Thesis for the degree of Doctor of Medicine

ETHANOL-INDUCED MODULATION OF DOPAMINE TRANSMISSION AND SYNAPTIC ACTIVITY IN STRIATAL SUBREGIONS

– focus on inhibitory receptors

Rhona Clarke

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UNIVERSITY OF GOTHENBURG

Addiction Biology Unit
Department of Psychiatry and Neurochemistry
Institute of Neuroscience and Physiology
The Sahlgrenska Academy, University of Gothenburg

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To my family

This thesis is based on the following research papers, which will be referred to in the text by their Roman numerals:

I. **Clarke RB**, Adermark L, Chau P, Söderpalm B, Ericson M. (2014). Increase in nucleus accumbens dopamine levels following local ethanol administration is not mediated by acetaldehyde. *Alcohol and Alcoholism*, 49:498-504

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IV. **Clarke RB**, Söderpalm B, Lotfi A, Ericson M, Adermark L (2015). Involvement of inhibitory receptors in regulating dopamine signalling and synaptic activity following acute ethanol exposure in striatal subregions. *Manuscript*.

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ABSTRACT

Background: Alcoholism is a chronic brain disease, affecting neurocircuitries involved in reward and learning. The rewarding effects of alcohol (ethanol) are believed to result from increased dopamine levels in the nucleus accumbens (nAc) via the mesolimbic system. The exact mechanisms through which this occurs are debated, but evidence from the current research group suggests that ethanol activates the mesolimbic system via a reciprocal connection between the nAc and the ventral tegmental area (VTA), involving the activation of glycine receptors (GlyRs) in the nAc. Research from other groups suggests that ethanol may activate the mesolimbic system via its primary metabolite, acetaldehyde, through direct actions in the VTA. The effects of acetaldehyde in the nAc-VTA-nAc neuronal circuitry however, have not been investigated. Dopamine signalling is also important in the dorsolateral striatum (DLS), an area involved in habit formation. The effects of ethanol on dopamine levels in this region are however poorly understood, as are the roles of inhibitory GlyRs and γ-amino-butyric acid type A ($GABA_A$) receptors, in mediating these effects. **Aims:** To explore the effects of ethanol (or acetaldehyde) on dopamine transmission and synaptic activity in the nAc and DLS of ethanol-naïve rats. Special emphasis is placed on the involvement of GlyRs and $GABA_A$ receptors. **Methods:** Dopamine transmission was studied using *in vivo* microdialysis in awake, adult Wistar rats. This method was also used for local administration of relevant drugs/substances. Synaptic activity was measured by *in vitro* field-potential recordings in coronal brain slices from juvenile and adult Wistar rats. **Results:** Local acetaldehyde administration did not increase nAc dopamine levels, nor did sequestering of ethanol-derived acetaldehyde affect the dopamine-elevating properties of ethanol. Results also showed that the dopamine-enhancing effects of ethanol were mediated by GlyRs in the nAc, but neither by GlyRs nor $GABA_A$ receptors in the DLS. Ethanol produced both enhancing and depressing effects on synaptic activity, which were dependent on the region studied, the age of the animal, as well as the concentration applied. The relative involvement of inhibitory receptors also differed in an age and region-specific manner. **Conclusions:** The results in this thesis indicate that acetaldehyde is not involved in the dopamine-enhancing effects of ethanol that are mediated via the reciprocal nAc-VTA-nAc neuronal circuitry. Furthermore, it is shown that changes in dopamine and synaptic activity induced by acute ethanol administration are modulated by inhibitory receptors in a subregion and age-specific manner. By pinpointing similarities and differences in response to alcohol between reward-related and habit-related parts of the brain this research may contribute to furthering the knowledge of how alcohol addiction develops and progresses.

LIST OF ABBREVIATIONS

5-HT – 5-hydroxytryptamine (serotonin)
6-OHDA – 6-hydroxydopamine
ADH – Alcohol dehydrogenase
ALDH – Acetaldehyde dehydrogenase
ANOVA – Analysis of variance
AP-5 – DL-2-amino-5-phosphonopentanoic acid
CI – Confidence interval
CNS – Central nervous system
CYP 2E1 – Cytochrome P_{450} 2E1
DMS 5 – Diagnostic and Statistical Manual of Mental Disorders 5th edition
DLS – Dorsolateral striatum
DMS – Dorsomedial striatum
GABA – γ-amino-butyric acid
HPLC – High performance liquid chromatography
i.p. – Intraperitoneally
MLA – Methyllycaconitine citrate
MSN – Medium spiny neuron
nAc – Nucleus accumbens
nAChR – Nicotinic acetylcholine receptor
NMDA – *N*-methyl-*D*-aspartate
NOS – Nitric oxide syntetase
NPY – Neuropeptide Y
PMBA – Phenylbenzene-ω-phosphono-α-amino acid
rvCP – Rostroventral caudate putamen
SEM – Standard error of the mean
shRNA – Small hairpin RNA
SN – Substantia nigra
SNc – Substantia nigra pars compacta
SNr – Substantia nigra pars reticulata
VTA – Ventral tegmental area
WHO – World Health Organisation

PREFACE

Alcohol is probably the oldest recreational drug used by man; the discovery of Neolithic beer jugs suggests that intentionally fermented beverages have existed for at least 12 000 years. To this day alcohol remains an integral part of most western societies and traditions, from the friendly Irish pub to the Scandinavian midsummer snaps. For most people, a moderate consumption of alcohol can enhance the quality of life, augment the flavour of delicious meals and intensify merriment, as well as relaxation. For a subset of consumers, however, alcohol intake becomes increasingly excessive and compulsive, despite serious adverse consequences; a pathological state known as addiction.

Although historically seen as the result of a flawed personality or unfortunate circumstance, addiction is increasingly viewed as a chronic brain disease, affecting areas involved in reward, learning and self-control. While the pathological agent may be obvious (i.e. alcohol itself) the neurobiological mechanisms through which the pathology progresses - from recreational drinking to compulsive use - remain to be elucidated. In addition, although several pharmacological treatment options are available, to date none have proven particularly effective. Therefore, as a basis for future pharmacotherapies, a greater understanding of the mechanisms of action of alcohol is needed.

This project studies the effects of alcohol in different parts of the striatum, an area of the brain highly relevant to reward and habit learning. Special focus is placed on the involvement of inhibitory neurotransmitter systems glycine and GABA. Hopefully, by elucidating subregional similarities and differences in the response to alcohol, a small piece of knowledge can be added to the great puzzle that is addiction, thus furthering our understanding of this debilitating illness.

April 2015

INTRODUCTION

Alcohol consumption in Sweden and the EU

Alcohol has long been an intrinsic part of many societies and traditions, in particular in western cultures. Production of alcoholic beverages constitutes a massive industry, with worldwide sales approaching 1 trillion dollars annually [1]. A moderate intake of alcoholic beverages may even provide certain health benefits, such as reducing the risk of coronary heart disease and type II diabetes [2], although this remains a controversial issue. The negative consequences of alcohol consumption, however, have been far more thoroughly investigated; in Europe alcohol is the third leading risk factor for disease and mortality, after tobacco use and hypertension [3]. Alcohol abuse and addiction are serious public health issues causing not only great suffering to the afflicted individual, but also leading to significant societal expenses. In Sweden alone the costs to society related to alcohol consumption have been estimated between and 66-150 billion SEK annually [4, 5].

The European Union has the highest consumption of alcohol in the world; in 2009 the average annual consumption among adults was estimated to 12.5 litres of pure alcohol [6], which corresponds to 27 g of alcohol - the rough equivalent of two standard drinks per day[1]. Consumption rates and patterns vary greatly between EU countries however, with Sweden ranking among those with the lowest consumption per capita (9.9 litres/year in 2013 [7]). Despite this, approximately 20% of men and 13% of women in Sweden are estimated to have a hazardous consumption[2] of alcohol [5].

Alcohol: abuse, addiction and dependence

While the term hazardous consumption usually refers to consuming alcohol in such an amount or manner as it may cause damage to one's health or put the individual at risk for developing addiction, the term alcohol abuse can defined as a maladaptive substance use that leads to clinically significant impairment or distress. At this point adverse outcomes have started to develop as a consequence of

[1] One standard drink in Sweden contains 12 g (1.5 cl) pure alcohol. This corresponds roughly to 12 cl wine, 33 cl beer (5%) or 4 cl spirits.
[2] Hazardous consumption is defined by Folkhälsoinstitutet as consuming more than 9 (if female) or 14 (if male) standard drinks per week, alternatively consuming more than 3 (if female) or 4 (if male) standard drinks per occasion.

drinking, and manifestations of alcohol abuse may thus be recurrent failure to fulfil major role obligations at school or work due to alcohol consumption.

Although most people have an intuitive notion of what addiction is and how it may be distinguished from abuse, a straightforward medical definition has proven elusive. There are no physiological markers or limit values, and diagnoses have therefore been based on clusters of symptoms described in diagnostic manuals (primarily the Diagnostic and Statistical Manual of Mental Disorders; DSM, issued by the American Psychiatric Association, and the International Classification of Disease; ICD 10, issued by the world Health Organization; WHO). Nevertheless, addiction can be described as a chronic, relapsing brain disease characterized by compulsive drug seeking or use, despite harmful consequences. It is estimated that more than 320 000 people in Sweden suffer from alcohol addiction [5].

In 1964 a WHO Expert committee introduced the term dependence to replace the term addiction. In its broadest sense, dependence refers to both psychological and physiological elements, and can be described as impaired control over drinking (psychological) as well as the exhibition of tolerance and withdrawal symptoms (physiological). In biologically oriented discussions the term dependence is often used to refer only to physiological dependence; however this view can be problematic, as the urge to drink may be present years after abstinence is achieved, when tolerance and withdrawal symptoms have long ceased.

While the previous edition (DSM-IV) made a clear distinction between alcohol abuse and alcohol dependence, the current edition integrates these two into a single disorder referred to as alcohol use disorder (AUD). According to DSM-5 [8], anyone meeting any two of the 11 criteria during the same 12-month period would receive a diagnosis of AUD. The severity of an AUD - mild, moderate, or severe - is based on the number of criteria met (Table 1). While the differentiation of diagnostic terms and criteria may have great merit in many circumstances, for the purpose of simplicity terms such as addiction, dependence, abuse and AUD may be used interchangeably throughout this thesis.

Addiction as a brain disease

Although historically marked with a certain stigma by many societies, addiction is exceedingly recognized as a chronic, relapsing brain disorder [9], characterized by a compulsive drug seeking and loss of control [10]. Genetic, developmental and environmental influences have all been identified as important contributing factors in the development of the disease, and some have proposed that there may be different subtypes of the disorder [11, 12], which in turn implies a need for differential treatment strategies.

Initial treatment usually involves dealing with acute withdrawal and detoxification, which is followed by various strategies designed to maintain the patient in remission and facilitate a lifestyle of long-term abstinence. These latter treatments often include various psychosocial interventions (such as Alcoholics Anonymous or counselling), pharmacotherapies, or a combination of both [13].

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

Table 1. The presence of at least 2 of these symptoms indicates an alcohol use disorder (AUD), according to the DSM-5. The severity of the AUD is defined by the number of symptoms presented (mild: 2-3 symptoms, moderate: 4-5 symptoms, severe: 6 or more symptoms).

The first drug to be approved for alcohol addiction was disulfiram (Antabus®). Disulfiram acts as a deterrent drug, which by increasing concentrations of the toxic alcohol metabolite acetaldehyde produces unpleasant symptoms upon alcohol ingestion. Other more modern pharmacotherapies include opioid receptor antagonists naltrexone (Naltrexone®) and nalmefene (Selincro®), as well as acamprosate (Campral®). None of these treatments are fully efficient however, illustrating the need for novel pharmacological treatment strategies. For this reason, a greater understanding of how alcohol affects regions of the brain and neurotransmitter systems involved in addiction is necessary.

Although several key features have been identified which differentiate the addicted brain from its non-addicted counterpart [14], the neurobiological mechanisms through which the pathology progresses from recreational use to compulsive use remain to be elucidated. From a neurobiological perspective, addiction appears to involve multiple neuronal circuits that interact and change over time. On the basis of imaging studies Volkow and colleagues have proposed a model in which four interconnected neurocircuitries are modified by drugs of abuse: 1) reward, 2) motivation/drive, 3) memory/learning and 4) cognitive control [14]. The concept of reward in particular is central to addiction biology, and virtually all drugs of abuse activate the mesolimbic pathway, which is part of the brain reward system. However, this system is also tightly connected with neurocircuitries involving learning and memory [15], and addiction is increasingly viewed as a pathological process of habit formation [16]. A key neurotransmitter in the study of addiction is dopamine, and dopamine signalling in different areas of the brain region known as the striatum appears to be important in different aspects of addiction such as reward and habit formation [17-19].

The role of dopamine in addiction

The brain reward system

From an evolutionary point of view, the ability of certain behaviours to induce sensations of pleasure and well-being has been absolutely essential; motivating the individual to engage in activities beneficial to the survival of the species, such as eating and mating. In the 1950s psychologists Olds and Milner serendipitously discovered that rats implanted with brain electrodes would repeatedly press a lever to receive electrical stimulation in some areas of the brain, while not in others [20]. Electrical stimulation targeted at the septum or nucleus accumbens (nAc) in particular would elicit intense lever-pressing and with time the rats' attention to natural rewards such as food, water and sex vanished, having been replaced with the electrical stimulus. The authors interpreted these powerful findings as having identified "reinforcing structures" in the brain. These areas were subsequently mapped anatomically and redefined as the brain reward system [21, 22].

Later studies found that animals will self-administer drugs abused by humans in a manner similar to that of electrical stimulation [23]. These finding suggested a common neuroanatomical substrate for natural rewards, electrical self-stimulation and drugs of abuse, with drugs of abuse eliciting more powerful rewards than natural rewards, in a sense "hijacking" the brain reward-system and possibly causing neuronal adaptations that may lead to addiction [24-26].

The mesolimbic dopamine system

Although multiple neurocircuitries are implicated in the rewarding effects of drugs of abuse, the major neurochemical pathway of the reward system is the mesocorticolimbic dopamine system [24, 27]. The mesocorticolimbic system is comprised of A10 dopaminergic neurons projecting from the ventral tegmental area (VTA) via the medial forebrain bundle to the nAc, frontal cortex, olfactory tubercle, septum, amygdala and hippocampus [28-30] (Fig 1). The VTA-nAc projection, often denoted the mesolimbic dopamine pathway, is considered the most central part of the reward system [28, 31].

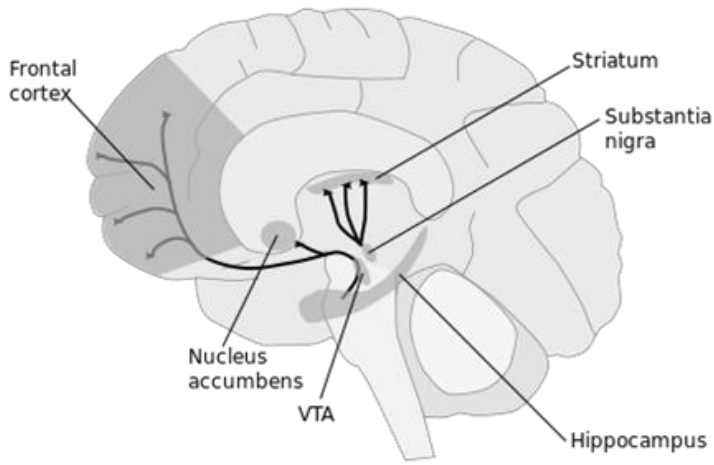

Figure 1. Schematic illustration of the mesocorticolimbic and nigrostriatal dopamine systems. Adapted with permission from the National Institutes of Health.

The dopamine neurons of the VTA display two different modes of firing; a single-spike firing mode (tonic firing), and a burst firing mode (phasic firing; [32, 33]). Typically, the dopamine neurons of the VTA are silent, or fire single spikes, however activation of this system results in a burst firing pattern, which in turn increases dopamine output in the nAc [33-35]. Levels of nAc dopamine are increased by natural rewards, such as food, water and sex, electrical brain stimulation, as well as by most drugs of abuse [24, 36, 37], with drugs of abuse often giving rise to dopamine elevations 3-5 times the size of natural rewards [38].

Dopamine - "pleasure molecule", reward predictor or neither?

The ability of natural rewards, electrical brain stimulation and drugs of abuse to elicit increased dopamine levels in the nAc has been interpreted as dopamine

mediating hedonic sensations of pleasure and "liking" (the "Hedonia hypothesis"; see [39] and [40] for discussion). Others have challenged this view, proposing that dopamine mediates the incentive saliency of rewards, modulating their motivational value and inducing a "wanting" rather than a "liking" (See [40, 41],[42]). Animal studies by Schultz and co-workers have shown that while midbrain dopaminergic neurons fire in response to natural rewards such as food, they do so only if the reward in unexpected [43]. By contrast, omission of an expected reward elicits suppression of the dopaminergic signal. These findings have led to the hypothesis that dopamine acts as a sort of learning signal, coding for a "reward-prediction error" [44].

Other factors complicating the role of dopamine in reward and addiction are the observations that 1) not all drugs of abuse increase accumbal dopamine levels[3] 2) lesions of the dopaminergic system generally fail to reduce ethanol self-administration [47, 48] and 3) aversive, unpleasant stimuli may also increase firing of dopaminergic neurons [33]. Most investigators would agree however, that the mesolimbic pathway is essential to reward in some way, even though the exact nature of the psychological reward function mediated by dopamine is still a matter of debate. Thus, for the sake of simplicity, throughout this thesis, the term reward will assume a tight interconnection with accumbal dopamine elevation.

The nigrostriatal dopamine system

The dorsal striatum receives dopaminergic innervation from the substantia nigra pars compacta (SNc; [29]; Fig. 1), with most dopaminergic cells from the SNc classified as belonging to the A9 cluster. This projection is known as the nigrostriatal dopamine system, and is a part of a much greater circuitry involving the basal ganglia and the regulation of movements [49] (Fig. 2).

Glutamatergic neurons from the motor cortex excite cells of the striatum, which in turn project along two separate pathways; the direct pathway and the indirect pathway. Activation of the direct pathway leads to a stimulatory effect by the thalamus on the cortex, ultimately resulting in a drive toward increased movement, whereas activation of the indirect pathway results in a decreased stimulation of the cortex by the thalamus, counteracting the effects of the direct pathway, inhibiting movement [50, 51].

Dopaminergic neurons of the SNc fire tonically, and the resulting dopamine in the dorsal striatum exerts excitatory effects via dopamine 1 (D1) receptors, stimulating the direct pathway, while inhibiting the indirect pathway via D2 receptors. Dopamine thus acts as a modulator, maintaining a balance between movement and inhibition of movement. Lack of balance in this delicate system leads to movement

[3] Notable exceptions being inhalants, barbiturates and benzodiazepines, which are also rewarding and often abused [28, 45, 46].

disorders, such as Parkinson's disease, which is characterized by a destruction of SNc dopaminergic neurons [52].

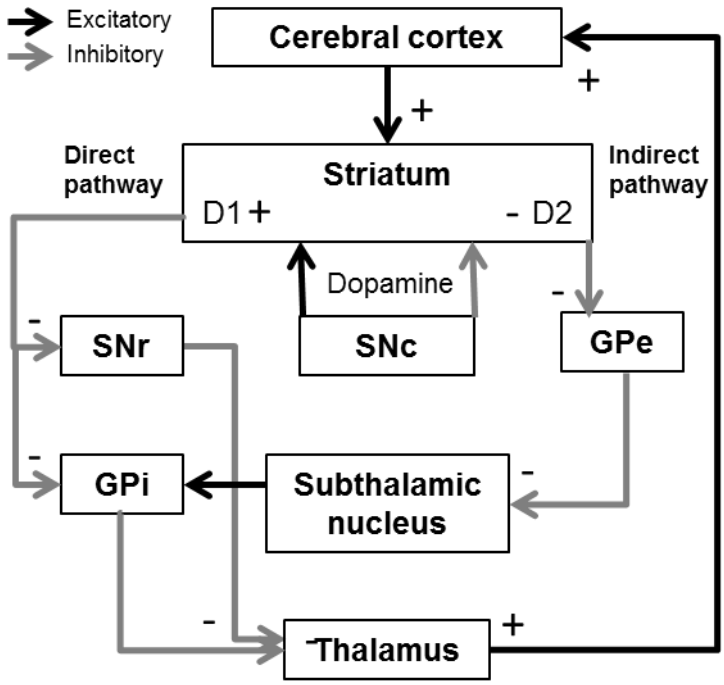

Figure 2. Simplified schematic of the direct and indirect pathways in the regulation of movement. SNc= substantia nigra pars compacta, SNr= substantia nigra pars reticulata, GPi= internal globus pallidus, GPe= external globus pallidus.

The role of dorsal striatal dopamine in addiction

Historically, the nigrostriatal dopamine system has been studied mainly for its role in regulating movement; however more recent findings support an important role for dorsal striatal dopamine signalling in learning processes relevant to addiction [19, 53]. It has been suggested that dopaminergic neurons originating in the medial SNc and projecting to the dorsomedial portions of the striatum (DMS) are critical for action-outcome learning and may be important for goal-directed drug-seeking [17, 19]. Dopamine projections from the lateral SNc to the dorsolateral striatum (DLS) on the other hand, appear to play an important role in habit formation [16, 17, 19, 54].

Persistent changes in striatal function during the progression of addiction may be caused by mechanisms of long-lasting synaptic plasticity [55]. In the striatum, such processes are strongly regulated by dopamine [56, 57], but are also influenced by other signalling systems targeted by drugs of abuse [16].

Alcohol and dopamine

Alcohol (or more specifically, ethanol), like most drugs of abuse, increases nAc dopamine levels. This occurs regardless of whether the drug is ingested [58, 59], administered systemically [36, 60, 61], or perfused locally in the nAc [62, 63]. However, the exact mechanisms by which ethanol increases nAc dopamine levels remain to be elucidated.

Even though the dorsal striatum appears to play an essential role in the development of addiction, ethanol-induced changes in dopamine transmission in this region have not been explored to the same extent as in the nAc, and results have often been conflicting. Systemic administration of ethanol has resulted in both increases [36, 64] and decreases [65] in dorsal striatal dopamine levels. In a similar manner, focal application of ethanol in the dorsal striatum has been shown to increase striatal dopamine in some studies [66, 67], whereas another study did not detect any significant changes [68].

The striatum

The basal ganglia is a collective term used to describe a network of deep lying cerebral nuclei including the striatum, globus pallidus, SN, and the subthalamic nucleus [51, 69]. The basal ganglia are strongly interconnected with other brain areas such as the cerebral cortex, thalamus and brainstem and are associated with a variety of functions such as control of voluntary movement, action selection, motivation, procedural learning, habit formation, cognition and emotion [16, 49, 53, 70].

The striatum (or caudate-putamen in primates; Fig. 3A) is the largest input nucleus to the basal ganglia, serving as an entry site for information flow from other areas of the brain. It receives glutamatergic inputs from virtually every area of the cortex, as well as from the thalamus. Other afferent projections include 5-hydroxytryptamine (serotonergic, 5-HT) inputs from the dorsal raphe nucleus [71, 72], noradrenergic innervation from the nucleus solitarius, locus coeruleus and the caudal ventrolateral medulla [73, 74], as well as dopaminergic inputs from the midbrain as discussed in previous sections. Striatal projection neurons, also called medium spiny neurons (MSNs), express γ-amino-butyric acid (GABA) and send efferent projections to the globus pallidus, SN and VTA. Through these connections the striatum influences all other basal ganglia regions, ultimately regulating cortical and subcortical targets.

Ethanol-induced changes in striatal synaptic output may thus affect the flow of information throughout the basal ganglia, possibly leading to neuronal adaptations that may underlie addiction. For instance, changes in striatal synaptic activity may influence the activity of midbrain dopaminergic neurons, thereby affecting local

dopamine concentrations, or may affect other striatal circuits relating to the development of addiction.

Subregions of the striatum

The striatum can be anatomically subdivided into a dorsal and a ventral (nAc) region; the former being mainly attributed with integrating sensorimotor information, whereas the latter is associated with reward and motivation [75]. Different regions of the striatum are believed to be involved in different aspects of the addictive disorder [17] (Fig. 3B). Although part of the striatal complex, the ventral striatum exhibits several unique features compared to the dorsal striatum, and is often considered a separate entity [76, 77]. The nAc can be anatomically subdivided into a shell and core region [78]. The core bears a greater resemblance to the dorsal striatum than the shell, and the shell is classified as part of the extended amygdala and is thus sometimes considered a limbic structure. The shell appears to be particularly important to initial drug actions, with addictive drugs having a greater effect on dopamine release in the shell than in the core [79-81] As discussed in previous sections, the nAc has long been the primary focus of addiction research, however increasing evidence would suggest that also the dorsal striatum has a role to play in the development of addictive disorders. The DMS (or caudate nucleus in primates) is associated with the control of goal-directed behaviours [82], and may thus influence goal-directed alcohol seeking. The DLS (putamen in primates) is important in habit formation and may thus participate in the development of habitual alcohol use [16, 19]. In addition, it has been suggested that as the addiction pathology progresses from reward-driven to habit driven drug-seeking behaviour, this behavioural progression associates with a neuroanatomical progression from ventral striatal to dorsal striatal control over drug-seeking behaviour [16]. Supporting this theory, the dorsal striatum appears to regulate the motivation to procure the drug in addicted humans [83].

