Thesis for the degree of Doctor of Medicine

Glycine receptors in the central nervous system – development, distribution, and relation to actions of alcohol

Susanne Jonsson

2012

UNIVERSITY OF GOTHENBURG

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

Cover by

Printed by

Previously published articles and figures were reprinted with permission from the publishers.

ISBN 978-91-628-8589-2

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. **Jonsson S**, Kerekes N, Hyytiä P, Ericson M, Söderpalm B. (2009) Glycine receptor expression in the forebrain of male AA/ANA rats Brain Res. 2009 Dec 11;1305 Suppl:S27-36

II. **Jonsson S**, Morud J, Pickering C, Adermark L, Ericson M and Söderpalm B (2012) Changes in glycine receptor expression in forebrain regions of the Wistar rat over development Brain Res. 2012 Mar 29;1446:12-21.

III. **Jonsson S**, Ericson M, Söderpalm B. (2012) The effects of long-term ethanol consumption on the expression of neurotransmitter receptor genes in the rat nucleus accumbens *Manuscript*

IV. **Jonsson S**, Adermark L, Stomberg R, Morud J, Ericson M and Söderpalm B (2012) Glycine receptors are involved in mesolimbic dopamine release induced by drugs of abuse *Manuscript*

TABLE OF CONTENT

ABSTRACT **8**

LIST OF ABBREVIATIONS **9**

PREFACE **11**

INTRODUCTION **12**

ALCOHOL ADDICTION 12

ALCOHOL ADDICTION AND GENES 13

ANIMAL MODELS OF ALCOHOL USE DISORDERS 14

BRAIN REWARD SYSTEM 17

MESOLIMBIC DOPAMINE SYSTEM 17

NUCLEUS ACCUMBENS 18

DOPAMINE AND ETHANOL 19

A PROPOSED NEURONAL CIRCUIT 20

ETHANOL AND LIGAND-GATED ION CHANNELS 22

$GABA_A$ 23

NMDA 24

nACh 24

5-HT3 25

THE GLYCINE RECEPTOR 25

ETHANOL AND THE GLYCINE RECEPTOR 28

AIM OF THESIS **30**

SPECIFIC AIMS: 30

EXPERIMENTAL DESIGN **31**

PAPER I 31

PAPER II 31

PAPER III 31

PAPER IV 31

MATERIALS AND METHODS **33**

ANIMALS 34

DRUGS AND CHEMICALS 34

Ethanol 34

Cocaine 35

Morphine 35

Nicotine 35

Strychnine 35

$^{\Delta 9}$-Tetrahydrocannabinol 35

2-hydroxypropyl-β-cyclodextrin 36
Tetrodotoxin 36
MEASUREMENTS OF ETHANOL INTAKE 36
Voluntary ethanol consumption 36
ANALYSIS OF mRNA 37
RNA extraction and cDNA synthesis 37
Quantitative Real-Time Polymerase Chain Reaction 38
Normalisation and data analysis 40
RT^2 Profiler array 40
BIOCHEMICAL ASSAY 41
In vivo microdialysis 41
Microdialysis technique 42
Neurochemical assay - dopamine 43
Neurochemical assay - taurine 43
PROTEIN EXPRESSION 44
Immunohistochemistry 44
Retrograde tracing 45
ELECTRICAL ACTIVITY 45
Electrophysiology 45
STATISTICAL ANALYSIS 46
RESULTS AND DISCUSSION **47**
PAPER I 47
PAPER II 49
PAPER III 52
PAPER IV 55
SUMMARY OF RESULTS **57**
GENERAL DISCUSSION **58**
SWEDISH SUMMARY **63**
ACKNOWLEDGEMENTS **68**
REFERENCES **70**

ABSTRACT

The widespread consumption of alcohol and the great socioeconomic impact of alcohol abuse and addiction have contributed to the extensive investigations of this substance. Despite great efforts there is still uncertainty concerning how alcohol exerts its effects and the mechanisms behind the transition from consumption to addiction. However, substantial evidence proposes involvement of the mesolimbic dopamine system in the rewarding and reinforcing effects of the drug. Alcohol is known to affect several neurotransmitter systems and the glycine receptor (GlyR) is among its primary targets. Previous studies from our group have strongly suggested that GlyRs in the nucleus accumbens (nAc, a key region in the mesolimbic dopamine system) are involved in the dopamine elevating and reinforcing effects of alcohol. Based on a number of studies a hypothesis of a neuronal circuit mediating these effects of alcohol has been proposed, where the GlyR is a key component. The aim of this thesis was therefore to further examine the GlyR and its role in the actions of ethanol. Gene expression of GlyR subunits was measured in animals selectively bred based on alcohol preference (Alko-Alcohol, AA, and Alko Non-Alcohol, ANA, rats) with and without exposure to alcohol (Paper I), during development (Paper II) and in response to long-term alcohol consumption (Paper III). The main method, quantitative polymerase chain reaction (qPCR), was complemented by monitoring of consumption behaviour (Paper I and III) and immunohistochemical studies (Paper II and III). The effect of accumbal GlyR blockade on the dopamine elevating effect of alcohol and other drugs of abuse was investigated using *in vivo* microdialysis (Paper IV). In this study immunohistochemistry and retrograde tracing were also utilised to explore the proposed neuronal circuit. The results of the work presented in this thesis suggest: (1) that based on gene expression the glycinergic system seems robust, (2) the disparate alcohol consumption of AA and ANA rats is not due to differences in forebrain GlyR gene expression, (3) α2 appears to be the dominating α-subunit in the rat brain and α2β should be the dominating GlyR receptor composition in the adult brain, (4) the commonly accepted developmental shift from α2 to α1β is not a general effect, (5) GlyRs are mainly located in the nAc shell-region, (6) accumbal GlyRs are involved in the dopamine-elevating effect of nicotine and tetrahydrocannabinol in addition to alcohol, and (7) the possible addition of the lateral septum in the neuronal circuit mediating ethanol's dopamine-elevating effect.

KEY WORDS; glycine receptor, nucleus accumbens, alcohol, gene expression, dopamine

LIST OF ABBREVIATIONS

β-cyclodextrin; 2-hydroxypropyl-β-cyclodextrin

AA; Alko Alcohol

ANA; Alko Non-Alcohol

AUD; alcohol use disorder

aVTA; anterior ventral tegmental area

BAL; blood alcohol level

Chrna5; nicotinic acetylcholine receptor α5 gene

CTB; cholera toxin B subunit

D_2; dopamine receptor 2

D_3; dopamine receptor 3

DAPI; 4',6-diamidino-2-phenylindole

DRD2; dopamine receptor 2 gene

DRD3; dopamine receptor 3 gene

GABA; γ-amino butyric acid

$GABA_A$ receptor; γ-amino butyric acid type A receptor

Gabra1; $GABA_A$ receptor α1 subunit gene

GlyR; glycine receptor

Grm5; metabotropic glutamate receptor 5 gene

HPLC; high-performance liquid chromatography

IHC; immunohistochemistry

i.p.; intraperitoneally

LDTg; laterodorsal tegmental nucleus

nAc; nucleus accumbens

nAChR; nicotinic acetylcholine receptor

nAChRa5; nicotinic acetylcholine receptor α5 subunit

NMDA; *N*-methyl -D-aspartate

NMDAR2a; *N*-methyl -D-aspartate receptor 2a gene

NMDAR2b; *N*-methyl -D-aspartate receptor 2b gene

NR2a; *N*-methyl -D-aspartate receptor subunit 2a

NR2b; *N*-methyl -D-aspartate receptor subunit 2b

OPA; *o*-phtaldialdehyde

Oprm; μ-opioid receptor gene

PCR; polymerase chain reaction

PFC; prefrontal cortex

PPTg; pedunculopontine tegmental nucleus

PS; population spikes

qPCR; quantitative real-time polymerase chain reaction

s.c.; subcutaneously

Sstr4; somatostatin receptor 4

Tacr3; tachykinin receptor 3

TBS; tris buffered saline

THC; $^{\Delta 9}$-tetrahydrocannabinol

TTX; tetrodotoxin

VTA; ventral tegmental area

PREFACE

9000. 125 000 000. 100.

The number of years that alcohol has been consumed, the number of humans estimated to be affected by alcohol use disorders worldwide, the annual alcohol-related cost in billions for the Swedish society alone (in SEK). The reasons for investigating alcohol related disorders are numerous indeed.

The image most often associated with the term "alcoholic" is a social outcast; unemployed, homeless and constantly inebriated. Although this might be a correct description of some individuals suffering from conditions collectively referred to as alcohol use disorders (AUDs), the vast majority of people with alcohol-related problems live normal lives and struggle with their addiction in private rather than in public. Despite the classification as a disease there is still a social stigma of alcohol addiction, contributing to the reluctance of people to acknowledge their problems, and to seek treatment for them. Thus, the number of unrecorded cases is presumed to be high and many are unaware of the severity of the problem.

For people with AUDs the positive, e.g. the anxiolytic and relaxing, effects of alcohol have been replaced by a compulsive need to consume the drug at any cost and usually in such quantities that the beneficial health effects are lost. What separates these individuals from those able to enjoy alcohol without developing a destructive behavioural pattern? No common factor for all addicted individuals has been found, probably because no such factor exists. That there is a genetic component is well known, and has been confirmed in a multitude of studies, but the exact nature of this genetic influence is unknown. Furthermore, knowledge of both alcohol's acute actions and the long-term alterations caused by alcohol consumption is insufficient but indicates that effects of this drug are multiple and complex. This is also reflected in the limited number of treatments available and the inability to predict and prevent development of the disease.

Despite the ancient and, practically, worldwide custom to consume alcohol we lack the knowledge and remedy to successfully treat many of those suffering from alcohol-related problems. For an alcohol researcher this is a challenge and an incentive, for someone suffering from an AUD it is a source of despair and frustration.

