethanol, stress sensitivity and anxiety-like behaviours (Palm et al., 2011, 2012; Theilmann et al., 2016; Tsuda et al., 2020)). For this reason, we have strived to always obtain animals from the same vendor, and of the same strain, for all experiments included in this thesis.

3.2 ANIMAL MODELS

Animal models, especially rodent models, have been used in medical research for a long time. However, research suggests that there is a discrepancy between the preclinical findings made using animal models, and the clinical benefit for the patient. This has led to attempts to better standardize both the planning and execution of animal experiments, with efforts like PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) providing guidelines to help researchers. PREPARE aims to assist researchers in three areas; formulation, dialogue between scientists and the animal facility, and quality control (Smith et al., 2018). Efforts have also been made, with the help of guidelines like ARRIVE (Animal Research: Reporting of *In Vivo* Experiments), to create a better

system by which research using animal models is reported, and thereby improve reproducibility.

While the focus of this thesis work has not been to perfectly emulate and model the complex human conditions of addiction and schizophrenia, it is important to discuss the manner in which we use animal models in psychiatric research. These conditions can be modelled in many different ways. One of the key elements to addiction is the protracted intake of the addictive substance, which can be achieved in animals in several ways. In papers I and II, two of those methods are utilised. In paper I, the animals are allowed to voluntarily consume ethanol for a long period of time, in order to obtain a high, and stable, ethanol intake, akin to binge drinking in humans (Sabino et al., 2013). In paper II, on the other hand, the drug (amphetamine) is passively administered to the animal via intraperitoneal injection. Contrasting these two methods of drug administration, the self-administration paradigm of paper I is the closest to the human condition. One drawback with self-administration is that some compounds possess a bitter taste when dissolved into the drinking water, thus making the animals less likely to consume pharmacologically relevant doses. Another drawback is the stability of the compound, where the prolonged time needed for the animal to voluntarily consume the drug might cause the compound to degrade over time, thereby making dosage difficult to calculate. Passive administration, in the form of injections, will deliver a set dose at predetermined intervals and a more predictable outcome.

Attempts have been made to create more comprehensive models for both alcohol use disorder and schizophrenia. For example, selective breeding for high alcohol-preferring rats have yielded strains of animals with a high preference for alcohol (Crabbe et al., 2010), thus potentially mimicking the high heritability (Bierut et al., 1998; Hardie, 2002) of alcohol use disorder. Efforts have been made to link genetic variations in these high-preferring strains to biomarkers seen in humans, thereby further validating the model (Landgren et al., 2011). Strides have also been made to improve schizophrenia models, with both transgenic

models such as the DISC-1 (Jaaro-Peled, 2009), Neuregulin1, and ErbB4 knock-outs (Jaaro-Peled et al., 2009; Mei and Xiong, 2008), as well as models attempting to simulate the pre- and perinatal risk-factors for schizophrenia, such as infection, brain trauma, and social isolation (Fone and Porkess, 2008; Lapiz et al., 2003; Lodge and Grace, 2009; Moore et al., 2006; Tseng et al., 2009).

3.3 BEHAVIOURAL METHODS

3.3.1 LOCOMOTOR MEASUREMENTS AND BEHAVIOURAL SENSITISATION

Behavioural sensitisation is a phenomenon described as an escalation in response to the same dose of a drug administered repeatedly. This increased sensitivity has been described as a reflection of neuroadaptations induced by the repeated drug administration (Robinson and Berridge, 1993), and behavioural measures can be used as a proxy to indirectly study these adaptations. In paper II, behavioural sensitisation by repeated administration of amphetamine was assessed using locomotor measurements, more specifically horizontal ambulatory and rearing (vertical) behaviours.

The equipment consists of an open arena, intersected by two separate layers of infrared beams, placed in a sound- and light-attenuated box. When an animal is placed in the arena, interrupting the beams where the animal is situated, it gives rise to a so-called beam break. These beam breaks are registered and interpreted by the software as movement, either horizontal (in the bottom layer) or vertical (in the top layer).

Locomotor measurements such as these are suggested to be linked to midbrain dopaminergic transmission, as many drugs produce an increase in locomotor behaviour, and the model has been a valuable tool for the study of neuroadaptations induced by repeated drug-intake (Steketee and Kalivas, 2011). However, the behaviour likely involves other neurotransmitters, alongside with dopamine. One study has found that pre-treatment with either dopaminergic or noradrenergic antagonists (haloperidol and prazosin, respectively) eliminated amphetamine-induced, but not phencyclidine-induced, locomotor hyperactivity (Kusljic et al., 2022). As amphetamine primarily affects dopaminergic and noradrenergic neurotransmission, and phencyclidine is an NMDA receptor antagonist, it can be assumed that drugs with different pharmacological effects affect locomotion and behavioural sensitisation in different ways.

In paper II, behavioural sensitisation of locomotor behaviour as a proxy of alterations in striatal dopaminergic transmission was further triangulated with the use of microdialysis experiments measuring extracellular dopamine, as well electrophysiological field potential recordings studying alterations in dopaminergic transmission with the help of the selective D2- and D3-agonist quinpirole.

3.3.2 ETHANOL CONSUMPTION MODEL

In paper I, a two-bottle-choice ethanol consumption model was used to test the efficacy of the combined treatment of varenicline and bupropion on ethanol intake in rats. The voluntary ethanol consumption model is used to model both non-pathological human ethanol intake, and alcohol use disorder (AUD). Two main paradigms are applied, intermittent ethanol consumption, and limited access ethanol consumption. For both paradigms, the ethanol is introduced at the beginning of the dark period, as rats are nocturnal and thus will be at their most active during dark period.

Intermittent ethanol consumption is a method that relies on the voluntary consumption of an ethanol solution of individually housed animals. The animals are presented with a two-bottle choice, where one bottle contains ordinary tap water, and the other the ethanol solution, which in this thesis had a concentration of 6 or 12 %.

As opposed to a continuous access paradigm, the animals have access to the ethanol solution in an intermittent manner, which is thought to further increase the ethanol consumption over time (Wise, 1973), and is suggested to mimic binge-drinking behaviour in humans (Sabino et al., 2013). Each drinking session lasts for 24 hours, and the sessions are interspersed by 24 or 48-hour periods during which the animals only have access to water.

When testing for treatment effect, the animals were instead subjected to a limited access paradigm. This meant that the animals only had access to the ethanol solution for eight hours each day, and prior to the addition of the bottles, each animal received their designated treatment. The

limited access paradigm was used to get a better time resolution in the measurements, to better assess possible differences between the treatment groups, and to ensure that the pharmacological treatment remains active during the drinking period.

One often-discussed issue with the method is the fact that it necessitates single housing of the animals. This is primarily due to the desire to observe and follow a single animal's consumption and preference, which cannot easily be done in standard group-housing conditions. As rats are social animals that normally live in large groups with defined hierarchical structures, single housing is known to have significant impact on the wellbeing of the animal (Arakawa, 2018; Olsson and Westlund, 2007). Isolation stress have also been shown to, at least temporarily, increase ethanol intake in rats (Vazquez-Leon et al., 2017). However, since psychological stress can impact alcohol craving (Breese et al., 2005), as well as act as a risk factor for relapse (Brown et al., 1995), it can be argued that the stress induced by social isolation is in fact beneficial to the ethanol consumption model, as it takes into account the effects that stress can have on humans with AUD.

3.4 IN VIVO MICRODIALYSIS

In vivo microdialysis is a method by which one can sample the contents of the extracellular fluid in a vast variety of tissues in awake and freely moving subjects (Chaurasia et al., 2007). In the work included in the present thesis, the method has been applied to the sampling of the extracellular space in brain tissue, with the main focus on dopamine.

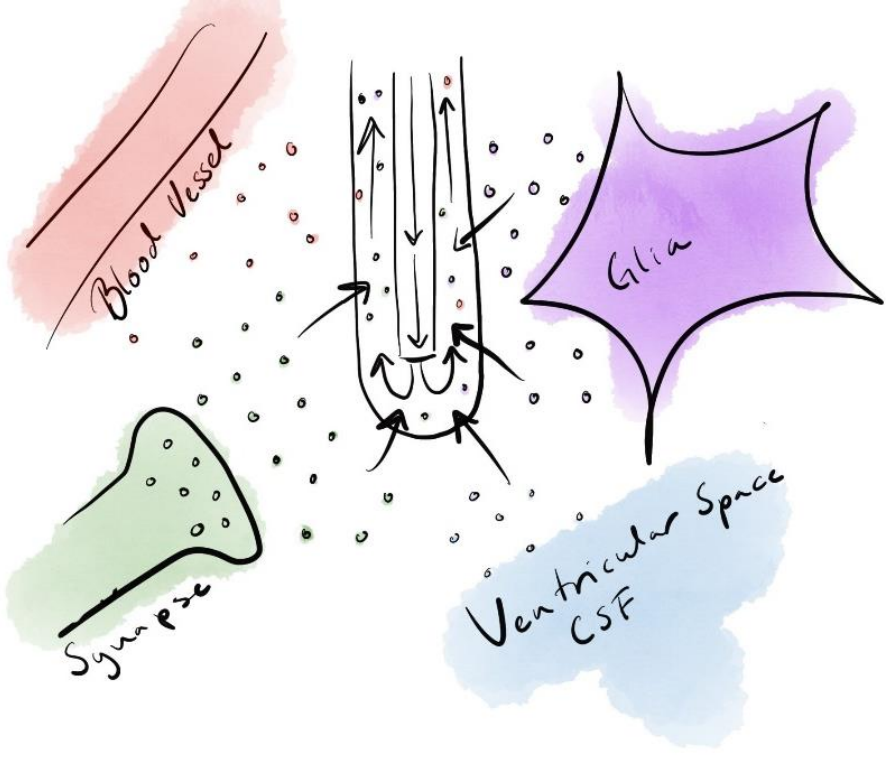


Figure 2: Schematic illustration of the different sources of analyte picked up by the microdialysis probe. Substances released from synapses (green) and glia cells (purple) constitute the majority of the analytes, but contributions can also come from substances that can pass over the blood brain barrier (red), or that are present in the cerebrospinal fluid (blue).

The principle of microdialysis is based on the concept of passive diffusion, where a soluble substance moves from an area of high concentration to an area of low concentration, often over some kind of membrane. This allows for sampling of the surrounding environment as well as delivery of pharmacologically active substance into the extracellular space, so called reversed microdialysis. In papers I, III and IV, regular microdialysis was used to indirectly measure extracellular concentrations of dopamine in the nAc and the DMS. In paper II, reversed microdialysis was used to deliver amphetamine dissolved in the perfusion solution directly into the nAc and the DMS, while simultaneously measuring dopamine.

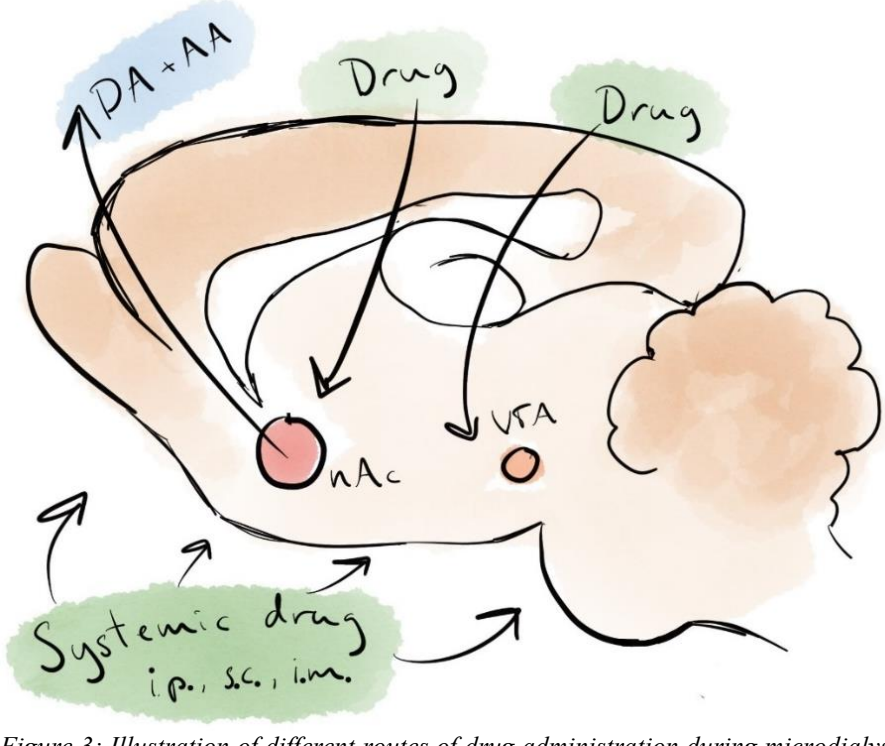


Figure 3: Illustration of different routes of drug administration during microdialysis. Substances can be administered via systemic injection, or locally, either through the microdialysis probe, or elsewhere in the brain using a guide cannula and local injections (exemplified by arrows pointing to the nucleus accumbens (nAc) or ventral tegmental area (VTA), respectively).