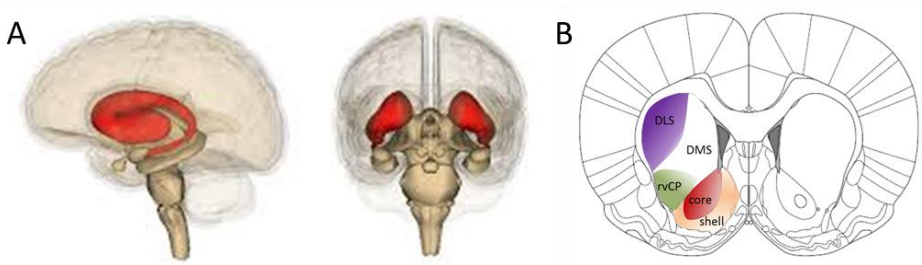

Figure 3. A) Lateral and anterior view of the human striatum (caudate-putamen), in red. Adapted with permission from Anatomography, Life Science Databases. B) Schematic drawing depicting striatal subregions involved in addiction; coronal view of rodent brain. DLS: dorsolateral striatum; DMS: dorsomedial striatum; rvCP: rostroventral caudate putamen. Red /orange sections refer to the core and shell regions of the nAc, respectively. Adapted from Paxinos and Watson, 2007 [84]).

It should also be mentioned that physiological dependence and the associated withdrawal are thought to constitute a motivational force that may contribute to relapse [85]. The rostroventral caudated putamen (rvCP) in particular, appears to play an important role in alcohol withdrawal [86].

Basic cytoarchitecture of the striatum

Roughly 95-97% of cells in the rodent striatum are GABAergic MSNs. The remaining cells have been classified as cholinergic interneurons, and there are at least three types of GABAergic interneurons [87, 88]. It has been suggested that the frequency of interneurons is greater in primate than in rodent, there comprising approximately 23% of striatal cells [89]. Studies by Ma and colleagues have shown that MSNs are larger, have a higher membrane capacitance and lower input resistance in the dorsolateral striatum, as compared to the nAc. In addition, MSNs in the shell region have fewer dendritic arbours, lower spine densities and may have up to 50% less total surface area than those in the core, suggesting that core neurons have a greater potential for collecting synaptic information [90].

Contrary to many brain regions such as the cortex, where cell organization is laminar in nature, the cytoarchitecture of the striatum appears more homogenous, and can be described as a "mosaic" [91]. The dorsal striatum is organized into small clusters of medium spiny neurons (MSNs) known as "patches" (or striosomes in primates) which are surrounded by the MSNs of the so called "matrix" compartment. This partitioning is done on a histological basis, but the patch-matrix compartmentalization also appears to reflect an input-output partitioning, with compartments being distinguished on the basis of afferents and projection targets, as well as receptor localization [91-93]. Whereas the patch-matrix compartmentalization of the dorsal striatum extends ventrally to the nAc core, cellular organization of the shell region appears more complex. It has been argued that the shell region may be comprised of one patch and one matrix compartment, alternatively that the shell region may be further categorized into various sub-regions with different immunohistochemical and input-output relationships [77, 94].

In contrast to the dorsal-to-ventral level of organization, an alternative, or complimentary, view of the functional organization of the striatum has been reviewed by Voorn and colleagues [95]. It is suggested that MSN density, neurochemical distribution as well as striatal inputs and outputs may conform to a dorsolateral-to-ventromedial gradient, which in turn may reflect overlaps in behavioural function between the classically defined dorsal and ventral striatum.

Striatal efferents

Based on neuropeptide content and projection targets the MSNs of the dorsal striatum can be classified into two broader groups: D1 receptor expressing MSNs that express substance P, dynorphin and project to the internal globus pallidus and substantia nigra pars reticulata (SNr, the direct pathway), and D2 MSNs that express enkephalin and project to the external globus pallidus (the indirect pathway)[91]. The matrix compartment contains equal numbers of D1 and D2 MSNs, while at least some patches appear to contain an overabundance of direct pathway neurons [92]. A subset of MSNs project to the SNc, and these are found exclusively in patches [91, 96]. Nucleus accumbens core MSNs projecting to the VTA express exclusively D1 receptors [97], thus resembling striatonigral MSNs, whereas core MSNs projecting to the pallidum may express either D1 or D2 receptors [98]. In the core, the distribution of D1 and D2 MSNs appear to be rather homogenous, whereas imaging studies of the nAc shell have shown that while D1 MSNs appear homogenously distributed, D2 MSNs are heterogeneously distributed, particularly in the medial and ventral portions of this region [99].

Intrastriatal connections

MSNs are characterized by large (180-260 μM) dendritic trees, which are covered with numerous spines. Axons are very long, giving off multiple collaterals near or within the dendritic field [100]. MSNs thus form a weak inhibitory network among themselves (feedback inhibition; [101]), but also synapse on cholinergic interneurons and GABAergic interneurons [88, 102] (Fig. 4).

Cholinergic interneurons are very large (cell bodies can exceed 40 μM diameter), aspiny cells, which comprise approximately 0.3-2 % of cells in the striatum [88, 103] . Although few in number, cholinergic interneurons have large axonal arbours, with each cholinergic cell containing about 500 000 axonal varicosities [104]. Axon collaterals in the dorsal striatum are largely restricted to the matrix compartment, where they target MSNs, although GABAergic interneurons and other cholinergic interneurons also receive cholinergic input [88, 105, 106]. Interestingly, cholinergic interneurons also appear to indirectly regulate the activity of MSNs by driving GABA release from dopaminergic terminals [107]. Cholinergic interneurons are typically tonically active, firing in a slow, regular pattern [108]. It has been observed that a synchronous pause in cell firing is observed in putative cholinergic interneurons following the presentation of reward or salience-related cues [109]. These responses in turn appear to be crucially dependent on input from both nigrostriatal dopaminergic projections and thalamostriatal glutamatergic projections [110, 111].

At least three types of striatal GABAergic interneurons have been identified, and they all produce a strong inhibitory postsynaptic potential in MSNs (feed-forward inhibition; [88]). The best characterized type expresses parvalbumin and is also

known as a fast-spiking interneuron (FSI; [112]). FSIs exert powerful monosynaptic inhibition of MSNs through multiple perisomatic synapses [113], and are also themselves coupled by dendritic gap junctions [114]. *In vitro*, these cells are typically hyperpolarized and silent, but appear continuously active in awake animals [115]. It has been suggested that cholinergic interneurons control the activity of FSIs by targeting excitatory somatodendritic nicotinic acetylcholine receptors (nAChRs), as well as inhibitory presynaptic muscarinic receptors, thus indirectly influencing the activity of striatal MSNs [105]. FSIs also receive glutamatergic afference from the cortex, and it has been suggested that cortical excitation of FSIs leads to feed-forward inhibition of MSNs [113].

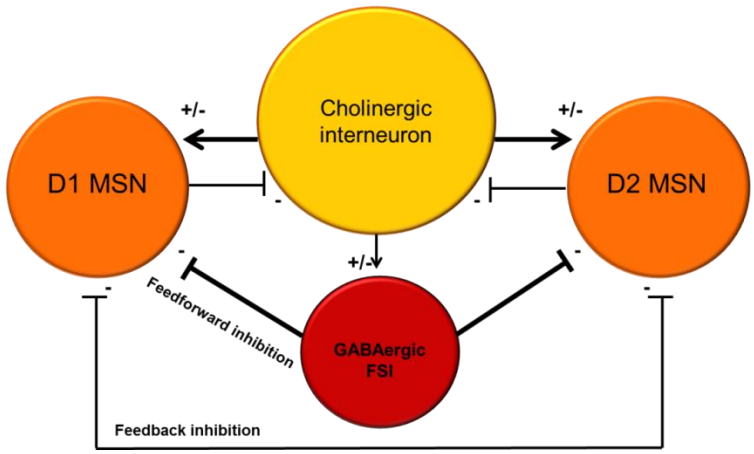

Figure 4. Schematic representation of the principal cells of the striatum and how they interconnect with each other.

A second class of GABAergic interneuron is the somatostatin, neuropeptide Y (NPY) and nitric oxide synthase (NOS) expressing interneuron. Electrophysiologically these cells are characterized by low threshold calcium spikes, and are sometimes termed persistent and low-threshold spike (LTS) neurons [116]. These cells receive both cholinergic and dopaminergic input [88]. A third class of GABAergic interneurons are the calretinin expressing interneurons. Little is known about the electrophysiological propertied of these neurons. In addition to these classically defined types, recent studies have revealed four novel types of GABAergic interneurons [117]. These interneurons are tyrosine hydroxylase positive, and each as its unique electrophysiological profile.

Striatal afferents

During resting conditions striatal projection neurons are hyperpolarized and silent but increased activity of many convergent cortical glutamatergic afferents induces

phasic firing of MSNs. However, although 80 % of the synapses in the striatum are glutamatergic [118], MSN activity is not solely dependent on excitatory transmission. As discussed in the previous section, MSN activity is modulated

		Origin of afferents	
		Dorsal striatum	**Ventral striatum**
Afferent neurotransmission	**Glutamate**	Cortex (Deep layer V+VI to patches, superficial layer V to matrix), thalamus, amygdala, STN	Prefrontal cortex, thalamus, amygdala, STN, Hippocampus, VTA
	GABA	Ventral pallidum	Ventral pallidum, VTA (to shell)
	Dopamine	SNc (Ventral to patches, dorsal to matix)	VTA (posteromedial to shell, lateral to core)
	5-HT	DRN (rostral regions)	DRN (Dorsal regions, lateral wings)
	Noradrenaline	-	Only to shell; nucelus solitarius, locus coeruleus, caudal ventreolateral medulla
Local neuromodulators	**Opioids: enkephalin, dynorphin, B-endorphins**	Present	Present
	GlyR agonists: glycine, taurine, B-alanine, Serine	Present	Present
	Endocannabionids	Present	Present

Table 2. Afferent projections to the dorsal and ventral striatum.

locally by cholinergic interneurons, as well as by feed-forward inhibition from GABAergic interneurons and feed-back inhibition via axon collaterals of other MSNs [88]. In addition, the striatum receives modulatory afferent input from midbrain dopaminergic neurons [29, 119] and 5-HT input from the dorsal raphe nucleus [71, 72]. The ventral striatum also receives GABAergic input from the VTA and ventral pallidum [120] and, interestingly, the nAc shell is the only region of the basal ganglia that receives a (moderate) noradrenergic innervation, originating from the nucleus solitarius, locus coeruleus and the caudal ventrolateral medulla [73, 74]. Glutamatergic and GABAergic transmission is also modulated by opioids [121], as well as by endocannabinoids released from MSNs [122]. Finally, activation of glycine receptors (GlyRs), which have several endogenous ligands, also appears to play an important role in modulating striatal neurotransmission [123] (Table 2).

In conclusion, the ventral and dorsal striatum exhibit many similarities, but also several differences, with regards to cell population, cytoarchitecture/morphology, as well as afferent and efferent circuitry, which in turn may be important to their unique roles in the development of addiction.

The pharmacology of ethanol

Alcohol produces a wide variety of effects in humans, many of them similar to those of other central nervous system (CSN) depressants such as sedatives, hypnotics and anaesthetic agents. At low blood concentrations (5-10 mM[4]) ethanol produces sensations of euphoria, anxiolysis and disinhibition. Higher concentrations produce impairment in motor function and slurred speech. When blood concentrations reach 43-65 mM vomiting can occur and the subject may fall into stupor. At concentrations above 109 mM there is a significant risk of death due to respiratory failure.

As suggested by its rich pharmacological profile, ethanol interacts with multiple receptor systems. In particular, ligand gated ion channels such as *N*-methyl-*D*-aspartate (NMDA), $GABA_A$, 5-HT3, nACh and Gly receptors are affected by ethanol [124-127]. In general, ethanol potentiates the function of $GABA_A$, Gly, 5-HT3 and nACh receptors [128-131], but inhibits NMDA receptor function [132, 133], although effects may vary depending on receptor subunit composition [126]. Ethanol is also known to inhibit the effects of L-type Ca_2^+ channels [134], activate G-protein-activated inwardly rectifying K^+ channels [135], as well modulate the endogenous opioid system [136]. As the main focus of this thesis is the

[4] 10 mM is equivalent to a blood alcohol concentration 0.43 ‰ (by mass) or 0.46 mg/ml blood.

involvement of GlyRs and $GABA_A$ receptors in the effects of ethanol, other relevant receptor systems will not be discussed further in this section.

Ethanol and $GABA_A$ receptors

GABA is the primary inhibitory neurotransmitter in the mammalian brain and activation of $GABA_A$ receptors usually decreases neuronal excitability. The $GABA_A$ receptor has a pentameric transmembrane structure with a central Cl^- selective pore. Binding of the endogenous ligand GABA triggers an opening the Cl^- pore, which usually results in Cl^- ions flowing into the cell. This, in turn, drives the neuron toward a state of polarization or hyperpolarization, thereby decreasing the probability of new action potentials.

$GABA_A$ receptors are the targets of benzodiazepines, barbiturates and anaesthetics [137], but they have also long been implicated in several effects of ethanol [138]. Notably, the sedative/hypnotic actions and anxiolytic effects of ethanol in laboratory animals can be blocked by GABA antagonists [139, 140] and several of the behavioural and cognitive consequences of alcohol consumption are suggested to be due to an involvement of this system [141-143]. Acute ethanol administration has generally been shown to potentiate $GABA_A$ receptor activity, however the overall effects of ethanol on GABAergic neurotransmission are complex, depending on receptor localization, brain region studied and concentration of ethanol applied [144-146]. In addition, effects of ethanol may vary depending on $GABA_A$ receptor subtype (for reviews and discussion see [147, 148]).

To date, 19 different $GABA_A$ receptor subunits have been found in humans. These can be classified into α(1-6), β(1-3), γ(1-3), δ, ε, π, θ and ρ(1-3)[5] subclasses [150]. Given the pentameric form of the receptor, the theoretical number of possible subunit combinations is astronomical. In reality however, the number of $GABA_A$ receptor subtypes appears restricted, with most pentamers containing two α-subunits, two β-subunits and one γ, δ or ε-subunit. The α1β2γ2 variant appears to be the most common one, comprising approximately 40% of all $GABA_A$ receptors [151]. It is well established that the subunit configuration of the $GABA_A$ receptor is important in determining its pharmacological and physiological properties [152]. There is still a great lack of knowledge however, as to the subcellular, cellular and regional distribution of different $GABA_A$ receptor subtypes in the brain, and their relative importance in ethanol-mediated effects.

While most $GABA_A$ receptor subtypes appear to be synaptic, δ-subunit containing $GABA_A$ receptors are believed to constitute the major class of extrasynaptic receptor subtype [153, 154]. These receptor subtypes appear sensitive to very low concentrations of GABA, enabling them to mediate a form of tonic inhibition

[5] The ρ receptor subunits co-assemble to form GABA ρ-receptors, formally classified as $GABA_C$ receptors [149].

[154], and some studies also suggest that this receptor subtype may be especially sensitive to ethanol [155, 156], although this may be disputed [148].

Ethanol and GlyRs

The amino acid glycine is, like GABA, an inhibitory neurotransmitter in the CNS [157, 158]. Although glycine does acts as a co-agonist at the excitatory glutamatergic NMDA receptor, it also has a specific inhibitory receptor [159, 160]. The inhibitory GlyR (also known as the strychnine-sensitive glycine receptor) is a ligand gated ion channel, which, like the $GABA_A$ receptor, enables Cl^- influx following the binding of its endogenous ligand. GlyRs are in particular abundance in the spinal cord, brain stem, cerebellum and retina. Here they are involved in the modulation of physiological processes such as respiration, sensory processing, pain and motor control [158, 161, 162] and disruptions of the glycinergic system have been implicated in spasticity and spinal cord degeneration [158].

The GlyR is formed by a pentameric complex of either homomeric (α) or heteromeric (αβ) subunit composition; the stoichiometry of the heteromer being 2α:3β [163]. The β-subunit is required for the receptor complex to cluster in synapses [164, 165], wherefore homomeric receptor variants are mainly found extrasynaptically [165]. Extrasynaptic/homomeric GlyRs appear to have slow activation rates, making them more suitable for paracrine or autocrine activation, rather than synaptic neurotransmission [166].

To date five subunits have been cloned from mammalian tissue; α1-α4 and β [167]. Although GlyRs were first identified in the spinal cord and brainstem, α1β heteromers appear to be uniformly expressed throughout the CNS [166]. Electrophysiological, immunohistochemical, western blot and in situ hybridization studies have also demonstrated the existence of GlyRs in the nAc [168-173] and dorsal striatum [168, 172, 174, 175]. The α2-subunits have been thought to be expressed mainly in embryonic and neonatal stages of development, with the predominant variant in the adult spinal cord being the α1β heteromer. However, α2β receptors have been shown to be the predominant variant in several forebrain regions of adult animals, suggesting that this is not a general developmental shift [168, 172, 176]. Splice variants of the α1-α3 subunits further add to receptor diversity [166, 177, 178].

To date, no striatal glycinergic neurons have been identified. However, being a ubiquitous amino acid, glycine is present in all mammalian body fluids and tissue proteins [157]. Additional possible sources of glycine include co-release with GABA [179, 180], as well as release from glia cells [180]. In addition to glycine, other endogenous amino acids such as taurine and β-alanine also have an affinity for the GlyR [166, 181], as does GABA [182].

An increasing amount of evidence supports an important role for the glycinergic system in the development of addiction (for reviews, see [127, 183]). Genetic studies of alcoholism have shown that many of the leading candidate genes code for subunits of $GABA_A$ receptors, but also for GlyRs [184]. Ethanol is known to potentiate GlyR function [185], possibly by enhancing GlyR mediated currents [186] [187]. In addition, ethanol increases extracellular levels of the endogenous GlyR ligand taurine [63, 188, 189], which alone has been shown to increase accumbal dopamine levels in a strychnine-dependent fashion [190], further promoting GlyR activation. GlyRs appear to be involved in the ethanol intake-reducing effect of the homotaurinate acamprosate [191], and administration of glycine reuptake inhibitors has produced a reduction in both ethanol preference and consumption in Wistar rats [192, 193].

Distribution of $GABA_A$ receptors and GlyRs in the striatum

Studies of the regional and cellular localization of $GABA_A$ receptors and GlyRs indicate a diversity of inhibitory receptor distribution in the striatum in both human [174, 175] and rodent [172, 194]. Studies in human tissue by Waldvogel and colleagues [175] have shown that on a regional level, $GABA_A$ receptor (most common subtypes) and GlyR distribution appears to follow the patch (striosome)/matrix subdivision of the striatum. The highest density of $GABA_A$ receptors were found in the striosomes, where GlyRs were not present, whereas the matrix contained lower levels of $GABA_A$ receptors and low levels of GlyRs. The authors suggest that this finding may be in line with the concept that the striosome/matrix compartmentalization reflects two different functional domains within the striatum, and that $GABA_A$ and GlyRs may have different roles to play in the processing of information that occurs within.

At a cellular level, $GABA_A$ receptors were expressed by most GABAergic interneurons (α1, β2/3, γ2 subunits), cholinergic interneurons (α3) and MSNs (α2, α3, β2/3, γ2 subunits). GlyRs were highly expressed (75%) on (presumed) cholinergic interneurons and were also present on subsets of FSIs and calretinin containing GABAergic interneurons [174]. No GlyRs were found on MSNs and a subset of GABAergic interneurons (somatostatin/NPY/NOS expressing; presumably LTS interneurons) showed no staining for either GlyRs or any of the major $GABA_A$ receptor subunits.

In rodent, extrasynaptic (α4β2δ) $GABA_A$ receptors are suggested to be expressed by striatal MSNs [195] and in the nAc α4βδ receptors have been found on both D1 and D2 receptor-expressing MSNs, where they mediate a strong tonic inhibition [196]. It has also been suggested that δ-containing $GABA_A$ receptors in the dorsomedial nAc shell (but not the core) are important in the reinforcing effects of oral alcohol [197]. GlyR staining in rodents have shown that GlyR positive cells appear to be situated primarily in the shell region of the nAc, and that the α2

subunit appears to be the most predominant, following the β-subunit, in both the nAc and in the dorsal striatum [172].

A proposed model for ethanol actions in the nAc

The framework for this thesis has been provided by previous work from the present research group, initiated in the 1990´s by Professor Bo Söderpalm. While it was known that ethanol increased nAc dopamine levels, the finding that systemic administration of mecamylamine, a nAChR antagonist, could completely counteract this effect [61], sparked further research into the role of central nAChRs in mediating the dopamine enhancing effects of ethanol. Subsequent microdialysis studies showed that mecamylamine applied locally in the anterior, but not posterior, VTA blocked the dopamine enhancing properties of systemic ethanol [198, 199]; suggesting that ethanol increases accumbal dopamine through interaction with nAChRs in the VTA. Direct microdialysis perfusion of ethanol into the VTA however, did not enhance dopamine levels, whereas ethanol administered in the nAc did; an effect which in turn could be counteracted by nAChR blockade in the anterior VTA [199-201]. These observations formed the basis for the hypothesis that ethanol may increase accumbal dopamine by acting primarily in the nAc itself, there producing effects which in turn influence the release of acetylcholine in the VTA; thereby increasing the activity of VTA dopaminergic neurons. One way for this to occur would be if ethanol decreased the activity of VTA-projecting MSNs [202], thereby causing a disinhibition of acetylcholine release in the VTA (see [183]). Possible pharmacological targets in this regard were inhibitory $GABA_A$ receptors or GlyRs, which as discussed in previous sections, are both modulated by ethanol. Following experiments showed that antagonism of GlyRs with strychnine in the nAc blocked ethanol-induced increases in accumbal dopamine [203], whereas local administration of $GABA_A$ receptor channel blocker picrotoxin did not [203, 204]. This pointed toward glycine and/or GlyRs in the nAc as important access points for ethanol to the brain reward system. Administration of glycine, however, increased accumbal dopamine levels only in some animals, while not others [205]. Endogenous GlyR agonist taurine, on the other hand, had been shown to increase in the nAc following systemic ethanol administration [189], and was subsequently shown to elevate dopamine levels in its own right; an effect which could be blocked with strychnine in the nAc and mecamylamine in the VTA [190]. Ensuing work confirmed an ethanol-induced decrease in nAc MSN activity [206], and implicated astrocytes as a potential source of ethanol-induced taurine [63].

Taken together, the findings discussed above have led to the formulation of the nAc-VTA-nAc neuronal circuitry hypothesis as a proposed mechanism of action for ethanol (Fig. 5). In brief, ethanol is believed to elevate extracellular levels of taurine in the nAc, which, in concert with ethanol, activates accumbal GlyRs. This in turn, via direct or indirect inhibition of VTA projecting MSNs, reduces the

GABAergic tone in the VTA. Subsequent increases in VTA acetylcholine may then activate nAChR on dopaminergic cell bodies, thus increasing dopamine levels in the nAc.

Most of the work involving the nAc-VTA-nAc neuronal circuitry described above was performed in ethanol-naïve animals, and may thus reflect processes that are relevant to the acutely rewarding effects of ethanol in the nAc. The DLS may also be of significance in acute ethanol reward [207], in addition to its importance in reward-guided learning and habit formation, which in turn may rely on interconnecting circuits with the ventral striatum [208]. However, the effects of ethanol on dorsal striatal dopamine signalling, as well as the potential importance of $GABA_A$ and GlyRs in this regard, are poorly understood.

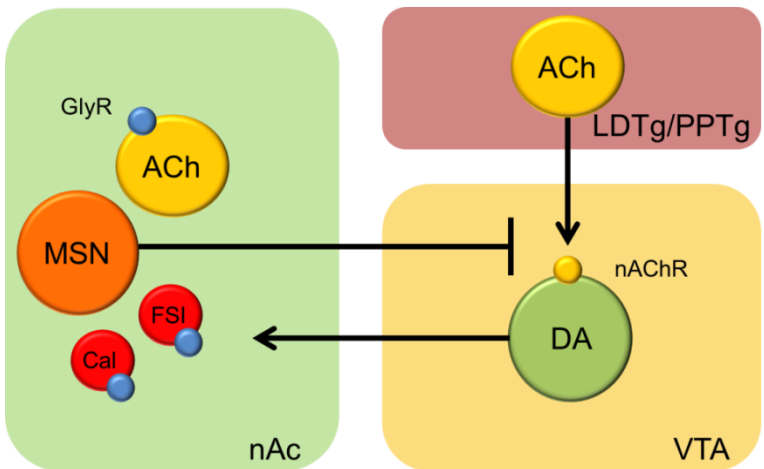

Figure 5. Proposed mechanism of action for ethanol: the nAc-VTA-nAc neuronal circuitry. In brief, ethanol modulates GlyR activation in the nAc, leading to a net decrease in the activity of VTA-projecting MSNs. This produces a disinhibition of nAChR-mediated activation of VTA dopaminergic neurons, which in turn increases accumbal dopamine levels. LDTg/PPTg = laterodorsal/pendunculopontince tegmental nucleus.