Göteborg, November 2012

INTRODUCTION

ALCOHOL ADDICTION

Alcohol is a legal, easily accessible and socially accepted drug. This status contributes to the widely spread consumption of ethanol, entailing that individuals vulnerable to addiction are likely to encounter this particular drug. As a drug ethanol is commonly consumed for its acute effects. In humans these effects are usually easy to distinguish; an initial pleasurable feeling, (including anxiolysis and elevated mood) and signs of psychomotor stimulation, e.g. increased talkativeness and social interaction. Should consumption continue this response is followed by more obvious signs of intoxication, such as lack of judgement, impaired motor function, sedation, and, eventually, coma. These are all temporary effects, passing as the drug is metabolised and blood alcohol levels recede. However, repeated ethanol consumption can also lead to chronic disorders including abuse and addiction, collectively referred to as Alcohol Use Disorders (AUDs). Approximately 125 million people worldwide are affected by these conditions (World Health Organization, Global burden of disease 2004 update) that usually take years to develop as an initial, voluntary, consumption is gradually replaced by a compulsive intake of alcohol. The transition from casual consumption to addiction is commonly divided into three steps; initiation of alcohol consumption, maintenance, and uncontrolled use, which is an indication of dependence and addiction. Development of an AUD involves both genetic and environmental factors and is not necessarily a linear process.

Alcohol addiction is classified as a chronic relapsing disease but for this diagnosis there are no absolute definitions, no physiological markers or limit values. The lack of a key insight into what causes alcohol addiction makes the diagnosis imperfect and based on clusters of symptoms described in diagnostic manuals (the upcoming 5th edition of Diagnostic and Statistical Manual of Mental Disorders (DSM 5) and the International Classification of Disease 10th edition (ICD 10)). The common diagnostic tool for AUDs in the clinic are the guidelines provided by these manuals, a set of criteria designed to describe different aspects of addiction, including physical dependence, loss of control, and craving. Based on what is known of the behaviour and condition of each individual an assessment is made to determine if that person meets the criteria for AUDs.

Although details are yet to be disclosed it is well established that several brain regions and circuits are involved and/or disrupted in drug addiction. Excessive and chronic ethanol consumption, as observed in abuse and dependence, has been linked to a number of systems in the brain, including acetylcholine (Soderpalm et al., 2000; Davis and de Fiebre,

2006), dopamine (Heinz, 2002), gamma-amino butyric acid (GABA; Wallner and Olsen, 2008; Kumar et al., 2009; Maccioni and Colombo, 2009), glutamate (Davis and Wu, 2001; Gass and Olive, 2008), serotonin (5-HT3; Lovinger, 1999), opiate (Drews and Zimmer, 2010), corticotropin releasing factor (Koob, 2010), substance P (George et al., 2008) and the hypothalamic-pituitary-adrenal axis (Richardson et al., 2008).

Considering the additional influence of genes (and epigenetics), environment, social and psychological factors, and interactions between them, heterogeneous manifestations of AUDs is to be expected. The variation observed in afflicted individuals has led to the proposal that there are several different types of the disorder (Cloninger et al., 1981; Lesch and Walter, 1996). The limited efficiency of available treatments (disulfiram, acamprosate and naltrexone) also implies that there are different subgroups in this population as only 20-30% of alcohol addicts respond to acamprosate or naltrexone (Ripley and Stephens, 2011). Consequently the opinion that a single compound is usually not sufficient to successfully treat an AUD, or all AUDs, is gaining ground. This has led to trials using a cocktail of substances in animal models, which show promise, and future prospects include tailor-made pharmacological treatments (Rezvani et al., 2000; Spanagel and Kiefer, 2008; Bell et al., 2012). A better understanding of the disorder and the basis of its different components is necessary, both to form a more cohesive theory of addiction and to produce more efficient treatment strategies.

ALCOHOL ADDICTION AND GENES

That there is a genetic component in alcohol-related behaviours has been repeatedly confirmed and heritability of alcohol addiction is estimated to be 50- 60% (McGue et al., 1999; Dick and Foroud, 2003). However, with the exception of genes involved in ethanol metabolism (see below), no causal relationships between single genes and addiction have been found. Rather results, and the nature of the condition, indicate the involvement of multiple genes, each with a weak individual effect but with a collective impact (Goldman et al., 2005).

The use of linkage studies, genome-wide expression analysis, selectively bred and inbred animals, transgenic and knock-out models has resulted in a large number of candidate genes, but to demonstrate their role in AUDs has proven difficult (Crabbe, 2008; Bjork et al., 2010; Farris and Miles, 2012). Thus far the only genes confirmed to affect the risk of alcohol addiction are variants of aldehyde dehydrogenase and liver alcohol

dehydrogenase, enzymes important in the metabolism of ethanol (Tu and Israel, 1995; Luczak et al., 2002; Pautassi et al., 2010). For multifaceted conditions such as AUDs there is the possibility that a single gene variant can result in a phenotype contributing to some aspect of addictive behaviour (Schumann, 2007). For example, a variant of the μ-opioid receptor appears to modulate the acute response to ethanol (King et al., 1997; Ray and Hutchison, 2004; Ramchandani et al., 2011). This is interesting since the same allelic variant of the gene for this receptor appears to predict response to treatment with the opioid receptor antagonist naltrexone (Oslin et al., 2003; Anton et al., 2008).

Apart from genes with direct influence (e.g. affecting ethanol metabolism) it has been proposed that genes with indirect influence (involved in personality traits or temperament) increase the risk of developing AUDs (Slutske et al., 2002; Sher et al., 2005; Elkins et al., 2006). While the importance of traits and temperament is still a matter of debate the effect of drug consumption is undisputed since there is no way to escape that development of addiction requires exposure to the drug. As with substance abuse in general the neurobiological grounds for alcohol disorders are believed to involve enduring (or permanent) adaptations in the central nervous system. Excessive consumption of ethanol (or other substances) has been suggested to induce these alterations, thus triggering abuse and addiction (Nestler and Aghajanian, 1997; Nestler, 2004; Volkow and Li, 2004). An example of these persisting changes is alterations in gene expression that may occur already a few hours after the first exposure to alcohol (Miles et al., 1991). Effects of chronic ethanol exposure on gene expression have been demonstrated in components of several neurotransmitter systems and in multiple brain areas (e.g. (Morrow et al., 1994; Devaud et al., 1995a; Follesa and Ticku, 1995; Ortiz et al., 2004; Simonyi et al., 2004). These effects may underlie changes in neurotransmission induced by ethanol and could contribute to the development of tolerance, dependence, craving, or other symptoms of abuse and addiction caused by altered brain function.

ANIMAL MODELS OF ALCOHOL USE DISORDERS

A majority of the symptoms used for diagnosing alcohol-related disorders are behavioural, due to the lack of reliable biological markers (see *Alcohol addiction*). Hence animal models of alcohol-related disorders struggle with the same difficulties as studies of other psychiatric disorders; to re-create conditions presumably unique to humans in animals, which per definition are not human. The great limitation of animal models is that it is not possible to

mimic the entire spectrum of the disease, only certain aspects. This has led to the development of an abundance of study designs aimed at modeling distinct features of drug abuse. These include, but are not restricted to, ethanol preference/self-administration models for studying initiation and maintenance of consumption, reinstatement models to measure craving and relapse (Shaham et al., 2003), and the alcohol deprivation model to investigate compulsive and uncontrolled ethanol intake (Spanagel and Holter, 1999). During these different stages of alcohol-related behavior it is presumed that the genes involved, effects of the drug, and the importance of environmental factors all change (Vengeliene et al., 2008; Spanagel, 2009; Bjork et al., 2010).

In an alcohol self-administration paradigm emphasis is on the positive reinforcement of the drug, the amount consumed is interpreted as a sign of the drug's reinforcing effect, and the route of administration is usually identical with that used by humans; oral consumption. Studies investigating the initiation phase have been helpful in identifying factors that may increase vulnerability to the reinforcing effects of ethanol. The brain regions believed to mediate these effects are evolutionary well preserved (see *Brain reward system*), making animal models suitable for studying the neurobiological underpinnings of ethanol consumption and reinforcement.

When trying to induce addiction-like behaviour in animals (and the underlying pathological mechanisms) the alcohol deprivation model, developed to mimic compulsive alcohol-seeking and alcohol-taking (Spanagel and Holter, 1999), has proven useful. After long periods of access to ethanol the drug is removed which results in a temporary increase in consumption when the drug is reintroduced – the alcohol deprivation effect (Sinclair and Senter, 1967). Studies using this approach appear to have successfully replicated the elusive compulsive/'loss of control' element of addiction by getting animals to consume ethanol despite addition of quinine to the solution, a substance normally causing a strong taste aversion in rats (Spanagel and Holter, 2000; Vengeliene et al., 2009)

Other factors suggested to be involved in the development of addiction are administration and exposure. By forced administration of ethanol, e.g. in a vapour chamber, dependence can be produced in a relatively short time and in addition to dose both duration and pattern of exposure can be controlled (Gilpin et al., 2009). However, it has been proposed that rats will develop addiction only after voluntary consumption, while forced administration will lead to physical dependence (Wolffgramm and Heyne, 1995). In line with this reasoning, active and passive administration of drugs of abuse have been reported to produce different neuroadaptations (Jacobs et al., 2003). Whereas the debate of voluntary versus forced

consumption is restricted to animal models, the effect of acute versus long-term drug exposure is as relevant for the human situation. The development of AUDs is often a prolonged process but even the first experiences of ethanol have been reported to affect the risk of developing addiction (Schuckit, 1994). Thus there are reasons for investigating both immediate and long-term effects of the drug. In animal models the distinction is usually made between acute and chronic exposure, where 'chronic' is often used to emphasize divergence from acute. As a result, the term chronic in studies of ethanol-related effects can refer to a period of days, weeks, months or years (e.g. (Devaud et al., 1995a; Charlton et al., 1997; Rage et al., 1998; Sarviharju et al., 2001), affecting the outcome and limiting comparisons between studies.