Because microdialysis relies on passive diffusion, no fluid is introduced to or removed from the sensitive tissue of the brain, meaning that there is no change in net fluid balance in the tissue. This prevents both dehydration of and mechanical damage to the brain, as sometimes seen in the older method of push-pull cannula sampling, where a small amount of liquid is injected into the tissue and then removed.

Microdialysis allows for high spatial resolution and, depending on the flowrate and requirements of the analysis method, moderate temporal resolution, meaning that it is possible to observe progressive changes in the extracellular environment in discreet sub-regions of the brain. Adjustments can be made to optimise for example sampling frequency, purity of the samples and to counteract low extracellular concentrations of the analyte. In the works included in this thesis, the flowrate is set to 2µl/min and the rate of sampling is every 20 minutes. This allows for ample time for the dopamine to accumulate in the perfusate solution, and yields enough sample for the subsequent HPLC analysis, as well as the reservation of a small amount of sample for preservation and future amino acid analysis, when desired. An increase in flow rate will allow for an increased sampling rate, but poses the risk of a smaller fraction of the analyte content passing over the membrane, thus requiring a higher sensitivity analysis method. Inversely, a lower flowrate can be applied in circumstances of low analyte concentrations, in order to ensure that a larger fraction of the analyte passes over the membrane. However, this will also enable larger molecules that would otherwise not have time to enter the probe to do so, potentially contaminating the samples.

The active space, the portion of the membrane that is left exposed and where the diffusion occurs, can be increased or reduced, as can the size of the pores in the semi-permeable membrane. An increase in active space will give a larger area over which the analyte can pass into the probe, but as with the lower flowrate, this increases the risk of a less pure sample. The same principle applies to an increase in pore-size, as

well as the risk of the pores clogging when larger molecules like peptides attempt to pass over the membrane.

Due to the relatively low temporal resolution, it is not possible to record fast events such as dopamine neuron burst firing, for which electrophysiological or voltammetry recordings are better suited. In the case of this thesis, the dopaminergic effects of interest are primarily a matter of bulk overflow, rather than direct synaptic transmission, making microdialysis an ideal sampling method.

3.5 ELECTROPHYSIOLOGICAL FIELD RECORDINGS

Electrophysiological field potential recordings were used to study changes in primarily excitatory neurotransmission before and after treatment with amphetamine. In brief, brain slices are placed in chambers circulated with oxygenated artificial cerebrospinal fluid (aCSF). Two electrodes are then inserted into the area of interest, one stimulating electrode, which applies an artificial stimulation at set intervals and strength, and one recording electrode. The recording electrode will detect changes in voltage, primarily connected to the influx of cations through AMPA receptors (fig 4). Changes in evoked potentials may thus primarily reflect alterations in glutamatergic signalling.

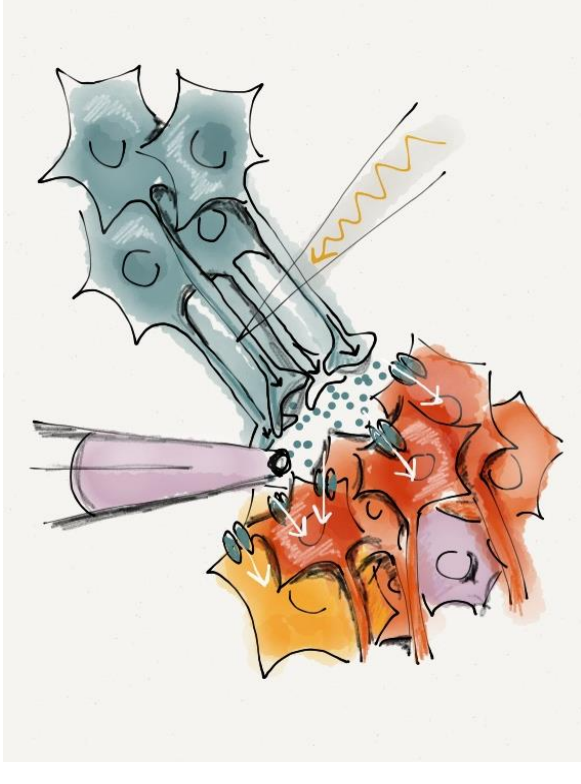


Figure 4: Schematic illustration of electrophysiological field recordings. The stimulation electrode, shown here with a yellow arrow representing the injected current, is placed along the projection into the target area and stimulates the nearby neurons in a manner akin to an artificial action potential. The recording electrode, here in purple, detects changes in ion charge in the surrounding tissue, which is then interpreted as a proxy of AMPAR mediated postsynaptic activation. Illustration by Louise Adermark.

Due to the innervation of glutamatergic afferents from cortex around 80% of the synapses in the striatum are glutamatergic (Ingham et al., 1998). Field potential recordings in the striatum thus primarily reflect AMPAR mediated postsynaptic currents, and pharmacological blockade of AMPAR erases the response elicited by the stimulating electrode (Lagstrom et al., 2019).

In paper II, microdialysis was used to study the effects of protracted amphetamine treatment on extracellular dopamine, and electrophysiological field recordings were utilized to more directly investigate the effects on synaptic transmission. Given the modulatory role of dopamine, and the considerable increases in striatal dopamine caused by amphetamine exposure, it was also of interest to study the acute dopaminergic effects on downstream signalling in amphetamine naïve and exposed rats. Thus, changes in evoked field potential amplitudes produced by repeated amphetamine exposure *in vivo*, or pharmacological manipulation via bath perfusion, was assessed *ex vivo*. In this extent, electrophysiological recordings may provide an overarching view of how the microcircuitry of the striatum is affected by protracted treatment.

Compared to electrophysiological whole cell recordings, field potential recordings cannot measure the spontaneous signalling of one specific cell, and an artificial activation is needed in order to elicit a response. However, this applied stimulation makes field potential recordings less sensitive to issues often arising in older animals, such as the density of dendritic spines and changes in the cell membrane, which can make it difficult to obtain a good signal in whole cell recordings. The setup used for the recordings presented in this thesis is equipped with four chambers, which makes it possible to measure responses in brain slices from control animals and treated animals simultaneously, which reduces variability between recording sessions.

Despite the fact that the recordings are performed *ex vivo* and in slices, meaning that afferent paths and feedback loops are severed, intact microcircuitry, and remaining fragments of synapses, can maintain

functional neurotransmitter release for some time. It is thus still possible to manipulate and study, for example, dopaminergic influence over MSNs.

The electrophysiological field recordings included in this thesis work were performed by Oona Lagström.

3.6 ETHICAL CONSIDERATIONS

All experiments included in this thesis work were approved by the Ethics Committee for Animal Experiments in Gothenburg, and planned and performed according to national laws and guidelines for the care and use of laboratory animals.

3.7 STATISTICS

The statistical analysis of the data presented in this thesis was performed using the software GraphPad Prism version 9.1.0 for Windows (GraphPad Software, San Diego, California, USA).

In papers I-IV, 2-way analysis of variance (ANOVA) with repeated measures was carried out for analysis of change over time (dopamine content in microdialysis samples, locomotion analysis and electrophysiological recordings) and input/output function. In papers I and IV, 1-way ANOVA was used for analysis of the alcohol deprivation effect and area under the curve analysis of dopamine content, respectively. For multiple comparisons, Tukey's post hoc test was used. T-tests were used when applicable in papers II and III. To analyse the correlation between basal nAc and DMS dopamine levels, the Pearson's correlation coefficient analysis was applied.

ANOVA is a statistical test used to analyse the difference between the means of two, or more, groups. The one-way ANOVA tests the data set according to one categorical value (e.g. treatment) affecting the result, whereas the two-way ANOVA tests according to two categorical values (e.g. treatment and time) affecting the outcome. The two-way ANOVA can thus give information of the interaction between the two categorical

values (Campbell, 2007). In this thesis, this interaction factor was used to describe the impact of brain region on the dopaminergic response to different treatments, that is to say, if the treatment elicited a different response over time, depending on the brain region. When performing multiple comparisons on the ANOVA results, the Tukey's honestly significant difference (HSD) test was used. The test essentially compares all possible pairs of means in a manner similar to that of the ordinary t-test, but with the added correction for family-wise error rate, that is to say the risk of making type-1 errors when performing multiple comparisons (Tukey, 1949).

The Student's t-test is amongst the most commonly used statistical tests, and it compares the means of two normally distributed data sets. The so called independent t-test is performed when the two data sets are obtained independently from one another, whereas the paired t-test is used when the data sets are matched and dependent on one another (Campbell, 2007). The paired t-test was applied for analysis of the double probe microdialysis data included in this thesis, where basal DA levels in the nAc and DMS within the same animal were compared. This dataset was also subjected to a correlation analysis, using the Pearson's correlation coefficient analysis. This analysis is a measure of the linear correlation between two sets of data, expressed as a ratio between the covariance of two variables. As applied in this thesis work, we wanted to determine if there was a correlation between the basal DA levels in the nAc and the DMS.

All of the analyses applied in this thesis work are parametric tests, meaning that they demand that the data is normally distributed. Normal distribution can be tested for in several ways, such as manual plotting of the data, to determine if it forms a bell-curve, thus indicating a normal distribution. It is also possible to perform a normality test, such as the Shapiro–Wilk test, where a p-value lower than 0.05 indicates that the data is not normally distributed. However, both of these tests require large sample sizes in order to provide a conclusive indication of the distribution of the data, which can be difficult, and ethically

problematic, to obtain in animal experiments. Instead, normal distribution is assumed based on substantial experience of the methods used, as well as the animals themselves. The rats used in this thesis are all outbred, meaning that the genetic variance within the population can be tentatively assumed to be relatively well distributed.

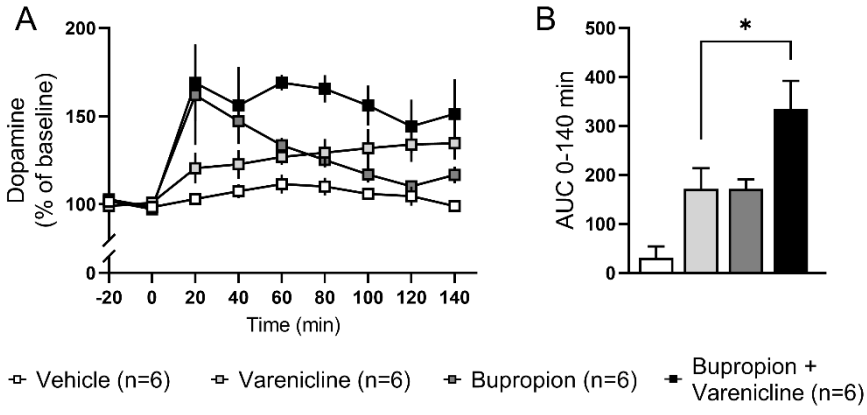
4 RESULTS AND DISCUSSION

Paper I: Combined administration of varenicline and bupropion produces additive effects on accumbal dopamine and abolishes the alcohol deprivation effect in rats

The overall aim for paper I was to investigate if the combination of the partial nAChR agonist varenicline and the dopamine and noradrenaline reuptake inhibitor bupropion could produce an additive effect on accumbal dopamine levels, and if the combination could reduce alcohol intake and relapse in a voluntary ethanol consumption model.

In short, microdialysis experiments were used to examine the dopamine elevating properties of varenicline and bupropion alone and in combination. The animals were equipped with custom-made microdialysis probes situated unilaterally in the nAc and were allowed to recuperate for 48h before the experiment took place. After a baseline had been established, vehicle, varenicline (1.5 mg/kg, s.c.), bupropion (2.5, 5 & 10 mg/kg, i.p.) or a combination of varenicline and each dose of bupropion was administered, and nAc dopamine was monitored for 140 minutes.

The microdialysis experiment showed that varenicline, as well as the 5 and 10 mg/kg doses of bupropion, effectively elevated nAc DA on their own (figure 1C, paper I). The addition of varenicline proved to produce an additive effect in combination with 5 mg/kg (Fig. 5 A&B) and 10 mg/kg bupropion. Of the two, the 5 mg/kg dose of bupropion was deemed to be the most suitable to combine with varenicline for the ethanol consumption study, as it provided the clearest additive effect.



F*igure 5: Varenicline (1.5 mg/kg) and bupropion (5 mg/kg) produce an additive effect on nAc dopamine levels. A) The addition of bupropion enhanced the dopamine elevating properties of varenicline. B) Area under the curve (AUC) analysis of the time following drug-administration showed a clear additive effect on the dopamine releasing properties of varenicline with the addition of bupropion. Data are presented as mean ± SEM. n = number of rats. *p<0.05*

We then moved on to investigate if this combination could reduce ethanol intake and relapse in animals' voluntary consuming ethanol.

The ethanol consumption study was dived into four main phases; 1) the intermittent ethanol consumption (screening) phase, which lasted for approximately 7 weeks; 2) the treatment phase, which lasted 5 days during which a limited access paradigm was applied; 3) the alcohol deprivation period, during which the animals only had access to water for two weeks; and finally 4) two additional days of limited access ethanol consumption to test the alcohol deprivation effect (ADE) (Fig. 6).