Potential role for acetaldehyde in ethanol reward

Acetaldehyde in the periphery vs acetaldehyde in the brain

Acetaldehyde is the first metabolite of ethanol. In the periphery, it is formed mainly in the liver from its parent compound via the enzymes alcohol dehydrogenase (ADH) I and cytochrome P_{450}2E1 (CYP 2E1). It is then converted to acetic acid by aldehyde dehydrogenase [209] (ALDH 2; Fig. 6). Being a toxic

substance, acetaldehyde is traditionally regarded as mediating aversive properties of ethanol; in essence being a "hangover molecule", responsible for symptoms such as nausea, flushing and headaches. The mechanism of action of alcohol deterrent disulfiram is based on inhibition of ALDH, which drastically increases concentrations of acetaldehyde upon alcohol ingestion, with severely unpleasant symptoms as a result. Interestingly, an increasing number of reports have indicated that acetaldehyde, while aversive in the periphery, may be rewarding centrally, and may even be responsible for the rewarding properties of alcohol [210-212]. As ADH I is not present in the brain [213], formation of central acetaldehyde has been a topic of some controversy. The findings that acetaldehyde-metabolizing enzymes in the brain increased following ethanol exposure in rats [214], and that brain-derived catalase was able to oxidize ethanol *in vitro* led to the suggestion that acetaldehyde may be formed directly in the brain via catalase [215]. Since then, formation of acetaldehyde from ethanol via catalase in the brain has been confirmed in multiple studies [216-218], and it is now generally assumed that approximately 60% of brain ethanol metabolism is mediated by catalase, 20 % via CYP 2E1 and the rest via other, as yet unknown mechanisms [218]. It has also been shown that there is great regional variation in catalase expression [219, 220], indicating that different areas of the brain may produce different (pharmacologically relevant) concentrations of acetaldehyde, following ethanol ingestion.

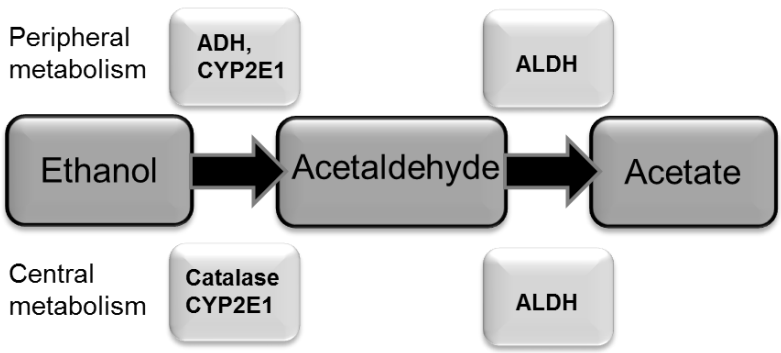

Figure 6. Schematic of the peripheral and central metabolism of ethanol and acetaldehyde.

Acetaldehyde – mediator of ethanol reward?

Acetaldehyde – like ethanol itself – induces several behaviours indicative of rewarding/reinforcing properties, such as conditioned place preference [221, 222] and self-administration [223-225]. In addition, results from both *in vitro* [226] and *in vivo* [227] studies have showed that acetaldehyde increases VTA dopaminergic neuronal activity, and intra-gastric, as well as intra-VTA administration of acetaldehyde produces increases in accumbal dopamine levels [226, 228, 229]. A

highly relevant question in this regard, is whether physiologically relevant concentrations of ethanol produce pharmacologically relevant brain concentrations of acetaldehyde, an issue complicated by technical difficulties in quantifying local acetaldehyde levels in the brain (see [210]).

In order to circumvent these impediments, many experimenters have sought to study the effects of ethanol under conditions when levels of produced acetaldehyde are kept to a minimum. Such strategies have employed the use of e.g. catalase inhibitors, acetaldehyde sequestering agents such as *D*-penicillamine, or, more recently, the use of lentiviral vectors encoding anti-catalase small hairpin RNA (shRNA), which effectively inhibit the expression of the catalase enzyme. Using these methods, it has been shown that the stimulatory effect of ethanol on dopaminergic VTA neurons can be abolished with the pre-treatment with catalase inhibitors [226] or *D*-penicillamine [229], and that the dopamine-enhancing effects of systemic ethanol can be prevented with sequestering of centrally formed acetaldehyde [229, 230], or with microinjections in the VTA with lentiviral vectors encoding anti-catalase shRNA [231].

Taken together, the studies described above suggest an important role for acetaldehyde in mediating ethanol effects, especially in the VTA. As acetaldehyde has been shown to interact with the GlyR [232], as well as increase levels of taurine in the nAc [233], the prospect that the ethanol effects in the nAc-VTA-nAc neuronal circuitry described in previous sections may, at least in part, be attributed to acetaldehyde provides an intriguing possibility. There have been no studies that we are aware of, however, investigating the effects of acetaldehyde administered locally in the nAc.

AIM OF THESIS

The overall aim of this thesis was to study the effects of ethanol, or its metabolite acetaldehyde, on dopamine release and synaptic activity in the striatal subregions nAc and DLS; with special emphasis on the roles of inhibitory GlyRs and $GABA_A$ receptors in mediating these effects.

Specific aims

Paper I. To determine whether increases in dopamine following local ethanol administration in the nAc are mediated by ethanol-metabolite acetaldehyde.

Paper II. To explore the roles of GlyRs and $GABA_A$ receptors in modulating synaptic activity and dopamine release in the nAc and DLS respectively, during baseline conditions.

Paper III. To define the roles of GlyRs and $GABA_A$ receptors in mediating effects of ethanol on synaptic activity in the DLS.

Paper IV. To investigate the roles of GlyRs and $GABA_A$ receptors in ethanol-mediated changes in dopamine release and synaptic activity in the nAc and DLS of adult animals.

MATERIALS & METHODS

Ethical considerations

All experiments were performed in accordance with the Declaration of Helsinki, and were approved by the Ethics Committee for Animal experiments, Gothenburg, Sweden. Diary numbers: 381/11, 214/14 and 266/12.

Animals

In microdialysis studies (Papers I, II and IV), and electrophysiological experiments with adult animals (Papers II and IV) only outbred male adult Wistar rats were used (weight range 250-400g). Animals were obtained from Taconic, Ejby, Denmark and housed four to a cage, with free access to tap water and standard rat feed (Lantmännen, Kimstad, Sweden). Rats were allowed to adapt to the novel environmental conditions (constant room temperature of 20°C, relative humidity 65 % and a regular light-dark cycle with lights on at 07:00 a.m. and off at 07:00 p.m.) for at least 5 days prior to any procedures. All surgeries and experiments were performed during the light phase of the cycle.

Electrophysiological experiments using juvenile animals (Papers II and III) were performed using both male and female outbred Wistar rats, ranging from 19-25 days of age (breeding performed at Gothenburg University, with rats originating from Charles River, Germany). Animals were not yet weaned, but were otherwise subjected to the same environmental conditions as described above. In a set of supplementary electrophysiological experiments (not published, but included in results section) aged rats (weight approximately 500 g) originating from the local breeder described above were used. All experiments were performed in ethanol-naïve animals.

In vivo microdialysis

In vivo microdialysis is a well-established sampling technique that allows for the continuous measurement of endogenous substances (e.g. neurotransmitters) present in the extracellular environment, in awake and freely-moving animals. A great advantage of the method is that it also enables the administration of substances (reversed microdialysis or retrodialysis), either at the site of sampling, or in other more distant areas of the brain. Both sampling and drug administration are made possible by the insertion of a microdialysis probe in the brain region(s) of

interest. The tip of the microdialysis probe is equipped with a semi-permeable membrane (the active space) and is perfused with a buffer (Ringer's solution) with an ion composition similar to that of the cerebral spinal fluid; in essence mimicking the function of a blood vessel. This allows for the passive diffusion of small molecules present in the extracellular fluid into the probe (the dialysate), which may then be collected for analysis. In a similar manner, substances dissolved in Ringer's solution (the perfusate) diffuse into the extracellular environment, allowing focal administration (Fig. 7). This, in turn, enables the simultaneous monitoring of the effects of locally applied drugs on physical parameters such as dopamine concentration.

The microdialysis probe

All microdialysis probes used in these experiments were a modified version of the *I*-shaped probe, custom made in our laboratory. The probe shaft, inlets and outlets were comprised of 20 gauge polyethylene tubing, with an inner/outer diameter of 1.09/0.38 mm (VWR, Sweden). To stabilize the probe construction, a tube of fused silica (Skandinaviska Genetec, Sweden) was inserted and extended 9 mm beyond the shaft. The fused silica was then covered with a semi-permeable dialysis membrane composed of a co-polymer of polyacrylonitrile and sodium methallyl sulfonate (Hospal-Gambro, Sweden). The dialysis membrane had an inner/outer diameter of 0.2/0.3 mm and a molecular cut-off of 20 kDa. The shafts of the

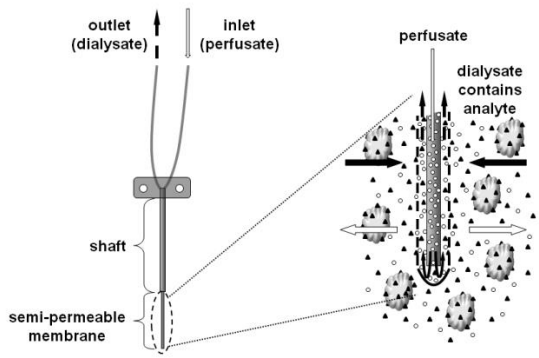

Figure 7. Schematic illustration of a microdialysis probe. Ringer's solution is perfused through the probe, allowing passive diffusion of molecules present in the extracellular environment into the probe, while substances dissolved in the Ringer's solution diffuse into the extracellular environment. Adapted with permission from Wikimedia.org.

probes were then covered with silicone glue (CAF 3; Rhodorsil Silicones, Nils Eksandh, Sweden), leaving an exposed tip of dialysis membrane (2.0 mm, the active space). Prior to implantation, the probes were washed with 70 % ethanol,

followed by Ringer's solution (2 μl/min; 30 and 120 minutes, respectively). The probe inlets and outlets were then sealed by heating, and probes were stored at +4 °C, for a maximum of 5 days prior to implantation. During manufacturing and implantation a glass rod was used as a holder.

Surgical procedure

Animals were anaesthetized with isoflurane (3.5-4.0 % in air; Forene®, Baxter, Sweden) and then fixed to a stereotactic instrument (David Kopf Instruments, Tujunga, CA, USA). A heating pad was used to prevent hypothermia and animals were injected with a local anaesthetic (buvipacaine; Marcain®; AstraZeneca, Sweden) at the incision site. Three to four holes were drilled through the scull; two for the use of anchoring screws and one or two for the insertion of microdialysis probes. The probes were lowered monolaterally into the nAc core/shell borderline region (a/p: +1.85, l/m: -1.4, d/v: -7.8), the DLS (a/p: +1.2, l/m: -3.4, d/v: -5.0) or the anterior VTA (a/p: -5.2, l/m: -0.7, d/v: -8.49; coordinates obtained from Paxinos & Watson 2007 and relative to the bregma and dura, respectively. A second analgesic was given (2.5 % ketoprofel gel; Ordudis®, Sanofi-aventis), and probes, as well as anchoring screws, were fixed to the scull using Harvard cement (Dental AB, Gothenburg, Sweden). The rats were injected subcutaneously with 0.9% NaCl (3 ml) to prevent dehydration and placed in individual cages, with free access to food and water. To ensure a good health status of the animals, rats were weighed after the surgical procedure, and then again prior to experimentation. Animals were allowed 48 h to recover before experiments were initiated.

Microdialysis procedure

On the day of the experiment individual swivels were attached to plastic collars on the animals, allowing them to move freely in their cages. Animals were perfused with Ringer's solution at a rate of 2 μl/min, using a microperfusion pump (Univentor-864 Syringe Pump, Agn Tho's AB, Lidingö, Sweden). Animals were perfused for one to two hours prior to baseline sampling, allowing for equilibration. Samples (40 μl) were then collected every 20 minutes and analysed on-line for dopamine content using a HPLC system with electrochemical detection. After a stable baseline had been confirmed (±10 %), drugs were perfused. In all experiments dopamine was the only substance quantified in the dialysate, and all drugs in the perfusate were dissolved in Ringer's solution.

Biochemical assays

Dialysate dopamine content was separated and quantified using high performance liquid chromatography (HPLC) with electrochemical detection. Two systems were used simultaneously. The first system (Papers I, II and IV) utilized a stainless steel

ion exchange column of 2 x 150 mm, operated at 32 °C and packed with Nucleosil SA (5 μm diameter; pore size 100 Å; Phenomenex Scandinavia, Västra Frölunda, Sweden), with a mobile phase (flow rate 0.3 ml/min) consisting of (in mM) 58 citric acid, 135 NaOH, 0.107 Na_2-EDTA, as well as 20 % methanol. The electrochemical detector of this system (Decade, Kovalent AB, Sweden) operated at 400mV versus the cell (Hy-REF). The second system (Papers I and IV) used a stainless steel reversed phase column of 2 x 50 mm, operated at 30 °C and packed with silica (3 μm diameter; pore size 100 Å; Phenomenex Scandinavia, Västra Frölunda, Sweden). The mobile phase for this system (flow rate 0.3 ml/min) consisted of 150 mM NaH_2PO_4, 4.76 mM citric acid, 3 mM sodium dodecyl sulphate, 50 μM EDTA, as well as 10 % methanol and 15 % acetonitrile. The electrochemical detector (Dionex, Västra Frölunda, Sweden) operated at 220 mV versus the cell. The limit of detection was calculated to 2.69 pA for the first system and 3.49 pA for the second system. An external standard containing 3.25 fmol/μl of dopamine was used to identify the dopamine peak, as well as to quantify dopamine concentrations in the dialysates

Verifcation of probe placements

At the end of the experiments animals were sacrificed, brains removed and immediately placed in fixative (Accustain Formaline-free fixative; Sigma-Aldrich) for 3-7 days, before probe placements were verified using a vibroslicer (Campden Instruments Ltd, Leicester, UK) or a cutting block (custom made after description by Heffner, Hartman and Seiden [234]). Only animals with correct probe placements and no visual defects (e.g. bleeding) were included in the study (Fig. 8).

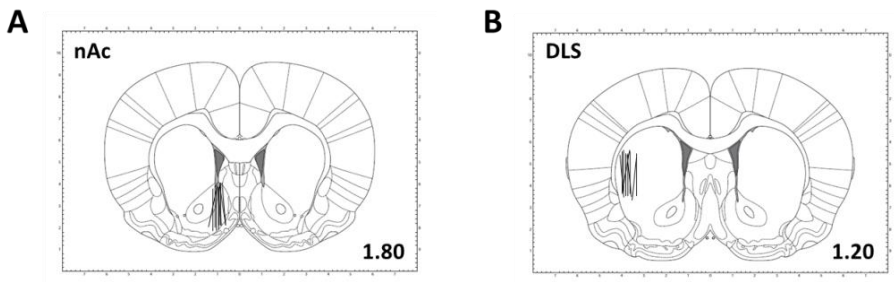

Figure 8. Coronal brain sections displaying representative accepted probe placements in the A) nAc and B) DLS. Numbers represent distance from bregma (mm), anterior/posterior axis. Adapted from Paxinos and Watson 2007 [84].

Electrophysiological experiments

Electrophysiology is the study of the electrical properties of biological tissues and cells; in the field of neuroscience including the electrical activity of neurons. The electrophysiological experiments conducted in this thesis were exclusively extracellular field potential recordings. This technique is performed in acutely isolated brain slices containing the brain region(s) of interest. A stimulation electrode (Fig. 9A) is positioned so as to activate pre-synaptic (primarily glutamatergic) afferents, resulting in an influx of (primarily) positively charged ions through glutamatergic receptors on post-synaptic cells. The resulting shift in electrical potential, as seen from the extracellular milieu, is measured by an extracellular recording electrode and is represented by the evoked population spike; PS. The negative wave of the PS represents the current "sink" detected extracellularly, as positive charges enter the surrounding cells. The PS amplitude provides a quantification of this effect, and reflects the synchronous activity of a large population of cells. By perfusing slices with agonists/antagonists of the receptor systems to be investigated, the impact of these receptor systems on striatal synaptic activity may be studied.

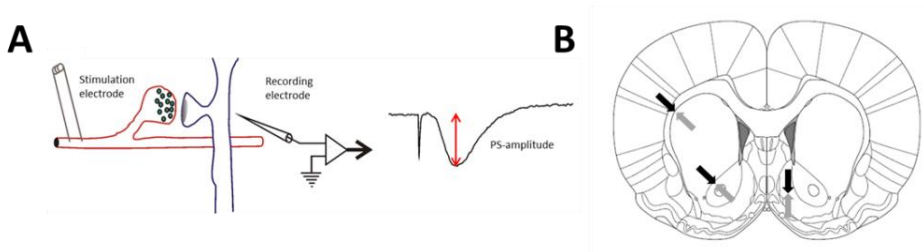

Figure 9. A) Schematic representation of the principal behind field potential recordings. Stimulation of glutamatergic afferents activates postsynaptic neurons and the resulting shift in electrical potential, the evoked population spike; PS, is measured by an extracellular recording electrode. Although only two cells are depicted here, the PS-amplitude reflects the synchronous activity of many cells. Image adapted with permission from wikimedia.org. B) Position of stimulation electrodes (black) and recording electrodes (grey) in the DLS, nAc core and nAc shell.

Changes in synaptic activity may be the result of altered presynaptic transmission (for instance, an increased release of glutamate), but may also result from changes in the postsynaptic cells (for instance, an increase in the number of glutamatergic receptors). In order to further investigate potential causes of evoked changes in PS amplitude, a paired-pulse facilitation protocol was implemented in Paper IV (see following sections).

Brain slice preparation

Animals were anaesthetized with isoflurane (Forene®, Baxter, Sweden) and decapitated. The brains were quickly removed and placed in a cutting solution consisting of a modified artificial cerebrospinal fluid (a-CSF), continuously bubbled with a mixture of 95% O_2/5% CO_2 gas. After a 5 min equilibration period the brain tissue was blocked at anterior and posterior ends and attached with histoacryl glue (Aesculap & Co., KG, Tuttlingen, Germany) to a Teflon pad and submerged into an ice-cold cutting solution. To obtain single hemisphere-slices containing the nAc or DLS the brain was separated along the midline with a razor and then sectioned coronally in 400 mm thick slices with a vibrating tissue sectioning system (Campden Instruments Ltd., Loughborough, England). Brain slices from juvenile animals were directly transferred to normal a-CSF, continuously bubbled with a mixture of 95% O_2/5% CO_2 gas and were then allowed to equilibrate for at least 1 h at room temperature. Slices from adult animals were first transferred to normal a-CSF kept at 30°C for 15 minutes, before equilibration at room temperature.

Striatal field potential recordings

One hemisphere of a slice was transferred to a recording chamber (four chambers in total) and perfused at a constant rate of 2.6 ml/min with pre-warmed aCSF kept at 30°C and continuously bubbled with a mixture of 95% O_2/5% CO_2 gas. For recordings the DLS (Papers II, III and IV), the stimulation electrodes were placed at the border of the subcortical white matter. For recordings in the nAc core (Paper II), the stimulation electrodes were placed close to the anterior commissure and dorsal to the recording electrode. In the nAc shell (Paper IV), the stimulation electrodes were placed in the medial shell region (Fig. 9 B).

In Papers II and III stimulation was delivered as 0.1 ms negative constant current pulses via a monopolar tungsten electrode (World Precision Instruments, FL, USA, type TM33B). Stimulus intensity was set to yield PS amplitudes approximately half the size of the maximal evoked responses, thus providing a margin for evoked increases or decreases in PS amplitude. Between the studied subregions there were no significant differences in half-maximal responses, which ranged from 0.14 to 1.3 mV, and were evoked with stimuli of 0.01–0.05 mA in intensity. Signals were amplified by a custom-made amplifier (gain 1000x), filtered at 3 kHz, digitized at 8 kHz (12-bit Analog-Digital converter with a maximum range of 10 V) and transferred to a PC for analysis. After monitoring a stable baseline for at least 10 min the appropriate treatment regimens were initiated.

If two synaptic responses are elicited in close succession, the second response can be either facilitated or depressed, depending on the initial release probability of the synapse population [235]. Changes in release probability can therefore be estimated

by calculating the ratio between the amplitude of the second and first PS (paired-pulse ration; PPR [236]). This, in turn, provides an indication of whether observed changes in PS amplitude are elicited by changes in pre-synaptic or post-synaptic signalling. In Paper IV a paired-pulse protocol was implemented, using paired-pulse stimulation with a 50 ms interpulse interval delivered every 20 s throughout the recording.

Drugs and solutions

Below are listed (alphabetically) the main drugs and chemicals used/administered in the experiments included in this thesis. All drugs perfused in microdialysis studies were dissolved in Ringer's solution, and substances injected systemically were dissolved in NaCl (0.9 %). All substances used in electrophysiological experiments were dissolved and perfused in a-CSF.

Acetaldehyde The first metabolite of ethanol (Sigma-Aldrich, Sweden).

a-CSF Artificial cerebrospinal fluid used as perfusion medium in electrophysiological studies. Content (in mM): 124 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4 and 10 D-glucose

AP-5 DL-2-amino-5-phosphonopentanoic acid, a competitive NMDA receptor antagonist (Tocris, UK).

Bicuculline A competitive $GABA_A$ receptor antagonist. Bicuculline methchloride was administered in microdialysis studies (Sigma-Aldrich, Sweden), in electrophysiological studies Bicuculline (Tocris, UK) was dissolved to 20 mM in DMSO and then diluted further in a-CSF.

Cutting solution Modified a-CSF consisting of (in mM) 194 sucrose, 30 NaCl, 4.5 KCl, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4 and 10 D-glucose

***D*-penicillamine** An acetaldehyde-sequestering agent (Sigma-Aldrich, Sweden).

Ethanol Diluted from Finsprit 95%, Kemetyl AB, Sweden

Mecamylamine A non-selective, non-competitive nAChR antagonist. In microdialysis studies Mecamylamine-HCl was acquired form

	Sigma-Aldrich, Sweden, in electrophysiological experiments from Sigma-Aldrich, USA.
MLA	Methyllycaconitine citrate, a selective α7- nAChR antagonist (Tocris, UK).
Nicotine	Administered subcutaneously (s.c.) as (-)- nicotine hydrogen tartrate, in 0.9 % NaCl neutralized with $NaHCO_3$ (Sigma-Aldrich, Sweden).
Picrotoxin	A non-competitive $GABA_A$ channel blocker. (Sigma-Aldrich, Sweden)
PMBA	Phenylbenzene-ω-phosphono-α-aminoacid, a non-competitive GlyR antagonist (Sigma-Aldrich, USA).
Ringer's solution	Perfusion medium in microdialysis studies. Composition (in mM): 140 NaCl, 1.2 $CaCl_2$, 3.0 KCl, 1.0 $MgCl_2$
Taurine	An endogenous amino acid-like compound that acts as a GlyR agonist (Sigma-Aldrich, Sweden).
Scopolamine	A competitive antagonist of muscarinic receptors (Scopolamine-HBr, Sigma-Aldrich, Sweden).
Strychnine	A competitive GlyR antagonist (Strychnine-HCl; Sigma-Aldrich, Sweden).

Data analysis and statistics

Microdialysis data

To obtain a stable baseline (± 10 %), at least 4 pre-drug samples were taken, and the average of the last two stable values was set to 100 % for each animal. Graphically, microdialysis data were presented over time as mean values ± the standard error of the mean (SEM), relative to baseline. As microdialysis experiments involved multiple treatment groups observed over several time points, statistical analysis was performed using two-way analysis of variance (ANOVA) with repeated measures, followed by a Bonferroni *post hoc* test when appropriate. Occasionally, microdialysis data was also presented graphically with bar charts displaying absolute dopamine concentrations or relative dopamine values at specific time points (± SEM), as compared to other relevant time points/treatment

groups. In these instances, one-way ANOVA or unpaired or paired t-tests were used for statistical analysis, as appropriate. In all statistical analyses a *p*-value of <0.05 was considered significant. Data was assembled in Microsoft Excel (Microsoft Inc.), graph assembly and data analysis was performed in Graphpad Prism (Graphpad Software Inc., USA).

Electrophyiological data

Population spikes were recorded every 20 seconds and amplitudes were combined to give a mean value for each minute. The means of the 10 last stable baseline values were set to 100 % for each slice. Time course figures were plotted as mean PS values compared to baseline ± SEM. Data was presented the text as the mean values of the PS at relevant time points, compared to individual baseline (usually calculated from the last 5 minutes of baseline sampling), with a 95 % confidence interval (CI). In experiments where paired pulse stimulation was used (Paper IV), the paired pulse ration (PPR) was calculated as the mean ratio of pulse 2 over pulse 1 at t = 25 to 30 min and presented in the text as % change with 95% CI, relative to baseline calculated at t = -5 to 0 min. PPR figures were plotted as relative change at t = 25 to 30 min, as compared to baseline values, with SEM. Two-tailed paired t-tests were used for statistical analysis, unless otherwise stated. A *p*-value of less than 0.05 was considered significant. Electrophysiological data was analysed with Clampex 10.1 (Molecular Devices, Foster City, CA), and assembled in Microsoft Excel. Statistical analysis and graph assembly was done in GraphPad Prism.