Similar to humans rodents display great variability in ethanol-related behaviour and in the 1940s it was discovered that ethanol preference is heritable in rodents (Williams et al., 1949). Bidirectional breeding of animals displaying extreme ethanol behaviours has since then yielded several high- and low-preferring strains (Mardones and Segovia-Riquelme, 1983; Crabbe, 2010). Despite breeding based on similar criteria there are differences in genetics, behaviour and neurochemistry between these rat lines (Turek et al., 2005; Bell et al., 2012; McBride et al., 2012; Roman et al., 2012). This indicates that an ethanol-preferring phenotype can arise via various pathways and may be a reflection of the subpopulations observed in humans suffering from AUDs (Bell et al., 2012). That high consumption/preference alone does not translate into addiction, which is characterised by loss of control over drinking, is a common critique concerning selectively bred animals. However, alcohol-preferring animals have shown behavioural, physiological and neurochemical similarities with subpopulations of humans addicted to alcohol (McBride et al., 1993a; Hietala et al., 1994; Volkow et al., 1996b; Tupala et al., 2001; Bell et al., 2012).

Despite the reductionist approach animal models have been useful in establishing the genetic influence on ethanol preference, in investigating the harmful effects of binge-drinking, in demonstrating that early exposure to ethanol increases the risk of developing AUDs, and for testing potential new pharmacotherapies (Bell et al., 2012). Different definitions of parameters, in combination with the many different models and methods used, contribute to the wealth of conflicting results reported in alcohol research. Thus, like the diagnostic guidelines, animal models are a useful but imperfect tool.

BRAIN REWARD SYSTEM

To eat, drink, socially interact and reproduce are elementary activities for all animals. These actions are experienced as rewarding and pleasant which is essential to motivate these behaviours and increase the chance of survival. The mechanism mediating this effect is believed to be an increment of the neurotransmitter dopamine in certain interconnected midbrain and forebrain regions following activation (Kelley and Berridge, 2002; Wise, 2008). These regions comprise what today is commonly referred to as the ’brain reward system’, an evolutionary ancient system present in all vertebrates. Due to its primitive properties this system has been well conserved and is astonishingly similar in different species, e.g. man and rat. The discovery of this reinforcing system was initiated in the 1950s when Olds and Milner published what would be groundbreaking results from a brain mapping study using electrical stimulation (Olds and Milner, 1954). They had found that animals would choose to stimulate some brain areas to an extreme extent, ignoring food and water and press the lever that resulted in stimulation until exhaustion. Following this finding it was shown that animals would self-administer drugs abused by humans in a manner similar to electrical stimulation (Schuster and Thompson, 1969), and that the reinforcing and rewarding effects of these substances exceeded those of natural rewards. Thus, by taking advantage of the function of the reward system, addictive drugs strongly motivate the continued use of potentially harmful substances. The shared effect on the reward system indicates that drugs of abuse have a common neuroanatomical substrate (Wise and Bozarth, 1987; Koob and Bloom, 1988).

MESOLIMBIC DOPAMINE SYSTEM

The central component in mediating reward and positive reinforcement (and associated behaviours) is proposed to be the bundle of dopaminergic neurons projecting from the ventral tegmental area (VTA) via the medial forebrain bundle to the nucleus accumbens (nAc) and the olfactory tubercle, septum, amygdala and hippocampus - the mesolimbic dopamine system (Koob, 1992; Ikemoto, 2007; Arias-Carrion et al., 2010). Although the reward pathway is not restricted to these areas and projections (Wise, 1998; Koob, 2003) the VTA-nAc pathway has received the most attention. Accumulating experimental evidence stress the importance of the VTA-nAc connection in mediating pleasurable feelings and in motivating behavior that will lead to reward (Le Moal and Simon, 1991; Kelley and Berridge, 2002; Tobler et al., 2005). Following chronic intake of addictive drugs, when the consumption has become compulsive and uncontrolled, the reward-related effects seem to be replaced by other, negative, driving

forces like anxiety and stress (Vengeliene et al., 2008; Koob and Volkow, 2010). The origin and quality of these alterations, and whether they involve the mesolimbic dopamine system remains to be ascertained (Spanagel and Heilig, 2005).

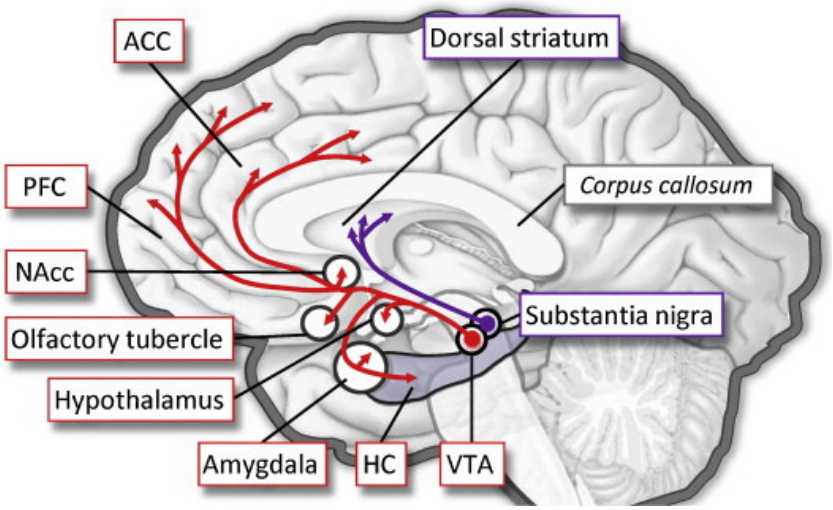


Figure 1. The mesolimbic dopamine system The VTA innervates a variety of regions via dopaminergic projections (in red), but the main components of the mesolimbic dopamine system are nucleus accumbens, amygdala, olfactory tubercle, the septal area and the hippocampus (Perogamvros and Schwartz, 2012). Abbreviations: ACC; anterior cingulate cortex, PFC; prefrontal cortex, NAcc; nucleus accumbens, HC; hippocampus, VTA; ventral tegmental area.

NUCLEUS ACCUMBENS

The activity of, and the dopamine increase in, the mesolimbic dopamine system is believed to mediate the reinforcing and pleasurable effects of natural rewards and addictive drugs alike in both humans and rodents (Gessa et al., 1985; Di Chiara and Imperato, 1988; Grenhoff and Svensson, 1989; Wise and Rompre, 1989; Di Chiara and North, 1992; Drevets et al., 2001; Boileau et al., 2003). The most pronounced dopamine increase following stimulation of this system is observed in the nAc, a region at the base of the forebrain which, together with the olfactory tubercle, forms the ventral striatum. It has been estimated that 95% of accumbal neurons in the rat (70% in primates) are GABAergic medium spiny projecting neurons, the remaining portion is made up of large aspiny cholinergic neurons and GABAergic interneurons (Kalivas et al., 1993; Heimer et al., 1997). The nAc is commonly divided into two subdivisions; core and shell, based on functional and anatomical differences (Brog et al., 1993; Heimer et al., 1997; Zahm, 1999). The nAc shell is a part of the extended amygdala and considered to be a limbic structure, appearing to be more diverse and sensitive to pharmacological stimuli than the core (Zahm, 1999). It is also the termination point of most of

the dopaminergic projections from the VTA (Ikemoto, 2007), thus many studies have focused on the shell and its implication in drug reinforcement. The core region strongly resembles the rest of the striatum, both in appearance and function (Groenewegen et al., 1996; Heimer et al., 1997). However, sensitisation to the dopamine increase induced by some, but not all, drugs of abuse has been observed in the nAc core (Cadoni and Di Chiara, 2000; Cadoni et al., 2000), a response presumably important for the transition to dependence (Di Chiara, 2000). Thus there is experimental evidence indicating that both subregions are relevant for the development of drug addiction.

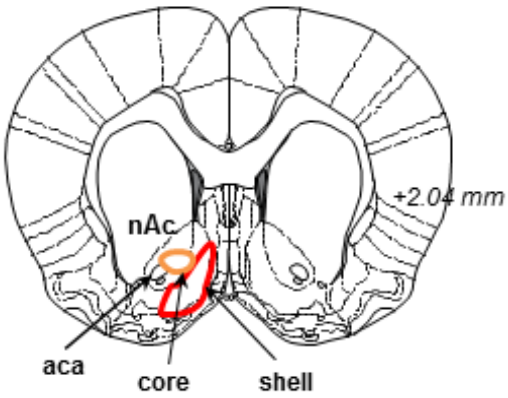


Figure 2. The nucleus accumbens
A coronal section of the rat brain showing the location of the nucleus accumbens. The division into core and shell is based on differences in organisation and function. While the core resembles the striatum the shell is a part of the extended amygdala. Abbreviations: aca; anterior commissure, nAc; nucleus accumbens. Adapted from Paxinos and Watson 2007.

DOPAMINE AND ETHANOL

First identified as a precursor of noradrenaline, dopamine was later granted the designation neurotransmitter (Carlsson et al., 1957; Carlsson, 1993). While its involvement in reward sensation is highly noticed in addiction research, dopamine is also critical for memory, motivation and executive function (Volkow et al., 2012). These functions may interact as learning about reinforcing stimuli is supposedly facilitated by a dopamine increase in nAc (Di Chiara, 1999). That drugs of abuse, despite different primary mechanisms, induce an increase in dopamine levels has made this effect a cornerstone of addiction research. Hence a mutual action of addictive drugs has resulted in a common starting point for studies investigating them.

Attempts to interfere with the accumbal dopamine increase, using selective lesions or antagonists, have successfully prevented the reinforcing effects of some drugs (nicotine, cocaine and amphetamine) (Roberts et al., 1980; Corrigall et al., 1992; Dani and Heinemann, 1996; Di Chiara et al., 2004), but not others (opiates) (Pettit et al., 1984; Koob and Bloom, 1988). For ethanol conflicting results have been reported (Kiianmaa et al., 1979;

Brown et al., 1982; Linseman, 1990; Levy et al., 1991; Fahlke et al., 1994), and it has been proposed that dopaminergic transmission is not essential in mediating ethanol's reinforcing actions (Linseman, 1990; Fahlke et al., 1994; Koob et al., 1994). However, there are other aspects of ethanol consumption and the proposed connection between ethanol and dopamine is not limited to extracellular levels in the nAc.