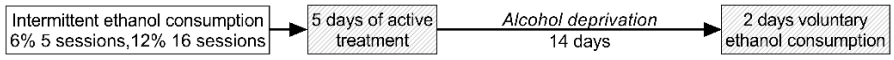


Figure 6: Illustration of the four phases of the ethanol consumption study. During the intermittent screening phase, the animals' ethanol consumption was monitored, and moving into the initial treatment phase, the animals with the highest consumption were selected and divided into four treatment groups (vehicle, bupropion 5 mg/kg, varenicline 1.5 mg/kg, and the combination). This phase was followed by a two-week alcohol deprivation period, during which the animals only had access to water, after which the alcohol was re-introduced for two days of alcohol deprivation effect testing.

Our results showed that alcohol consumption was not significantly depressed by treatment of either compound during the initial treatment phase (Fig. 2C, paper I), however, following the two-week alcohol deprivation period, all animals exhibited a clear ADE, except for the animals treated with the combination (Fig. 7 A&B).

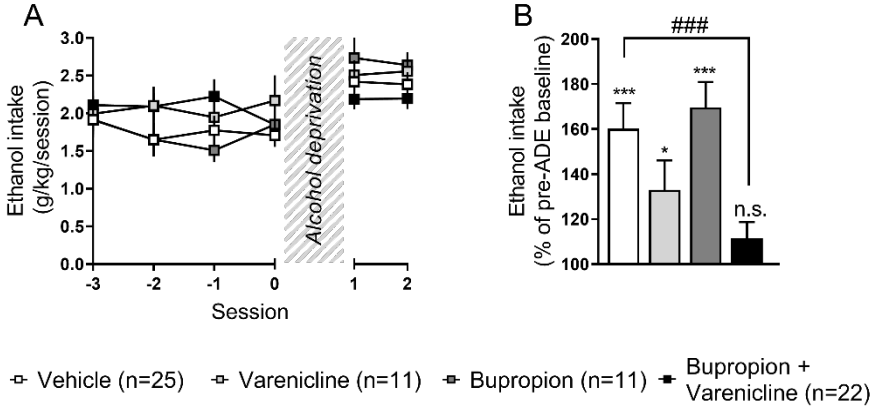


*Figure 7: Combination of varenicline (1.5 mg/kg) and bupropion (5 mg/kg) elimininates the alcohol deprivation effect (ADE). A) Time course graph illustrating baseline ethanol intake before and after the alcohol deprivation period. B) Only the combination treatment successfully eliminated the ADE seen in the other three groups. Data are presented as mean ± SEM, n = number of rats, * p <0.05, *** p <0.001, significant compared with baseline. ###, P <0.001, significant as compared with vehicle-treated control*

The data obtained in this study showed that both varenicline and bupropion are capable of elevating nAc DA, which is in line with previous studies (Ericson et al., 2009; Nomikos et al., 1989). One of the

novel findings was that the addition of bupropion could further enhance the DA elevating properties of varenicline. This is speculated to be related to an increase in DA neuronal activity and release brought on by varenicline, in combination with the reduced DA uptake mediated by bupropion, resulting in enhanced nAc DA.

Abnormal DA transmission and activity has been connected to increased alcohol intake and craving, in animal models as well as human studies (Ericson et al., 2020; Weiss et al., 1996). It has therefore been suggested that an attempt to normalise, or stabilise, this DA system might help reduce alcohol treatment. Varenicline, which is currently the most efficient smoking cessation treatment available (Aubin et al., 2011), has also been shown to reduce alcohol intake in both animal models (Steensland et al., 2007) as clinical studies (de Bejczy et al., 2015; Mitchell et al., 2012; Vatsalya et al., 2015). Assuming that this effect is primarily due to the dopaminergic effect, it could possibly be bolstered by the additional effect brought on by bupropion. While we did not see an immediate effect on the ethanol consumption during the initial treatment phase, the combination of the effect did successfully eliminate the ADE, a model with high predictive value for efficacy in treatment of AUD (Spanagel and Holter, 2000).

The fact that neither substance alone affected ADE suggests a synergistic effect between the two, which is supported by the additive effect seen in the microdialysis studies. However, it should be noted that since dopamine was not monitored concurrently with ethanol consumption, we cannot say for certain that it is this additive effect that results in the suppression of the ADE.

One notable issue is the observed α4β2 nAChR-blocking effects of bupropion (Warner and Shoaib, 2005), which could, in theory, interfere with the partial agonism of varenicline. However, both the higher nAChR affinity of varenicline, and the additive effect on nAc dopamine, suggests that this effect is negligible in the current model.

In conclusion, the combination of varenicline and bupropion produces an additive effect on accumbal DA and eliminates the ADE in rats. The results obtained here suggest that the combination of these two drugs could provide a valuable improvement to the currently available treatments of AUD in humans.

Paper II: Subregion-specific effects on striatal neurotransmission and dopamine signalling by acute and repeated amphetamine exposure

The aim of this study was to investigate how neurotransmission in nAc and DMS is altered following acute local administration or repeated systemic amphetamine exposure. Special emphasis was on the dopamine system, and studies were performed using both *in vivo* microdialysis and *ex vivo* electrophysiology.

For acute microdialysis experiments, amphetamine (1-100 μM) was locally perfused via the microdialysis probe. While a similar dopamine elevation was observed in the nAc and DMS at a lower concentration of amphetamine, a significantly higher increase was observed in the nAc at higher concentrations (Fig. 8). The underlying mechanism for the higher increase in DA at higher amphetamine concentrations remains to be determined but may be linked to differences in DAT expression, or to other characteristics of dopaminergic neurons in the VTA and substantia nigra pars compacta (Montero et al., 2021; Richter et al., 2020).

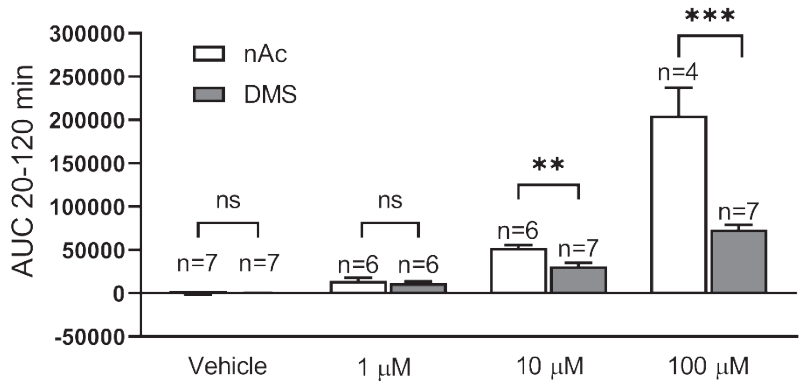


*Figure 8: Local administration of amphetamine produces a more substantial dopamine elevation in the nAc compared to the DMS. Area under the curve describing the effects of locally administered amphetamine (1 – 100 μM) in the nAc and DMS. Both the 10 and 100 μM dose resulted in a significantly higher dopamine elevation in the nAc, as compared to the DMS. Data are presented as mean ± SEM, n = number of rats, ** P <0.01, *** p <0.001*

In the next set of experiments, animals were discontinuously exposed to amphetamine (2.0 mg/kg, i.p.) for a total of five days over one week. Animals were then left in their home cages for additionally two weeks before the experiments were conducted.

In repeatedly exposed animals, microdialysis experiments revealed a reduced endogenous tone of dopamine selectively in the nAc. Amphetamine challenge furthermore produced a more substantial elevation of dopamine in the nAc of animals that had received amphetamine two weeks earlier, while no potentiation was observed in the DMS. The reduced dopamine levels observed in the nAc might be a neurobiological underpinning of the negative state, which may occur after a period of repeated drug exposure (Koob and Le Moal, 2001, 2008).

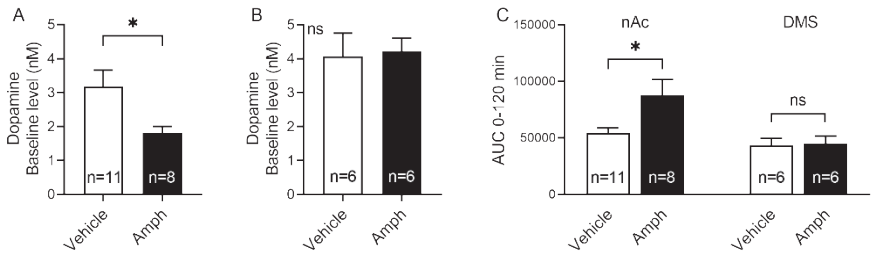


*Figure 9: In vivo microdialysis, basal dopamine and dopamine response to local amphetamine challenge after repeated systemic amphetamine injections. A-B) Amphetamine treated animals had lower basal dopamine levels in the nAc, as compared to vehicle treated controls, but no difference was found in the DMS. C) Area under the curve (AUC) analysis of the dopamine response to a local dopamine challenge (10µM) shows an increased sensitivity in the nAc, but not in the DMS. Data are presented as mean ± SEM, n = number of rats, * p <0.05*

To further outline neurophysiological changes in response to repeated amphetamine exposure, electrophysiological field potential recordings were performed in the nAc and DMS. When monitoring evoked field potentials during continuous inhibition of $GABA_A$ receptors, accumbal response amplitude was significantly depressed in brain slices from rats previously receiving amphetamine (Fig. 10). Paired pulse ratio was not significantly modulated between groups, suggesting that the effect on evoked potentials is primarily postsynaptic. Evoked field potential amplitude primarily reflects activation of AMPA receptors, and the effect may thus be linked to changes in the expression of AMPA receptor subunits (Chojnacki et al., 2020; Yu et al., 2005).

Field potential recordings further revealed a change in the responsiveness to the DA D2 receptor agonist quinpirole. While quinpirole produced synaptic depression in vehicle-exposed animals, synaptic output was rather increased in the nAc of animals previously receiving amphetamine (Fig. 10 C). While the underlying mechanism was not outlined here, the effect might be linked to changes in the DA tone and connected to changes in the expression of D2 receptors at different synaptic inputs. It should be noted that bicuculline was omitted in experiments where quinpirole was in the bath, and changes in the inhibitory tone may thus have contributed to the effect.

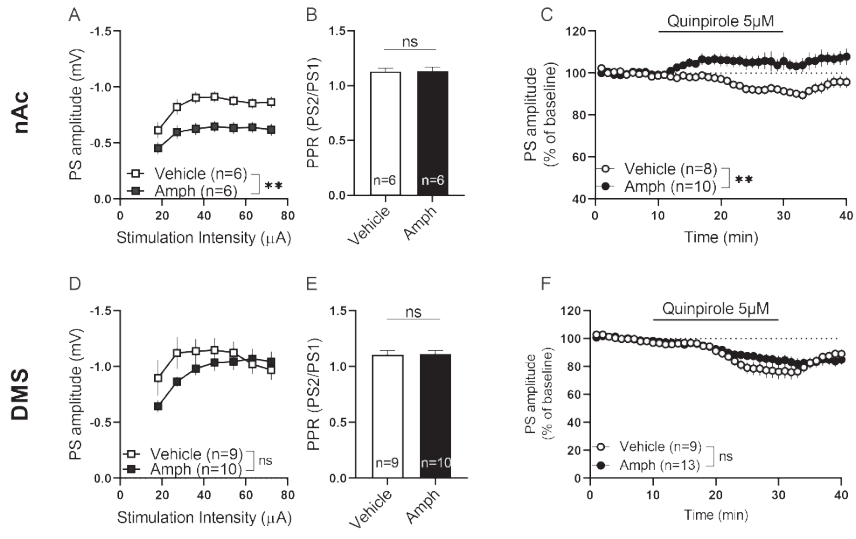


*Figure 10: Electrophysiological field potential recordings. A, D) Repeated exposure to amphetamine followed by abstinence significantly depressed evoked field potential amplitudes in the nAc, with a similar trend at lower stimulation intensities in the DMS. B, E) Changes in evoked potentials appear to be connected to postsynaptic transformations since there was no effect on the paired pulse ration when comparing the two brain regions. C, F) Bath perfusion of D2 receptor agonist quinpirole was significantly modulated in the nAc of brain slices from rats previously receiving amphetamine. n=number of animals. Data are presented as mean values ± SEM, n=number of rats, ** $p < .01$*

Taken together, the data presented in paper II suggests that repeated exposure to amphetamine followed by abstinence produces sustained neuroadaptations in the nAc that especially impinge on DA signalling. Not only is the endogenous dopamine level reduced, the responsiveness to amphetamine and downstream signalling via DA D2 receptors are also transformed. These sustained changes in DA signalling could contribute to a negative state during drug abstinence but may also play a key role in the establishment of amphetamine addiction.

Paper III: Differential dopamine release by psychosis-generating and non-psychosis-generating addictive substances in the nucleus accumbens and dorsomedial striatum

The overall aim of study III was to compare sub-region specific effects on DA induced by psychosis generating and psychosis-non-generating drugs of abuse. Our theory was that psychosis-generating drugs would especially increase DA in the DMS, while psychosis-non-generating drugs of abuse would not. The study was conducted using reversed microdialysis using a double-probe, allowing simultaneous sampling in the nAc and DMS from the same animal.