Methodological considerations

Microdialysis method

Microdialysis constitutes a powerful tool for monitoring and manipulating *in vivo* neurotransmission locally; allowing sampling from the extracellular environment in awake and freely moving animals. Effects of systemic treatment can be monitored in specific brain regions, and the use of multiple dialysis probes allows for the study of discreet neurocircuitries, by administering treatment in one brain region and monitoring effects in another. While this technique does not provide absolute extracellular concentrations of the analyte in question (se discussion below), it does reflect the extracellular milieu; although it should be kept in mind that microdialysis measurements probably better monitor changes in volume transmission, rather than fast, direct synaptic transmission, due to the size of the probe, as well as the relatively poor temporal resolution of this method.

One of the greatest problems with the microdialysis method, however, is the risk of tissue damage. As probe implantation is an invasive procedure, it is important to allow the animal sufficient time to recover from the surgery, as well as ensure that sampling will not be too influenced by leakage from damaged tissue. However, as the procedure may also activate microglia cells and induce scarring and necrosis (which in turn may reduce probe function), the latency period between surgery and dialysis must not be too long. 24-48 hours is usually considered an optimal recovery period [237, 238] and for this reason, animals were allowed to recover for 48 h before experiments were initiated. In addition, animals showing visual defects (e.g. bleeding) upon inspection of probe placements were excluded from the studies. Nevertheless, the possibility that bleeding or damage to the extracellular milieu caused by the probe may influence dialysis measurements cannot be ruled out.

Another challenge of the microdialysis method is choosing the concentrations of drugs to administer, as it is very difficult to estimate to which degree substances will pass over the dialysis membrane to the extracellular environment (the excovery). Similarly, the quantity of analyte measured only represents a fraction of the actual extracellular concentration (the extraction fraction or relative recovery). Both the recovery and excovery for different substances vary, depending on a variety of factors such as perfusion rate, the surface area of the dialysis membrane and temperature [239]. Characteristics of the substance itself, e.g. molecular weight, shape and tendency to bind to the membrane and/or tubing are also important. In addition, substances delivered by microdialysis diffuse along a concentration gradient [240] once in the extracellular environment, further complicating estimation of yielded *in situ* concentrations. Based on previous (unpublished) findings from our laboratory, the administration of 300 mM of ethanol via the perfusate was expected to yield a concentration of approximately 45-60 mM (i.e. an excovery of 15-20 %) immediately outside the probe [200]; a concentration range well within what may be expected following intoxicating levels of ethanol. Concentrations of other substances used in the experiments described here have been carefully chosen, taking into consideration an estimated excovery of 10-20 %, known EC50 values, as well as experience and knowledge from our own laboratory or from colleagues. Still, it cannot be excluded that concentrations of substances administered in the present experiments in actuality yielded far greater or lower *in situ* concentrations than were expected. For this reason, stated concentrations of administered substances in this thesis always refer to perfusate concentrations. Similarly, measured concentrations of dopamine in the dialysate are always stated as uncorrected for estimated extraction fraction/relative recovery.

Field potential recordings

Given the complexity of striatal afference and intra-striatal connections, the net striatal output response to a given stimuli is often very difficult to predict. Local

field potential recordings allow the study of the electrical activity of a large population of cells, which, contrary to the case in whole-cell recordings, have their intracellular composition intact. This enables tentative conclusions to be drawn with regards to the importance of selected receptor systems in contributing to the net striatal output.

Having said this, it is important to keep in mind that many afferent and efferent connections are cut during slice preparation; thus the results studied in field potential recordings may not necessarily reflect the actual outcome in the intact animal. Another disadvantage is that it is not known exactly which cells, or which afferents, contribute to the field response, which may complicate interpretation of results. The cortico-striatal glutamatergic projections are positioned in such a way that it is a fairly straight-forward matter to position stimulation electrodes so as to stimulate preferentially these afferents, when performing recordings in the DLS. In the nAc, glutamatergic afferents are distributed quite differently (see [95]), making electrode placement more challenging in this region. It is thus possible, that afferents other than glutamate are activated to a greater extent than in the DLS when recording in this region.

Core vs shell

As discussed in previous sections, the shell and core regions of the nAc differ with regards to afference, efference and cytoarchitecture. This in turn, may be indicative of the two regions having different roles to play in the development of addiction; indeed some studies indicate that this may be the case (see review [241]). While electrophysiological experiments included in this thesis were performed in either the shell or core region of the nAc, in most microdialysis experiments the dialysis probe was placed in the core-shell borderline region; suggesting sampling from and drug perfusion in both regions.

There have been several reports demonstrating that addictive drugs preferentially increase dopamine signalling in the nAc shell region, rather than in the core [79-81]. However, the coordinates used in our experiments have proven successful in our hands when demonstrating the dopamine elevating effects of ethanol after systemic (i.p.), local (nAc perfusion) and oral (self-administration) administration [58, 61, 63]. In the present studies we wanted to explore the possible involvement of acetaldehyde (Paper I), as well as inhibitory receptors (Paper IV), in mediating the dopamine elevating response of ethanol previously observed when administered in this region, which is why we considered it important that the same co-ordinates were used. Still, it cannot be excluded that the effects on dopamine in the nAc shell were "diluted", delayed or opposed by those in the core in these experiments, and that different result may have been obtained, had the probes been placed more exclusively in either region.

Experimental design

Paper I

To determine whether acetaldehyde alone could increase accumbal dopamine levels animals were administered acetaldehyde or ethanol (300 mM) in the nAc via reversed *in vivo* microdialysis, and dopamine levels were analysed using HPLC. To determine if ethanol-derived acetaldehyde was necessary for accumbal dopamine increase a subset of animals were pre-treated with acetaldehyde-sequestering agent *D*-Penicillamine (i.p.) prior to ethanol perfusion.

Paper II

To investigate the importance of GlyRs and $GABA_A$ receptors in modulating nAc core and DLS synaptic activity, local field potential recordings were performed in coronal brain slices from juvenile and adult animals. Slices were treated with GlyR and $GABA_A$ receptor antagonists, as well as cholinergic antagonists. GlyR and $GABA_A$ receptor antagonists were administered in the nAc core and DLS via reversed microdialysis in adult animals, to determine the importance of GlyRs and $GABA_A$ receptors in modulating basal dopamine levels in these regions. To investigate any involvement of the nAc-VTA-nAc neuronal circuitry in mediating dopamine-enhancing effects by antagonists in the nAc core, nAChR antagonist mecamylamine was administered in the VTA in some experiments.

Paper III

The effects of ethanol on synaptic activity in the DLS of juvenile and adult animals were investigated with field potential recordings in coronal brain slices. The involvement of different receptor systems in mediating ethanol-induced responses was studied by pre-treating slices with antagonists of GlyRs, $GABA_A$ receptors, NMDA receptors, as well as nAChRs and muscarinic receptors, prior to ethanol-exposure. In all experiments slices were pre-treated with antagonists for at least 20 minutes prior to ethanol exposure, and antagonists remained in the bath during ethanol perfusion and throughout the experiment.

Paper IV

To study differences between the nAc and DLS with regards to the involvement of GlyRs and $GABA_A$ receptors in ethanol-induced changes in dopamine, ethanol was administered locally in these regions via reversed microdialysis in animals pre-treated with GlyR or $GABA_A$ receptor antagonists. The effects of the same antagonists on ethanol-induced changes in synaptic activity were investigated in field potential recordings in the nAc shell and DLS of adult rats, using coronal brain slices.

RESULTS & DISCUSSION

Paper I

Increase in nucleus accumbens dopamine levels following local ethanol administration is not mediated by acetaldehyde

To determine the effects of acetaldehyde alone on accumbal dopamine levels, acetaldehyde (1, 10, 100 or 200 μM) was perfused locally in the nAc via reversed microdialysis in male adult Wistar rats. Results showed that acetaldehyde did not significantly alter accumbal dopamine levels at any concentration given (Fig. 10). Control animals perfused with ethanol (300 mM) in the same region exhibited an increase in accumbal dopamine levels, which is consistent with previous findings [62, 63, 200].

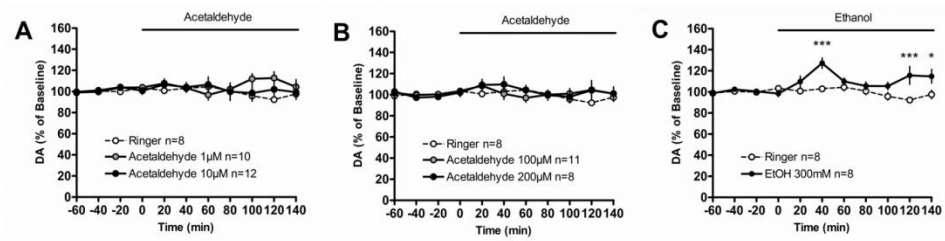

Figure 10. Local administration of acetaldehyde did not increase accumbal dopamine levels.

Generally, brain acetaldehyde concentrations following EtOH intoxication have been estimated to be in the low micromolar range, or lower [210, 242]. Assuming an excovery as low as 5-20 % in our experimental setup, the acetaldehyde concentrations used in the present study should theoretically generate *in situ* concentrations ranging from 50 nM to 40 μM. This is within the range of what may be expected following physiologically relevant levels of ethanol, that is self-administered in the VTA [225], that has resulted in increases in accumbal dopamine following administration in the VTA [226], [228], and that has been shown to activate GlyRs [232]. However, the magnitude of acetaldehyde brain levels produced by pharmacologically relevant concentrations of ethanol, as well as their pharmacological relevance, remains a controversial issue [210]. In addition, microinjection studies indicate that dopamine release following acetaldehyde may display an inverted "U-shaped" dose-response curve [228]. It is therefore possible that the concentrations of acetaldehyde used in the present experiment, despite the wide range, may have been insufficient. In order to circumvent this issue, in a final study, rats were administered ethanol locally in the nAc, following pre-treatment

with the acetaldehyde sequestering agent *D*-penicillamine (50 mg/kg, i.p.). *D*-penicillamine was administered according to a dose regimen that was identical, or very similar to, dose regimens that have proven effective in preventing ethanol-derived activation of VTA dopaminergic neurons and ethanol-induced increases in accumbal dopamine following systemic alcohol administration [229], as well as in preventing ethanol-induced place-preference [224] and behavioural stimulation induced by systemic [243], or intra-VTA microinjections of ethanol [244]. Yet in the present study *D*-penicillamine was not effective in attenuating nAc dopamine increases following local ethanol perfusion (Fig. 11).

In conclusion, the data presented here suggest that the increase in accumbal dopamine following local ethanol administration is not mediated by acetaldehyde.

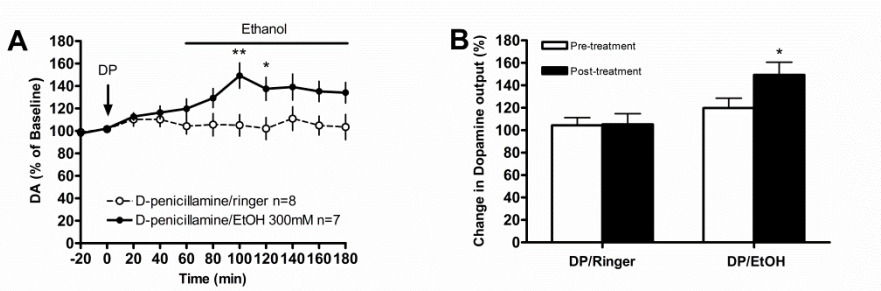

Figure 11. (A) Sequestering of acetaldehyde with *D*-penicillamine does not inhibit ethanol-induced increases in nAc dopamine. (B) Change in dopamine output at t= 100 min relative to time-point of ethanol administration.

Paper II

Subregion-specific modulation of excitatory input and dopaminergic output in the striatum by tonically activated GlyRs and $GABA_A$ receptors

In order to determine the importance of $GABA_A$ receptors in modulating basal synaptic activity in the DLS and nAc core, local field potential recordings were performed in brain slices from juvenile/young adult (9 weeks of age) rats treated with competitive $GABA_A$ receptor antagonist bicuculline or non-competitive receptor channel blocker picrotoxin. Both antagonists produced an increase in PS amplitude in both regions, with no differences between juvenile and adult animals (Fig 12. A, B). The relative increase in PS amplitude following treatment with $GABA_A$ receptor antagonists was significantly higher in the DLS than in the nAc core. These findings suggest that $GABA_A$ receptors exhibit a tonic inhibitory control over synaptic activity in both regions, possibly exerting a stronger influence in the DLS as compared to the nAc core.

In order to determine the roll of GlyRs in modulating striatal synaptic activity slices were treated with competitive GlyR antagonist strychnine or non-competitive antagonist PMBA (Fig. 12 C, D). Both antagonists increased PS amplitude in both regions. The increase was significantly larger in the nAc core than in the DLS in slices treated with strychnine, indicating that GlyR-mediated inhibition of synaptic activity is more pronounced in this region than in the DLS. This difference was however not replicated in slices treated with PMBA.

To investigate the role of cholinergic and $GABA_A$ receptors in mediating strychnine-induced increases in PS-amplitude slices from juvenile animals were pre-treated with non-competitive, non-selective nAChR-antagonist mecamylamine, competitive, non-selective muscarinic receptor antagonist scopolamine and bicuculline in the nAc core, as well as with bicuculline in the DLS. Strychnine-induced increases in PS-amplitude in the core were not affected by scopolamine, were partially attenuated by mecamylamine and were completely blocked by bicuculline, indicating that GlyRs depress striatal synaptic activity through modulation of cholinergic and GABAergic neurotransmission..

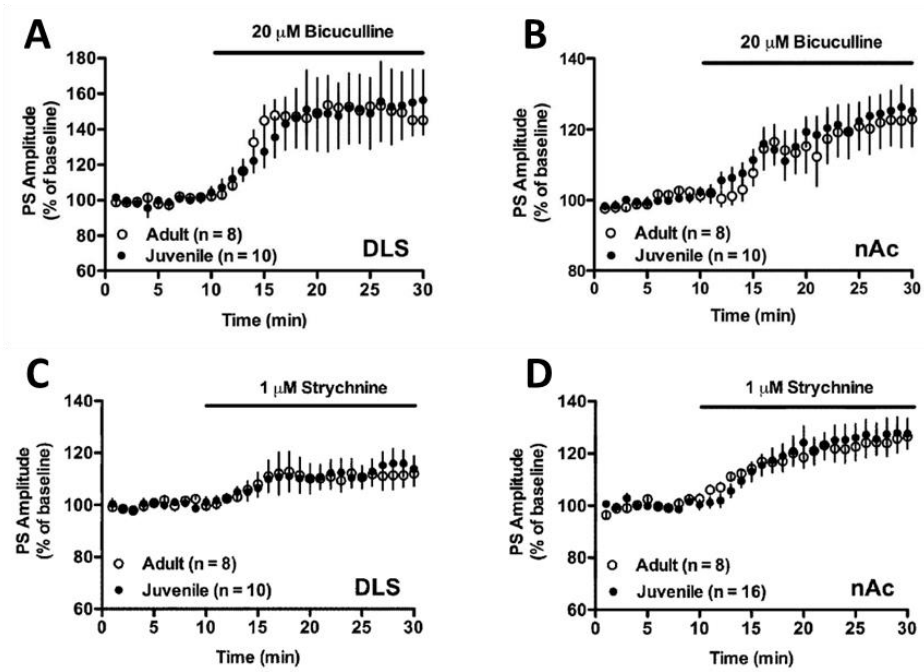

Figure 12. $GABA_A$ receptor inhibition with bicuculline increased PS amplitude in both the A) DLS and B) nAc core. GlyR inhibition with strychnine increased PS amplitude in both the C) DLS and D) nAc core. No differences were found between adult and juvenile animals.

Local microdialysis perfusion with bicuculline increased extracellular dopamine levels in both striatal subregions, although higher concentrations were required to achieve this effect in the DLS (Fig. 13 A, B). These results indicate that $GABA_A$ receptors exert a tonic inhibitory influence over dopaminergic transmission in the striatum, possibly with a more pronounced effect in the nAc than in the DLS. To determine if the dopamine-increasing effects of bicuculline were mediated by the nAc-VTA-nAc circuitry a subset of bicuculline-treated animals were perfused with mecamylamine in the VTA. Inhibition of VTA nAChRs however, did not attenuate bicuculline-induced elevation of dopamine (Fig. 13 C), indicating that $GABA_A$ receptor inhibition modulates accumbal dopamine locally, or via neurocircuitries other than the nAc-VTA-nAc-loop. Strychnine (200 μM) decreased dopamine levels in both regions (Fig 13. D), indicating that GlyRs are important in upholding striatal dopamine levels, a finding which is in line with previous reports [173].

In conclusion, the data presented here suggest that $GABA_A$ receptors and GlyRs in the DLS and nAc are tonically activated and influence striatal synaptic transmission and dopamine output in a partly sub-region specific manner.

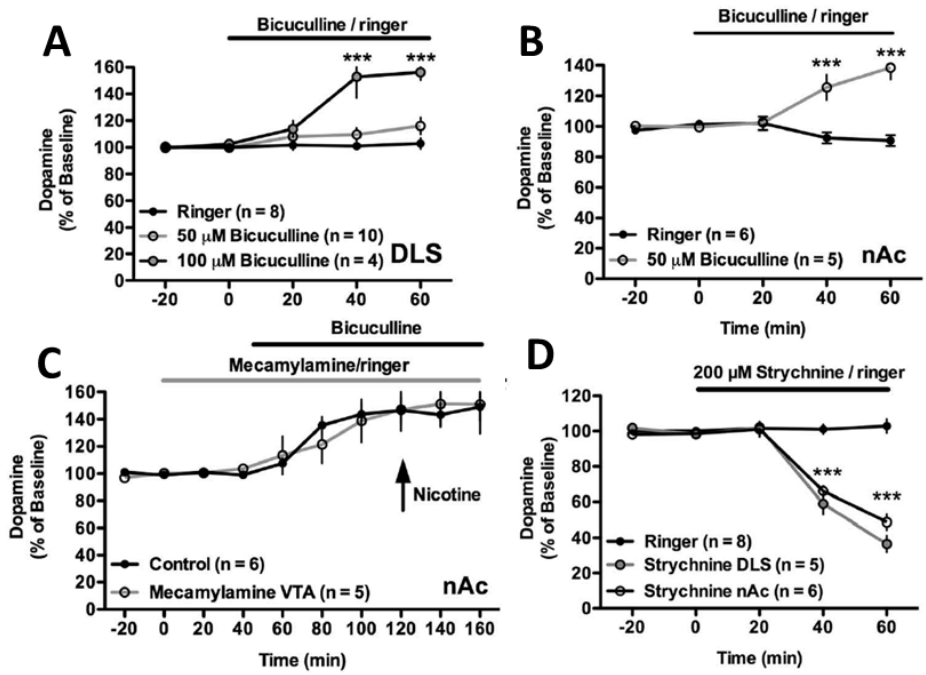

Figure 13. Bicuculline perfused via microdialysis increased dopamine levels in the A) DLS and B) nAc. C) Co-perfusion with mecamylamine in the VTA did not attenuate bicuculline-induced increases in accumbal dopamine. D) Strychnine decreased dopamine in both subregions.

Paper III

Ethanol-induced modulation of synaptic output from the dorsolateral striatum in rat is regulated by cholinergic interneurons

The findings in paper II indicated a role for both GlyRs and $GABA_A$ receptors in modulating baseline synaptic activity in the striatum. To investigate the effects of ethanol *per se* on striatal synaptic activity , as well as the involvement of GlyRs and $GABA_A$ receptors in mediating these effects, electrophysiological field potential recordings were performed DLS-slices from juvenile Wistar rats treated with a range of ethanol concentrations (20, 50, 80 and 100 mM).

Results showed that 50 mM of ethanol, but no other applied concentration, induced a significant decrease in PS amplitude in juvenile rats (Fig. 14 A). Following ethanol wash-off, a significant increase in PS amplitude was seen following all ethanol concentrations, save the lowest (Fig. 14 B). These results indicate possible dual effects of ethanol on striatal synaptic activity, the balance of which varies with the concentration applied; one acute effect that depresses PS-amplitude and that is discontinued once ethanol is removed, as well an opposing long-term effect that facilitates excitatory transmission and that is sustained following ethanol wash-out. We proceeded to further evaluate possible receptor systems involved in these processes, focusing on effects produced by 50 mM ethanol.

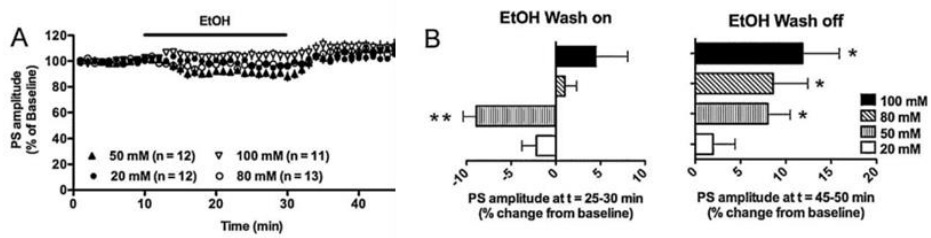

Figure 14. A) Only 50 mM ethanol decreased PS amplitude in the DLS of juvenile rats. B) PS amplitude was increased following ethanol wash-off.

Ethanol is known to inhibit the activity of NMDA receptors [133], but has also reported to produce biphasic effects on NMDA receptor-mediated currents, including a long-term facilitation [245]. It was thus possible that ethanol-induced changes in NMDA receptor activity may underlie the modulations of PS amplitude produced by ethanol in the slices. Pre-treatment with competitive NMDA receptor antagonist DL-2-amino-5-phosphonopentanoic acid (AP-5) however, did not inhibit the ethanol-induced decrease in PS-amplitude, nor the subsequent increase in PS-amplitude following ethanol wash-out, indicating that NMDA receptors were not involved in the effects produced by ethanol.

Pre-treatment with GlyR inhibitors strychnine and PMBA eliminated the ethanol-induced depression, as well as increases in PS amplitude following wash-out (Fig. 15), indicating an important role for GlyRs in mediating ethanol-induced modulation of PS amplitude.

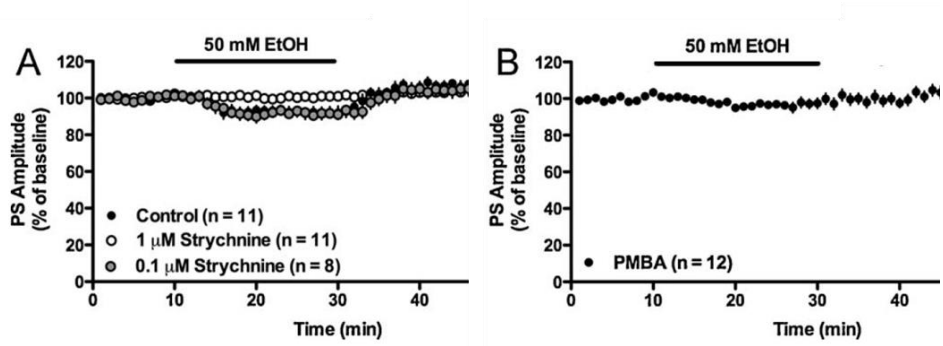

Figure 15. Pre-treatment with A) strychnine or B) PMBA blocked ethanol-induced modulation of PS amplitude.

To investigate the importance of cholinergic transmission in mediating ethanol effects slices were pre-treated with mecamylamine or scopolamine. Mecamylamine prevented both the acute depression and long-term facilitation in striatal output produced by ethanol, and ethanol-induced modulation of PS amplitude was also blocked by selective α7 nAChR antagonist methylly-caconitine citrate (MLA). This would suggest that nAChRs are involved in mediating ethanol effects. Scopolamine, on the other hand, did not prevent ethanol-induced depression of striatal output, however the long-term facilitating effect of ethanol on striatal output was blocked by scopolamine.

The experiments with scopolamine suggested that while muscarinic receptors may not mediate the depressing effects of ethanol on synaptic activity, they may be involved in the increase in PS amplitude observed following ethanol wash-out. To explore this possibility, slices were pre-treated with scopolamine and then perfused with 100 mM ethanol, which was a concentration that alone did not affect PS amplitude. In scopolamine-treated slices 100 mM ethanol significantly decreased PS amplitude (Fig. 17 B). These results suggest that muscarinic receptors are involved in the long-term facilitating effects of ethanol on synaptic activity, and by inhibiting muscarinic receptors the acute, PS amplitude-depressing effects of ethanol were unveiled, even at higher ethanol concentrations.

$GABA_A$ receptor involvement in the effects of ethanol was explored by pre-treating slices with bicuculline or picrotoxin. Both $GABA_A$ receptor antagonists inhibited ethanol-induced modulation of PS amplitude, indicating that ethanol effects on striatal synaptic activity are dependent on $GABA_A$ receptor activation (Fig. 16).