Like all neurotransmitters dopamine exerts its effects via a receptor. For dopamine five receptors, D_1-D_5, have been identified and these are separated into D_1-like (D_1 and D_5) and D_2-like (D_2, D_3 and D_5) receptors based on structure, pharmacology and biochemistry (Beaulieu and Gainetdinov, 2011). Agonists and antagonists of D_1, D_2 and D_3 have all been able to alter ethanol consumption in both non-selected (Pfeffer and Samson, 1988; Russell et al., 1996; Cohen et al., 1998) and ethanol-preferring rats (Dyr et al., 1993; Russell et al., 1996; McBride and Li, 1998). Furthermore, cue-induced reinstatement of alcohol-seeking behavior has been reduced by administration of antagonists for these three receptors (Liu and Weiss, 2002; Vengeliene et al., 2006). Of the dopamine receptor subtypes D_2 is the one presumably involved in the rewarding effects of ethanol (Stefanini et al., 1992; McBride et al., 1993b; Nowak et al., 2000). Interestingly this receptor variant has also been implicated in alcohol addiction as decreased D_2 levels have been observed in alcohol addicts and could be a predisposing factor for abuse (Volkow et al., 1996a; Volkow et al., 1996b; Volkow et al., 2002; Heinz et al., 2004). Conversely, high D_2 levels might have a protective effect against alcohol abuse as overexpression of accumbal D_2 receptors in rats reduces both ethanol intake and preference (Thanos et al., 2001). Despite the wealth of evidence concerning the importance of dopamine it should be noted that endogenous opioids, GABA, serotonin and glutamate have also been implicated in ethanol's reinforcing effects (Engel et al., 1992; Koob, 1996).

A PROPOSED NEURONAL CIRCUIT

There are several theories concerning how ethanol interacts with the mesolimbic DA-system, the hypothesis presented here is based on results from a compilation of experiments performed by the present research group.

In a series of studies the role of ventral tegmental nicotinic acetylcholine receptors (nAChRs) was investigated (reviewed in Soderpalm et al., 2000), concluding that activation of these receptors is implicated in ethanol's dopamine activating and reinforcing effects. This was illustrated by the ability of both systemic and local injections of a nAChR

antagonist (mecamylamine) to inhibit the dopamine-increasing effect of systemic ethanol (Blomqvist et al., 1993; Blomqvist, 1996; Blomqvist et al., 1997). Interestingly, ethanol administered into the nAc, but not into the VTA, elevated dopamine levels (Ericson et al., 2003; Lof et al., 2007c). This dopamine elevation could be blocked by application of mecamylamine in the VTA, an action that also resulted in reduced voluntary ethanol intake and preference (Ericson et al., 1998; Ericson et al., 2003; Ericson et al., 2008). Collectively these results implied that activation of nAChRs in anterior VTA (aVTA) is preceded by an ethanol effect in the nAc (Larsson et al., 2004; Lof et al., 2007a; Ericson et al., 2008).

The initial attempts to identify this action involved type A γ-amino butyric acid ($GABA_A$) receptors in the nAc (Zetterstrom and Fillenz, 1990; Molander and Soderpalm, 2005b; Lof et al., 2007b). When this failed the attention was turned to another of ethanol's primary targets; the glycine receptor (GlyR). It was demonstrated that functional GlyRs are present in the nAc, are able to modify dopamine output and are involved in the reinforcing and dopamine-activating effects of ethanol (Molander and Soderpalm, 2005b, a). In addition, local administration of the GlyR antagonist strychnine prevented ethanol-induced dopamine elevation in nAc and increased ethanol intake (Molander et al., 2005; Molander and Soderpalm, 2005a). After fitting the results together the proposed sequence of events between the ethanol-GlyR interaction and the accumbal dopamine increase is as follows;

GlyRs in the nAc, presumably on GABAergic neurons projecting to the aVTA, are activated by ethanol. This activation leads to reduced inhibition of cholinergic neurons from the laterodorsal/pedunculopontine tegmental nuclei (LDTg/PPTg) by the GABAergic projection neurons and acetylcholine is released. Through activation of nAChRs, presumptively on dopaminergic neurons projecting to nAc, dopamine is released in the nAc.

In addition, the reducing effect of glycine re-uptake inhibitors on ethanol consumption in rats further supports the involvement of GlyRs in ethanol reinforcement (Molander et al., 2007; Lido et al., 2012). A series of studies investigating the mechanism of action for acamprosate, an anti-relapse substance, also corroborates the theory of this neuronal circuit (Chau, 2011).

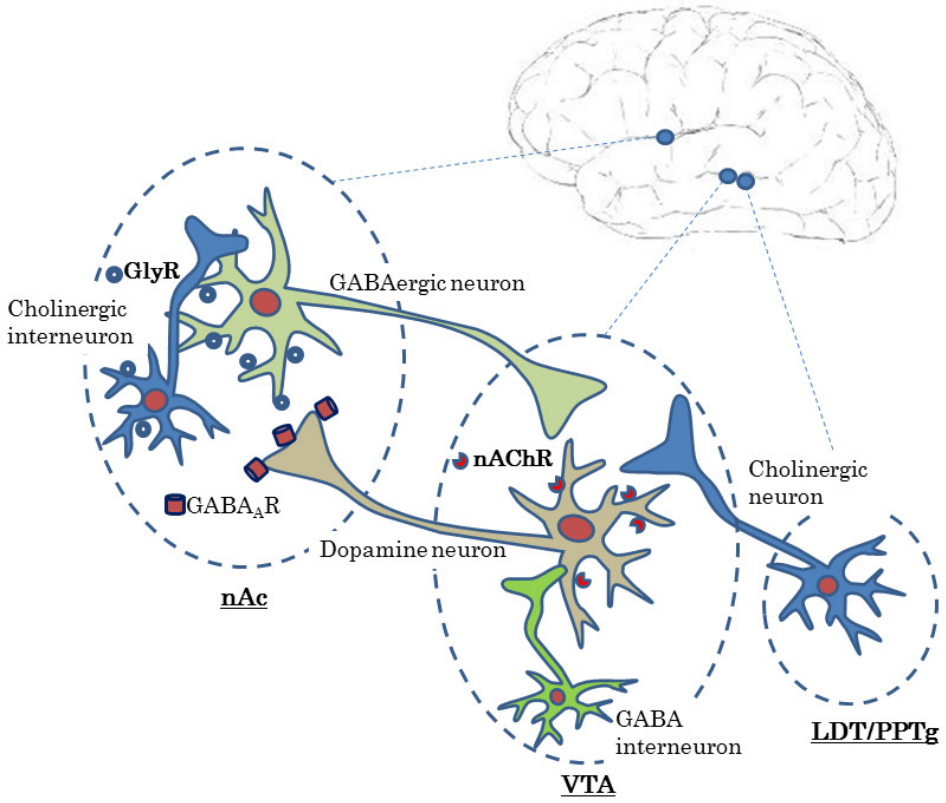


Figure 3. A neuronal circuit mediating ethanol's dopamine activating effects A schematic illustration showing the elements involved in the proposed neuronal circuit mediating the dopamine-elevating and reinforcing effects of ethanol. Inhibition of GABAergic neurons projecting from nAc to VTA, due to ethanol activation of GlyRs on these neurons, leads to acetylcholine release in the VTA from neurons originating in LDTg/PPTg. This activates nAChRs, presumably on dopaminergic neurons projecting back to the nAc, causing a release of dopamine in the nAc. Modified from Chau 2011. Abbreviations: $GABA_AR$; GABA A type receptors, GlyR; glycine receptor, LDTg/PPTg; laterodorsal/pedunculopontine tegmental nuclei, nAc; nucleus accumbens, nAChR; nicotinic acetylcholine receptor, VTA; ventral tegmental area.

ETHANOL AND LIGAND-GATED ION CHANNELS

For ethanol to exert its effects access to the brain is necessary. This is achieved as the small and soluble ethanol molecule easily penetrates the blood-brain barrier. While the specificity of other drugs of abuse has given name to their respective receptor, e.g. the *nicotinic* acetylcholine receptor, ethanol has been shown to be a promiscuous ligand with several primary targets; $GABA_A$, 5-hydroxytryptamine (serotonin, 5-HT_3), glycine, *N*-methyl-D-aspartate (NMDA) and nACh receptors (Lovinger et al., 1989; Mihic et al., 1997; Lovinger, 1999; Mihic, 1999; Narahashi et al., 1999) to mention a few. As ligand-gated ion channels these receptors share a pentameric transmembrane structure and the binding of a ligand is required for them to open or close. By binding to the ion channels ethanol potentiates the function of $GABA_A$, glycine, 5-HT_3 and nACh receptors but inhibits NMDA receptor

function (Lovinger, 1997; Harris et al., 2008). In addition, ethanol activates G-protein activated inwardly rectifying K^+-channels (GIRKs) (Kobayashi et al., 1999; Lewohl et al., 1999) and inhibits dihydropyridine-sensitive L-type Ca^{2+} channels (Wang et al., 1994). These interactions occur at physiologically relevant concentrations (5-100 mM in blood and brain) and the altered receptor functions lead to dose-dependent symptoms of intoxication (Valenzuela, 1997; Bjork et al., 2010).

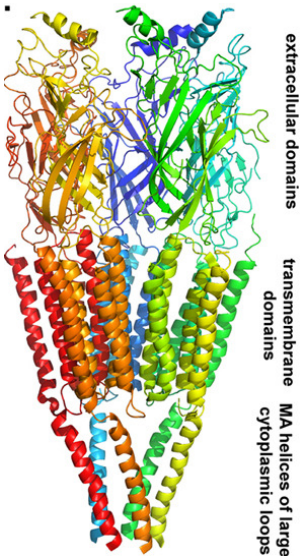


Figure 4. Structure of the ligand-gated ion channel This type of ion channels includes receptors for glycine, NMDA, GABA and serotonin but the archetype is the nAChR, seen here. Each subunit has an extracellular ligand-binding portion and a transmembrane domain with four helixes (Wells, 2008).