Our results show that psychosis-generating psychostimulants, such as amphetamine and cocaine, increased dopamine levels to a similar extent in both nAc and the DMS. The similar increase in DA is also in line with the proposed mechanism of action, suggesting that psychostimulants act by inhibiting dopamine uptake in terminal regions (Fleckenstein et al., 2007; Venton et al., 2006). Importantly, the most striking difference was found when analysing the effect by the non-psychosis generating stimulant nicotine, a compound commonly used by individuals diagnosed with schizophrenia (Kumari and Postma, 2005; Sagud et al., 2019). In these experiments, we found a robust increase in dopamine levels in the nAc while dopamine levels in the DMS were not significantly affected.

For morphine and ethanol, which are not considered psychosis-generating, an increase in DA was detected in both nAc and DMS. However, the increase in DA had a slightly slower onset and lower amplitude in the nAc as compared to the DMS. This finding is partially in line with previous studies (Vena et al., 2016), and indicates that these compounds may affect neurotransmission in a partially brain regions specific manner.

Tetrahydrocannabinol (THC) did not produce a significant DA elevation in any of the two brain regions studied. This is somewhat surprising considering the psychosis generating properties of THC. However, cannabinoid receptors, which is the main target of THC, are

widely expressed on striatal medium spiny neurons and glutamatergic terminals from cortex (Hohmann and Herkenham, 2000). The psychosis generating property of THC may thus be downstream from the DA signalling, and more directly linked to changes in striatal neurotransmission (Burns, 2013).

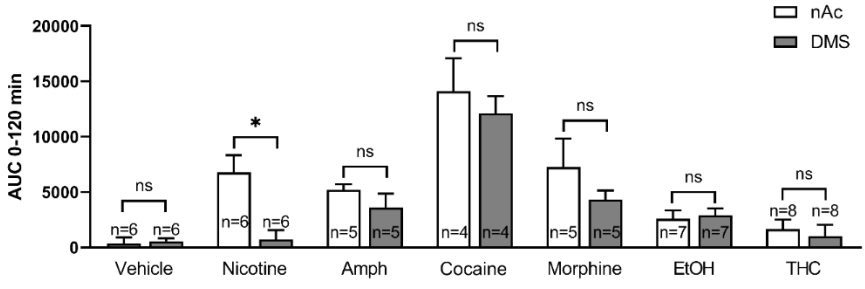


*Figure 11: In vivo microdialysis data, area under the curve (AUC) analysis of the dopamine elevation in the nAc and the DMS following systemic administration of NaCl (vehicle), nicotine, amphetamine (amph), cocaine, morphine, ethanol (EtOH) and THC. All treatments produced relatively similar elevations of extracellular dopamine in both sub-regions, aside from nicotine, which produced a substantially more robust dopamine elevation in the nAc, as compared to the DMS. n=number of animals. Data are presented as mean values ± SEM, n=number of rats, ** p < .01*

One of the major novelties in this paper was the use of a double-probe to monitor dopamine levels in two different brain sub regions from the same animal. Our data clearly show that DA can be measured selectively in either brain regions, without a possible DA overflow. Thus, this technique can be used to retrieve more reliable data for brain-region comparisons, and to minimize the number of experimental animals required for the experiments.

The doses used in this study were selected in order to produce modest dopamine elevations in the nAc, and the results garnered here were in line with previous findings (Adermark et al., 2015; Frank et al., 2008; Kankaanpaa et al., 1998; Nisell et al., 1994; Rada et al., 1991). However, there are fewer studies of the effects of addictive substances on DMS dopamine, making the findings in this area more difficult to verify. Studies in which the dorsal striatum is investigated often focus

on the entire dorsal striatum, or the dorsolateral striatum. Due to the nuances in structure and function of the subregions in the dorsal striatum, we believe that this distinction is important, and can explain discrepancies between these findings and others.

Perhaps the most central finding in paper III was the selectivity of the effects on striatal dopamine by nicotine, especially as compared to other central stimulants cocaine and amphetamine. While cocaine and amphetamine act directly on dopaminergic terminals, nicotine exerts its effect by stimulation of nAChR, likely on both dopaminergic neurons and on glutamatergic afferents (Charpantier et al., 1998; Schilstrom et al., 2003; Yan et al., 2018), in the VTA. It is possible to speculate that these dopaminergic and glutamatergic neurons in the VTA, which project preferentially to the nAc and PFC, have a different expression from those in the substantia nigra, from which the majority of dopaminergic input to the DMS comes.

These speculative differences in dopamine innervation could also be responsible for the qualitative differences in dopamine elevation in the nAc and DMS brought on by ethanol and morphine. Both ethanol and morphine are thought to indirectly elevate dopamine through different pathways, suggesting that differences in glycine receptor (in the case of ethanol (Soderpalm and Ericson, 2013; Soderpalm et al., 2017)) and mu-opioid (morphine (Johnson and North, 1992)) receptor pattern could result in the slower and slightly lower effect on DMS dopamine which was observed in this study.

The data presented in paper III partially supports our theory that psychosis-generating drugs increase DA levels in the DMS, while psychosis-non-generating drugs do not. However, these recordings were all performed in drug naïve animals, and the response to these compounds may change with repeated exposure. While many factors contribute to the risk of developing drug-induced psychosis, the data presented here supports the idea of increased DA in the DMS, a brain sub-region corresponding to the human associative striatum.

Paper IV: Sub-region-specific modulation of striatal dopamine in Wistar rats. Manuscript

The overall idea of this research is to find pharmacological tools to selectively elevate DA levels in the nAc, without raising DA in the DMS. We speculate that this would be an appropriate approach to not only reduce negative symptomology but also the risk of drug relapse, without increasing positive symptomatology. The aim of the last project was thus to assess if a combination of pharmacological manipulations could act additively to selectively elevate DA levels in nAc.

In paper III, we had shown that nicotine selectively increases DA in the nAc and that the response to ethanol differed over time. In the first set of experiments, we thus performed *in vivo* microdialysis in the nAc and DMS during exposure to either ethanol or nicotine, or a combination of the two compounds. While these single-probe experiments did not demonstrate a robust separation between the two groups during exposure of either ethanol or nicotine, animals treated with both compounds demonstrated an additive effect on DA levels in the nAc, but not in the DMS (Fig. 12) In fact, in contrast to the additive effect on accumbal DA, which is in agreement with previous research conducted by the research group (Ericson et al., 2009), a combination of ethanol and nicotine produced a significantly lower increase in DA as compared to nicotine alone in the DMS. These effects may be connected to the property of the DA inputs and/or differences in how microcircuits in striatal sub-regions regulate DA output.

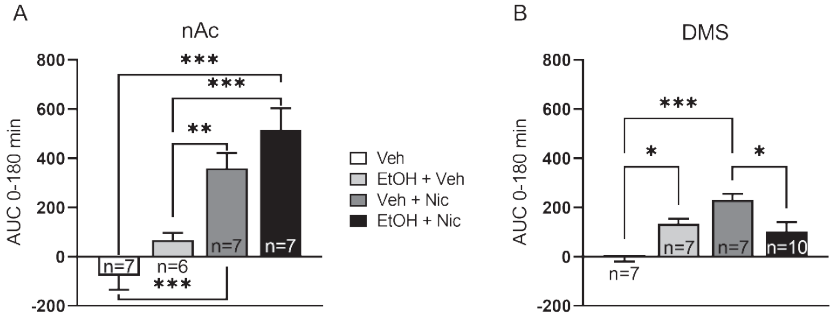


*Figure 12: Area under the curve (AUC) analysis of microdialysis data. A-B) The addition of nicotine produces an additive effect of the dopamine elevating effect of EtOH in the nAc, whereas the combination appears to have an attenuating effect on nicotine-induced dopamine elevation in the DMS. Values are presented as means ± SEM. n = number of rats. *p < 0.05, **p < 0.01, ***p < 0.001.*

Since it would not be recommended to treat patients with a combination of ethanol and nicotine, we then moved on to pharmacological substances that might partially mimic the effects by ethanol and nicotine. The research group has a long history of studies supporting a key role for glycine receptor activation in the dopamine elevating property of ethanol, and the glycine transporter blocker Org24598 was thus used to mimic the effect by ethanol. The smoking cessation agent varenicline (Champix®) is a partial agonist of the nAChRs and was thus used to mimic the effect by nicotine. In these second sets of experiments, we employed the double-probe to simultaneous monitor DA levels in the DMS and nAc from the same rat, and treated animals with Org24598 and varenicline, alone or in combination.

Similar to the data for experiment one, there was no significant separation between groups when monitoring DA levels during administration of either Org24598 or varenicline alone. When combining the two substances, however, there was a robust elevation of DA in the nAc, while DA levels in the DMS were not significantly elevated as compared to vehicle treated control.

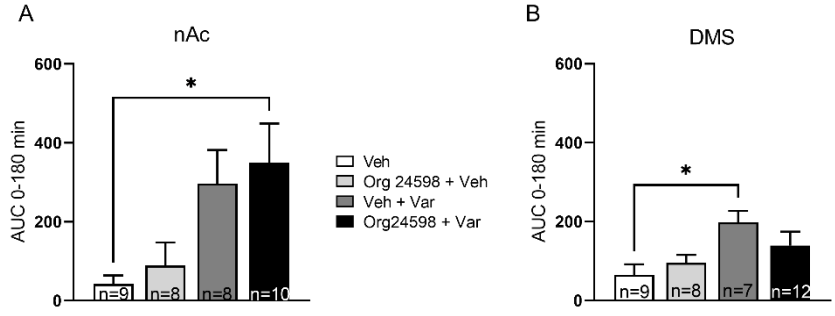


*Figure 13: Area under the curve (AUC) analysis of double-probe microdialysis data. A-B) The combination of the glycine transporter inhibitor Org24598 and the partial nicotinic acetylcholine receptor agonist varenicline produced a significant elevation in nAc dopamine, but had no effect on DMS dopamine. In fact, the addition of Org24598 to varenicline appeared to negate the varenicline-induced dopamine elevation seen in the DMS. Values are presented as means ± SEM. n = number of rats. *p < 0.05, **p < 0.01, ***p < 0.001.*

The data presented in manuscript IV show that we can combine pharmacological agents acting on the DA system to selectively increase DA in the nAc. This line of thinking may open up for new treatment strategies to relieve negative symptomatology in schizophrenia. Even though more research is required to assess tentative tolerance development and the long-term effects by these treatments on endogenous DA levels, we hypothesise that brain regions selective regulation of DA opens up for new pharmacological interventions.

5 GENERAL DISCUSSION AND CONCLUSION

Dopamine plays a crucial part not only in several physiological processes and functions (reward, memory, motor control), but also in many different pathological conditions (addiction, Parkinson's disease, schizophrenia). It has been suggested that low dopaminergic tone in the ventral striatum, a central part of the reward pathway, is connected to both addiction, where the low tone is postulated to be the driving force behind the substance use and/or a consequence thereof, and to schizophrenia, where a hypodopaminergic state (Juckel et al., 2006; Wolf et al., 2014) is thought to be connected to negative and cognitive symptomatology. Add to that the fact that substance use disorders and schizophrenia are highly linked in terms of a high prevalence of co-morbidity (Chiang et al., 2019; Hunt et al., 2018; Khokhar et al., 2018; Voce et al., 2019; Voce et al., 2018). Taken together, this suggests that if one could revert, or combat, low dopaminergic tone in the ventral striatum (specifically the nucleus accumbens, nAc), one could potentially find a path of treatment for both conditions.

In the first paper, we aimed at experimentally addressing the long-standing hypothesis that a low dopaminergic state in the nAc drives intake of addictive drugs, in this case alcohol, by employing two available pharmacological agents which have the potential to produce a robust dopamine elevation with a relatively slow onset, and which have been proven safe in the clinic without significant addictive potentials.

Dopamine is closely connected to a few main pathways in the brain, primarily the mesolimbic, mesocortical, nigrostriatal and tuberoinfundibular pathways, with clusters of dopaminergic neurons, which project out to target areas in other parts of the brain (Ikemoto, 2007; Ikemoto, 2010; Pierce and Kumaresan, 2006). The focus of this thesis was the mesolimbic and the nigrostriatal pathways. The mesolimbic pathway consists of dopaminergic neurons which project *i.e.* to the ventral striatum, whereas the nigrostriatal pathway consists of projections from the substantial nigra pars compacta to the dorsal striatum (Smith and Bolam, 1990; Tritsch et al., 2012). The associative striatum, i.e. a part of the dorsal striatum, is highly implicated as the focus point of positive symptomatology of schizophrenia, where, unlike for the negative and cognitive symptoms, the culprit is rather a

hyperdopaminergic state (Abi-Dargham et al., 1998; Conn et al., 2020; Katthagen et al., 2020). It is therefore of great importance, especially in the case of schizophrenia, that any attempt to elevate nAc dopamine does not also increase dopamine in the dorsal striatum, and thereby risk worsening positive symptoms. The second aim for this thesis work was therefore to investigate the possibility of selectively increasing dopamine in the nAc, without also causing an increase in associative striatum (the dorsomedial striatum, DMS), in a rat model.