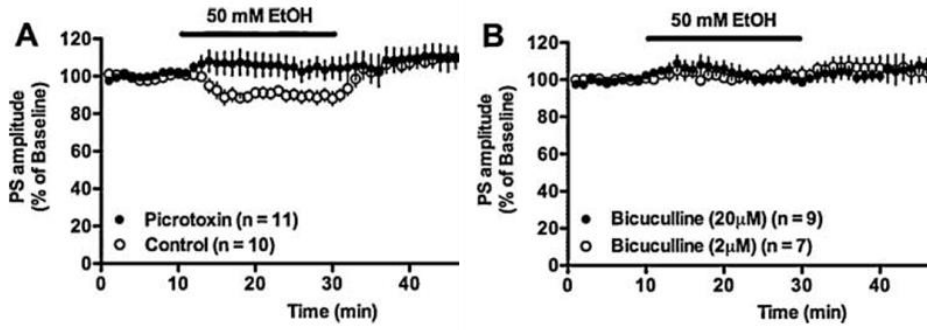

Figure 16. Pre-treatment with a) picrotoxin or B) bicuculline blocked ethanol-induced modulation of PS amplitude

To investigate possible age-differences slices from adult animals were perfused with 50 mM ethanol. Contrary to the case with juveniles, 50 mM ethanol increased PS-amplitude in slices from adult rats and PS amplitude was also enhanced following ethanol wash-off (Fig. 17 A). One possible explanation for this age-discrepancy is that the long-term facilitating effects of ethanol on striatal activity are more pronounced in adult animals. Supporting this notion, it was shown that PS amplitude was significantly depressed by ethanol and returned to baseline levels following ethanol wash-out in slices from adult animals pre-treated with scopolamine.

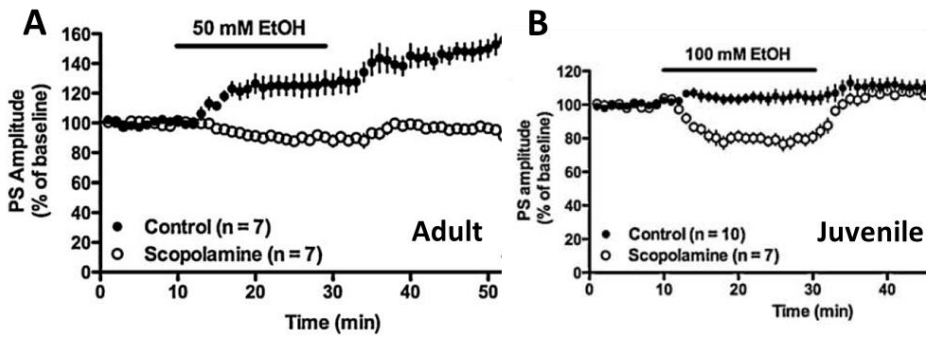

Figure 17. A) In slices from adult animals 50 mM ethanol induced an increase in PS amplitude, which could be blocked with scopolamine. B) When pre-treating slices from juvenile animals with scopolamine application of 100 mM ethanol, which alone did not depress PS amplitude, induced a decrease in synaptic activity.

In conclusion, ethanol appears to produce dual, counteracting actions on striatal output that are modulated by GlyRs, $GABA_A$ receptors and cholinergic receptors. The results also suggest that the balance of ethanol's opposing effects on synaptic activity are dependent on the concentration of ethanol applied, as well as the age of

the animal; with juvenile animals being more susceptible to the depressing effects of ethanol on synaptic activity.

Paper IV

Involvement of inhibitory receptors in regulating dopamine signalling and synaptic activity following acute ethanol exposure in striatal subregions

The findings in paper III indicated that the effects of ethanol on synaptic activity in the DLS of juvenile animals were dependent on GlyRs and $GABA_A$ receptors, and may be age-dependent. We now wished to further explore the role of these receptors in modulating ethanol-induced changes in dopamine efflux in striatal subregions. As most experiments in Paper III were performed in the DLS in slices from juvenile animals, we also wished to confirm these results with recordings in adult animals in both the nAc and DLS.

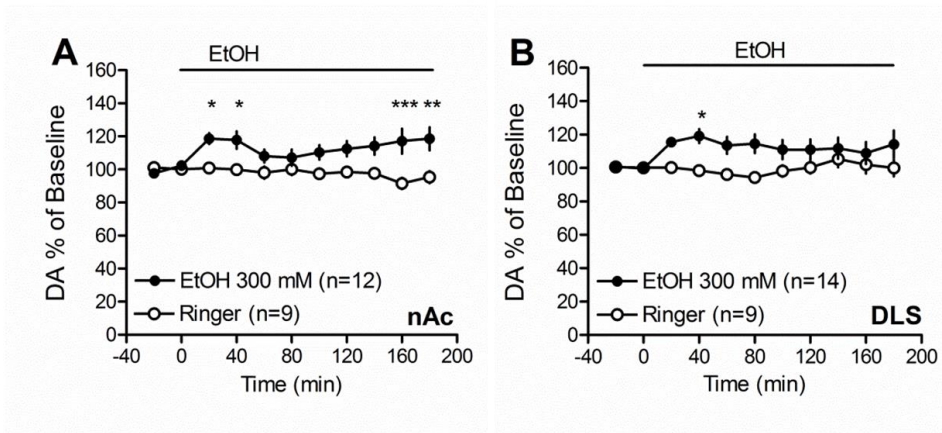

Figure 18. Local ethanol perfusion increases dopamine levels in the A) nAc and B) DLS.

Microdialysis experiments showed that ethanol (300 mM) perfused locally increased dopamine levels in both the nAc shell and DLS of adult male Wistar rats (Fig. 18). In the nAc shell dopamine-induced increases in dopamine were inhibited by strychnine (Fig. 19 A, B), which was in line with previous findings in Paper I, as well as other publications [63, 200], further supporting an important role for accumbal GlyRs in modulating ethanol-induced increases in dopamine.

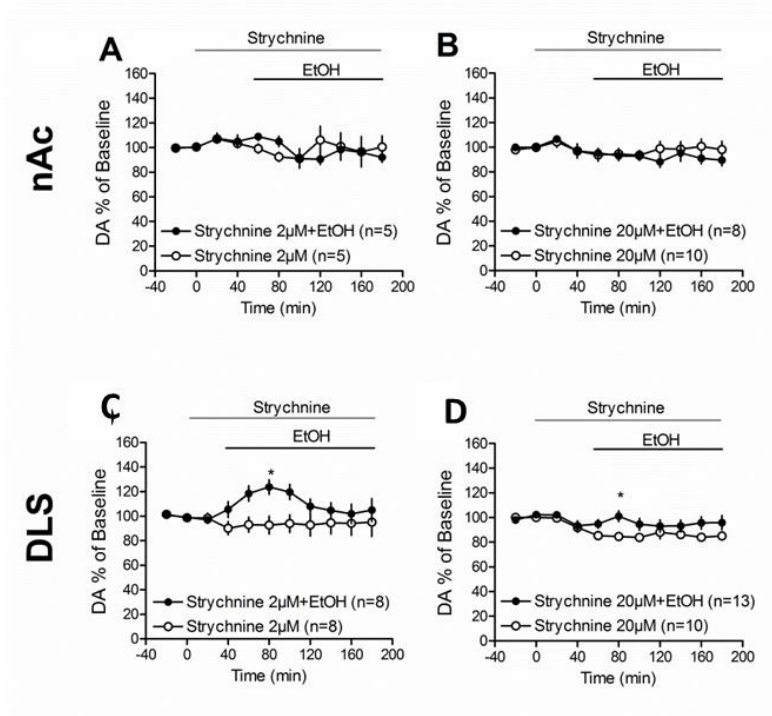

Figure 19. Ethanol-induced increases in dopamine were inhibited by A) 2 μM and B) 20 μM strychnine in the nAc shell, but neither C) 2 μM nor D) 20 μM inhibited ethanol-induced dopamine increases in the DLS.

Ethanol-induced increases in accumbal dopamine were not blocked by a low (5 μM) concentration of bicuculline, but were inhibited by pre-treatment with higher bicuculline concentrations (20 and 50 μM; Fig. 20 A-C). As IC_{50} concentrations of bicuculline are usually reported to lie in the low micromolar range, it was possible that a perfusate concentration of 5 μM had resulted in pharmacologically irrelevant *in situ* concentrations. Pre-treatment with 5 μM of bicuculline however, inhibited decreases in dopamine induced by perfusion of $GABA_A$ receptor agonist muscimol (10-75 μM; Fig. 20, insert), showing that this concentration was sufficient to inhibit $GABA_A$ receptor activation. The results obtained with co-perfusion of ethanol and 5 μM bicuculline corroborate previous findings with local picrotoxin, which failed to block ethanol-induced accumbal dopamine elevations [203, 204], indicating that $GABA_A$ receptors in the nAc are not important to this mechanism.

However, pre-treatment with two higher concentrations of bicuculline (20 μM and 50 μM), inhibited ethanol-induced dopamine elevation. It should be noted though, that the highest concentration of bicuculline (50 μM) in itself significantly increased accumbal dopamine levels, and although 20 μM of bicuculline did not

significantly affect dopamine efflux, a trend toward an increase can be discerned. Thus, it is difficult to ascertain whether the inhibition of ethanol-induced dopamine increases obtained with these concentrations of bicuculline were due to physiological antagonism, or if they represent a pharmacological antagonism of $GABA_A$ receptors involved in the dopamine-enhancing effects of ethanol.

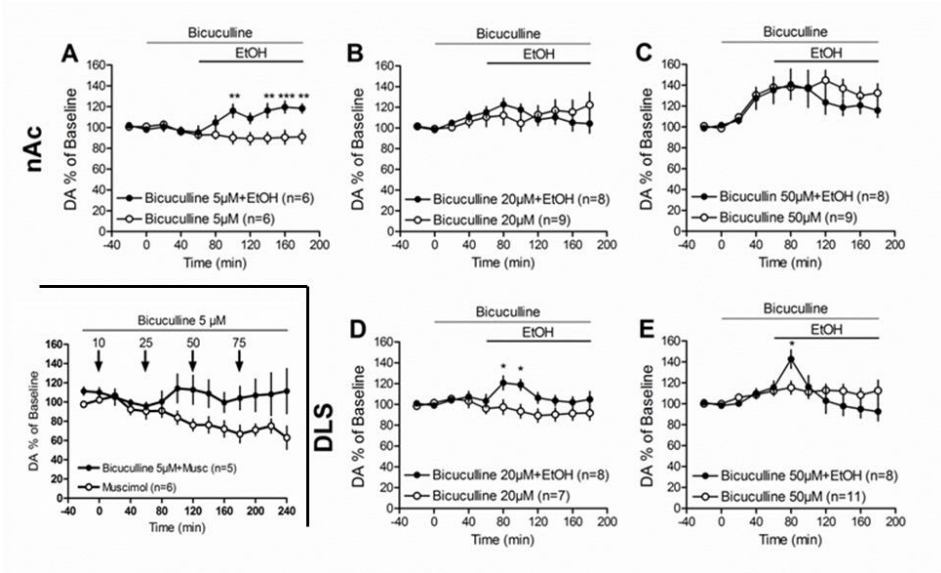

Figure 20. In the nAc, pre-treatement with A) 5 μM bicuculline did not inhibit ethanol-induced increases in dopamine, whereas pre-treatment with B9 20 μM or C) 50 μM bicuculline did. In the DLS neither D) 20 μM nor E) 50 μM bicuculline pre-treatment inhibited ethanol-induced increases in dopamine. Insert: 5μM bicuculline effectively inhibited decreases in accumbal dopamine induced by increasing concentrations of muscimol.

In the DLS local ethanol perfusion resulted in an increase in dopamine levels (Fig. 18 B), which was in line with previous findings from one research group [66, 67]. Pre-treatment with strychnine (Fig. 19C, D) or bicuculline (Fig. 20 D, E) did not inhibit the ethanol-induced dopamine increase, indicating that neither GlyRs nor $GABA_A$ receptors are important to the dopamine-elevating effects of ethanol in this region.

Field-potential recordings in the nAc shell showed that ethanol (50 mM) produced an increase in PS amplitude. PPR was decreased following ethanol perfusion, indicating that ethanol increase PS amplitude by increasing pre-synaptic glutamate release. Pre-treatment with both strychnine and bicuculline completely blocked ethanol-mediated increases in PS amplitude, indicating that both GlyRs and $GABA_A$ receptors are involved in ethanol-induced increases in glutamatergic transmission (Fig. 21 A-C).

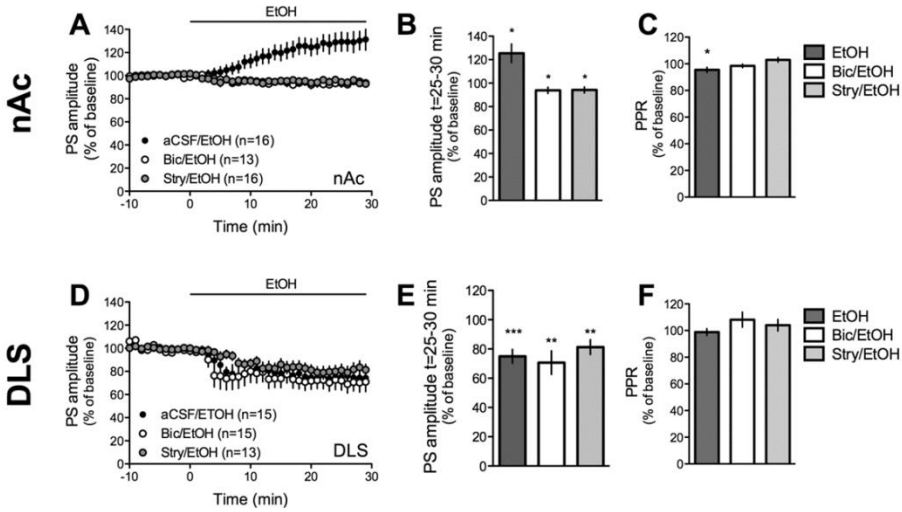

Figure 21. Field-potential recordings showed that 50 mM ethanol A) increased PS amplitude in the nAc shell in a strychnine and bicuculline sensitive manner, whereas D) PS amplitude was decreased in a strychnine and bicuculline insensitive manner in the DLS. B) and C) show changes in PS amplitude at t= 25-30 min relative to baseline and C) and F) show changes is PPR following ethanol in both regions.

In the DLS ethanol significantly depressed PS amplitude. This finding is in line with results from juvenile slices (Paper III), but contradicts findings with slices from adult animals in Paper III, where PS amplitude was increased following 50 mM ethanol perfusion. One possible explanation for this discrepancy was that adult animals used in Paper III had been older than animals used in Paper IV, suggesting age-related differences. In order to confirm this hypothesis additional field-potential experiments were performed (not published or included in manuscript), which showed that in aged animals (weight approximately 500 g) 50 mM ethanol produced a significant increase in PS amplitude (Fig. 22). PPR was unchanged following ethanol perfusion, indicating that ethanol decreased DLS synaptic activity by modulating the post-synaptic response. Ethanol-induced decreases in PS amplitude were not blocked by strychnine or bicuculline (Fig. 21 D-F). This contradicts findings in juvenile animals in Paper III, and suggests a possible age-dependent difference in the importance of these receptor systems in mediating ethanol-induces changes in striatal output.

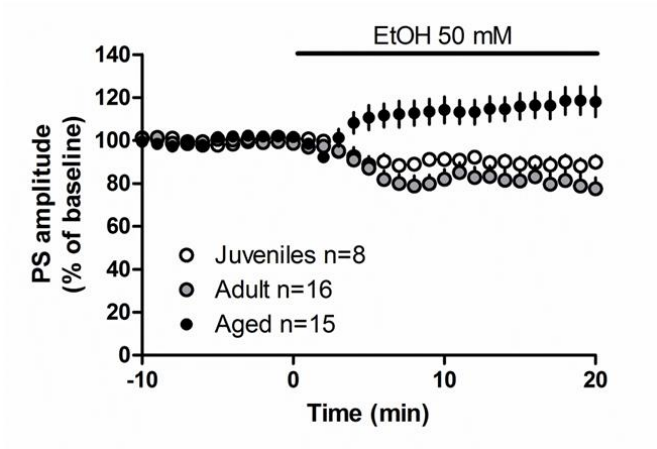

Figure 22. Age-related differences in ethanol-induced changes in PS amplitude in the DLS.

CONCLUSIONS & GENERAL DISCUSSION

The role of acetaldehyde in ethanol reward

The notion that ethanol-metabolite acetaldehyde may be aversive in the periphery, while rewarding in the brain [212] proposes acetaldehyde as a mediator of ethanol reward, in particular in individuals who may have a high tolerance to the aversive, peripheral actions, coupled with a high sensitivity to the centrally rewarding ones. The research performed in our laboratory has largely centred on glycine receptors, and their importance in mediating the dopamine-elevating properties of ethanol through the nAc-VTA-nAc neuronal circuit previously described. As acetaldehyde has been shown to interact with the GlyR [232], as well as increase levels of taurine in the nAc [233], the prospect that the effects of locally applied ethanol in the nAc may, at least in part, be attributed to acetaldehyde provided an intriguing possibility that needed to be addressed.

The issue of acetaldehyde's role in the rewarding properties of its parent compound has been controversial, and there are a number of confounding factors complicating the interpretation of early studies. Firstly, while administration of acetaldehyde alone increases accumbal dopamine levels [226, 228, 229], so does aversive stimuli [246, 247]. Secondly, while self-administration and conditioned place-preference studies do suggest that acetaldehyde alone has rewarding properties [221, 225], the ultimate question is whether physiologically relevant levels of acetaldehyde were used. Thirdly, technical caveats involved in the quantification of acetaldehyde brain concentrations [210] have necessitated the use of agents which manipulate ethanol and/or acetaldehyde metabolism, e.g. catalase inhibitors, which involve problems with specificity [211], as well as other issues.

The introduction of novel pharmacological tools such as acetaldehyde-sequestering agents and lentiviral vectors has provided new means to investigate the role of ethanol-derived brain acetaldehyde in alcohol reward. Sequestering agents such as *D*-penicillamine allow for the inactivation of centrally formed ethanol-derived acetaldehyde, without interfering with the metabolism of ethanol itself. Lentiviral vectors in turn, may be designed to encode anti-catalase shRNA, effectively and selectively inhibiting the expression of the catalase enzyme. Using the latter method, Karahanian and colleagues convincingly demonstrate an important role for ethanol-derived acetaldehyde in the VTA [231, 248, 249]. In these studies voluntary ethanol consumption, as well as post-deprivation binge drinking and ethanol-induced increases in dopamine, were greatly reduced in ethanol-naïve animals injected in the VTA with lentiviral vectors encoding anti-catalase shRNA or ALDH-2, leading to a decrease in VTA acetaldehyde levels by minimizing catalase activity or increasing acetaldehyde clearance.

Considering these findings, the negative results obtained in the present study were somewhat surprising. None of the concentrations of locally administered acetaldehyde, despite encompassing a wide range, increased accumbal dopamine. In addition, acetaldehyde-sequestering agent *D*-penicillamine did not attenuate ethanol-induced increases in nAc dopamine, despite following a dose regimen proven effective in inhibiting ethanol actions in a variety of behavioural models of reward [222, 243]. These results would indicate that neither acetaldehyde alone, nor ethanol-derived acetaldehyde, is important in activating the nAc-VTA-nAc neuronal circuitry.

The studies by Karahanian and colleagues discussed above, further pinpoint the VTA as a region of particular importance in acetaldehyde action. One possible explanation for the lack of acetaldehyde-related effects in the nAc in the present study may be related to the great heterogeneity in the expression of catalase in different brain regions [220, 250]. This, in turn, suggests that local levels of acetaldehyde following alcohol consumption may vary greatly, with larger quantities being produced in areas rich in aminergic neurons, such as the VTA [219]. The nAc, however, contains low/undetectable catalase levels [219]. It is thus possible that following alcohol consumption pharmacologically relevant levels of acetaldehyde are not normally present in the nAc, and that the dopamine-enhancing effects of ethanol are mediated by other mechanisms in this region, e.g. via GlyRs. As the actions of acetaldehyde did not appear essential to the nAc-VTA-nAc circuitry, the role of this compound was not investigated further in the subsequent studies included in this thesis.

Future perspectives in acetaldehyde research

The role of acetaldehyde in alcohol reward is an important issue, as it in essence implies that ethanol is merely a pro-drug. Should this be the case, the ramifications would include a significant paradigm shift in alcohol research, as well as in the clinical situation. The use of alcohol-deterrent disulfiram (Antabus®), which acts by increasing acetaldehyde levels in both the periphery and in the brain, may be put into question. In addition, the encouraging findings by Karahanian *et al* in naïve animals suggest that new therapeutic avenues should be explored in the treatment of AUDs, such as central acetaldehyde sequestering agents. An important challenge, of course, is finding substances that may inactivate acetaldehyde in the brain, but not in the periphery.

A vital point however, is that while the manipulations of ethanol/acetaldehyde metabolism in the VTA via lentiviral vectors performed by Karahanian and colleagues were effective in preventing drinking in ethanol-naïve animals, they did not reduce alcohol intake in animals in which a chronic drinking behaviour had already been established [248, 249]. This suggests that factors other than VTA

acetaldehyde may be involved in the perpetuation of alcohol self-administration. Indeed, additional experiments showed that by changing the contextual cues of alcohol, catalase-depleted animals discontinued their drinking behaviour, relative to controls [249]. Conversely, only changing the cues without targeting pharmacological reward did not alter drinking behaviour in controls. This would indicate that blunting ethanol reward alone is not enough to combat addiction; additional behavioural reconditioning is probably also required.

The actions of ethanol alone (and/or taurine) in the nAc appear to represent different mechanisms entirely by which accumbal dopamine increase, but also other possible upstream, parallel or downstream processes produced by alcohol, are implemented. A large body of evidence supports the nAc as an important point of origin in activating the mesolimbic dopamine system [183], and deep brain stimulation of the nAc has been shown to alleviate alcohol dependency, supporting a role for the nAc also in addiction therapy [251]. It is therefore likely that the dual importance of the nAc and the VTA reflects two separate pathways by which ethanol/acetaldehyde increases accumbal dopamine, which are not necessarily mutually exclusive. Therefore, pharmacological strategies aimed at interfering with alcohol actions at either site may be seen as complementary, and highly relevant in the treatment of addiction.

Inhibitory receptors modulate baseline neurotransmission

Before exploring the role of GlyRs and $GABA_A$ receptors in mediating ethanol-induced changes in synaptic activity and dopamine output in striatal subregions, it was necessary to assess the relevance of these receptor systems during baseline conditions, when no ethanol was present. While about 80 % of synapses in the striatum are glutamatergic, [118] results from Paper II show that both GlyRs and $GABA_A$ receptors exert a powerful inhibitory influence over excitatory transmission in both the nAc core and DLS, with no differences between juvenile and adult animals. In addition, it would appear that $GABA_A$ receptor-mediated inhibition of synaptic activity is more pronounced in the DLS than in the nAc core, while the opposite may be true for GlyRs, although discrepancies between experiments with strychnine and PMBA make the latter conclusion less certain.

The differences in the relative effects of the antagonists in the nAc core and DLS may be related to regional differences in local connectivity, glycinergic/GABAergic tone or GlyR/$GABA_A$ receptor localisation. For instance, it has been shown that MSNs in the ventral striatum receive more inhibitory input from local circuits than the dorsal striatal MSNs [90], which may explain the difference in $GABA_A$ antagonist sensitivity observed in Paper II. To our knowledge, glycinergic neurons have not been found in the brain, however glycine, as well as GlyR agonists taurine and β-alanine, have all been detected by microdialysis in the nAc during baseline

conditions [63, 252]. It is thus probable that GlyRs in the striatum are tonically active, exerting a net inhibitory tone on synaptic activity. While $GABA_A$ receptors are present on virtually all striatal cells, MSNs do not appear to express GlyRs [165, 174, 175], suggesting that GlyR-mediated inhibition of these neurons may be more indirect than GABAergic inhibition. GlyRs are predominantly located on cholinergic interneurons and on subpopulations of GABAergic interneurons in both the dorsal and ventral striatum [174, 253, 254]. It is thus plausible that GlyR-mediated inhibition of striatal output is mediated via changes in cholinergic and/or GABAergic neurotransmission. The experiments in Paper II show that strychnine-induced increases in PS amplitude were mediated by $GABA_A$ receptors, as well as nicotinic, but not muscarinic, acetylcholine receptors in the nAc core. Thus, it is plausible that GlyRs located on GABAergic, as well as cholinergic interneurons, influence striatal synaptic activity in a manner that leads to a net depression of striatal output during conditions present in slice recordings. Unfortunately, as no paired-pulse protocol was implemented in Paper II, it is difficult to ascertain whether the net inhibitory control mediated by $GABA_A$ and GlyRs over striatal output is due to inhibition of presynaptic glutamatergic afferents, or if it involves modulation of the post-synaptic response. Further experimentation is required in order to address this issue.

While GlyRs and $GABA_A$ receptors appear to exert a net inhibitory control over synaptic activity in slice recordings in both the nAc core and the DLS, baseline dopamine release appears to be oppositely modulated by the two receptor systems. Microdialysis studies in Papers II and IV show that local antagonism of GlyRs with strychnine decreases dopamine levels in a similar manner in both the nAc and the DLS, indicating that GlyRs are important in upholding striatal dopamine levels [173]. On the other hand, inhibition of $GABA_A$ receptors with bicuculline increased dopamine levels in both subregions, indicating that $GABA_A$ receptors exert an inhibitory influence over striatal dopamine levels. The dopamine-reducing effects of $GABA_A$ receptor agonist muscimol in Paper IV further support reports from our group and others that $GABA_A$ receptor activation in the nAc reduces accumbal dopamine [255-257].