$GABA_A$

Characteristic symptoms of ethanol can be linked to effects on specific receptors, e.g. the anxiolytic, sedative and motor-impairing effects of ethanol involve facilitation of GABAergic neurotransmission (Ticku, 1990). $GABA_A$ receptors, primarily located in the postsynaptic membrane, mediate rapid neurotransmission in the mammalian CNS. For ethanol's effect on $GABA_A$ receptors time of exposure appears to be relevant, as short-term consumption may increase receptor function while long-term consumption has the opposite effect (Morrow et al., 1990; Mihic, 1999; Davies, 2003). Another important aspect seems to be receptor configuration since different subunits appear to respond to diverse ethanol concentrations and continuing ethanol exposure may cause alterations in subunit composition (Mhatre and Ticku, 1993; Mihic et al., 1994; Devaud et al., 1995b; Wallner et al., 2003; Olsen et al., 2007). The variety of subunits contributes to the diversity of the receptor and certain subtypes have been associated with distinct actions, e.g. δ-subunit containing receptors in the nAc shell are suggested to be important for the reinforcing effects of ethanol (Nie et al., 2011). Modifications in both mRNA and protein expression of $GABA_A$ receptor subunits have been observed in many regions after chronic ethanol consumption (see Kumar et al., 2009). It is

presumed that these and other effects of ethanol on GABAergic receptors form the basis of the alcohol withdrawal syndrome, a condition successfully treated using positive modulators of $GABA_A$ receptor function, such as benzodiazepines (Mayo-Smith, 1997; Lejoyeux et al., 1998; Bayard et al., 2004).

NMDA

Similar to the exposure-dependent effects on $GABA_A$ receptors, function of the NMDA receptor is acutely inhibited by ethanol, but after chronic ethanol exposure both receptor expression and function are increased (Iorio et al., 1992; Dodd et al., 2000; Krystal et al., 2003; Gass and Olive, 2008). These effects, together with the alterations in subunit expression also observed, possibly contribute to tolerance and withdrawal (Snell et al., 1993; Hoffman, 1995; Snell et al., 1996). Moreover, diverse NMDA receptor responses to ethanol have been observed in various brain regions, presumably due to the variety of subunits creating receptors with distinct pharmacological properties (Allgaier, 2002). Several acute and chronic effects of ethanol are likely to be mediated by NMDA receptors, e.g. cognitive deficits and neuronal degradation (Dodd et al., 2000; Kumari and Ticku, 2000; Woodward, 2000). Results from studies using NMDA receptor antagonists indicate that self-administration of ethanol, and the reinforcing effects of the drug, might be regulated by glutamate neurotransmission in the nAc (Rassnick et al., 1992; Biala and Kotlinska, 1999). Furthermore, the anti-craving substance acamprosate has been shown to inhibit the NMDA receptor (Zeise et al., 1993; Allgaier et al., 2000), an action that may be involved in the effect of this compound.

nACh

By acting as a co-agonist at the nAChR ethanol enhances the effect of acetylcholine, and nicotine if present (Marszalec et al., 1999). Central nAChRs have been implicated in the dopamine increasing effect ethanol has in the mesolimbic dopamine system (Soderpalm et al., 2000). However, the subunit composition of the receptors involved has not been determined. Studies using subtype specific antagonists have suggested that α3β2, α6 and/or β3 (Larsson and Engel, 2004; Jerlhag et al., 2006b; Jerlhag et al., 2006a), but not α4β2 or α7 (Le et al., 2000; Ericson et al., 2003), subtypes are of importance for the increased dopaminergic activity following ethanol exposure. In studies using human subjects it has been demonstrated that the stimulatory and pleasurable effects of ethanol are reduced by nAChR blockade (Blomqvist et al., 2002; Chi and de Wit, 2003; Young et al., 2005). It has also been suggested that ventral tegmental nAChRs mediate the dopamine activating and reinforcing properties of

ethanol cues (Lof et al., 2007c). Thus, nAChRs seem to have impact on ethanol reinforcement and voluntary intake.

5-HT3

Like dopamine, serotonin is proposed to be vital for initiation of ethanol reinforcement (Engel et al., 1992; Vengeliene et al., 2008). Ethanol potentiates 5-HT_3 actions through direct interaction with the 5-HT_3 receptor (Lovinger, 1991; Lovinger and White, 1991), and findings from knock-out mouse models and pharmacological manipulations of the 5-HT_3 system suggest that voluntary ethanol consumption is affected by serotonin (Engel et al., 1998; Zhou et al., 1998; Vengeliene et al., 2008).

Although influenced by multiple factors, the time of exposure, drug concentration and subunit composition seem to be of particular importance for ethanol's interactions with its targets (Grant, 1994; Vengeliene et al., 2008). The precise mechanism for how ethanol interacts with ligand-gated ion channels is still uncertain and probably varies depending on receptor type and posttranslational alterations (Mihic and Harris, 1996; Harris, 1999; Spanagel, 2009). Due to differences in ethanol sensitivity of receptor subunits, and the distribution of these subunits, there are regional variations in drug effect in the brain (Spanagel, 2009). As a consequence of ethanol's primary interactions a number of secondary, indirect, effects involving multiple neurotransmitter and neuropeptide systems are initiated. Altogether these events form a complex pharmacological effect expressed as the diverse behavioural response associated with ethanol consumption and intoxication.

THE GLYCINE RECEPTOR

Glycine, the smallest of the amino acids, was recognised as a neurotransmitter in the 1960s and the system mediating its transmission is the second inhibitory system in the CNS (Aprison and Werman, 1965; Betz and Becker, 1988). Although a co-agonist at the NMDA receptor glycine also has a specific, strychnine-sensitive, receptor (Curtis et al., 1968; Young and Snyder, 1973). Apart from glycine the endogenous ligands of this receptor also include taurine and β-alanine, and a co-agonist action of GABA has been reported (Lu et al., 2008). In the CNS of mammals GlyRs are most abundantly expressed in the spinal cord, brainstem, retina and cerebellum and are involved in modulation of essential physiological functions like

respiration, sensory processing and motor control (Betz and Becker, 1988; Yevenes and Zeilhofer, 2011a).

The transmembrane GlyR comprises a Cl^- selective pore opening as a ligand binds to the receptor, making it possible for chloride ions to diffuse over the membrane. Normally this leads to hyperpolarization and inhibition of signalling. However, in environments with high intracellular Cl^- levels, e.g. during development, GlyR activation is excitatory, as it results in depolarization (Ito and Cherubini, 1991; Lynch, 2004; Kirsch, 2006).

The great similarity between the GlyR α1-subunit and the nAChR rendered inclusion of the GlyR in the cys-loop superfamily of ligand-gated ion channels (Grenningloh et al., 1987; Lynch, 2009). Structurally, GlyRs are either α-homomers or αβ-heteromers with a subunit stoichiometry of 2α3β (Grudzinska et al., 2005). Currently the existence of five different subunits, α1-4 and β, have been identified and found in the mammalian brain (Malosio et al., 1991a; Matzenbach et al., 1994; Harvey et al., 2000; Lynch, 2004). While α1-3 and β-subunits are widely spread in the spinal cord and brain, α4 expression is very modest but has been located in the retina (Malosio et al., 1991a; Matzenbach et al., 1994; Heinze et al., 2007). Identified splice variants of the α1-α3 subunits further add to this diversity (Kuhse et al., 1991; Malosio et al., 1991b; Nikolic et al., 1998; Lynch, 2004; Le-Corronc et al., 2011).

Similar to other ion channels the characteristics of the GlyR is determined by its subunit composition, affecting both function and localisation (Malosio et al., 1991a; Laube et al., 2002; Deleuze et al., 2005). Qualities like kinetic properties (Mangin et al., 2003), affinity for agonists (Kuhse et al., 1990; Pribilla et al., 1992; Schmieden et al., 1992; Mascia et al., 1996b; Li and Slaughter, 2007; Chen et al., 2009), and antagonists all appear to vary depending on subunit combinations (Pribilla et al., 1992; Han et al., 2004; Yang et al., 2007). Yet the subunits also display great similarities, homology in amino acid sequence is 80-90% for the α-subunits and few compounds are specific enough to distinguish between them (Grenningloh et al., 1990; Lynch, 2009). Separation of heteromers and homomers is more easily achieved as sensitivity to picrotoxin inhibition is much higher in homomers, irrespective of α-subunit (Pribilla et al., 1992). Localisation is another way of differentiating between receptor types as it appears that heteromeric GlyRs are located in the synapse whereas homomeric GlyRs are found extrasynaptically (Deleuze et al., 2005). This is due to fundamental differences between α and β subunits. While the β-subunit alone is unable to form functional receptors it binds to gephyrin, a postsynaptic anchoring protein, which enables synaptic clustering (Bormann et al., 1993; Kirsch and Betz, 1995; Handford et al.,

1996; Kirsch et al., 1996; Waldvogel et al., 2010). The ligand-binding α-subunits lack this quality but are capable of forming receptors on their own, even by co-assembly of α1 and α2 subunits (Kuhse et al., 1993). Since β-subunits are needed for synaptic distribution and α1 is supposedly the most abundantly expressed α-subunit in adult animals there is a general assumption that α1β is the predominant GlyR configuration in synapses (Becker et al., 1988; Malosio et al., 1991a), mediating most of the glycinergic neurotransmission. Functionally the slow activation rate of homomeric/extrasynaptic GlyRs is proposed to prohibit activation by fast neurotransmitter release in a synapse, making them more suitable for paracrine or autocrine activation than for synaptic neurotransmission (Mangin et al., 2003; Muller et al., 2008; Le-Corronc et al., 2011).