To these ends we used the sampling method of *in vivo* microdialysis, a method that allows for sampling of the extracellular milieu of a tissue, in this case the nAc and the DMS, in order to answer three broad research questions;

- ***Can robust but controlled elevation of dopamine levels in the nAc reduce ethanol intake in a rat model predictive of clinical effect in man?***
- ***Can the pharmacology of drugs of abuse give clues to the treatment of negative symptoms of schizophrenia?***
- ***Can selective pharmacological elevation of dopamine levels in the nAc but not in the DMS be achieved?***

In paper I, we show that when combining the smoking cessation aid varenicline and the antidepressant bupropion, an additive effect on nAc dopamine is achieved. This is theorised to be due to the different pathways by which these compounds elevate dopamine. Varenicline is a partial agonist at the α4β2 subtypes of the nicotinic acetylcholine receptors which produces a modest but sustained dopamine release in the nAc (Ericson et al., 2009; Feduccia et al., 2014), whereas bupropion weakly blocks the dopamine/noradrenaline reuptake, thereby elevating dopamine at the synapse. Combined, this resulted in an additive sustained elevation in nAc dopamine (Paper I, figure 1). Varenicline has been tested in clinical trials targeting alcohol use disorder with positive outcomes (de Bejczy et al., 2015; Litten et al., 2013), and in order to test if the addition of bupropion could potentiate this effect, we carried out an ethanol consumption study in rats. This experiment showed that the combination of the two treatments completely eliminated the alcohol

deprivation effect, which has a high predictive validity for efficacy in man (Spanagel and Holter, 2000).

Paper I provided valuable information regarding the assumption that the rescue of a presumed compromised mesolimbic dopamine system can be beneficial in treating alcohol use disorder. While we did not examine the accumbal dopamine levels in the animals that had been given access to ethanol, it has been suggested that protracted ethanol consumption does affect dopamine transmission (Karkhanis et al., 2015). The findings of paper I thus helped in formulating the plans for the following studies, in which the aim was to selectively interact with a compromised mesolimbic dopamine system.

In paper II, we aimed to study both the acute effects of amphetamine, as well as those of protracted amphetamine administration on dopamine transmission in the nAc and the DMS. For this purpose, we performed *ex vivo* electrophysiological field potential recordings in addition to the *in vivo* microdialysis experiments. The animals were either drug-naïve, or had previously undergone a five-day amphetamine treatment followed by 14 days of withdrawal. The experiments showed that the nAc was, overall, more sensitive to amphetamine challenge, with higher dopamine elevations induced by locally administered amphetamine in high concentrations, both acutely and after repeated exposure, but also with an apparent adaptive down-regulation of baseline dopamine levels. Further, a reversal of direction of the electrophysiological effect of bath application of the dopamine D2 agonist quinpirole was observed after repeated amphetamine. Interestingly, such an effect has previously been observed on *ex vivo* electrophysiology also after repeated nicotine administration (Morud et al., 2016).

These findings indicate that repeated subchronic exposure for amphetamine results both in reduced presynaptic dopamine activity (decreased baseline dopamine release) and in reduced or maybe even reversed post-synaptic responsivity of dopamine D2 receptors. If translated to the human situation this may produce a state where a low baseline dopamine activity results in craving for the drug but where a

subsequent amphetamine intake despite clearly raising dopamine levels will produce a weak or qualitatively different response, since the quality of the dopamine D2 receptor activation has changed.

In paper III, we investigated the effects of several known psychosis-generating substances (amphetamine, cocaine and THC) on nAc and DMS dopamine, and compared them to the effects of non-psychosis-generating substances (nicotine, ethanol and morphine). The most crucial findings were in the comparisons between the central stimulants, where both amphetamine and cocaine resulted in robust and nearly identical effects on both nAc and DMS dopamine, whereas nicotine almost solely had an effect in the nAc. As amphetamine and cocaine both act directly on the dopamine terminals, by interfering with dopamine uptake, and nicotine acts primarily on the dopamine soma, directly or indirectly, we speculate that the manner in which a substance affects dopamine plays a part in its psychosis-generating potential. This is further supported by the findings of ethanol and morphine, which are both generally considered to have a low psychosis-generating potential. While both substances did produce significant elevations in both nAc and DMS dopamine (Paper III, figure 3), statistical analysis showed that they did so in a differential manner, with a slower and lower effect on DMS dopamine.

A discrepancy between the findings from papers II and III becomes evident when looking at the differences in dopamine response to amphetamine challenge, where in paper II, the local challenge elicited a stronger response in the nAc, than in the DMS, at higher doses, which was not seen after systemic administration in paper III. However, this is most likely explained by not only the differences in route of administration, but also in dose range. While the local concentration of amphetamine surrounding the probe after systemic administration can only be speculated upon, it is reasonable to assume that it is substantially lower than after local administration. This is further substantiated by that the lowest concertation (1 μM) produced a similar dopamine elevation as systemic administration of amphetamine 0.5 mg/kg.

Finally, in paper IV (manuscript), we aimed to combine the findings in previous studies and propose a way of selectively elevating nAc dopamine, with little to no effect on DMS dopamine. To this end, we repeated previous experiments that showed that combining ethanol and nicotine produces an additive effect on nAc dopamine, and we showed that the combination had no such effect in DMS. As ethanol and nicotine are both addictive and harmful substances, we then attempted to mimic the dopamine elevating properties of these compounds with pharmacological agents. Again, we used varenicline as a substrate for nicotine, and the glycine transport inhibitor Org24598 to mimic one aspect of the proposed pathways by which ethanol elevates dopamine, namely by interactions with the glycine receptor. Again, we saw an additive effect in the nAc, but no significant effect on DMS dopamine.

5.1 FUTURE PERSPECTIVES

The positive findings from Paper IV suggest a potential route by which one can selectively elevate dopamine in the nAc, thus supporting the ground hypothesis of the thesis. However, in order to validate the usefulness of this proposed treatment, it has to be tested in behavioural animal models. An ethanol consumption study investigating the added benefit of Org24598 to the already tested combination of varenicline and bupropion is currently underway, which will provide us with valuable information on the benefit of the additive effect of Org24598 on varenicline in a substance use disorder-model. To test the effect on negative symptoms in schizophrenia, as well as ensuring that there are no adverse effects on positive symptomatology, several tests need to be carried out. Anhedonia models are often used to model negative symptoms, alongside social interaction methods (Millan et al., 2014; Ward et al., 2015). Conditioned avoidance response (CAR) is a method used to evaluate the antipsychotic efficacy of a drug with high predictive validity (Smith et al., 2004). We suggest that it could be possible to utilise this method to screen for potential interference from the combination of varenicline and Org24598 by pre-treating animals with

an antipsychotic treatment (by which the avoidance behaviour is quenched), and then co-administer the combination, to see whether the behaviour is recovered or not.

While it should be acknowledged that ours and others hypothesis regarding a low dopaminergic tone in the nAc as a major driving force not only in addictive behaviours but also in producing negative symptomatology in schizophrenia remains to be firmly established, this thesis work has provided some clues as to how dopaminergic pathways may be regulated in a brain-subregion-specific manner. Through this work, we now have received tools to further test our theories. If selectively elevating baseline DA levels in the nAc can act to reduce alcohol relapse or dampen negative symptoms, then this line of research may have an immense societal impact.

In conclusion, the findings presented in this study suggest that selective elevation of nucleus accumbens dopamine can be achieved through a combined approach, and that this could hold potential as a way of treating negative symptoms in schizophrenia.

6 ACKNOWLEDGEMENTS

I am so grateful for all the help and kindness I have received over the years, and I would like to extend my deepest gratitude to you all.

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REFERENCES

Abi-Dargham, A., Gil, R., Krystal, J., Baldwin, R. M., Seibyl, J. P., Bowers, M., van Dyck, C. H., Charney, D. S., Innis, R. B., Laruelle, M., 1998. Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. Am J Psychiatry 155, 761-767.

Adermark, L., Morud, J., Lotfi, A., Jonsson, S., Soderpalm, B., Ericson, M., 2015. Age-contingent influence over accumbal neurotransmission and the locomotor stimulatory response to acute and repeated administration of nicotine in Wistar rats. Neuropharmacology 97, 104-112.

Ahmed, S. H., Koob, G. F., 2004. Changes in response to a dopamine receptor antagonist in rats with escalating cocaine intake. Psychopharmacology (Berl) 172, 450-454.

Alheid, G. F., Heimer, L., 1988. New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid, and corticopetal components of substantia innominata. Neuroscience 27, 1-39.

Allen, A. T., Heaton, E. C., Shapiro, L. P., Butkovich, L. M., Yount, S. T., Davies, R. A., Li, D. C., Swanson, A. M., Gourley, S. L., 2022. Inter-individual variability amplified through breeding reveals control of reward-related action strategies by Melanocortin-4 Receptor in the dorsomedial striatum. Commun Biol 5, 116.

American Psychiatric Association, A. P. A. D. S. M. T. F., 2013. Diagnostic and statistical manual of mental disorders : DSM-5. American Psychiatric Association, Arlington, VA.

Arakawa, H., 2018. Ethological approach to social isolation effects in behavioral studies of laboratory rodents. Behav Brain Res 341, 98-108.

Aubin, H. J., Karila, L., Reynaud, M., 2011. Pharmacotherapy for smoking cessation: present and future. Curr Pharm Des 17, 1343-1350.

Balleine, B. W., O'Doherty, J. P., 2010. Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action. Neuropsychopharmacology 35, 48-69.

Barth, K. S., Malcolm, R. J., 2010. Disulfiram: an old therapeutic with new applications. CNS Neurol Disord Drug Targets 9, 5-12.

Belujon, P., Grace, A. A., 2017. Dopamine System Dysregulation in Major Depressive Disorders. Int J Neuropsychopharmacol 20, 1036-1046.

Belujon, P., Jakobowski, N. L., Dollish, H. K., Grace, A. A., 2016. Withdrawal from Acute Amphetamine Induces an Amygdala-Driven Attenuation of Dopamine Neuron Activity: Reversal by Ketamine. Neuropsychopharmacology 41, 619-627.

Bertler, A., Rosengren, E., 1959. Occurrence and distribution of dopamine in brain and other tissues. Experientia 15, 10-11.

Bierut, L. J., Dinwiddie, S. H., Begleiter, H., Crowe, R. R., Hesselbrock, V., Nurnberger, J. I., Jr., Porjesz, B., Schuckit, M. A., Reich, T., 1998. Familial transmission of substance dependence: alcohol, marijuana, cocaine, and habitual smoking: a report from the Collaborative Study on the Genetics of Alcoholism. Arch Gen Psychiatry 55, 982-988.

Breese, G. R., Chu, K., Dayas, C. V., Funk, D., Knapp, D. J., Koob, G. F., Le, D. A., O'Dell, L. E., Overstreet, D. H., Roberts, A. J., Sinha, R., Valdez, G. R., Weiss, F., 2005. Stress enhancement of craving during sobriety: a risk for relapse. Alcohol Clin Exp Res 29, 185-195.

Brown, S. A., Vik, P. W., Patterson, T. L., Grant, I., Schuckit, M. A., 1995. Stress, vulnerability and adult alcohol relapse. J Stud Alcohol 56, 538-545.

Burns, J., 2013. Pathways from Cannabis to Psychosis: A Review of the Evidence. Frontiers in Psychiatry 4.

Calipari, E. S., Bagot, R. C., Purushothaman, I., Davidson, T. J., Yorgason, J. T., Pena, C. J., Walker, D. M., Pirpinias, S. T., Guise, K. G., Ramakrishnan, C., Deisseroth, K., Nestler, E. J., 2016. In vivo imaging identifies temporal signature of D1 and D2 medium spiny neurons in cocaine reward. Proc Natl Acad Sci U S A 113, 2726-2731.

Campbell, M. J., 2007. Medical statistics : a textbook for the health sciences. Chichester : Wiley.

Campos-Jurado, Y., Marti-Prats, L., Moron, J. A., Polache, A., Granero, L., Hipolito, L., 2020. Dose-dependent induction of CPP or

CPA by intra-pVTA ethanol: Role of mu opioid receptors and effects on NMDA receptors. Prog Neuropsychopharmacol Biol Psychiatry 100, 109875.

Carboni, E., Tanda, G. L., Frau, R., Di Chiara, G., 1990. Blockade of the noradrenaline carrier increases extracellular dopamine concentrations in the prefrontal cortex: evidence that dopamine is taken up in vivo by noradrenergic terminals. J Neurochem 55, 1067-1070.

Carlsson, A., 1959. The occurrence, distribution and physiological role of catecholamines in the nervous system. Pharmacol Rev 11, 490-493.

Carlsson, A., Lindqvist, M., 1963. Effect of Chlorpromazine or Haloperidol on Formation of 3methoxytyramine and Normetanephrine in Mouse Brain. Acta Pharmacol Toxicol (Copenh) 20, 140-144.

Carlsson, A., Lindqvist, M., Magnusson, T., 1957. 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature 180, 1200.

Charpantier, E., Barneoud, P., Moser, P., Besnard, F., Sgard, F., 1998. Nicotinic acetylcholine subunit mRNA expression in dopaminergic neurons of the rat substantia nigra and ventral tegmental area. Neuroreport 9, 3097-3101.