Previous research indicates that GlyRs influence accumbal dopamine levels indirectly, through modulating the activity of dopaminergic neurons in the VTA via the nAc-VTA-nAc neuronal circuit [173, 190, 198]. The dopamine-enhancing effects of bicuculline in the nAc were however not attenuated by nAChR blockade in the VTA with mecamylamine, suggesting that GABA exerts an inhibitory control over accumbal dopamine efflux locally, perhaps via $GABA_A$ receptors located on dopamine terminals, or on cholinergic interneurons, which have been shown to regulate both dopamine and GABA release from dopaminergic terminals in the nAc [107, 258]. It can however not be excluded that distal neurocircuitries other than the nAc-VTA-nAc connection investigated here may be involved in these effects.

While GABAergic influence over synaptic activity appears to be stronger in the DLS than in the nAc core, $GABA_A$ receptor-mediated modulation of dopamine efflux appears stronger in the nAc than in the DLS, as a higher concentration of bicuculline was required in order to influence DLS dopamine levels in both Paper II and IV. This may be related to differences in local connectivity, GABAergic tone or $GABA_A$ receptor localisation as previously discussed, but it is also possible that both GlyR and $GABA_A$ receptor mediated influences on DLS dopamine may involve interactions between the striatum and the SN, akin to the reciprocal connection between the nAc and VTA.

Inhibitory receptors modulate ethanol actions

Field-potential recordings from the DLS in slices from both juvenile and adult animals (Paper III) suggest that ethanol exerts dual, counteracting effects on synaptic activity. The balance of these opposing effects appeared to be dependent on the concentration of ethanol applied, as well as the age of the animal. It is suggested that juveniles and younger adults are more susceptible to the inhibitory influences of ethanol on DLS synaptic activity, resulting with a decrease in PS amplitude which is reversed above baseline when ethanol is removed, while in older animals ethanol produces a net increase in striatal output which is seen directly upon application and elevated further following wash-out.

In juvenile slices (Paper III) GlyRs, nAChRs and $GABA_A$ receptors appear involved in both the depressing and enhancing effects of ethanol on PS amplitude in the DLS. Muscarinergic receptors appear important primarily in the latter in slices from both juvenile and older animals. Cholinergic interneurons exert an excitatory influence over MSN activity directly, possibly via M1 receptors (which are excitatory in D2 MSNs) [259], but they also modulate GABAergic feed-forward inhibition, possibly via nAChRs, which exerts a powerful inhibitory influence over MSN activity [101, 260]. In addition, cholinergic interneurons also regulate neurotransmitter release from glutamatergic afferents, possibly via inhibitory M2 receptors [261]. Glutamatergic transmission, in turn, influences the activity of all cells in the striatum. Ethanol effects on GlyRs and $GABA_A$ receptors may thus influence both inhibitory and excitatory microcircuits which shape the net striatal output. Unfortunately, as no paired-pulse protocol was implemented in Paper III, it remains to be determined if the effects of ethanol in slices from juvenile animals are mediated by changes in presynaptic glutamatergic release or by postsynaptic modulation.

In Paper IV it was shown that the decrease in DLS PS amplitude following acute ethanol was related to modulation of the postsynaptic response, rather than direct influences on presynaptic glutamate release in slices from (young) adult animals. Contrary to slices from juvenile animals, however, the ethanol effect on PS

amplitude was not modulated by strychnine or bicuculline, indicating (somewhat surprisingly) that neither GlyRs nor $GABA_A$ receptors appear to be important in mediating the depressant effects of ethanol in slices from (young) adult animals. Instead, ethanol actions may perhaps be attributed to inhibition of NMDA receptors [133, 262] or modulations of cholinergic transmission via receptor systems other than Gly or $GABA_A$ [259]. Field-potential recordings from the nAc shell in slices from (young) adult rats showed that in this region ethanol induced an increase in PS amplitude, which was attributed to an increase in presynaptic glutamate release. The ethanol-induced increase in PS amplitude in the nAc shell was blocked by both strychnine and bicuculline, indicating that both GlyR and $GABA_A$ receptor activity may influence glutamatergic neurotransmission in this region, perhaps by attenuating cholinergic inhibition of glutamate release by decreasing the activity of cholinergic interneurons.

Microdialysis experiments in Paper IV confirmed once again that ethanol-induced increases in accumbal dopamine are dependent on GlyR activation. Additional microdialysis in the nAc shell aimed to investigate the role of $GABA_A$ receptors in mediating ethanol-induced increases in dopamine in this region. The results obtained with co-perfusion of ethanol and a low (5 μM) concentration of bicuculline corroborate previous findings with local picrotoxin, which failed to block ethanol-induced accumbal dopamine elevations [203, 204], suggesting that $GABA_A$ receptors do not mediate the dopamine-increasing effects of locally applied ethanol in the nAc.

While pre-treatment with higher, baseline-perturbing concentrations of bicuculline contradicted these findings, the ability of 5 μM bicuculline to attenuate the effects of $GABA_A$ agonist muscimol confirmed that this was in fact an effective concentration. It thus seems likely that while nAc $GABA_A$ receptors are influential in modulating dopamine levels during baseline conditions, they are not important in mediating the dopamine-increasing effects of ethanol. Rather, previous research suggests that ethanol, by enhancing nAc $GABA_A$ receptor mediated inhibition, decreases dopamine levels [127, 204]. When combining the present and previously published data regarding local effects of ethanol in the nAc, the weight of evidence indicates that ethanol, by engagement of GlyRs and depression of MSN activity, releases dopamine by recruiting a nAc-VTA-nAc neuronal circuitry. Later on, inhibitory $GABA_A$ receptors on dopamine terminals may be engaged, resulting in varying degrees of counteraction of the dopamine elevation [127, 183, 206].

Despite the importance of DLS dopamine transmission in habit formation, few studies have investigated the effects of ethanol on dopamine transmission in the DLS. In Paper IV it was observed that local ethanol perfusion increases DLS dopamine levels. This finding may be the result of local actions of ethanol in the DLS (e.g. on dopaminergic terminals), but may also be attributed to ethanol affecting the activity of striato-nigral MSNs, which in turn may influence the activity of SNc dopaminergic neurons, resulting in increased dopamine release.

Additional experiments, exploring the role of the SN in mediating the dopamine enhancing effects of striatal ethanol perfusion are needed in order to answer this question.

The dopamine-enhancing effects of ethanol in the DLS were not inhibited by either strychnine or bicuculline. This would suggest that while both GlyRs and $GABA_A$ receptors are involved in modulating DLS dopamine levels during baseline conditions, none of these receptor systems appear to be important in mediating the dopamine-increasing effects of ethanol. While somewhat surprising, these results are strengthened by the observation that neither receptor system appeared to be involved in the ethanol-induced decreases in PS amplitude in this region. Clearly, additional experimentation is needed in order to determine the mechanisms/receptor systems involved in ethanol effects on DLS dopamine release, however considering the importance of cholinergic interneurons in regulating synaptic activity, nAChRs and/or muscarinic receptors constitute likely targets.

Striatal inhibitory receptors – future perspectives

Despite apparent similarities in cell population, cytoarchitechture, and involvement of inhibitory receptor systems in modulating dopamine transmission and synaptic activity during baseline conditions, the dorsal and ventral striatum differ markedly with regards to the involvement of inhibitory receptors in modulating ethanol-induced changes in dopamine transmission and synaptic activity. Though it is not possible to discern if, or in which way, this subregional heterogeneity reflects the different roles of the ventral and dorsal striatum in the development of addiction, the results presented and discussed in this thesis offer a "starting point", as all experiments were performed in drug-naïve animals. A natural continuation of this project will thus be to compare the findings presented here with those discovered in addicted animals.

While it may be perilous to extrapolate findings from slice recordings to the intact animal, or indeed the human addict, the age-related differences observed in ethanol effects on PS amplitude in the DLS also constitute an interesting finding. The results suggest that ethanol may exert differential, age-related effects in an area of the brain important to habit-formation. These findings may thus be of importance in understanding age-related sensitivity to alcohol [263] which in turn bears relevance to the question of why early onset of drinking increases the risk of developing alcoholism [264]. It remains to be seen if age-related differences in synaptic activity are detected in the nAc following ethanol, or indeed other drugs of abuse.

In conclusion, while experiments in this thesis exploring the roles of GlyRs and $GABA_A$ receptors in ethanol actions in the striatum do not directly provide new avenues to pharmacological treatments of addiction, they do provide a foundation on which future research can be based. By pinpointing similarities and differences between reward-related and habit-related regions of the brain in response to alcohol, this research may aid in elucidating the functional roles of these two regions in addictive disorders, thus furthering the knowledge of how this pathology develops and progresses.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Mänskligheten har avsiktligt framställt alkoholhaltiga drycker sedan stenåldern och än idag är alkoholen en självklar del av det västerländska kulturarvet, från den gemytliga irländska puben till den traditionsenliga skandinaviska midsommarsnapsen. För de allra flesta ger en måttlig alkoholkonsumtion en förhöjd livskvalitet, men för en del blir konsumtionen tvångsmässig – ett sjukligt tillstånd känt som beroende. Det är inte känt varför vissa individer lättare utvecklar ett beroende, och det är heller inte känt genom vilka biologiska verkningsmekanismer ett beroende fortskrider från ett njutningsbaserat, tillfälligt bruk, till ett vanemässigt, och slutligen tvångsmässigt intag. De farmakologiska behandlingsalternativen för alkoholism är idag otillräckliga, och mer kunskap om hur alkoholen utövar sina effekter i hjärnan är därför nödvändig.

Beroende kan betraktas som en kronisk hjärnsjukdom, där viktiga kretsar som styr belöning, motivation, inlärning/vanebildning och självbehärskning har förvanskats. Olika delar av hjärnan tros vara viktiga för dessa olika funktioner, och den här avhandlingen fokuserar på att studera effekter av alkohol i delar av hjärnans belöningssystem (nucleus accumbens), samt i ett annat område av hjärnan som är viktigt för inlärning av vanor (dorsolaterala striatum).

Hjärnans belöningssystem (mesolimbiska systemet) är en uråldrig struktur vars evolutionära syfte troligen varit att förmedla upplevelser av välbehag för handlingar som är viktiga för artens fortlevnad, såsom att äta, dricka och ha sex (s.k. ”naturliga belöningar”). Aktiveringen av mesolimbiska systemet resulterar i en frisättning av signalsubstansen dopamin i nucleus accumbens. Denna dopaminökning är starkt kopplad till den subjektiva upplevelsen av belöning/eufori. Alkohol, liksom nästan alla beroendeframkallande droger, aktiverar hjärnans belöningssystem, troligen på ett sätt som förmedlar en mycket en starkare upplevelse av njutning än de naturliga belöningarna.

Det är inte klarlagt på exakt vilket sätt alkohol ökar frisättningen av dopamin i nucleus accumbens, även om flera teorier finns. Forskning från vår grupp har visat att alkohol troligtvis verkar via en neuronal krets som går från nucleus accumbens till VTA (ventral tegmental area), och sedan tillbaka till nucleus accumbens, och att glycinreceptorer i nucleus accumbens är viktiga för denna krets funktion. En alternativ hypotes är att alkohol verkar direkt i VTA via en av sina nedbrytningsprodukter, acetaldehyd. Acetaldehyd är en toxisk molekyl, som tros förmedla illamående, och andra bakfyllesymtom, men som eventuellt kan ha belönande egenskaper i hjärnan. Det finns mycket forskning som studerat effekterna av acetaldehyd i VTA, men hittills har ingen studerat dess effekter på dopaminnivåer när den administreras i nucleus accumbens.

De belönande effekterna av alkohol kan inte ensamma förklara uppkomsten av den komplexa beroendesjukdomen. Även processer som är viktiga för inlärning och vanebildning tycks påverkas av beroendeframkallande droger, inklusive alkohol. Ett viktigt hjärnområde i detta avseende är dorsolaterala striatum, som i hög grad förknippas med inlärning av vanor. Även här spelar dopaminsignalering en viktig funktion, men hittills är det få studier som har utforskat hur alkohol påverkar dopaminnivåer i detta område.

Syftet med den här avhandlingen har varit att studera hur alkohol (eller acetaldehyd) påverkar dopaminsignalering och cellaktivitet i de två regionerna nucleus accumbens och dorsolaterala striatum. Speciellt fokus har lagts vid att undersöka hur glycinreceptorer och receptorer för gamma-amino-smörsyra, typ A ($GABA_A$) kan vara involverade i förmedlandet av dessa effekter. Dessa receptorer tillhör de två viktigaste hämmande receptorsystemen och generellt har de en ”bromsande” verkan på signalering i hjärnan. Frågorna vi har ställt oss är:

1. Har acetaldehyd en dopaminhöjande effekt i nucleus accumbens när den administreras direkt i detta område? (Artikel I).
2. Reglerar glycin-och $GABA_A$ receptorer cellaktivitet och dopaminsignalering på ett likartat sätt i nucleus accumbens och dorsolaterala striatum, när alkohol inte är närvarande? (Artikel II).
3. Är effekterna av alkohol på cellaktivitet och dopaminsignalering olika i de båda hjärnregionerna, och vilken betydelse har glycin- och $GABA_A$ receptorer i förmedlandet av dessa effekter? (Artiklar III och IV).

För att studera dopaminnivåer i de olika hjärnområdena har mikrodialys använts. Med denna metod kan man mäta halterna av dopamin, men även administrera substanser direkt i enskilda hjärnområden i vakna råttor. För att studera påverkan på cellaktivitet har elektrofysiologska fältmätningar gjorts. Med denna metod studeras elektiska signaler i skivor av rått hjärna, som i sin tur reflekterar nettoeffekten av olika behandlingar på den elektriska aktiviteten i en stor population celler i specifika hjärnområden.

Resultaten visade att acetaldehyd inte ökade halterna av dopamin i nucleus accumbens vid lokal administrering, oavsett om substansen administrerades ensamt eller härrörde från nedbrytning av alkohol. Detta i sin tur antyder att acetaldehyd inte är viktigt för de dopaminhöjande effekterna av alkohol i den neuronala krets som går via nucleus accumbens till VTA, och tillbaka till nucleus accumbens. Detta betyder dock inte att acetaldehyd inte kan ha betydelse för alkoholens belönande effekter i andra delar av hjärnan, och mer forskning behövs för att klargöra om huruvida substanser som hämmar bildandet av acetaldehyd i hjärnan kan utgöra potentiella läkemedel mot alkoholism.

Resultaten visade även att både glycin-och $GABA_A$ receptorer är viktiga för reglerandet av cellaktivitet och dopaminnivåer i både nucleus accumbens och

dorsolaterala striatum, och att effekterna av dessa receptorsystem var likartade i båda regioner, när alkohol inte var närvarande. När alkohol tillfördes syntes dock tydliga skillnader mellan hjärnregionerna. Alkohol ökade halterna av dopamin i både nucleus accumbens och dorsolaterala striatum, men medan glycinreceptorer var viktiga för denna effekt i nucleus accumbens verkade varken glycinreceptorer eller $GABA_A$ receptorer förmedla denna effekt i dorsolaterala striatum. Studier på cellaktivitet visade även att alkohol verkade producera två olika, motriktade effekter, varav den ena ökade cellaktivitet, medan den andra minskade cellaktivitet. Balansen mellan dessa två motriktade krafter, samt betydelsen av glycin- och $GABA_A$ receptorer i detta sammanhang, verkade variera beroende på djurets ålder, men även beroende på vilken hjärnregion som studerades och vilken koncentration alkohol som administrerades.

Dessa resultat tyder på tydliga ålderspecifika, men även regionspecifika skillnader, när det gäller effekterna av alkohol på cellaktivitet och dopaminsignalering, samt vilken betydelse glycin-och $GABA_A$ receptorer har för dessa effekter. De åldersberoende effekterna av alkohol antyder en eventuellt ökad känslighet hos yngre djur för de cellaktivitetssänkande effekterna av alkoholens i en del av hjärnan som är viktig för vanebildning, vilket kan vara av betydelse för att förstå hur en tidig debutålder kan öka risken att utveckla alkoholism. Då alla försök utfördes i djur som inte behandlats med alkohol tidigare utgör dessa resultat även en ”grund”, utifrån vilken framtida forskning kan utgå. Viktiga framtida frågeställningar är om/hur glycin-och $GABA_A$ receptorer förmedlar alkoholeffekter i de studerade hjärnregionerna på liknande sätt i djur som utvecklat ett beroende.

Sammanfattningsvis belyser dessa studier både likheter och skillnader i alkoholeffekter mellan hjärnområden kopplade till belöning respektive vanebildning. Förhoppningsvis kan innehållet i denna avhandling bidra till en ökad förståelse för de biologiska mekanismer som kan ligga till grund för progressionen från ett njutningsbaserat alkoholintag till ett vanemässigt beroende.

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REFERENCES

1. Systembolaget, *Lanseringsplan 1/2008*. 2008.
2. Hendriks, H.F. and van Tol, A., *Alcohol.* Handb Exp Pharmacol, 2005(170): p. 339-61.
3. WHO, *Global health risks*. 2009.
4. Johnson, A., *Hur mycket kostar supen? Update 2003; 150 miljarder kostar supen.* 2000: Sober.
5. Socialdepartementet, *Bättre insatser vid missbruk och beroende.* Statens Offentliga Utredningar, 2011. **SOU**(2011:35).
6. WHO, *Alcohol in the European Union - Consumption, harm and policy approaches.* 2012.
7. CAN, *Alkoholkonsumptionen i Sverige 2013*. 2014.
8. APA, *Diagnostic and Statistical Manual of Mental Disorders DSM-5*. 5th ed. 2013: American Psychiatric Association.
9. McLellan, A.T., Lewis, D.C., O'Brien, C.P., and Kleber, H.D., *Drug dependence, a chronic medical illness: implications for treatment, insurance, and outcomes evaluation.* Jama, 2000. **284**(13): p. 1689-95.
10. Koob, G.F. and Le Moal, M., *Drug addiction, dysregulation of reward, and allostasis.* Neuropsychopharmacology, 2001. **24**(2): p. 97-129.
11. Cloninger, C.R., *Neurogenetic adaptive mechanisms in alcoholism.* Science, 1987. **236**(4800): p. 410-6.
12. Babor, T.F., Hofmann, M., DelBoca, F.K., Hesselbrock, V., Meyer, R.E., Dolinsky, Z.S., and Rounsaville, B., *Types of alcoholics, I. Evidence for an empirically derived typology based on indicators of vulnerability and severity.* Arch Gen Psychiatry, 1992. **49**(8): p. 599-608.
13. Garbutt, J.C., West, S.L., Carey, T.S., Lohr, K.N., and Crews, F.T., *Pharmacological treatment of alcohol dependence: a review of the evidence.* Jama, 1999. **281**(14): p. 1318-25.
14. Volkow, N.D., Fowler, J.S., and Wang, G.J., *The addicted human brain: insights from imaging studies.* J Clin Invest, 2003. **111**(10): p. 1444-51.
15. Hyman, S.E., Malenka, R.C., and Nestler, E.J., *Neural mechanisms of addiction: the role of reward-related learning and memory.* Annu Rev Neurosci, 2006. **29**: p. 565-98.
16. Gerdeman, G.L., Partridge, J.G., Lupica, C.R., and Lovinger, D.M., *It could be habit forming: drugs of abuse and striatal synaptic plasticity.* Trends Neurosci, 2003. **26**(4): p. 184-92.
17. Chen, G., Cuzon Carlson, V.C., Wang, J., Beck, A., Heinz, A., Ron, D., Lovinger, D.M., and Buck, K.J., *Striatal involvement in human alcoholism and alcohol consumption, and withdrawal in animal models.* Alcohol Clin Exp Res, 2011. **35**(10): p. 1739-48.
18. Yin, H.H. and Knowlton, B.J., *The role of the basal ganglia in habit formation.* Nat Rev Neurosci, 2006. **7**(6): p. 464-76.
19. Yin, H.H., Ostlund, S.B., and Balleine, B.W., *Reward-guided learning beyond dopamine in the nucleus accumbens: the integrative functions of cortico-basal ganglia networks.* Eur J Neurosci, 2008. **28**(8): p. 1437-48.
20. Olds, J. and Milner, P., *Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain.* J Comp Physiol Psychol, 1954. **47**(6): p. 419-27.
21. German, D.C. and Bowden, D.M., *Catecholamine systems as the neural substrate for intracranial self-stimulation: a hypothesis.* Brain Res, 1974. **73**(3): p. 381-419.

22. Milner, P.M., *Brain-stimulation reward: a review.* Can J Psychol, 1991. **45**(1): p. 1-36.
23. Schuster, C.R. and Thompson, T., *Self administration of and behavioral dependence on drugs.* Annu Rev Pharmacol, 1969. **9**: p. 483-502.
24. Wise, R.A. and Rompre, P.P., *Brain dopamine and reward.* Annu Rev Psychol, 1989. **40**: p. 191-225.
25. Wise, R.A. and Bozarth, M.A., *A psychomotor stimulant theory of addiction.* Psychol Rev, 1987. **94**(4): p. 469-92.
26. Koob, G.F. and Le Moal, M., *Drug abuse: hedonic homeostatic dysregulation.* Science, 1997. **278**(5335): p. 52-8.
27. Engel, J. and Carlsson, A., *Catecholamines and behavior.* Curr Dev Psychopharmacol, 1977. **4**: p. 1-32.
28. Koob, G.F., *Drugs of abuse: anatomy, pharmacology and function of reward pathways.* Trends Pharmacol Sci, 1992. **13**(5): p. 177-84.
29. Ikemoto, S., *Dopamine reward circuitry: Two projection systems from the ventral midbrain to the nucleus accumbens–olfactory tubercle complex.* Brain Research Reviews, 2007. **56**(1): p. 27-78.
30. Dahlstrom, A. and Fuxe, K., *Localization of monoamines in the lower brain stem.* Experientia, 1964. **20**(7): p. 398-9.
31. Koob, G.F., *Neural mechanisms of drug reinforcement.* Ann N Y Acad Sci, 1992. **654**: p. 171-91.
32. Grenhoff, J., Aston-Jones, G., and Svensson, T.H., *Nicotinic effects on the firing pattern of midbrain dopamine neurons.* Acta Physiol Scand, 1986. **128**(3): p. 351-8.
33. Marinelli, M. and McCutcheon, J.E., *Heterogeneity of dopamine neuron activity across traits and states.* Neuroscience, 2014. **282c**: p. 176-197.
34. Gonon, F.G., *Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry.* Neuroscience, 1988. **24**(1): p. 19-28.
35. Wightman, R.M. and Zimmerman, J.B., *Control of dopamine extracellular concentration in rat striatum by impulse flow and uptake.* Brain Res Brain Res Rev, 1990. **15**(2): p. 135-44.
36. Di Chiara, G. and Imperato, A., *Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats.* Proc Natl Acad Sci U S A, 1988. **85**(14): p. 5274-8.
37. Pontieri, F.E., Tanda, G., and Di Chiara, G., *Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the "shell" as compared with the "core" of the rat nucleus accumbens.* Proc Natl Acad Sci U S A, 1995. **92**(26): p. 12304-8.
38. Wise, R.A., *Brain reward circuitry: insights from unsensed incentives.* Neuron, 2002. **36**(2): p. 229-40.
39. Wise, R.A. and Bozarth, M.A., *Brain mechanisms of drug reward and euphoria.* Psychiatr Med, 1985. **3**(4): p. 445-60.
40. Berridge, K.C. and Robinson, T.E., *What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience?* Brain Res Brain Res Rev, 1998. **28**(3): p. 309-69.
41. Kringelbach, M.L. and Berridge, K.C., *Towards a functional neuroanatomy of pleasure and happiness.* Trends Cogn Sci, 2009. **13**(11): p. 479-87.
42. Berridge, K.C., *The debate over dopamine's role in reward: the case for incentive salience.* Psychopharmacology (Berl), 2007. **191**(3): p. 391-431.