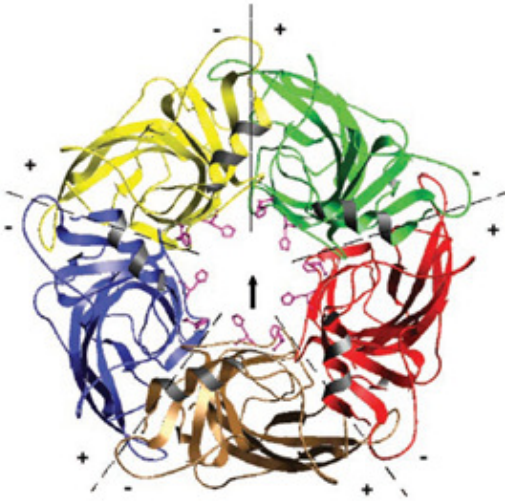


Figure 5. The glycine receptor
A model of the ligand-binding domain of an α1GlyR homomer. The five subunits needed for a functional receptor (here represented by different nuances) create the pore of the ion channel, opening in response to ligand-binding (Nevin et al., 2003).

Besides the transition from excitatory to inhibitory function, another developmental shift is often mentioned in the literature; the replacement of the previously dominating 'neonatal' α2-homomers by 'adult' α1β-heteromers (Becker et al., 1988; Hoch et al., 1989; Akagi et al., 1991; Lynch, 2004). In rats this transformation is completed 2-3 weeks after birth and should involve alterations in GlyR function and signalling due to the different properties of these receptor subtypes (Malosio et al., 1991a; Watanabe and Akagi, 1995; Singer et al., 1998), but the actual impact of this change is difficult to determine.

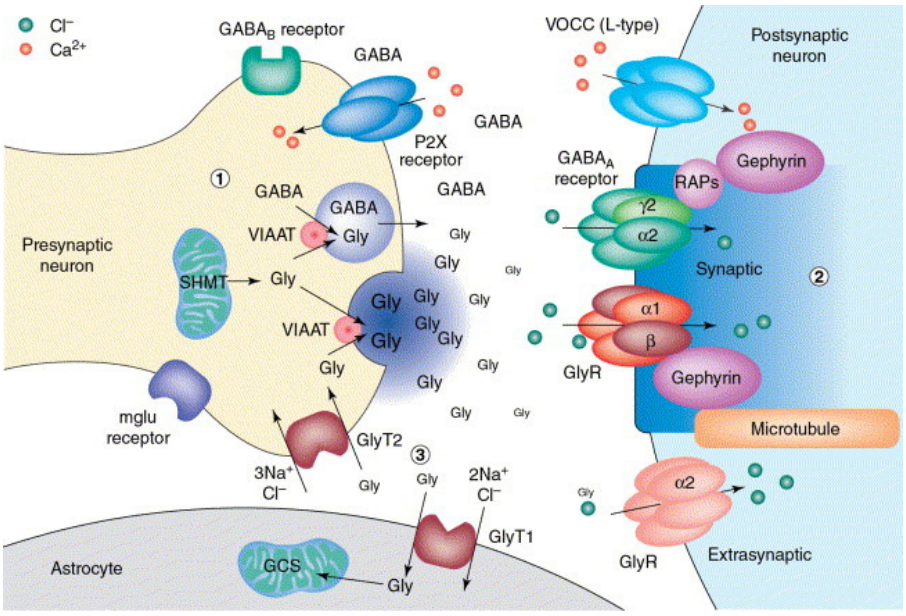


Figure 6. A simplified illustration of a synapse with glycinergic signalling Glycine is released from vesicles into the synaptic cleft, activating postsynaptic GlyRs. The β-subunit binds gephyrin, allowing the formation of synaptic receptors while homomeric receptors assemble at extrasysnaptic sites, relying on paracrine/autocrine activation (Laube et al., 2002).

ETHANOL AND THE GLYCINE RECEPTOR

While drugs primarily acting through the GlyR are scarce receptor actions are modulated by several compounds in addition to its endogenous ligands (Laube et al., 2002). Of the drugs abused by humans, cannabinoids and ethanol appear to affect GlyR function (Lynch, 2004; Yevenes and Zeilhofer, 2011b, a). By binding to a site on the GlyR ethanol potentiates the response, an effect observed in multiple parts of the CNS (Aguayo et al., 1996; Jiang and Ye, 2003; Eggers and Berger, 2004). Several studies have also indicated that ethanol's effect on GlyRs is partly indirect via ethanol-sensitive proteins (Mascia et al., 1998; Jiang and Ye, 2003; Yevenes et al., 2008). Subtype-specific variations may occur as it has been proposed that α1 homomers are more sensitive to ethanol than α2 homomers, particularly at low concentrations (below 100 mMol) (Mascia et al., 1996b; Perkins et al., 2008; Yevenes et al., 2010). Similarly decreased sensitivity to ethanol in neonatal (mainly α2 homomers) GlyRs relative to α1 GlyRs has been reported in neuronal preparations (Eggers et al., 2000; Sebe et al., 2003).

Compared to $GABA_A$ and NMDA receptors, no specific symptom of ethanol abuse or addiction has been linked to the GlyR. Rather the involvement of GlyRs in the ability of ethanol to increase dopamine levels indicates that actions on this receptor are of importance in the initiation and maintenance of ethanol consumption. Similar to ethanol, the

administration of GlyR ligands glycine, taurine and β-alanine all increase dopamine levels in the nAc (Molander and Soderpalm, 2005b; Ericson et al., 2006; Ericson et al., 2010). These effects are blocked by strychnine, confirming that the elevations are mediated via GlyRs. Furthermore taurine has been suggested to be of importance for obtaining dopamine release in response to ethanol administration (Ericson et al., 2006; Adermark et al., 2011c; Ericson et al., 2011). GlyRs are also involved in the ethanol intake reducing effect of the homotaurinate acamprosate (Chau et al., 2010), an effect reversed by GlyR blockade with strychnine. Another interference with the glycinergic system has been shown to robustly reduce both ethanol preference and consumption in Wistar rats (Molander et al., 2007; Lido et al., 2012). This was achieved by preventing reuptake of glycine in the extracellular space using selective glycine reuptake inhibitors. This strategy also succeeded in reducing ethanol intake following an alcohol deprivation period, without any indications of tolerance development (Molander et al., 2007; Vengeliene et al., 2010; Lido et al., 2012).

AIM OF THESIS

This thesis aimed to further investigate the glycine receptor, its involvement in alcohol-related behaviour and in the proposed neuronal circuit mediating the reinforcing effects of alcohol.

SPECIFIC AIMS:

Paper I

To examine the relative expression of mRNA encoding GlyR subunits in different brain areas and relate it to ethanol consumption in selectively bred animals.

Paper II

To study age-related changes in mRNA expression of GlyR subunits in various brain areas.

Paper III

To investigate the effect of continuous, and voluntary, long-term ethanol intake on ethanol consumption behaviour and the expression of neurotransmitter-related genes.

Paper IV

To explore the previously proposed neuronal circuit through which ethanol modulates dopamine release in the nucleus accumbens, and to investigate whether accumbal GlyRs are involved in mediation of the mesolimbic dopamine activating effects of other addictive drugs.

EXPERIMENTAL DESIGN

PAPER I

This study was designed to investigate the potential involvement of GlyRs in the diverse ethanol consumption behaviours observed in selectively bred animals. High preferring Alko Alcohol (AA) and low preferring Alko Non-Alcohol (ANA) rats were divided into groups with and without access to ethanol. This allowed us to determine whether differences in gene expression were induced by ethanol consumption or due to a pre-existing (genetic) difference. Expression of genes encoding GlyR subunits (α1-3 and β) was analysed in eight different brain regions, with focus on mesolimbic areas.

PAPER II

Results from Paper I contradicted the proposed developmental shift in subunit expression from neonatal α2 homomers to adult α1β heteromers in rodents. This, in combination with the role of GlyRs in ethanol's dopamine-increasing effect, the varying ethanol sensitivity of GlyR subunits and the risks associated with early ethanol exposure, led to a study of developmental changes in GlyR expression. Tissue from different brain regions of animals aged 2 (neonatal), 21 (juvenile), and 60 (adult) days was analysed and gene expression was compared to determine age-related changes.

PAPER III

Long-term ethanol consumption is likely to induce changes in many neurotransmitter systems, changes that may contribute to the transition from controlled to compulsive intake. With single-housed animals voluntarily consuming ethanol for extended periods of time (2, 4 or 10 months) this study was designed to mimic this aspect of the development of addiction. Expression of neurotransmission-related genes (receptors, regulators etc.) was monitored, in addition to drinking behaviour, to see if chronic consumption alone would be enough to induce addiction-like alterations.

PAPER IV

Involvement of accumbal GlyRs in the dopamine-activating effect of ethanol has previously been demonstrated, primarily by abolishing the dopamine increase usually induced by ethanol

through blockade of these receptors. GlyRs in the nAc have been proposed to be part of a neuronal circuit through which ethanol modulates dopamine release and immunohistochemistry and retrograde tracing were used to map elements of this proposed circuit. Accumbal dopamine elevation is characteristic for all drugs of abuse. To determine if GlyRs are involved also in the effect of other drugs on accumbal dopamine levels systemic administration of different addictive drugs was combined with local receptor blockade.

MATERIALS AND METHODS

The materials and methods used in this thesis were as follows:

ANIMALS

- Alko Alcohol (AA) and Alko Non-Alcohol (ANA) rats, adult males (Paper I)
- Male and female Wistar rats aged 2, 21 and 60 days (Paper II)
- Male Wistar rats, adolescent-adult (Paper III)
- Male Wistar rats, adult (Paper IV)

DRUGS AND CHEMICALS (Paper I, III and IV)

MEASUREMENTS OF ETHANOL INTAKE

Voluntary ethanol consumption (Papers I and III)

ANALYSIS OF mRNA

Quantitative real-time PCR (Papers I-III)

PROTEIN ANALYSIS

- Immunohistochemistry (Papers II and IV)
- Retrograde tracing (Paper IV)

ELECTRICAL ACTIVITY

Electrophysiology, field potential recordings (Paper IV)

BIOCHEMICAL ASSAYS

In vivo microdialysis (Paper IV)

STATISTICS (Papers I-IV)

In the following section the methods used in this thesis are briefly described and discussed. For further details the reader is referred to the individual papers and the references cited therein.