Chaurasia, C. S., Muller, M., Bashaw, E. D., Benfeldt, E., Bolinder, J., Bullock, R., Bungay, P. M., DeLange, E. C., Derendorf, H., Elmquist, W. F., Hammarlund-Udenaes, M., Joukhadar, C., Kellogg, D. L., Jr., Lunte, C. E., Nordstrom, C. H., Rollema, H., Sawchuk, R. J., Cheung, B. W., Shah, V. P., Stahle, L., Ungerstedt, U., Welty, D. F., Yeo, H., 2007. AAPS-FDA workshop white paper: microdialysis principles, application and regulatory perspectives. Pharm Res 24, 1014-1025.

Chiang, M., Lombardi, D., Du, J., Makrum, U., Sitthichai, R., Harrington, A., Shukair, N., Zhao, M., Fan, X., 2019. Methamphetamine-associated psychosis: Clinical presentation, biological basis, and treatment options. Hum Psychopharmacol 34, e2710.

Chojnacki, M. R., Jayanthi, S., Cadet, J. L., 2020. Methamphetamine pre-exposure induces steeper escalation of methamphetamine self-administration with consequent alterations in hippocampal glutamate AMPA receptor mRNAs. Eur J Pharmacol 889, 173732.

Ciliax, B. J., Drash, G. W., Staley, J. K., Haber, S., Mobley, C. J., Miller, G. W., Mufson, E. J., Mash, D. C., Levey, A. I., 1999. Immunocytochemical localization of the dopamine transporter in human brain. J Comp Neurol 409, 38-56.

Coffin, P. O., Santos, G. M., Das, M., Santos, D. M., Huffaker, S., Matheson, T., Gasper, J., Vittinghoff, E., Colfax, G. N., 2013. Aripiprazole for the treatment of methamphetamine dependence: a randomized, double-blind, placebo-controlled trial. Addiction 108, 751-761.

Conn, K. A., Burne, T. H. J., Kesby, J. P., 2020. Subcortical Dopamine and Cognition in Schizophrenia: Looking Beyond Psychosis in Preclinical Models. Front Neurosci 14, 542.

Corbit, L. H., Muir, J. L., Balleine, B. W., 2001. The role of the nucleus accumbens in instrumental conditioning: Evidence of a functional dissociation between accumbens core and shell. J Neurosci 21, 3251-3260.

Cotzias, G. C., Van Woert, M. H., Schiffer, L. M., 1967. Aromatic amino acids and modification of parkinsonism. N Engl J Med 276, 374-379.

Crabbe, J. C., Phillips, T. J., Belknap, J. K., 2010. The complexity of alcohol drinking: studies in rodent genetic models. Behav Genet 40, 737-750.

de Bejczy, A., Lof, E., Walther, L., Guterstam, J., Hammarberg, A., Asanovska, G., Franck, J., Isaksson, A., Soderpalm, B., 2015. Varenicline for treatment of alcohol dependence: a randomized, placebo-controlled trial. Alcohol Clin Exp Res 39, 2189-2199.

Der-Avakian, A., Markou, A., 2010. Withdrawal from chronic exposure to amphetamine, but not nicotine, leads to an immediate and enduring deficit in motivated behavior without affecting social interaction in rats. Behav Pharmacol 21, 359-368.

Durieux, P. F., Schiffmann, S. N., de Kerchove d'Exaerde, A., 2012. Differential regulation of motor control and response to dopaminergic drugs by D1R and D2R neurons in distinct dorsal striatum subregions. EMBO J 31, 640-653.

Elmslie, K. S., Yoshikami, D., 1985. Effects of kynurenate on root potentials evoked by synaptic activity and amino acids in the frog spinal cord. Brain Res 330, 265-272.

Erhardt, S., Schwieler, L., Engberg, G., 2003. Kynurenic acid and schizophrenia. Adv Exp Med Biol 527, 155-165.

Erhardt, S., Schwieler, L., Nilsson, L., Linderholm, K., Engberg, G., 2007. The kynurenic acid hypothesis of schizophrenia. Physiol Behav 92, 203-209.

Ericson, M., Lof, E., Stomberg, R., Chau, P., Soderpalm, B., 2008. Nicotinic acetylcholine receptors in the anterior, but not posterior, ventral tegmental area mediate ethanol-induced elevation of accumbal dopamine levels. J Pharmacol Exp Ther 326, 76-82.

Ericson, M., Lof, E., Stomberg, R., Soderpalm, B., 2009. The smoking cessation medication varenicline attenuates alcohol and nicotine interactions in the rat mesolimbic dopamine system. J Pharmacol Exp Ther 329, 225-230.

Ericson, M., Molander, A., Lof, E., Engel, J. A., Soderpalm, B., 2003. Ethanol elevates accumbal dopamine levels via indirect activation of ventral tegmental nicotinic acetylcholine receptors. Eur J Pharmacol 467, 85-93.

Ericson, M., Ulenius, L., Andren, A., Jonsson, S., Adermark, L., Soderpalm, B., 2020. Different dopamine tone in ethanol high- and low-consuming Wistar rats. Addict Biol 25, e12761.

Evans, A. H., Lees, A. J., 2004. Dopamine dysregulation syndrome in Parkinson's disease. Current Opinion in Neurology 17, 393-398.

Fanshawe, T. R., Halliwell, W., Lindson, N., Aveyard, P., Livingstone-Banks, J., Hartmann-Boyce, J., 2017. Tobacco cessation interventions for young people. Cochrane Database Syst Rev 11, CD003289.

Feduccia, A. A., Simms, J. A., Mill, D., Yi, H. Y., Bartlett, S. E., 2014. Varenicline decreases ethanol intake and increases dopamine release via neuronal nicotinic acetylcholine receptors in the nucleus accumbens. Br J Pharmacol 171, 3420-3431.

Fleckenstein, A. E., Volz, T. J., Riddle, E. L., Gibb, J. W., Hanson, G. R., 2007. New insights into the mechanism of action of amphetamines. Annu Rev Pharmacol Toxicol 47, 681-698.

Foddai, M., Dosia, G., Spiga, S., Diana, M., 2004. Acetaldehyde Increases Dopaminergic Neuronal Activity in the VTA. Neuropsychopharmacology 29, 530-536.

Fone, K. C., Porkess, M. V., 2008. Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders. Neurosci Biobehav Rev 32, 1087-1102.

Ford, C. P., 2014. The role of D2-autoreceptors in regulating dopamine neuron activity and transmission. Neuroscience 282, 13-22.

Fowler, J. S., Volkow, N. D., Wang, G. J., Pappas, N., Logan, J., MacGregor, R., Alexoff, D., Shea, C., Schlyer, D., Wolf, A. P., Warner, D., Zezulkova, I., Cilento, R., 1996. Inhibition of monoamine oxidase B in the brains of smokers. Nature 379, 733-736.

Frank, S. T., Krumm, B., Spanagel, R., 2008. Cocaine-induced dopamine overflow within the nucleus accumbens measured by in vivo microdialysis: a meta-analysis. Synapse 62, 243-252.

Goldstein, N., Carty, J. R. E., Betley, J. N., 2022. Specificity of Varenicline in Blocking Mesolimbic Circuit Activation to Natural and Drug Rewards. Neuroscience 483, 40-51.

Gonzales, R. A., Weiss, F., 1998. Suppression of ethanol-reinforced behavior by naltrexone is associated with attenuation of the ethanol-induced increase in dialysate dopamine levels in the nucleus accumbens. J Neurosci 18, 10663-10671.

Gorelick, D. A., Wilkins, J. N., 2006. Bromocriptine treatment for cocaine addiction: association with plasma prolactin levels. Drug Alcohol Depend 81, 189-195.

Grace, A. A., Bunney, B. S., 1984a. The control of firing pattern in nigral dopamine neurons: burst firing. J Neurosci 4, 2877-2890.

Grace, A. A., Bunney, B. S., 1984b. The control of firing pattern in nigral dopamine neurons: single spike firing. J Neurosci 4, 2866-2876.

Gremel, C. M., Costa, R. M., 2013. Orbitofrontal and striatal circuits dynamically encode the shift between goal-directed and habitual actions. Nat Commun 4, 2264.

Hardie, T. L., 2002. The genetics of substance abuse. AACN Clin Issues 13, 511-522.

Heilig, M., Egli, M., 2006. Pharmacological treatment of alcohol dependence: target symptoms and target mechanisms. Pharmacol Ther 111, 855-876.

Hipolito, L., Sanchez, M. J., Polache, A., Granero, L., 2007. Brain metabolism of ethanol and alcoholism: an update. Curr Drug Metab 8, 716-727.

Hohmann, A. G., Herkenham, M., 2000. Localization of cannabinoid CB(1) receptor mRNA in neuronal subpopulations of rat striatum: a double-label in situ hybridization study. Synapse 37, 71-80.

Hughes, J. R., Hatsukami, D. K., Mitchell, J. E., Dahlgren, L. A., 1986. Prevalence of smoking among psychiatric outpatients. Am J Psychiatry 143, 993-997.

Hunt, G. E., Large, M. M., Cleary, M., Lai, H. M. X., Saunders, J. B., 2018. Prevalence of comorbid substance use in schizophrenia spectrum disorders in community and clinical settings, 1990-2017: Systematic review and meta-analysis. Drug Alcohol Depend 191, 234-258.

Husky, M. M., Paliwal, P., Mazure, C. M., McKee, S. A., 2007. Gender differences in association with substance use diagnoses and smoking. J Addict Med 1, 161-164.

Ikemoto, S., 2007. Dopamine reward circuitry: Two projection systems from the ventral midbrain to the nucleus accumbens–olfactory tubercle complex. Brain Research Reviews 56, 27-78.

Ikemoto, S., 2010. Brain reward circuitry beyond the mesolimbic dopamine system: a neurobiological theory. Neurosci Biobehav Rev 35, 129-150.

Ikemoto, S., Yang, C., Tan, A., 2015. Basal ganglia circuit loops, dopamine and motivation: A review and enquiry. Behav Brain Res 290, 17-31.

Ingham, C. A., Hood, S. H., Taggart, P., Arbuthnott, G. W., 1998. Plasticity of synapses in the rat neostriatum after unilateral lesion of the nigrostriatal dopaminergic pathway. J Neurosci 18, 4732-4743.

Ito, R., Hayen, A., 2011. Opposing roles of nucleus accumbens core and shell dopamine in the modulation of limbic information processing. J Neurosci 31, 6001-6007.

Jaaro-Peled, H., 2009. Gene models of schizophrenia: DISC1 mouse models. Prog Brain Res 179, 75-86.

Jaaro-Peled, H., Hayashi-Takagi, A., Seshadri, S., Kamiya, A., Brandon, N. J., Sawa, A., 2009. Neurodevelopmental mechanisms of schizophrenia: understanding disturbed postnatal brain maturation through neuregulin-1-ErbB4 and DISC1. Trends Neurosci 32, 485-495.

Jablensky, A., 2000. Epidemiology of schizophrenia: the global burden of disease and disability. Eur Arch Psychiatry Clin Neurosci 250, 274-285.

Johnson, S. W., North, R. A., 1992. Opioids excite dopamine neurons by hyperpolarization of local interneurons. J Neurosci 12, 483-488.

Johnstone, E. C., Crow, T. J., Frith, C. D., Carney, M. W., Price, J. S., 1978. Mechanism of the antipsychotic effect in the treatment of acute schizophrenia. Lancet 1, 848-851.

Jonas, D. E., Amick, H. R., Feltner, C., Bobashev, G., Thomas, K., Wines, R., Kim, M. M., Shanahan, E., Gass, C. E., Rowe, C. J., Garbutt, J. C., 2014. Pharmacotherapy for adults with alcohol use disorders in outpatient settings: a systematic review and meta-analysis. JAMA 311, 1889-1900.

Juckel, G., Schlagenhauf, F., Koslowski, M., Filonov, D., Wustenberg, T., Villringer, A., Knutson, B., Kienast, T., Gallinat, J., Wrase, J., Heinz, A., 2006. Dysfunction of ventral striatal reward prediction in schizophrenic patients treated with typical, not atypical, neuroleptics. Psychopharmacology (Berl) 187, 222-228.

Kankaanpaa, A., Meririnne, E., Lillsunde, P., Seppala, T., 1998. The acute effects of amphetamine derivatives on extracellular serotonin and dopamine levels in rat nucleus accumbens. Pharmacol Biochem Behav 59, 1003-1009.

Karakaya, S., Kipp, M., Beyer, C., 2007. Oestrogen regulates the expression and function of dopamine transporters in astrocytes of the nigrostriatal system. J Neuroendocrinol 19, 682-690.

Karkhanis, A. N., Rose, J. H., Huggins, K. N., Konstantopoulos, J. K., Jones, S. R., 2015. Chronic intermittent ethanol exposure reduces presynaptic dopamine neurotransmission in the mouse nucleus accumbens. Drug Alcohol Depend 150, 24-30.

Katthagen, T., Kaminski, J., Heinz, A., Buchert, R., Schlagenhauf, F., 2020. Striatal Dopamine and Reward Prediction Error Signaling in Unmedicated Schizophrenia Patients. Schizophr Bull 46, 1535-1546.

Kesby, J. P., Chang, A., Markou, A., Semenova, S., 2018. Modeling human methamphetamine use patterns in mice: chronic and binge methamphetamine exposure, reward function and neurochemistry. Addict Biol 23, 206-218.