43. Schultz, W., *Dopamine neurons and their role in reward mechanisms.* Curr Opin Neurobiol, 1997. **7**(2): p. 191-7.
44. Hollerman, J.R. and Schultz, W., *Dopamine neurons report an error in the temporal prediction of reward during learning.* Nat Neurosci, 1998. **1**(4): p. 304-9.
45. Balster, R.L., *Neural basis of inhalant abuse.* Drug Alcohol Depend, 1998. **51**(1-2): p. 207-14.
46. Wise, R.A., *The role of reward pathways in the development of drug dependence.* Pharmacol Ther, 1987. **35**(1-2): p. 227-63.
47. Fahlke, C., Hansen, S., Engel, J.A., and Hard, E., *Effects of ventral striatal 6-OHDA lesions or amphetamine sensitization on ethanol consumption in the rat.* Pharmacol Biochem Behav, 1994. **47**(2): p. 345-9.
48. Rassnick, S., Stinus, L., and Koob, G.F., *The effects of 6-hydroxydopamine lesions of the nucleus accumbens and the mesolimbic dopamine system on oral self-administration of ethanol in the rat.* Brain Res, 1993. **623**(1): p. 16-24.
49. Obeso, J.A., Rodriguez-Oroz, M.C., Benitez-Temino, B., Blesa, F.J., Guridi, J., Marin, C., and Rodriguez, M., *Functional organization of the basal ganglia: therapeutic implications for Parkinson's disease.* Mov Disord, 2008. **23 Suppl 3**: p. S548-59.
50. Albin, R.L., Young, A.B., and Penney, J.B., *The functional anatomy of basal ganglia disorders.* Trends Neurosci, 1989. **12**(10): p. 366-75.
51. Parent, A. and Hazrati, L.N., *Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop.* Brain Res Brain Res Rev, 1995. **20**(1): p. 91-127.
52. Galeffi, F., Bianchi, L., Bolam, J.P., and Della Corte, L., *The effect of 6-hydroxydopamine lesions on the release of amino acids in the direct and indirect pathways of the basal ganglia: a dual microdialysis probe analysis.* Eur J Neurosci, 2003. **18**(4): p. 856-68.
53. Graybiel, A.M., *Building action repertoires: memory and learning functions of the basal ganglia.* Curr Opin Neurobiol, 1995. **5**(6): p. 733-41.
54. Packard, M.G. and Knowlton, B.J., *Learning and memory functions of the Basal Ganglia.* Annu Rev Neurosci, 2002. **25**: p. 563-93.
55. Yin, H.H., Park, B.S., Adermark, L., and Lovinger, D.M., *Ethanol reverses the direction of long-term synaptic plasticity in the dorsomedial striatum.* Eur J Neurosci, 2007. **25**(11): p. 3226-32.
56. Calabresi, P., Maj, R., Pisani, A., Mercuri, N.B., and Bernardi, G., *Long-term synaptic depression in the striatum: physiological and pharmacological characterization.* J Neurosci, 1992. **12**(11): p. 4224-33.
57. Kerr, J.N. and Wickens, J.R., *Dopamine D-1/D-5 receptor activation is required for long-term potentiation in the rat neostriatum in vitro.* J Neurophysiol, 2001. **85**(1): p. 117-24.
58. Ericson, M., Blomqvist, O., Engel, J.A., and Söderpalm, B., *Voluntary ethanol intake in the rat and the associated accumbal dopamine overflow are blocked by ventral tegmental mecamylamine.* European Journal of Pharmacology, 1998. **358**(3): p. 189-196.
59. Weiss, F., Lorang, M.T., Bloom, F.E., and Koob, G.F., *Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants.* J Pharmacol Exp Ther, 1993. **267**(1): p. 250-8.
60. Campbell, A.D. and McBride, W.J., *Serotonin-3 receptor and ethanol-stimulated dopamine release in the nucleus accumbens.* Pharmacol Biochem Behav, 1995. **51**(4): p. 835-42.
61. Blomqvist, O., Engel, J.A., Nissbrandt, H., and Soderpalm, B., *The mesolimbic dopamine-activating properties of ethanol are antagonized by mecamylamine.* Eur J Pharmacol, 1993. **249**(2): p. 207-13.
62. Yoshimoto, K., McBride, W.J., Lumeng, L., and Li, T.K., *Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens.* Alcohol, 1992. **9**(1): p. 17-22.

63. Adermark, L., Clarke, R.B., Olsson, T., Hansson, E., Soderpalm, B., and Ericson, M., *Implications for glycine receptors and astrocytes in ethanol-induced elevation of dopamine levels in the nucleus accumbens.* Addict Biol, 2011. **16**(1): p. 43-54.
64. Imperato, A. and Di Chiara, G., *Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol.* J Pharmacol Exp Ther, 1986. **239**(1): p. 219-28.
65. Budygin, E.A., Phillips, P.E., Robinson, D.L., Kennedy, A.P., Gainetdinov, R.R., and Wightman, R.M., *Effect of acute ethanol on striatal dopamine neurotransmission in ambulatory rats.* J Pharmacol Exp Ther, 2001. **297**(1): p. 27-34.
66. Wozniak, K.M., Pert, A., and Linnoila, M., *Antagonism of 5-HT3 receptors attenuates the effects of ethanol on extracellular dopamine.* Eur J Pharmacol, 1990. **187**(2): p. 287-9.
67. Wozniak, K.M., Pert, A., Mele, A., and Linnoila, M., *Focal application of alcohols elevates extracellular dopamine in rat brain: a microdialysis study.* Brain Res, 1991. **540**(1-2): p. 31-40.
68. Wang, Y., Palmer, M.R., Cline, E.J., and Gerhardt, G.A., *Effects of ethanol on striatal dopamine overflow and clearance: an in vivo electrochemical study.* Alcohol, 1997. **14**(6): p. 593-601.
69. Bolam, J.P., Hanley, J.J., Booth, P.A., and Bevan, M.D., *Synaptic organisation of the basal ganglia.* J Anat, 2000. **196 (Pt 4)**: p. 527-42.
70. Belin, D., Jonkman, S., Dickinson, A., Robbins, T.W., and Everitt, B.J., *Parallel and interactive learning processes within the basal ganglia: relevance for the understanding of addiction.* Behav Brain Res, 2009. **199**(1): p. 89-102.
71. Van Bockstaele, E.J., Biswas, A., and Pickel, V.M., *Topography of serotonin neurons in the dorsal raphe nucleus that send axon collaterals to the rat prefrontal cortex and nucleus accumbens.* Brain Res, 1993. **624**(1-2): p. 188-98.
72. Waselus, M., Galvez, J.P., Valentino, R.J., and Van Bockstaele, E.J., *Differential projections of dorsal raphe nucleus neurons to the lateral septum and striatum.* J Chem Neuroanat, 2006. **31**(4): p. 233-42.
73. Berridge, C.W., Stratford, T.L., Foote, S.L., and Kelley, A.E., *Distribution of dopamine beta-hydroxylase-like immunoreactive fibers within the shell subregion of the nucleus accumbens.* Synapse, 1997. **27**(3): p. 230-41.
74. Delfs, J.M., Zhu, Y., Druhan, J.P., and Aston-Jones, G.S., *Origin of noradrenergic afferents to the shell subregion of the nucleus accumbens: anterograde and retrograde tract-tracing studies in the rat.* Brain Res, 1998. **806**(2): p. 127-40.
75. Pennartz, C.M., Berke, J.D., Graybiel, A.M., Ito, R., Lansink, C.S., van der Meer, M., Redish, A.D., Smith, K.S., and Voorn, P., *Corticostriatal Interactions during Learning, Memory Processing, and Decision Making.* J Neurosci, 2009. **29**(41): p. 12831-8.
76. Herkenham, M., Edley, S.M., and Stuart, J., *Cell clusters in the nucleus accumbens of the rat, and the mosaic relationship of opiate receptors, acetylcholinesterase and subcortical afferent terminations.* Neuroscience, 1984. **11**(3): p. 561-93.
77. Zahm, D.S. and Brog, J.S., *On the significance of subterritories in the "accumbens" part of the rat ventral striatum.* Neuroscience, 1992. **50**(4): p. 751-67.
78. Zaborszky, L., Alheid, G.F., Beinfeld, M.C., Eiden, L.E., Heimer, L., and Palkovits, M., *Cholecystokinin innervation of the ventral striatum: a morphological and radioimmunological study.* Neuroscience, 1985. **14**(2): p. 427-53.
79. Lecca, D., Cacciapaglia, F., Valentini, V., Acquas, E., and Di Chiara, G., *Differential neurochemical and behavioral adaptation to cocaine after response contingent and noncontingent exposure in the rat.* Psychopharmacology (Berl), 2007. **191**(3): p. 653-67.

80. Lecca, D., Cacciapaglia, F., Valentini, V., and Di Chiara, G., *Monitoring extracellular dopamine in the rat nucleus accumbens shell and core during acquisition and maintenance of intravenous WIN 55,212-2 self-administration.* Psychopharmacology (Berl), 2006. **188**(1): p. 63-74.
81. Lecca, D., Cacciapaglia, F., Valentini, V., Gronli, J., Spiga, S., and Di Chiara, G., *Preferential increase of extracellular dopamine in the rat nucleus accumbens shell as compared to that in the core during acquisition and maintenance of intravenous nicotine self-administration.* Psychopharmacology (Berl), 2006. **184**(3-4): p. 435-46.
82. Balleine, B.W. and O'Doherty, J.P., *Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action.* Neuropsychopharmacology, 2010. **35**(1): p. 48-69.
83. Volkow, N.D., Fowler, J.S., Wang, G.J., Swanson, J.M., and Telang, F., *Dopamine in drug abuse and addiction: results of imaging studies and treatment implications.* Arch Neurol, 2007. **64**(11): p. 1575-9.
84. Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates.* (2007): Academic Press Inc.
85. Wise, R.A. and Koob, G.F., *The development and maintenance of drug addiction.* Neuropsychopharmacology, 2014. **39**(2): p. 254-62.
86. Chen, G. and Buck, K.J., *Rostroventral caudate putamen involvement in ethanol withdrawal is influenced by a chromosome 4 locus.* Genes Brain Behav, 2010. **9**(7): p. 768-76.
87. Rymar, V.V., Sasseville, R., Luk, K.C., and Sadikot, A.F., *Neurogenesis and stereological morphometry of calretinin-immunoreactive GABAergic interneurons of the neostriatum.* J Comp Neurol, 2004. **469**(3): p. 325-39.
88. Tepper, J.M. and Bolam, J.P., *Functional diversity and specificity of neostriatal interneurons.* Curr Opin Neurobiol, 2004. **14**(6): p. 685-92.
89. Graveland, G.A. and DiFiglia, M., *The frequency and distribution of medium-sized neurons with indented nuclei in the primate and rodent neostriatum.* Brain Res, 1985. **327**(1-2): p. 307-11.
90. Ma, Y.Y., Cepeda, C., Chatta, P., Franklin, L., Evans, C.J., and Levine, M.S., *Regional and cell-type-specific effects of DAMGO on striatal D1 and D2 dopamine receptor-expressing medium-sized spiny neurons.* ASN Neuro, 2012. **4**(2).
91. Gerfen, C.R., *The neostriatal mosaic: multiple levels of compartmental organization.* Trends Neurosci, 1992. **15**(4): p. 133-9.
92. Crittenden, J.R. and Graybiel, A.M., *Basal Ganglia disorders associated with imbalances in the striatal striosome and matrix compartments.* Front Neuroanat, 2011. **5**: p. 59.
93. Gerfen, C.R., Herkenham, M., and Thibault, J., *The neostriatal mosaic: II. Patch- and matrix-directed mesostriatal dopaminergic and non-dopaminergic systems.* J Neurosci, 1987. **7**(12): p. 3915-34.
94. Voorn, P., Gerfen, C.R., and Groenewegen, H.J., *Compartmental organization of the ventral striatum of the rat: immunohistochemical distribution of enkephalin, substance P, dopamine, and calcium-binding protein.* J Comp Neurol, 1989. **289**(2): p. 189-201.
95. Voorn, P., Vanderschuren, L.J., Groenewegen, H.J., Robbins, T.W., and Pennartz, C.M., *Putting a spin on the dorsal-ventral divide of the striatum.* Trends Neurosci, 2004. **27**(8): p. 468-74.
96. Fujiyama, F., Sohn, J., Nakano, T., Furuta, T., Nakamura, K.C., Matsuda, W., and Kaneko, T., *Exclusive and common targets of neostriatofugal projections of rat striosome neurons: a single neuron-tracing study using a viral vector.* Eur J Neurosci, 2011. **33**(4): p. 668-77.

97. Le Moine, C. and Bloch, B., *D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum.* J Comp Neurol, 1995. **355**(3): p. 418-26.
98. Lu, X.Y., Ghasemzadeh, M.B., and Kalivas, P.W., *Expression of D1 receptor, D2 receptor, substance P and enkephalin messenger RNAs in the neurons projecting from the nucleus accumbens.* Neuroscience, 1998. **82**(3): p. 767-80.
99. Gangarossa, G., Espallergues, J., de Kerchove d'Exaerde, A., El Mestikawy, S., Gerfen, C.R., Herve, D., Girault, J.A., and Valjent, E., *Distribution and compartmental organization of GABAergic medium-sized spiny neurons in the mouse nucleus accumbens.* Front Neural Circuits, 2013. **7**: p. 22.
100. Plenz, D. and Kitai, S.T., *Up and down states in striatal medium spiny neurons simultaneously recorded with spontaneous activity in fast-spiking interneurons studied in cortex-striatum-substantia nigra organotypic cultures.* J Neurosci, 1998. **18**(1): p. 266-83.
101. Tepper, J.M., Wilson, C.J., and Koos, T., *Feedforward and feedback inhibition in neostriatal GABAergic spiny neurons.* Brain Res Rev, 2008. **58**(2): p. 272-81.
102. Bolam, J.P., Ingham, C.A., Izzo, P.N., Levey, A.I., Rye, D.B., Smith, A.D., and Wainer, B.H., *Substance P-containing terminals in synaptic contact with cholinergic neurons in the neostriatum and basal forebrain: a double immunocytochemical study in the rat.* Brain Res, 1986. **397**(2): p. 279-89.
103. Kreitzer, A.C., *Physiology and pharmacology of striatal neurons.* Annu Rev Neurosci, 2009. **32**: p. 127-47.
104. Zhou, F.M., Wilson, C.J., and Dani, J.A., *Cholinergic interneuron characteristics and nicotinic properties in the striatum.* J Neurobiol, 2002. **53**(4): p. 590-605.
105. Koos, T. and Tepper, J.M., *Dual cholinergic control of fast-spiking interneurons in the neostriatum.* J Neurosci, 2002. **22**(2): p. 529-35.
106. Lenz, J.D. and Lobo, M.K., *Optogenetic insights into striatal function and behavior.* Behav Brain Res, 2013. **255**: p. 44-54.
107. Nelson, A.B., Hammack, N., Yang, C.F., Shah, N.M., Seal, R.P., and Kreitzer, A.C., *Striatal cholinergic interneurons Drive GABA release from dopamine terminals.* Neuron, 2014. **82**(1): p. 63-70.
108. Wilson, C.J., Chang, H.T., and Kitai, S.T., *Firing patterns and synaptic potentials of identified giant aspiny interneurons in the rat neostriatum.* J Neurosci, 1990. **10**(2): p. 508-19.
109. Aosaki, T., Tsubokawa, H., Ishida, A., Watanabe, K., Graybiel, A.M., and Kimura, M., *Responses of tonically active neurons in the primate's striatum undergo systematic changes during behavioral sensorimotor conditioning.* J Neurosci, 1994. **14**(6): p. 3969-84.
110. Reynolds, J.N. and Wickens, J.R., *The corticostriatal input to giant aspiny interneurons in the rat: a candidate pathway for synchronising the response to reward-related cues.* Brain Res, 2004. **1011**(1): p. 115-28.
111. Aosaki, T., Graybiel, A.M., and Kimura, M., *Effect of the nigrostriatal dopamine system on acquired neural responses in the striatum of behaving monkeys.* Science, 1994. **265**(5170): p. 412-5.
112. Koos, T. and Tepper, J.M., *Inhibitory control of neostriatal projection neurons by GABAergic interneurons.* Nat Neurosci, 1999. **2**(5): p. 467-72.
113. Bennett, B.D. and Bolam, J.P., *Synaptic input and output of parvalbumin-immunoreactive neurons in the neostriatum of the rat.* Neuroscience, 1994. **62**(3): p. 707-19.
114. Kita, H., Kosaka, T., and Heizmann, C.W., *Parvalbumin-immunoreactive neurons in the rat neostriatum: a light and electron microscopic study.* Brain Res, 1990. **536**(1-2): p. 1-15.

115. Berke, J.D., *Functional properties of striatal fast-spiking interneurons.* Front Syst Neurosci, 2011. **5**: p. 45.
116. Kawaguchi, Y., Wilson, C.J., Augood, S.J., and Emson, P.C., *Striatal interneurones: chemical, physiological and morphological characterization.* Trends Neurosci, 1995. **18**(12): p. 527-35.
117. Ibanez-Sandoval, O., Tecuapetla, F., Unal, B., Shah, F., Koos, T., and Tepper, J.M., *Electrophysiological and morphological characteristics and synaptic connectivity of tyrosine hydroxylase-expressing neurons in adult mouse striatum.* J Neurosci, 2010. **30**(20): p. 6999-7016.
118. Wilson, C.J., *GABAergic inhibition in the neostriatum.* Prog Brain Res, 2007. **160**: p. 91-110.
119. Joel, D. and Weiner, I., *The connections of the dopaminergic system with the striatum in rats and primates: an analysis with respect to the functional and compartmental organization of the striatum.* Neuroscience, 2000. **96**(3): p. 451-74.
120. Van Bockstaele, E.J. and Pickel, V.M., *GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain.* Brain Res, 1995. **682**(1-2): p. 215-21.
121. Jiang, Z.G. and North, R.A., *Pre- and postsynaptic inhibition by opioids in rat striatum.* J Neurosci, 1992. **12**(1): p. 356-61.
122. Gerdeman, G.L., Ronesi, J., and Lovinger, D.M., *Postsynaptic endocannabinoid release is critical to long-term depression in the striatum.* Nat Neurosci, 2002. **5**(5): p. 446-51.
123. Adermark, L., Clarke, R.B., Ericson, M., and Soderpalm, B., *Subregion-Specific Modulation of Excitatory Input and Dopaminergic Output in the Striatum by Tonically Activated Glycine and GABA(A) Receptors.* Front Syst Neurosci, 2011. **5**: p. 85.
124. Vengeliene, V., Bilbao, A., Molander, A., and Spanagel, R., *Neuropharmacology of alcohol addiction.* Br J Pharmacol, 2008. **154**(2): p. 299-315.
125. Spanagel, R., *Alcoholism: A Systems Approach From Molecular Physiology to Addictive Behavior.* Physiological Reviews, 2009. **89**(2): p. 649-705.
126. Harris, R.A., *Ethanol actions on multiple ion channels: which are important?* Alcohol Clin Exp Res, 1999. **23**(10): p. 1563-70.
127. Soderpalm, B. and Ericson, M., *Neurocircuitry involved in the development of alcohol addiction: the dopamine system and its access points.* Curr Top Behav Neurosci, 2013. **13**: p. 127-61.
128. Mihic, S.J., *Acute effects of ethanol on GABAA and glycine receptor function.* Neurochem Int, 1999. **35**(2): p. 115-23.
129. Harris, R.A., Trudell, J.R., and Mihic, S.J., *Ethanol's molecular targets.* Sci Signal, 2008. **1**(28): p. re7.
130. Lovinger, D.M. and Zhou, Q., *Alcohols potentiate ion current mediated by recombinant 5-HT3RA receptors expressed in a mammalian cell line.* Neuropharmacology, 1994. **33**(12): p. 1567-72.
131. Aistrup, G.L., Marszalec, W., and Narahashi, T., *Ethanol modulation of nicotinic acetylcholine receptor currents in cultured cortical neurons.* Mol Pharmacol, 1999. **55**(1): p. 39-49.
132. Hoffman, P.L., Rabe, C.S., Moses, F., and Tabakoff, B., *N-methyl-D-aspartate receptors and ethanol: inhibition of calcium flux and cyclic GMP production.* J Neurochem, 1989. **52**(6): p. 1937-40.
133. Lovinger, D.M., White, G., and Weight, F.F., *Ethanol inhibits NMDA-activated ion current in hippocampal neurons.* Science, 1989. **243**(4899): p. 1721-4.

134. Wang, X., Wang, G., Lemos, J.R., and Treistman, S.N., *Ethanol directly modulates gating of a dihydropyridine-sensitive Ca2+ channel in neurohypophysial terminals.* J Neurosci, 1994. **14**(9): p. 5453-60.
135. Kobayashi, T., Ikeda, K., Kojima, H., Niki, H., Yano, R., Yoshioka, T., and Kumanishi, T., *Ethanol opens G-protein-activated inwardly rectifying K+ channels.* Nat Neurosci, 1999. **2**(12): p. 1091-7.
136. Drews, E. and Zimmer, A., *Modulation of alcohol and nicotine responses through the endogenous opioid system.* Prog Neurobiol, 2010. **90**(1): p. 1-15.
137. Tallman, J.F., Paul, S.M., Skolnick, P., and Gallager, D.W., *Receptors for the age of anxiety: pharmacology of the benzodiazepines.* Science, 1980. **207**(4428): p. 274-81.
138. Gilman, G.a., *The pharmacological basis of therapeutics.*
139. Cott, J., Carlsson, A., Engel, J., and Lindqvist, M., *Suppression of ethanol-induced locomotor stimulation by GABA-like drugs.* Naunyn Schmiedebergs Arch Pharmacol, 1976. **295**(3): p. 203-9.
140. Liljequist, S. and Engel, J., *Effects of GABAergic agonists and antagonists on various ethanol-induced behavioral changes.* Psychopharmacology (Berl), 1982. **78**(1): p. 71-5.
141. Ueno, S., et al., *Alcohol actions on GABA(A) receptors: from protein structure to mouse behavior.* Alcohol Clin Exp Res, 2001. **25**(5 Suppl ISBRA): p. 76s-81s.
142. Davies, M., *The role of GABAA receptors in mediating the effects of alcohol in the central nervous system.* J Psychiatry Neurosci, 2003. **28**(4): p. 263-74.
143. Korpi, E.R., *Role of GABAA receptors in the actions of alcohol and in alcoholism: recent advances.* Alcohol Alcohol, 1994. **29**(2): p. 115-29.
144. Aguayo, L.G., Peoples, R.W., Yeh, H.H., and Yevenes, G.E., *GABA(A) receptors as molecular sites of ethanol action. Direct or indirect actions?* Curr Top Med Chem, 2002. **2**(8): p. 869-85.
145. Kumar, S., Porcu, P., Werner, D.F., Matthews, D.B., Diaz-Granados, J.L., Helfand, R.S., and Morrow, A.L., *The role of GABA(A) receptors in the acute and chronic effects of ethanol: a decade of progress.* Psychopharmacology (Berl), 2009. **205**(4): p. 529-64.
146. Weiner, J.L. and Valenzuela, C.F., *Ethanol modulation of GABAergic transmission: the view from the slice.* Pharmacol Ther, 2006. **111**(3): p. 533-54.
147. Lovinger, D.M. and Homanics, G.E., *Tonic for what ails us? high-affinity GABAA receptors and alcohol.* Alcohol, 2007. **41**(3): p. 139-43.
148. Korpi, E.R., et al., *Does ethanol act preferentially via selected brain GABAA receptor subtypes? the current evidence is ambiguous.* Alcohol, 2007. **41**(3): p. 163-76.
149. Enz, R. and Cutting, G.R., *Molecular composition of GABAC receptors.* Vision Res, 1998. **38**(10): p. 1431-41.
150. Korpi, E.R. and Sinkkonen, S.T., *GABA(A) receptor subtypes as targets for neuropsychiatric drug development.* Pharmacol Ther, 2006. **109**(1-2): p. 12-32.
151. McKernan, R.M. and Whiting, P.J., *Which GABAA-receptor subtypes really occur in the brain?* Trends Neurosci, 1996. **19**(4): p. 139-43.
152. Barnard, E.A., et al., *Molecular biology of the GABAA receptor.* Adv Exp Med Biol, 1988. **236**: p. 31-45.
153. Whiting, P.J., *GABA-A receptor subtypes in the brain: a paradigm for CNS drug discovery?* Drug Discov Today, 2003. **8**(10): p. 445-50.
154. Brickley, S.G. and Mody, I., *Extrasynaptic GABA(A) receptors: their function in the CNS and implications for disease.* Neuron, 2012. **73**(1): p. 23-34.
155. Santhakumar, V., Wallner, M., and Otis, T.S., *Ethanol acts directly on extrasynaptic subtypes of GABAA receptors to increase tonic inhibition.* Alcohol, 2007. **41**(3): p. 211-21.