ANIMALS

In Paper I adult male Alko Alcohol (AA) and Alko Non-Alcohol (ANA) rats (300-460 g), developed for high and low ethanol consumption through selective breeding, were used. Animals were single-housed and kept under inverted light/dark conditions (lights on/off at 7.00 PM/AM). For Paper II group-housed male and female Wistar rats aged 2, 21 and 60 days were kept under regular light/dark conditions (lights on/off at 7.00 AM/PM). Male Wistar rats (130-150 g) (Taconic, Denmark) were employed for Paper III. Similar to Paper I these animals were single-housed and kept under inverted light/dark conditions (lights on/off at 10.00 PM/AM). For Paper IV male Wistar rats (270-320 g) were group-housed four per cage under regular light/dark conditions (lights on/off at 7.00 AM/PM). After surgery these animals were placed in separate cages for two days until the day of the experiment. The arrival weights correspond in age to adult (Paper I and IV) or adolescent (Paper III) animals according to the breeder. Outbred animals were used (Papers II-IV) as a population with more diverse behaviour more accurately models a general population. All animals were given one week to adjust to the controlled environment of the animal facilities (22ºC and 65% humidity) before experiments commenced. Food and tap water were available *ad libitum* for the entire duration of all experiments. All experiments presented in this thesis were approved by the Ethics Committee for Animal Experiments, Gothenburg, Sweden.

DRUGS AND CHEMICALS

All drugs for systemic injection were dissolved in saline (0.9% NaCl) before administration intraperitoneally (i.p.) or subcutaneously (s.c.) at a volume of 2 or 5(ethanol) ml/kg. Locally perfused substances were dissolved in Ringer solution consisting of (in mmol / l): 140 NaCl, 1.2 $CaCl_2$, 3.0 KCl, and 1.0 $MgCl_2$. For electrophysiological experiments all drugs were diluted in artificial cerebrospinal fluid and administered in the bath (Adermark et al., 2011a). With the exception of ethanol all drugs were used exclusively in Paper IV

Ethanol

For voluntary alcohol consumption studies (Paper I and III) ethanol (95% Kemetyl AB, Haninge, Sweden) was dissolved in tap water to the concentration chosen for consumption, 2-10% (Paper I) or 6% (Paper III). For Paper III a 6% solution was used based on the results of Fahlke and colleagues (Fahlke et al., 1994), indicating that for consumption in adult male Wistar rats this concentration would be optimal. For systemic administration (Paper IV)

ethanol was dissolved to a concentration of 15% and administered i.p. at a dose of 2.5 g/kg. This dose has been shown to induce a robust DA increase in previous studies from our laboratory (e.g. Ericson et al., 2011). In electrophysiological experiments slices were exposed to a concentration of 50 mM ethanol.

Cocaine

Cocaine is a stimulant that inhibits the reuptake of dopamine, serotonin and noradrenaline (Koe, 1976; Ritz et al., 1987; Florin et al., 1994). Cocaine (Apoteket AB, Sweden) was administered i.p. at a dose of 15 mg/kg.

Morphine

The opiate morphine is a potent analgesic drug acting directly on the CNS, primarily via the μ-opioid receptor. Morphine (Apoteket AB, Sweden) was administered i.p. at a dose of 5 mg/kg.

Nicotine

Nicotine hydrogen tartrate salt (Sigma-Aldrich, Sweden), a lipophilic nicotinic acetylcholine receptor agonist, was dissolved in saline and neutralised with sodium bicarbonate. The solution was injected s.c. at a dose of 0.4 mg/kg, the nicotine dose is expressed as free base.

Strychnine

The plant alkaloid strychnine is a competitive antagonist at GlyRs (Sigma-Aldrich, Sweden), but has also been reported to interfere with the activity of other receptors (Matsubayashi et al., 1998; Garcia-Colunga and Miledi, 1999). A dose (20 μM) that by itself did not affect accumbal dopamine was administered in the nAc via reversed microdialysis. For electrophysiological experiments strychnine was diluted to a concentration of 1 μM.

$^{\Delta 9}$-Tetrahydrocannabinol

$^{\Delta 9}$-tetrahydrocannabinol (THC) is the component primarily responsible for the psychoactive effects of cannabis (Sigma-Aldrich, USA). From a concentration of 25 mg/ml (in ethanol) THC was dissolved in 2-hydroxypropyl-β-cyclodextrin (β-cyclodextrin) and a dose of 3 mg/kg was administered i.p. Similar to strychnine, a concentration of 1 μM was used for electrophysiological experiments.

2-hydroxypropyl-β-cyclodextrin

The aqueous solubility of THC was increased by dissolving the compound in 45% w/v β-cyclodextrin (Sigma-Aldrich).

Tetrodotoxin

Tetrodotoxin (TTX) is a potent neurotoxin preventing neurons from firing action potentials by irreversibly blocking the sodium channels involved in this process (Sigma-Aldrich). TTX was dissolved to a concentration of 1.0 μM in Ringer solution and locally administered in the nAc via reversed microdialysis.

MEASUREMENTS OF ETHANOL INTAKE

Voluntary ethanol consumption

Since it appears that the voluntary aspect of ethanol consumption is of importance for the development of alcohol-related disorders in humans this aspect was important to consider when selecting an animal model. Furthermore, voluntary consumption is proposed to be required for the development of addiction in rats whereas forced administration supposedly induces physical dependence, but not addiction (Wolffgramm and Heyne, 1995). The two-bottle preference model (where animals have continuous access to both water and an ethanol solution) was used to study effects of ethanol consumption, both in animals selectively bred based on ethanol preference and in outbred animals. Like humans, rodents display individual drinking behaviours and vary in their preference for ethanol consumption (Wolffgramm and Heyne, 1995). Provided that experimental conditions remain unaltered these individual patterns are preserved, thus we expected to see a range in ethanol consumption including both high- and low-consumers when using outbred animals (Paper III). In addition both housing conditions and concentration of the drug also affect consumption (Wolffgramm and Heyne, 1995). While single housing is a measure that may increase drug intake in general, the preferred concentration of drug varies between individuals and cannot be manipulated by the researcher to the same extent (Wolffgramm, 1990). For the selectively-bred AA and ANA animals (Paper I) the ethanol concentration was gradually increased (2-4-6-10%) over the course of two weeks after which animals had access to a 10% ethanol solution for the remaining four weeks of the study. In the long-term (2-10 months) consumption study (Paper III) the concentration of 6% was chosen based on a previous study where this concentration

was preferred by male Wistar rats (Fahlke et al., 1994). Body weight was monitored once a week, while ethanol and water consumption were measured twice a week. The parameters used to quantify ethanol intake were consumption and preference. Ethanol consumption was measured as grams of ethanol consumed per kilogram body weight per day (g/kg/day). Ethanol preference was defined as percentage of total fluid intake per day (%). The aim of the present studies was to examine changes in gene expression and consumption behaviour induced by ethanol exposure rather than intoxication. Thus, that animals exposed to the two-bottle preference model generally do not consume ethanol in a binge-drinking manner, or to the extent of overt intoxication, was not considered a limitation.

ANALYSIS OF mRNA

RNA extraction and cDNA synthesis

To avoid potential effects on mRNA expression animals were not anaesthetised before decapitation. Brains were dissected using a brain matrix and under strictly RNase free conditions (Heffner et al., 1980). The tissue was kept cold during the dissection and dissected areas were placed in RNase free Eppendorf tubes, frozen on dry ice and kept at -80°C until further processing. Samples were homogenised in a monophasic solution of phenol and guanidine thiocyanate (QIAzol Lysis Reagent). Extraction of RNA was performed according to the manufacturer's protocols using Qiagen kits with silica-membrane purification. Measures were taken to remove residual amounts of DNA, in the RNA extraction (DNase treatment) and/or in the cDNA synthesis (gDNA Wipeout Buffer). Genomic DNA contamination is a known problem with the phenol extraction method but this does not always disturb the PCR analysis. In addition, the PCR Arrays contained control primers to quantify how much gDNA contamination a given sample contained (see *RT^2 Profiler array*). RNA concentrations of all samples were determined with a SmartSpec Plus spectrophotometer (BioRad Laboratories). Based on these readings the amount of material used for cDNA synthesis was determined. Following the manufacturer's instructions Qiagen kits were used for the reverse transcription of RNA to cDNA after which samples were diluted with RNase free water.

Quantitative Real-Time Polymerase Chain Reaction

Quantification of mRNA is one way to determine alterations in gene expression. Polymerase Chain Reaction (PCR) is a fast, specific and sensitive method and the use of it has increased rapidly the last couple of decades. Its mechanism is based on the events leading to duplication of the genetic material in a dividing cell where alterations in temperature have replaced most of the enzymes involved. Basically three steps; denaturation, annealing and extension, constitutes a cycle during which the genetic target sequence is amplified. In the denaturation step the increased temperature causes separation of the DNA strands, allowing annealing of specific primer sequences to the DNA target sequence as the temperature is decreased. The temperature is then increased to where activity of the DNA polymerase is optimal and the enzyme produces a DNA sequence complementary to the target region/template. Hence, the only double-stranded DNA sequences in each reaction are copies of the target sequence. By adding a nucleic acid stain, in this case SYBR Green, fluorescent light is emitted and the amplification of the transcript can be quantified. For each cycle the number of target copies, and the corresponding SYBR Green emission, increases. In real-time PCR a measurement is made after each cycle, and comparisons between samples are based on the number of cycles needed to reach a pre-set threshold value (C_t). Thus, the C_t value reflects the initial amount of the target sequence in a sample. A melting curve analysis was performed at the end of each PCR run to ensure that each sample contained a single product and that this product was not the result of primer-to-primer binding (primer dimers). Apart from the original number of transcripts in the sample several other factors may influence the results of a PCR; primer efficiency, run-to-run differences and the general level of gene expression in an individual. To detect and adjust for these variations standard curves were performed to determine the efficiency of each primer pair, identical calibrator samples were included in each PCR run as internal controls, and the expression of several reference genes (genes with stable expression under different experimental conditions) was analysed and used for normalisation of target gene expression. Samples without template (negative controls) were also included in every run to exclude contamination. All samples, including controls, were analysed in duplicates to increase the certainty of the results and facilitate discovery of possible errors and abnormal variations. In the present studies commercially available primers, protocols and kits have been used in accordance with the manufacturer's instructions. Furthermore controls for contamination, efficiency, sample-to-sample and run-to-run differences have been applied to increase the reliability of the results presented. Data points were excluded based on low

expression (a C_t-value of 40 cycles or more is automatically labelled by the LC 480 software as an uncertain value) or if the melting curve analysis indicated contamination.