Kessler, M., Terramani, T., Lynch, G., Baudry, M., 1989. A glycine site associated with N-methyl-D-aspartic acid receptors: characterization and identification of a new class of antagonists. J Neurochem 52, 1319-1328.

Khokhar, J. Y., Dwiel, L. L., Henricks, A. M., Doucette, W. T., Green, A. I., 2018. The link between schizophrenia and substance use disorder: A unifying hypothesis. Schizophr Res 194, 78-85.

Koob, G. F., Le Moal, M., 1997. Drug abuse: hedonic homeostatic dysregulation. Science 278, 52-58.

Koob, G. F., Le Moal, M., 2001. Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24, 97-129.

Koob, G. F., Le Moal, M., 2008. Addiction and the brain antireward system. Annu Rev Psychol 59, 29-53.

Kranzler, H. R., Van Kirk, J., 2001. Efficacy of naltrexone and acamprosate for alcoholism treatment: a meta-analysis. Alcohol Clin Exp Res 25, 1335-1341.

Kuhn, J., Lenartz, D., Huff, W., Lee, S. H., Koulousakis, A., Klosterkoetter, J., Sturm, V., 2009. Remission of alcohol dependency following deep brain stimulation of the nucleus accumbens: valuable therapeutic implications? BMJ Case Rep 2009.

Kumari, V., Postma, P., 2005. Nicotine use in schizophrenia: the self medication hypotheses. Neurosci Biobehav Rev 29, 1021-1034.

Kusljic, S., van den Buuse, M., Gogos, A., 2022. Reassessment of amphetamine- and phencyclidine-induced locomotor hyperactivity as a model of psychosis-like behavior in rats. J Integr Neurosci 21, 17.

Lagstrom, O., Danielsson, K., Soderpalm, B., Ericson, M., Adermark, L., 2019. Voluntary ethanol intake produces sub-region specific neuroadaptations in striatal and cortical areas of Wistar rat. Alcohol Clin Exp Res.

Landgren, S., Engel, J. A., Hyytia, P., Zetterberg, H., Blennow, K., Jerlhag, E., 2011. Expression of the gene encoding the ghrelin receptor in rats selected for differential alcohol preference. Behav Brain Res 221, 182-188.

Lapiz, M. D., Fulford, A., Muchimapura, S., Mason, R., Parker, T., Marsden, C. A., 2003. Influence of postweaning social isolation in the rat on brain development, conditioned behavior, and neurotransmission. Neurosci Behav Physiol 33, 13-29.

Leucht, S., Cipriani, A., Spineli, L., Mavridis, D., Orey, D., Richter, F., Samara, M., Barbui, C., Engel, R. R., Geddes, J. R., Kissling, W., Stapf, M. P., Lassig, B., Salanti, G., Davis, J. M., 2013. Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. Lancet 382, 951-962.

Leucht, S., Corves, C., Arbter, D., Engel, R. R., Li, C., Davis, J. M., 2009. Second-generation versus first-generation antipsychotic drugs for schizophrenia: a meta-analysis. Lancet 373, 31-41.

Lhost, J., More, S., Watabe, I., Louber, D., Ouagazzal, A. M., Liberge, M., Amalric, M., 2021. Interplay Between Inhibitory Control and Behavioural Flexibility: Impact of Dorsomedial Striatal Dopamine Denervation in Mice. Neuroscience 477, 25-39.

Liao, R. M., Chang, Y. H., Wang, S. H., Lan, C. H., 2000. Distinct accumbal subareas are involved in place conditioning of amphetamine and cocaine. Life Sci 67, 2033-2043.

Lido, H. H., Ericson, M., Marston, H., Soderpalm, B., 2011. A role for accumbal glycine receptors in modulation of dopamine release by the glycine transporter-1 inhibitor org25935. Front Psychiatry 2, 8.

Litten, R. Z., Ryan, M. L., Fertig, J. B., Falk, D. E., Johnson, B., Dunn, K. E., Green, A. I., Pettinati, H. M., Ciraulo, D. A., Sarid-Segal, O.,

Kampman, K., Brunette, M. F., Strain, E. C., Tiouririne, N. A., Ransom, J., Scott, C., Stout, R., Group, N. S., 2013. A double-blind, placebo-controlled trial assessing the efficacy of varenicline tartrate for alcohol dependence. J Addict Med 7, 277-286.

Lodge, D. J., Grace, A. A., 2009. Gestational methylazoxymethanol acetate administration: a developmental disruption model of schizophrenia. Behav Brain Res 204, 306-312.

Lu, J., Cheng, Y., Xie, X., Woodson, K., Bonifacio, J., Disney, E., Barbee, B., Wang, X., Zaidi, M., Wang, J., 2021. Whole-Brain Mapping of Direct Inputs to Dopamine D1 and D2 Receptor-Expressing Medium Spiny Neurons in the Posterior Dorsomedial Striatum. eNeuro 8.

Läkemedelskommittén, 2022. REKlistan 2022. In: Götalandsregionen, L. i. V., (Ed).

Ma, T., Huang, Z., Xie, X., Cheng, Y., Zhuang, X., Childs, M. J., Gangal, H., Wang, X., Smith, L. N., Smith, R. J., Zhou, Y., Wang, J., 2022. Chronic alcohol drinking persistently suppresses thalamostriatal excitation of cholinergic neurons to impair cognitive flexibility. J Clin Invest 132.

Malcolm, R., Herron, J., Sutherland, S. E., Brady, K. T., 2001. Adverse outcomes in a controlled trial of pergolide for cocaine dependence. J Addict Dis 20, 81-92.

Malcolm, R., Kajdasz, D. K., Herron, J., Anton, R. F., Brady, K. T., 2000. A double-blind, placebo-controlled outpatient trial of pergolide for cocaine dependence. Drug Alcohol Depend 60, 161-168.

Martinez, D., Broft, A., Foltin, R. W., Slifstein, M., Hwang, D. R., Huang, Y., Perez, A., Frankle, W. G., Cooper, T., Kleber, H. D., Fischman, M. W., Laruelle, M., 2004. Cocaine dependence and d2 receptor availability in the functional subdivisions of the striatum: relationship with cocaine-seeking behavior. Neuropsychopharmacology 29, 1190-1202.

Martinez, D., Gil, R., Slifstein, M., Hwang, D. R., Huang, Y., Perez, A., Kegeles, L., Talbot, P., Evans, S., Krystal, J., Laruelle, M., Abi-Dargham, A., 2005. Alcohol dependence is associated with blunted dopamine transmission in the ventral striatum. Biol Psychiatry 58, 779-786.

McCutcheon, R., Beck, K., Jauhar, S., Howes, O. D., 2018. Defining the Locus of Dopaminergic Dysfunction in Schizophrenia: A Meta-analysis and Test of the Mesolimbic Hypothesis. Schizophr Bull 44, 1301-1311.

McCutcheon, R. A., Abi-Dargham, A., Howes, O. D., 2019. Schizophrenia, Dopamine and the Striatum: From Biology to Symptoms. Trends Neurosci 42, 205-220.

McKee, S. A., Falba, T., O'Malley, S. S., Sindelar, J., O'Connor, P. G., 2007. Smoking status as a clinical indicator for alcohol misuse in US adults. Arch Intern Med 167, 716-721.

McLellan, A. T., Lewis, D. C., O'Brien, C. P., Kleber, H. D., 2000. Drug dependence, a chronic medical illness: implications for treatment, insurance, and outcomes evaluation. JAMA 284, 1689-1695.

Mei, L., Xiong, W. C., 2008. Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. Nat Rev Neurosci 9, 437-452.

Mei, Y. Y., Wu, D. C., Zhou, N., 2018. Astrocytic Regulation of Glutamate Transmission in Schizophrenia. Front Psychiatry 9, 544.

Melis, M., Diana, M., Enrico, P., Marinelli, M., Brodie, M. S., 2009. Ethanol and acetaldehyde action on central dopamine systems: mechanisms, modulation, and relationship to stress. Alcohol 43, 531-539.

Millan, M. J., Fone, K., Steckler, T., Horan, W. P., 2014. Negative symptoms of schizophrenia: clinical characteristics, pathophysiological substrates, experimental models and prospects for improved treatment. Eur Neuropsychopharmacol 24, 645-692.

Mitchell, J. M., Teague, C. H., Kayser, A. S., Bartlett, S. E., Fields, H. L., 2012. Varenicline decreases alcohol consumption in heavy-drinking smokers. Psychopharmacology (Berl) 223, 299-306.

Miyake, N., Miyamoto, S., Jarskog, L. F., 2012. New serotonin/dopamine antagonists for the treatment of schizophrenia: are we making real progress? Clin Schizophr Relat Psychoses 6, 122-133.

Molander, A., Soderpalm, B., 2005. Glycine receptors regulate dopamine release in the rat nucleus accumbens. Alcohol Clin Exp Res 29, 17-26.

Montagu, K. A., 1957. Catechol compounds in rat tissues and in brains of different animals. Nature 180, 244-245.

Montero, T., Gatica, R. I., Farassat, N., Meza, R., Gonzalez-Cabrera, C., Roeper, J., Henny, P., 2021. Dendritic Architecture Predicts in vivo Firing Pattern in Mouse Ventral Tegmental Area and Substantia Nigra Dopaminergic Neurons. Front Neural Circuits 15, 769342.

Moore, H., Jentsch, J. D., Ghajarnia, M., Geyer, M. A., Grace, A. A., 2006. A neurobehavioral systems analysis of adult rats exposed to methylazoxymethanol acetate on E17: implications for the neuropathology of schizophrenia. Biol Psychiatry 60, 253-264.

Moore, T. H., Zammit, S., Lingford-Hughes, A., Barnes, T. R., Jones, P. B., Burke, M., Lewis, G., 2007. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet 370, 319-328.

Morud, J., Adermark, L., Perez-Alcazar, M., Ericson, M., Soderpalm, B., 2016. Nicotine produces chronic behavioral sensitization with changes in accumbal neurotransmission and increased sensitivity to re-exposure. Addict Biol 21, 397-406.

Mueser, K. T., McGurk, S. R., 2004. Schizophrenia. Lancet 363, 2063-2072.

Nestler, E. J., Kenny, P. J., Russo, S. J., Schaefer, A., 2020. Neural and Neuroendocrine Control of the Internal Milieu. Nestler, Hyman & Malenka's Molecular Neuropharmacology: A Foundation for Clinical Neuroscience, 4e. McGraw-Hill, New York, NY.

Neve, K. A., Seamans, J. K., Trantham-Davidson, H., 2004. Dopamine receptor signaling. J Recept Signal Transduct Res 24, 165-205.

Newton, T. F., Reid, M. S., De La Garza, R., Mahoney, J. J., Abad, A., Condos, R., Palamar, J., Halkitis, P. N., Mojisak, J., Anderson, A., Li, S. H., Elkashef, A., 2008. Evaluation of subjective effects of aripiprazole and methamphetamine in methamphetamine-dependent volunteers. Int J Neuropsychopharmacol 11, 1037-1045.

Nisell, M., Nomikos, G. G., Svensson, T. H., 1994. Systemic nicotine-induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. Synapse 16, 36-44.

Nomikos, G. G., Damsma, G., Wenkstern, D., Fibiger, H. C., 1989. Acute effects of bupropion on extracellular dopamine concentrations in rat striatum and nucleus accumbens studied by in vivo microdialysis. Neuropsychopharmacology 2, 273-279.

O'Malley, S. S., Zweben, A., Fucito, L. M., Wu, R., Piepmeier, M. E., Ockert, D. M., Bold, K. W., Petrakis, I., Muvvala, S., Jatlow, P., Gueorguieva, R., 2018. Effect of Varenicline Combined With Medical Management on Alcohol Use Disorder With Comorbid Cigarette Smoking: A Randomized Clinical Trial. JAMA Psychiatry 75, 129-138.

Olsson, I. A. S., Westlund, K., 2007. More than numbers matter: The effect of social factors on behaviour and welfare of laboratory rodents and non-human primates. Applied Animal Behaviour Science 103, 229-254.

Omelchenko, N., Sesack, S. R., 2007. Glutamate synaptic inputs to ventral tegmental area neurons in the rat derive primarily from subcortical sources. Neuroscience 146, 1259-1274.

Palm, S., Roman, E., Nylander, I., 2011. Differences in voluntary ethanol consumption in Wistar rats from five different suppliers. Alcohol 45, 607-614.

Palm, S., Roman, E., Nylander, I., 2012. Differences in basal and ethanol-induced levels of opioid peptides in Wistar rats from five different suppliers. Peptides 36, 1-8.

Peroutka, S. J., Synder, S. H., 1980. Relationship of neuroleptic drug effects at brain dopamine, serotonin, alpha-adrenergic, and histamine receptors to clinical potency. Am J Psychiatry 137, 1518-1522.

Pierce, R. C., Kumaresan, V., 2006. The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? Neurosci Biobehav Rev 30, 215-238.

Potvin, S., Sepehry, A. A., Stip, E., 2006. A meta-analysis of negative symptoms in dual diagnosis schizophrenia. Psychol Med 36, 431-440.