156. Olsen, R.W., Hanchar, H.J., Meera, P., and Wallner, M., *GABAA receptor subtypes: the "one glass of wine" receptors.* Alcohol, 2007. **41**(3): p. 201-9.
157. Aprison, M.H. and Werman, R., *The distribution of glycine in cat spinal cord and roots.* Life Sci, 1965. **4**(21): p. 2075-83.
158. Betz, H. and Becker, C.M., *The mammalian glycine receptor: biology and structure of a neuronal chloride channel protein.* Neurochem Int, 1988. **13**(2): p. 137-46.
159. Langosch, D., Becker, C.M., and Betz, H., *The inhibitory glycine receptor: a ligand-gated chloride channel of the central nervous system.* Eur J Biochem, 1990. **194**(1): p. 1-8.
160. Curtis, D.R., Hosli, L., and Johnston, G.A., *A pharmacological study of the depression of spinal neurones by glycine and related amino acids.* Exp Brain Res, 1968. **6**(1): p. 1-18.
161. Yevenes, G.E. and Zeilhofer, H.U., *Allosteric modulation of glycine receptors.* Br J Pharmacol, 2011. **164**(2): p. 224-36.
162. Rajendra, S., Lynch, J.W., and Schofield, P.R., *The glycine receptor.* Pharmacol Ther, 1997. **73**(2): p. 121-46.
163. Grudzinska, J., Schemm, R., Haeger, S., Nicke, A., Schmalzing, G., Betz, H., and Laube, B., *The beta subunit determines the ligand binding properties of synaptic glycine receptors.* Neuron, 2005. **45**(5): p. 727-39.
164. Langosch, D., Herbold, A., Schmieden, V., Borman, J., and Kirsch, J., *Importance of Arg-219 for correct biogenesis of alpha 1 homooligomeric glycine receptors.* FEBS Lett, 1993. **336**(3): p. 540-4.
165. Waldvogel, H.J., Baer, K., Eady, E., Allen, K.L., Gilbert, R.T., Mohler, H., Rees, M.I., Nicholson, L.F., and Faull, R.L., *Differential localization of gamma-aminobutyric acid type A and glycine receptor subunits and gephyrin in the human pons, medulla oblongata and uppermost cervical segment of the spinal cord: an immunohistochemical study.* J Comp Neurol, 2010. **518**(3): p. 305-28.
166. Le-Corronc, H., Rigo, J.-M., Branchereau, P., and Legendre, P., *GABA(A) receptor and glycine receptor activation by paracrine/autocrine release of endogenous agonists: more than a simple communication pathway.* Molecular neurobiology, 2011. **44**(1): p. 28.
167. Lynch, J.W., *Molecular structure and function of the glycine receptor chloride channel.* Physiol Rev, 2004. **84**(4): p. 1051-95.
168. Jonsson, S., Kerekes, N., Hyytia, P., Ericson, M., and Soderpalm, B., *Glycine receptor expression in the forebrain of male AA/ANA rats.* Brain Res, 2009. **1305 Suppl**: p. S27-36.
169. Martin, G. and Siggins, G.R., *Electrophysiological evidence for expression of glycine receptors in freshly isolated neurons from nucleus accumbens.* J Pharmacol Exp Ther, 2002. **302**(3): p. 1135-45.
170. Sato, K., Kiyama, H., and Tohyama, M., *Regional distribution of cells expressing glycine receptor alpha 2 subunit mRNA in the rat brain.* Brain Res, 1992. **590**(1-2): p. 95-108.
171. Fujita, M., Sato, K., Sato, M., Inoue, T., Kozuka, T., and Tohyama, M., *Regional distribution of the cells expressing glycine receptor beta subunit mRNA in the rat brain.* Brain Res, 1991. **560**(1-2): p. 23-37.
172. Jonsson, S., Morud, J., Pickering, C., Adermark, L., Ericson, M., and Soderpalm, B., *Changes in glycine receptor subunit expression in forebrain regions of the Wistar rat over development.* Brain Res, 2012. **1446**: p. 12-21.
173. Molander, A. and Soderpalm, B., *Glycine receptors regulate dopamine release in the rat nucleus accumbens.* Alcohol Clin Exp Res, 2005. **29**(1): p. 17-26.
174. Waldvogel, H.J., Baer, K., Allen, K.L., Rees, M.I., and Faull, R.L., *Glycine receptors in the striatum, globus pallidus, and substantia nigra of the human brain: an immunohistochemical study.* J Comp Neurol, 2007. **502**(6): p. 1012-29.

175. Waldvogel, H.J., Baer, K., Gilbert, R.T., Gai, W., Rees, M.I., and Faull, R.L., *The cellular localisation of GABAA and glycine receptors in the human basal ganglia in "The Basal Ganglia IX"*. Advances in Behavioural Biology. Vol. 58. 2009: Springer.
176. Weltzien, F., Puller, C., O'Sullivan, G.A., Paarmann, I., and Betz, H., *Distribution of the glycine receptor beta-subunit in the mouse CNS as revealed by a novel monoclonal antibody.* J Comp Neurol, 2012. **520**(17): p. 3962-81.
177. Kuhse, J., Kuryatov, A., Maulet, Y., Malosio, M.L., Schmieden, V., and Betz, H., *Alternative splicing generates two isoforms of the alpha 2 subunit of the inhibitory glycine receptor.* FEBS letters, 1991. **283**(1): p. 73.
178. Malosio, M.L., Grenningloh, G., Kuhse, J., Schmieden, V., Schmitt, B., Prior, P., and Betz, H., *Alternative splicing generates two variants of the alpha 1 subunit of the inhibitory glycine receptor.* Journal of Biological Chemistry, 1991. **266**(4): p. 2048.
179. Dumoulin, A., Triller, A., and Dieudonne, S., *IPSC kinetics at identified GABAergic and mixed GABAergic and glycinergic synapses onto cerebellar Golgi cells.* J Neurosci, 2001. **21**(16): p. 6045-57.
180. Hernandes, M.S. and Troncone, L.R., *Glycine as a neurotransmitter in the forebrain: a short review.* J Neural Transm, 2009. **116**(12): p. 1551-60.
181. Pan, Z.H. and Slaughter, M.M., *Comparison of the actions of glycine and related amino acids on isolated third order neurons from the tiger salamander retina.* Neuroscience, 1995. **64**(1): p. 153-64.
182. Singer, J.H., *GABA is an endogenous ligand for synaptic glycine receptors.* Neuron, 2008. **57**(4): p. 475-7.
183. Soderpalm, B., Lof, E., and Ericson, M., *Mechanistic studies of ethanol's interaction with the mesolimbic dopamine reward system.* Pharmacopsychiatry, 2009. **42 Suppl 1**: p. S87-94.
184. Trudell, J.R., Messing, R.O., Mayfield, J., and Harris, R.A., *Alcohol dependence: molecular and behavioral evidence.* Trends Pharmacol Sci, 2014. **35**(7): p. 317-23.
185. Perkins, D.I., Trudell, J.R., Crawford, D.K., Alkana, R.L., and Davies, D.L., *Molecular targets and mechanisms for ethanol action in glycine receptors.* Pharmacol Ther, 2010. **127**(1): p. 53-65.
186. Mascia, M.P., Machu, T.K., and Harris, R.A., *Enhancement of homomeric glycine receptor function by long-chain alcohols and anaesthetics.* Br J Pharmacol, 1996. **119**(7): p. 1331-6.
187. Farley, N.M. and Mihic, S.J., *Allosteric modulation of the glycine receptor activated by agonists differing in efficacy.* Brain Res, 2015.
188. Ericson, M., Chau, P., Clarke, R.B., Adermark, L., and Söderpalm, B., *Rising taurine and ethanol concentrations in nucleus accumbens interact to produce dopamine release after ethanol administration.* Addiction biology, 2011. **16**(3): p. 377-385.
189. De Witte, P., Dahchour, A., and Quertemont, E., *Acute and chronic alcohol injections increase taurine in the nucleus accumbens.* Alcohol Alcohol Suppl, 1994. **2**: p. 229-33.
190. Ericson, M., Molander, A., Stomberg, R., and Soderpalm, B., *Taurine elevates dopamine levels in the rat nucleus accumbens; antagonism by strychnine.* Eur J Neurosci, 2006. **23**(12): p. 3225-9.
191. Chau, P., Hoifodt-Lido, H., Lof, E., Soderpalm, B., and Ericson, M., *Glycine receptors in the nucleus accumbens involved in the ethanol intake-reducing effect of acamprosate.* Alcohol Clin Exp Res, 2010. **34**(1): p. 39-45.
192. Molander, A., Lido, H.H., Lof, E., Ericson, M., and Soderpalm, B., *The glycine reuptake inhibitor Org 25935 decreases ethanol intake and preference in male wistar rats.* Alcohol Alcohol, 2007. **42**(1): p. 11-8.

193. Lido, H.H., Marston, H., Ericson, M., and Soderpalm, B., *The glycine reuptake inhibitor Org24598 and acamprosate reduce ethanol intake in the rat; tolerance development to acamprosate but not to Org24598.* Addict Biol, 2012. **17**(5): p. 897-907.
194. Fujiyama, F., Fritschy, J.M., Stephenson, F.A., and Bolam, J.P., *Synaptic localization of GABA(A) receptor subunits in the striatum of the rat.* J Comp Neurol, 2000. **416**(2): p. 158-72.
195. Hoestgaard-Jensen, K., Dalby, N.O., Krall, J., Hammer, H., Krogsgaard-Larsen, P., Frolund, B., and Jensen, A.A., *Probing alpha4betadelta GABAA receptor heterogeneity: differential regional effects of a functionally selective alpha4beta1delta/alpha4beta3delta receptor agonist on tonic and phasic inhibition in rat brain.* J Neurosci, 2014. **34**(49): p. 16256-72.
196. Maguire, E.P., Macpherson, T., Swinny, J.D., Dixon, C.I., Herd, M.B., Belelli, D., Stephens, D.N., King, S.L., and Lambert, J.J., *Tonic inhibition of accumbal spiny neurons by extrasynaptic alpha4betadelta GABAA receptors modulates the actions of psychostimulants.* J Neurosci, 2014. **34**(3): p. 823-38.
197. Nie, H., Rewal, M., Gill, T.M., Ron, D., and Janak, P.H., *Extrasynaptic delta-containing GABAA receptors in the nucleus accumbens dorsomedial shell contribute to alcohol intake.* Proc Natl Acad Sci U S A, 2011. **108**(11): p. 4459-64.
198. Blomqvist, O., Ericson, M., Engel, J.A., and Soderpalm, B., *Accumbal dopamine overflow after ethanol: localization of the antagonizing effect of mecamylamine.* Eur J Pharmacol, 1997. **334**(2-3): p. 149-56.
199. Ericson, M., et al., *Nicotinic acetylcholine receptors in the anterior, but not posterior, ventral tegmental area mediate ethanol-induced elevation of accumbal dopamine levels.* The Journal of pharmacology and experimental therapeutics, 2008. **326**(1): p. 76-82.
200. Ericson, M., et al., *Ethanol elevates accumbal dopamine levels via indirect activation of ventral tegmental nicotinic acetylcholine receptors.* European Journal of Pharmacology, 2003. **467**(1): p. 85-93.
201. Lof, E., Ericson, M., Stomberg, R., and Soderpalm, B., *Characterization of ethanol-induced dopamine elevation in the rat nucleus accumbens.* Eur J Pharmacol, 2007. **555**(2-3): p. 148-55.
202. Walaas, I. and Fonnum, F., *Biochemical evidence for gamma-aminobutyrate containing fibres from the nucleus accumbens to the substantia nigra and ventral tegmental area in the rat.* Neuroscience, 1980. **5**(1): p. 63-72.
203. Molander, A. and Soderpalm, B., *Accumbal strychnine-sensitive glycine receptors: an access point for ethanol to the brain reward system.* Alcohol Clin Exp Res, 2005. **29**(1): p. 27-37.
204. Löf, E., Ericson, M., Stomberg, R., and Söderpalm, B., *Characterization of ethanol-induced dopamine elevation in the rat nucleus accumbens.* European Journal of Pharmacology, 2007a. **555**(2): p. 148-155.
205. Molander, A., Löf, E., Stomberg, R., Ericson, M., and Söderpalm, B., *Involvement of accumbal glycine receptors in the regulation of voluntary ethanol intake in the rat.* Alcoholism, clinical and experimental research, 2005. **29**(1): p. 38-45.
206. Burkhardt, J.M. and Adermark, L., *Locus of onset and subpopulation specificity of in vivo ethanol effect in the reciprocal ventral tegmental area-nucleus accumbens circuit.* Neurochem Int, 2014. **76**: p. 122-30.
207. Wise, R.A., *Roles for nigrostriatal--not just mesocorticolimbic--dopamine in reward and addiction.* Trends Neurosci, 2009. **32**(10): p. 517-24.
208. Belin, D. and Everitt, B.J., *Cocaine seeking habits depend upon dopamine-dependent serial connectivity linking the ventral with the dorsal striatum.* Neuron, 2008. **57**(3): p. 432-41.

209. Deng, X.S. and Deitrich, R.A., *Putative role of brain acetaldehyde in ethanol addiction.* Curr Drug Abuse Rev, 2008. **1**(1): p. 3-8.
210. Correa, M., Salamone, J.D., Segovia, K.N., Pardo, M., Longoni, R., Spina, L., Peana, A.T., Vinci, S., and Acquas, E., *Piecing together the puzzle of acetaldehyde as a neuroactive agent.* Neurosci Biobehav Rev, 2012. **36**(1): p. 404-30.
211. Quertemont, E., Tambour, S., and Tirelli, E., *The role of acetaldehyde in the neurobehavioral effects of ethanol: A comprehensive review of animal studies.* Progress in Neurobiology, 2005. **75**(4): p. 247-274.
212. Israel, Y., Rivera-Meza, M., Karahanian, E., Quintanilla, M.E., Tampier, L., Morales, P., and Herrera-Marschitz, M., *Gene specific modifications unravel ethanol and acetaldehyde actions.* Front Behav Neurosci, 2013. **7**: p. 80.
213. Galter, D., Carmine, A., Buervenich, S., Duester, G., and Olson, L., *Distribution of class I, III and IV alcohol dehydrogenase mRNAs in the adult rat, mouse and human brain.* Eur J Biochem, 2003. **270**(6): p. 1316-26.
214. Amit, Z., Brown, Z.W., and Rockman, G.E., *Possible involvement of acetaldehyde, norepinephrine and their tetrahydroisoquinoline derivatives in the regulation of ethanol seld-administration.* Drug and alcohol dependence, 1977. **2**(5-6): p. 495-500.
215. Cohen, G., Sinet, P.M., and Heikkila, R., *Ethanol oxidation by rat brain in vivo.* Alcohol Clin Exp Res, 1980. **4**(4): p. 366-70.
216. Aragon, C.M. and Amit, Z., *Differences in ethanol-induced behaviors in normal and acatalasemic mice: systematic examination using a biobehavioral approach.* Pharmacol Biochem Behav, 1993. **44**(3): p. 547-54.
217. Aragon, C.M., Rogan, F., and Amit, Z., *Ethanol metabolism in rat brain homogenates by a catalase-H2O2 system.* Biochem Pharmacol, 1992. **44**(1): p. 93-8.
218. Zimatkin, S.M., Pronko, S.P., Vasiliou, V., Gonzalez, F.J., and Deitrich, R.A., *Enzymatic mechanisms of ethanol oxidation in the brain.* Alcoholism, clinical and experimental research, 2006. **30**(9): p. 1500-1500.
219. Zimatkin, S.M. and Lindros, K.O., *Distribution of catalase in rat brain: aminergic neurons as possible targets for ethanol effects.* Alcohol Alcohol, 1996. **31**(2): p. 167-74.
220. Moreno, S., Mugnaini, E., and Ceru, M.P., *Immunocytochemical localization of catalase in the central nervous system of the rat.* J Histochem Cytochem, 1995. **43**(12): p. 1253-67.
221. Smith, B.R., Amit, Z., and Splawinsky, J., *Conditioned place preference induced by intraventricular infusions of acetaldehyde.* Alcohol, 1984. **1**(3): p. 193-195.
222. Peana, A.T., Enrico, P., Assaretti, A.R., Pulighe, E., Muggironi, G., Nieddu, M., Piga, A., Lintas, A., and Diana, M., *Key role of ethanol-derived acetaldehyde in the motivational properties induced by intragastric ethanol: a conditioned place preference study in the rat.* Alcohol Clin Exp Res, 2008. **32**(2): p. 249-58.
223. Myers, W.D., Ng, K.T., and Singer, G., *Intravenous self-administration of acetaldehyde in the rat as a function of schedule, food deprivation and photoperiod.* Pharmacology, Biochemistry and Behavior, 1982. **17**(4): p. 807-811.
224. Peana, A.T., Muggironi, G., and Diana, M., *Acetaldehyde-reinforcing effects: a study on oral self-administration behavior.* Front Psychiatry, 2010. **1**: p. 23.
225. Rodd-Henricks, Z.A., Melendez, R.I., Zaffaroni, A., Goldstein, A., McBride, W.J., and Li, T.-K., *The reinforcing effects of acetaldehyde in the posterior ventral tegmental area of alcohol-preferring rats.* Pharmacology, Biochemistry and Behavior, 2002. **72**(1): p. 55-64.
226. Melis, M., Enrico, P., Peana, A.T., and Diana, M., *Acetaldehyde mediates alcohol activation of the mesolimbic dopamine system.* The European journal of neuroscience, 2007. **26**(10): p. 2824-2833.

227. Foddai, M., Dosia, G., Spiga, S., and Diana, M., *Acetaldehyde increases dopaminergic neuronal activity in the VTA.* Neuropsychopharmacology, 2004. **29**(3): p. 530-536.
228. Deehan, G.A., Jr., Engleman, E.A., Ding, Z.M., McBride, W.J., and Rodd, Z.A., *Microinjections of acetaldehyde or salsolinol into the posterior ventral tegmental area increase dopamine release in the nucleus accumbens shell.* Alcohol Clin Exp Res, 2013. **37**(5): p. 722-9.
229. Enrico, P., Sirca, D., Mereu, M., Peana, A.T., Lintas, A., Golosio, A., and Diana, M., *Acetaldehyde sequestering prevents ethanol-induced stimulation of mesolimbic dopamine transmission.* Drug Alcohol Depend, 2009. **100**(3): p. 265-71.
230. Sirca, D., Enrico, P., Mereu, M., Peana, A.T., and Diana, M., *L-cysteine prevents ethanol-induced stimulation of mesolimbic dopamine transmission.* Alcohol Clin Exp Res, 2011. **35**(5): p. 862-9.
231. Karahanian, E., Quintanilla, M.E., Tampier, L., Rivera-Meza, M., Bustamante, D., Gonzalez-Lira, V., Morales, P., Herrera-Marschitz, M., and Israel, Y., *Ethanol as a prodrug: brain metabolism of ethanol mediates its reinforcing effects.* Alcohol Clin Exp Res, 2011. **35**(4): p. 606-12.
232. Mascia, M.P., Maiya, R., Borghese, C.M., Lobo, I.A., Hara, K., Yamakura, T., Gong, D.H., and Beckstead, M.J., *Does Acetaldehyde Mediate Ethanol Action in the Central Nervous System?* Alcohol Clin Exp Res, 2001. **25**(11): p. 1570-1575.
233. Ward, R.J., Colantuoni, C., Dahchour, A., Quertemont, E., and De Witte, P., *Acetaldehyde-induced Changes in the Monoamine and Amino Acid Extracellular Microdialysate Content of the Nucleus Accumbens.* Neuropharmacology, 1997. **36**(2): p. 225-232.
234. Heffner, T.G., Hartman, J.A., and Seiden, L.S., *A rapid method for the regional dissection of the rat brain.* Pharmacol Biochem Behav, 1980. **13**(3): p. 453-6.
235. Hanse, E. and Gustafsson, B., *Paired-pulse plasticity at the single release site level: an experimental and computational study.* J Neurosci, 2001. **21**(21): p. 8362-9.
236. Branco, T. and Staras, K., *The probability of neurotransmitter release: variability and feedback control at single synapses.* Nat Rev Neurosci, 2009. **10**(5): p. 373-83.
237. Di Chiara, G., Tanda, G., and Carboni, E., *Estimation of in-vivo neurotransmitter release by brain microdialysis: the issue of validity.* Behav Pharmacol, 1996. **7**(7): p. 640-657.
238. Westerink, B.H., Damsma, G., Rollema, H., De Vries, J.B., and Horn, A.S., *Scope and limitations of in vivo brain dialysis: a comparison of its application to various neurotransmitter systems.* Life Sci, 1987. **41**(15): p. 1763-76.
239. Chefer, V.I., Thompson, A.C., Zapata, A., and Shippenberg, T.S., *Overview of brain microdialysis.* Curr Protoc Neurosci, 2009. **Chapter 7**: p. Unit7.1.
240. Parent, M., Bush, D., Rauw, G., Master, S., Vaccarino, F., and Baker, G., *Analysis of amino acids and catecholamines, 5-hydroxytryptamine and their metabolites in brain areas in the rat using in vivo microdialysis.* Methods, 2001. **23**(1): p. 11-20.
241. Di Chiara, G., *Nucleus accumbens shell and core dopamine: differential role in behavior and addiction.* Behav Brain Res, 2002. **137**(1-2): p. 75-114.
242. Deitrich, R.A., *Acetaldehyde: deja vu du jour.* J Stud Alcohol, 2004. **65**(5): p. 557-72.
243. Font, L., Miquel, M., and Aragon, C.M., *Prevention of ethanol-induced behavioral stimulation by D-penicillamine: a sequestration agent for acetaldehyde.* Alcohol Clin Exp Res, 2005. **29**(7): p. 1156-64.
244. Marti-Prats, L., Sanchez-Catalan, M.J., Hipolito, L., Orrico, A., Zornoza, T., Polache, A., and Granero, L., *Systemic administration of D-penicillamine prevents the locomotor activation after intra-VTA ethanol administration in rats.* Neurosci Lett, 2010. **483**(2): p. 143-7.

245. Wang, J., Carnicella, S., Phamluong, K., Jeanblanc, J., Ronesi, J.A., Chaudhri, N., Janak, P.H., Lovinger, D.M., and Ron, D., *Ethanol induces long-term facilitation of NR2B-NMDA receptor activity in the dorsal striatum: implications for alcohol drinking behavior.* J Neurosci, 2007. **27**(13): p. 3593-602.
246. Salamone, J.D., *The involvement of nucleus accumbens dopamine in appetitive and aversive motivation.* Behav Brain Res, 1994. **61**(2): p. 117-33.
247. Abercrombie, E.D., Keefe, K.A., DiFrischia, D.S., and Zigmond, M.J., *Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex.* J Neurochem, 1989. **52**(5): p. 1655-8.
248. Karahanian, E., Rivera-Meza, M., Tampier, L., Quintanilla, M.E., Herrera-Marschitz, M., and Israel, Y., *Long-term inhibition of ethanol intake by the administration of an aldehyde dehydrogenase-2 (ALDH2)-coding lentiviral vector into the ventral tegmental area of rats.* Addict Biol, 2014.
249. Quintanilla, M.E., Tampier, L., Karahanian, E., Rivera-Meza, M., Herrera-Marschitz, M., and Israel, Y., *Reward and relapse: complete gene-induced dissociation in an animal model of alcohol dependence.* Alcohol Clin Exp Res, 2012. **36**(3): p. 517-22.
250. Brannan, T.S., Maker, H.S., and Raes, I.P., *Regional distribution of catalase in the adult rat brain.* J Neurochem, 1981. **36**(1): p. 307-9.
251. Kuhn, J., Lenartz, D., Huff, W., Lee, S., Koulousakis, A., Klosterkoetter, J., and Sturm, V., *Remission of alcohol dependency following deep brain stimulation of the nucleus accumbens: valuable therapeutic implications?* J Neurol Neurosurg Psychiatry, 2007. **78**(10): p. 1152-3.
252. Lido, H.H., Stomberg, R., Fagerberg, A., Ericson, M., and Soderpalm, B., *The glycine reuptake inhibitor org 25935 interacts with basal and ethanol-induced dopamine release in rat nucleus accumbens.* Alcohol Clin Exp Res, 2009. **33**(7): p. 1151-7.
253. Darstein, M., Landwehrmeyer, G.B., Kling, C., Becker, C.M., and Feuerstein, T.J., *Strychnine-sensitive glycine receptors in rat caudatoputamen are expressed by cholinergic interneurons.* Neuroscience, 2000. **96**(1): p. 33-9.
254. Darstein, M., Loschmann, P.A., Knorle, R., and Feuerstein, T.J., *Strychnine-sensitive glycine receptors inducing [3H]-acetylcholine release in rat caudatoputamen: a new site of action of ethanol?* Naunyn Schmiedebergs Arch Pharmacol, 1997. **356**(6): p. 738-45.
255. Zetterstrom, T. and Fillenz, M., *Local administration of flurazepam has different effects on dopamine release in striatum and nucleus accumbens: a microdialysis study.* Neuropharmacology, 1990. **29**(2): p. 129-34.
256. Löf, E., Chau, P.P., Stomberg, R., and Soderpalm, B., *Ethanol-induced dopamine elevation in the rat--modulatory effects by subchronic treatment with nicotinic drugs.* Eur J Pharmacol, 2007b. **555**(2-3): p. 139-47.
257. Ferraro, L., Tanganelli, S., O'Connor, W.T., Antonelli, T., Rambert, F., and Fuxe, K., *The vigilance promoting drug modafinil increases dopamine release in the rat nucleus accumbens via the involvement of a local GABAergic mechanism.* Eur J Pharmacol, 1996. **306**(1-3): p. 33-9.
258. Zhou, F.M., Liang, Y., and Dani, J.A., *Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum.* Nat Neurosci, 2001. **4**(12): p. 1224-9.
259. Blomeley, C.P., Cains, S., Smith, R., and Bracci, E., *Ethanol affects striatal interneurons directly and projection neurons through a reduction in cholinergic tone.* Neuropsychopharmacology, 2011. **36**(5): p. 1033-46.
260. Luo, R., Janssen, M.J., Partridge, J.G., and Vicini, S., *Direct and GABA-mediated indirect effects of nicotinic ACh receptor agonists on striatal neurones.* J Physiol, 2013. **591**(Pt 1): p. 203-17.

261. Smolders, I., Bogaert, L., Ebinger, G., and Michotte, Y., *Muscarinic modulation of striatal dopamine, glutamate, and GABA release, as measured with in vivo microdialysis.* J Neurochem, 1997. **68**(5): p. 1942-8.
262. Kumari, M. and Ticku, M.K., *Regulation of NMDA receptors by ethanol.* Prog Drug Res, 2000. **54**: p. 152-89.
263. Philpot, R.M., Badanich, K.A., and Kirstein, C.L., *Place conditioning: age-related changes in the rewarding and aversive effects of alcohol.* Alcohol Clin Exp Res, 2003. **27**(4): p. 593-9.
264. DeWit, D.J., Adlaf, E.M., Offord, D.R., and Ogborne, A.C., *Age at first alcohol use: a risk factor for the development of alcohol disorders.* Am J Psychiatry, 2000. **157**(5): p. 745-50.