Rada, P., Mark, G. P., Pothos, E., Hoebel, B. G., 1991. Systemic morphine simultaneously decreases extracellular acetylcholine and increases dopamine in the nucleus accumbens of freely moving rats. Neuropharmacology 30, 1133-1136.

Richter, A., Reinhard, F., Kraemer, B., Gruber, O., 2020. A high-resolution fMRI approach to characterize functionally distinct neural pathways within dopaminergic midbrain and nucleus accumbens during reward and salience processing. Eur Neuropsychopharmacol 36, 137-150.

Robinson, T. E., Berridge, K. C., 1993. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 18, 247-291.

Rolls, E. T., 2012. Glutamate, obsessive-compulsive disorder, schizophrenia, and the stability of cortical attractor neuronal networks. Pharmacol Biochem Behav 100, 736-751.

Russo, S. J., Nestler, E. J., 2013. The brain reward circuitry in mood disorders. Nat Rev Neurosci 14, 609-625.

Sabino, V., Kwak, J., Rice, K. C., Cottone, P., 2013. Pharmacological characterization of the 20% alcohol intermittent access model in Sardinian alcohol-preferring rats: a model of binge-like drinking. Alcohol Clin Exp Res 37, 635-643.

Saddoris, M. P., Cacciapaglia, F., Wightman, R. M., Carelli, R. M., 2015. Differential Dopamine Release Dynamics in the Nucleus Accumbens Core and Shell Reveal Complementary Signals for Error Prediction and Incentive Motivation. J Neurosci 35, 11572-11582.

Sagud, M., Mihaljevic Peles, A., Pivac, N., 2019. Smoking in schizophrenia: recent findings about an old problem. Curr Opin Psychiatry 32, 402-408.

Schaub, A. C., Kirschner, M., Schweinfurth, N., Mahlmann, L., Kettelhack, C., Engeli, E. E., Doll, J. P. K., Borgwardt, S., Lang, U. E., Kaiser, S., Walter, M., Herdener, M., Wrege, J., Schmidt, A., 2021. Neural mapping of anhedonia across psychiatric diagnoses: A transdiagnostic neuroimaging analysis. Neuroimage Clin 32, 102825.

Schilstrom, B., Rawal, N., Mameli-Engvall, M., Nomikos, G. G., Svensson, T. H., 2003. Dual effects of nicotine on dopamine neurons mediated by different nicotinic receptor subtypes. Int J Neuropsychopharmacol 6, 1-11.

Schulteis, G., Markou, A., Cole, M., Koob, G. F., 1995. Decreased brain reward produced by ethanol withdrawal. Proc Natl Acad Sci U S A 92, 5880-5884.

Seiler, J. L., Cosme, C. V., Sherathiya, V. N., Schaid, M. D., Bianco, J. M., Bridgemohan, A. S., Lerner, T. N., 2022. Dopamine signaling in the dorsomedial striatum promotes compulsive behavior. Curr Biol 32, 1175-1188 e1175.

Sekiguchi, H., Pavey, G., Dean, B., 2019. Altered levels of dopamine transporter in the frontal pole and dorsal striatum in schizophrenia. NPJ Schizophr 5, 20.

Shen, L. H., Liao, M. H., Tseng, Y. C., 2012. Recent advances in imaging of dopaminergic neurons for evaluation of neuropsychiatric disorders. J Biomed Biotechnol 2012, 259349.

Shen, W. W., 1999. A history of antipsychotic drug development. Compr Psychiatry 40, 407-414.

Smith, A., Li, M., Becker, S., Kapur, S., 2004. A Model of Antipsychotic Action in Conditioned Avoidance: A Computational Approach. Neuropsychopharmacology 29, 1040-1049.

Smith, A. D., Bolam, J. P., 1990. The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. Trends Neurosci 13, 259-265.

Smith, A. J., Clutton, R. E., Lilley, E., Hansen, K. E. A., Brattelid, T., 2018. PREPARE: guidelines for planning animal research and testing. Lab Anim 52, 135-141.

Soderpalm, B., Ericson, M., 2013. Neurocircuitry involved in the development of alcohol addiction: the dopamine system and its access points. Curr Top Behav Neurosci 13, 127-161.

Soderpalm, B., Lido, H. H., Ericson, M., 2017. The Glycine Receptor-A Functionally Important Primary Brain Target of Ethanol. Alcohol Clin Exp Res 41, 1816-1830.

Soyka, M., Chick, J., 2003. Use of acamprosate and opioid antagonists in the treatment of alcohol dependence: a European perspective. Am J Addict 12, s69-s80.

Spagnolo, P. A., Ramchandani, V. A., Schwandt, M. L., Zhang, L., Blaine, S. K., Usala, J. M., Diamond, K. A., Phillips, M. J., George, D. T., Momenan, R., Heilig, M., 2014. Effects of naltrexone on neural and subjective response to alcohol in treatment-seeking alcohol-dependent patients. Alcohol Clin Exp Res 38, 3024-3032.

Spanagel, R., Holter, S. M., 2000. Pharmacological validation of a new animal model of alcoholism. J Neural Transm (Vienna) 107, 669-680.

Srisurapanont, M., Jarusuraisin, N., 2002. Opioid antagonists for alcohol dependence. Cochrane Database Syst Rev, CD001867.

Stahl, S. M., 2018. Beyond the dopamine hypothesis of schizophrenia to three neural networks of psychosis: dopamine, serotonin, and glutamate. CNS Spectr 23, 187-191.

Steensland, P., Simms, J. A., Holgate, J., Richards, J. K., Bartlett, S. E., 2007. Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, selectively decreases ethanol consumption and seeking. Proc Natl Acad Sci U S A 104, 12518-12523.

Steketee, J. D., Kalivas, P. W., 2011. Drug wanting: behavioral sensitization and relapse to drug-seeking behavior. Pharmacol Rev 63, 348-365.

Tepper, J. M., Bolam, J. P., 2004. Functional diversity and specificity of neostriatal interneurons. Curr Opin Neurobiol 14, 685-692.

Thapar, A., Cooper, M., Eyre, O., Langley, K., 2013. What have we learnt about the causes of ADHD? J Child Psychol Psychiatry 54, 3-16.

Theilmann, W., Kleimann, A., Rhein, M., Bleich, S., Frieling, H., Loscher, W., Brandt, C., 2016. Behavioral differences of male Wistar rats from different vendors in vulnerability and resilience to chronic mild stress are reflected in epigenetic regulation and expression of p11. Brain Res 1642, 505-515.

Tizabi, Y., Bai, L., Copeland, R. L., Jr., Taylor, R. E., 2007. Combined effects of systemic alcohol and nicotine on dopamine release in the nucleus accumbens shell. Alcohol Alcohol 42, 413-416.

Trifu, S. C., Kohn, B., Vlasie, A., Patrichi, B. E., 2020. Genetics of schizophrenia (Review). Exp Ther Med 20, 3462-3468.

Tritsch, N. X., Ding, J. B., Sabatini, B. L., 2012. Dopaminergic neurons inhibit striatal output through non-canonical release of GABA. Nature 490, 262-266.

Tseng, K. Y., Chambers, R. A., Lipska, B. K., 2009. The neonatal ventral hippocampal lesion as a heuristic neurodevelopmental model of schizophrenia. Behav Brain Res 204, 295-305.

Tsuda, M. C., Mahdi, S., Namchuk, A., Wu, T. J., Lucki, I., 2020. Vendor differences in anxiety-like behaviors in female and male Sprague Dawley rats. Physiol Behav 227, 113131.

Tukey, J. W., 1949. Comparing Individual Means in the Analysis of Variance. Biometrics 5, 99-114.

Uno, Y., Coyle, J. T., 2019. Glutamate hypothesis in schizophrenia. Psychiatry Clin Neurosci 73, 204-215.

Valenta, J. P., Job, M. O., Mangieri, R. A., Schier, C. J., Howard, E. C., Gonzales, R. A., 2013. mu-opioid receptors in the stimulation of mesolimbic dopamine activity by ethanol and morphine in Long-Evans rats: a delayed effect of ethanol. Psychopharmacology (Berl) 228, 389-400.

van Os, J., Kapur, S., 2009. Schizophrenia. Lancet 374, 635-645.

Vatsalya, V., Gowin, J. L., Schwandt, M. L., Momenan, R., Coe, M. A., Cooke, M. E., Hommer, D. W., Bartlett, S., Heilig, M., Ramchandani, V. A., 2015. Effects of Varenicline on Neural Correlates of Alcohol Salience in Heavy Drinkers. Int J Neuropsychopharmacol 18.

Vazquez-Leon, P., Martinez-Mota, L., Quevedo-Corona, L., Miranda-Paez, A., 2017. Isolation stress and chronic mild stress induced immobility in the defensive burying behavior and a transient increased ethanol intake in Wistar rats. Alcohol 63, 43-51.

Vena, A. A., Mangieri, R., Gonzales, R. A., 2016. Regional Analysis of the Pharmacological Effects of Acute Ethanol on Extracellular Striatal Dopamine Activity. Alcohol Clin Exp Res 40, 2528-2536.

Venton, B. J., Seipel, A. T., Phillips, P. E., Wetsel, W. C., Gitler, D., Greengard, P., Augustine, G. J., Wightman, R. M., 2006. Cocaine increases dopamine release by mobilization of a synapsin-dependent reserve pool. J Neurosci 26, 3206-3209.

Voce, A., Burns, R., Castle, D., Calabria, B., McKetin, R., 2019. Is there a discrete negative symptom syndrome in people who use methamphetamine? Compr Psychiatry 93, 27-32.

Voce, A., McKetin, R., Burns, R., Castle, D., Calabria, B., 2018. The relationship between illicit amphetamine use and psychiatric symptom profiles in schizophrenia and affective psychoses. Psychiatry Res 265, 19-24.

Volkow, N. D., Fowler, J. S., Wolf, A. P., Schlyer, D., Shiue, C. Y., Alpert, R., Dewey, S. L., Logan, J., Bendriem, B., Christman, D., et al., 1990. Effects of chronic cocaine abuse on postsynaptic dopamine receptors. Am J Psychiatry 147, 719-724.

Volkow, N. D., Wang, G. J., Fowler, J. S., Logan, J., Hitzemann, R., Ding, Y. S., Pappas, N., Shea, C., Piscani, K., 1996. Decreases in dopamine receptors but not in dopamine transporters in alcoholics. Alcohol Clin Exp Res 20, 1594-1598.

Ward, R. D., Winiger, V., Higa, K. K., Kahn, J. B., Kandel, E. R., Balsam, P. D., Simpson, E. H., 2015. The impact of motivation on cognitive performance in an animal model of the negative and cognitive symptoms of schizophrenia. Behav Neurosci 129, 292-299.

Warner, C., Shoaib, M., 2005. How does bupropion work as a smoking cessation aid? Addict Biol 10, 219-231.

Watson, J., Guzzetti, S., Franchi, C., Di Clemente, A., Burbassi, S., Emri, Z., Leresche, N., Parri, H. R., Crunelli, V., Cervo, L., 2010. Gamma-hydroxybutyrate does not maintain self-administration but induces conditioned place preference when injected in the ventral tegmental area. Int J Neuropsychopharmacol 13, 143-153.

Weiss, F., Parsons, L. H., Schulteis, G., Hyytia, P., Lorang, M. T., Bloom, F. E., Koob, G. F., 1996. Ethanol self-administration restores withdrawal-associated deficiencies in accumbal dopamine and 5-hydroxytryptamine release in dependent rats. J Neurosci 16, 3474-3485.

Wiesbeck, G. A., Weijers, H. G., Lesch, O. M., Glaser, T., Toennes, P. J., Boening, J., 2001. Flupenthixol decanoate and relapse prevention in alcoholics: results from a placebo-controlled study. Alcohol Alcohol 36, 329-334.

Wise, R. A., 1973. Voluntary ethanol intake in rats following exposure to ethanol on various schedules. Psychopharmacologia 29, 203-210.

Wolf, D. H., Satterthwaite, T. D., Kantrowitz, J. J., Katchmar, N., Vandekar, L., Elliott, M. A., Ruparel, K., 2014. Amotivation in schizophrenia: integrated assessment with behavioral, clinical, and imaging measures. Schizophr Bull 40, 1328-1337.

Yan, Y., Peng, C., Arvin, M. C., Jin, X. T., Kim, V. J., Ramsey, M. D., Wang, Y., Banala, S., Wokosin, D. L., McIntosh, J. M., Lavis, L. D., Drenan, R. M., 2018. Nicotinic Cholinergic Receptors in VTA Glutamate Neurons Modulate Excitatory Transmission. Cell Rep 23, 2236-2244.

Yin, H. H., Ostlund, S. B., Knowlton, B. J., Balleine, B. W., 2005. The role of the dorsomedial striatum in instrumental conditioning. Eur J Neurosci 22, 513-523.

Yu, M. F., Chien, C. L., Lee, W. T., Yin, H. S., 2005. Effects of acute amphetamine administration on AMPA-mediated synaptic activity and expression of AMPA receptor subunit 2 of brain neurons. J Mol Neurosci 25, 171-181.

Zhao, S., Chen, H., 2020. Modeling the epidemic dynamics and control of COVID-19 outbreak in China. Quant Biol, 1-9.