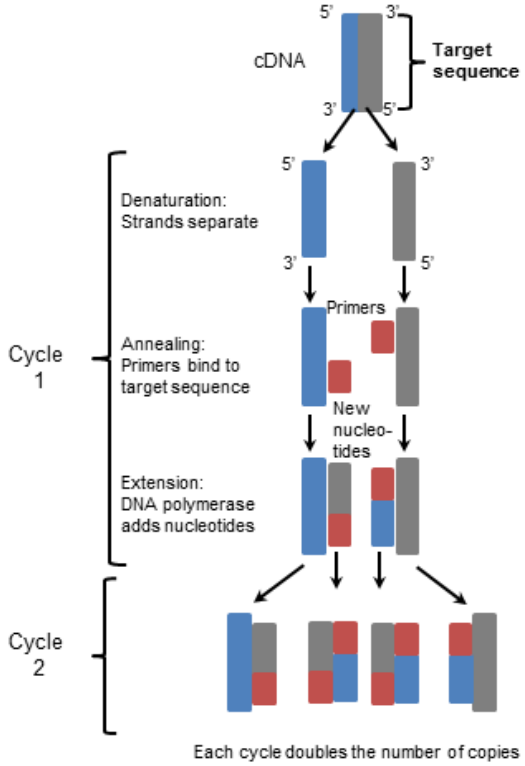


Figure 7. Polymerase chain reaction This technique multiplies genetic material by the same mechanisms as in a cell division but replaces enzymes with temperature changes. To copy the desired gene a primer pair specific for the target sequence is added to the reaction. Heat is used to separate double-stranded cDNA, allowing the primer to bind to the target and DNA polymerase to add nucleotides. A new double-strand is created, and then separated to allow repetition of the procedure. Under ideal conditions the number of copies is doubled in a reaction. After each cycle the amount of double-stranded material in the sample is measured to determine if the preset limit, the Ct-value, has been reached.

The accuracy and sensitivity of PCR is dependent on the specificity of the primers. In the present work QuantiTect Primer assays were used (Qiagen). Although not disclosing the exact location and sequence of these pre-designed primer pairs, Qiagen gives an approximate location on their website and guarantees their specificity. To avoid amplification of genomic DNA these primers are designed to cross exon/exon borders where possible. Quantitative real-time PCR was carried out in 96-well plates using a LightCycler®480 Real Time PCR System (Roche Applied Science). Each sample contained 10 µl 2xQuantiTect SYBR Green Master Mix (Paper I) or 10 µl 2xQuantiFast SYBR Green Master Mix (Paper II, III), 6 µl RNase free water, 2 µl primer assay, and 2 µl cDNA, resulting in a total volume of 20 µl. Optimisation of reaction and cycling conditions were not performed since QuantiTect- and QuantiFast-SYBR Green PCR kits are ready-to-use (Qiagen). The PCR protocol used always consisted of 45 cycles but differed in set-up for QuantiTect SYBR Green (denaturation 94 °C for 15 s, annealing 55 °C for 20 s, extension 72 °C for 20 s) and QuantiFast SYBR Green (denaturation at 95 °C for 10 s, and combined annealing/extension at 60 °C for 30 s). For every run

LightCycler® 480 software (version 1.5) data were exported as text files and imported into Microsoft Excel for further statistical analysis.

Normalisation and data analysis

The LightCycler analysis module calculated the C_t-value for each sample and the mean C_t value of each duplicate was used for computing the results. The mathematical model utilised to calculate the relative quantification of a target gene in comparison to reference genes was presented by Pfaffl (Pfaffl, 2001). Briefly, the relative expression ratio of a target gene is decided by a relation between the sample of interest and a control, and in comparison with reference genes. As seen in the equation below, the efficiencies (E) of the PCR runs for target and reference and the crossing point deviations (ΔCP) are also required.

$$\text{Ratio} = \frac{(E_{target})^{\Delta CP\ target\ (control-sample)}}{(E_{ref})^{\Delta CP\ ref\ (control-sample)}}$$

Each sample value was normalised using the geometric mean of reference genes; these values, in combination with the efficiency of each primer pair, constituted the specific control for each calculation. The geometric mean was used since it is a better control for outlying values and differences between genes. Grubbs test was employed to exclude outliers (GraphPad Software, Inc.). A normalisation factor based on multiple reference genes was used to increase accuracy and reliability of the data (Vandesompele et al., 2002). Although the use of a single reference gene is often observed in the literature it is associated with a certain risk as housekeeping genes, commonly utilised as controls, can vary in expression and affect results (Thellin et al., 1999; Vandesompele et al., 2002).

RT² Profiler array

The Rat Neurotransmitter Receptors and Regulators RT² Profiler™ PCR Array (SA Biosciences/Qiagen) was used to extend the investigation of ethanol's effect on gene expression. This pre-designed array contains 84 genes involved in modulation of neurotransmitter-related actions (biosynthesis, uptake, transport, regulation of neurotransmitter levels, and signalling through receptors) (for a complete list of genes

included in the array; www.sabiosciences.com/genetable.php?pcatn=PARN-060A). Among the specific receptors for neurotransmitters included in the array were acetylcholine, dopamine, GABA, glutamate, and glycine, all implicated in the (primary) actions of ethanol (Grant, 1994; Lovinger, 1997; Vengeliene et al., 2008). In an array the expression of multiple genes in one sample is analysed whereas the expression of one gene in multiple individuals is analysed in a regular replicate PCR. The advantage of this method is the ability to screen the expression of a hypothesis-driven selection of genes, resulting in a large amount of information in relation to the work effort. However, the increased uncertainty of the results, based on readings from a single well instead of duplicates, should be taken into consideration. As well as the possible need for further statistical analysis and additional PCR runs to confirm the results. 2x RT^2 SYBR Green Master mix and RNAse free water were added to the samples before addition to the array plates in which each well already contained an optimised primer assay. The 10 minute activation period at 95°C was followed by 45 cycles; 15 s at 95°C and 60 s at 60°C. C_t-values were determined by the LC 480 Software Version 1.5 (Roche Applied Science) and all raw data was analysed with RT² Profiler PCR ArrayData Analysis Template v3.3 (http://www.sabiosciences.com/pcrarraydataanalysis.php).

BIOCHEMICAL ASSAY

In vivo microdialysis

In vivo microdialysis is a method allowing investigations of the extracellular environment in awake and freely-moving animals. In the present study this method was used to monitor the effect of systemically and locally administered compounds on neurotransmitter and amino acid levels (dopamine and taurine) in the nAc. This was done by inserting a probe with a semi-permeable membrane in the tissue, allowing the probe to function in a manner comparable to a blood capillary. Analytes flow over the membrane via passive diffusion determined by the concentration gradient and the pore size of the membrane. Due to the constant flow through the probe equilibrium is not established. Thus, concentrations in dialysate samples are lower than *in vivo* concentrations (recovery), 5-10% for dopamine in our laboratory (unpublished data). For taurine the recovery has not been established and the data presented here are not corrected for recovery. Insertion of the probe is an invasive action that damages the tissue, activates microglia cells and later induces scaring and necrosis. Due to the possible influence of these factors on the results, 24-48 hours post-surgery is considered

optimal for performing microdialysis experiments. Since samples were collected and analysed every 20 minutes instant changes in neurotransmitter levels were not estimated. Although presumed that alterations in extracellular levels are primarily caused by changes in synaptic release this is not possible to determine with microdialysis.

Microdialysis technique

Two days prior to the experiment the dialysis probes were surgically implanted. When the rats had been anaesthetised with isofluorane they were mounted into a stereotaxic instrument. To prevent hypothermia during the surgery animals were also placed on a heating pad. Holes were drilled for the placement of two anchoring screws and for an I-shaped dialysis probe (custom made in the laboratory). The dialysis probe was lowered into the nAc (A/P:+1.85, M/L: -1.4 mm relative to bregma, V/D:-7.8 mm relative to dura; Paxinos and Watson, 2007).

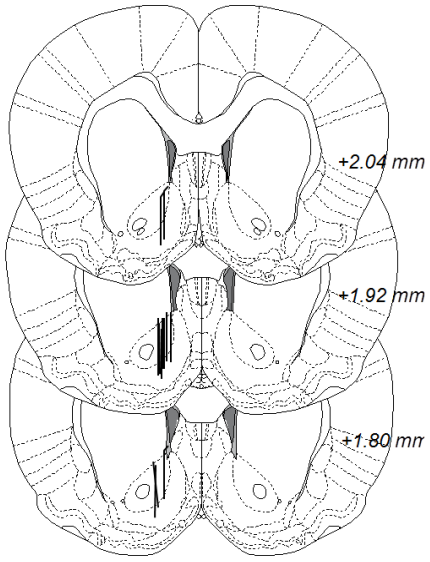


Figure 8. Probe placement Coronal sections of the rat brain showing a representative sample of verified probe placements in the rat nucleus accumbens. Adapted from Paxinos and Watson 2007.

The exposed length of the dialysis membrane was 2 mm and the nAc dialysis probes were placed in the core-shell border region (suggesting that sampling was done in both the core and the shell of the nAc). After positioning, both the probes and the anchoring screws were fixed to the skull with Harvard cement (DAB Dental AB, Gothenburg, Sweden). Following two days of recovery the dialysis experiment was performed. The sealed inlet and outlet were cut open and connected to a microperfusion pump (U-864 Syringe Pump AgnTho's, Lidingö, Sweden) via a swivel, allowing the animal to move around freely. The probes were perfused with Ringer solution at a rate of 2 µl/minute and dialysate samples (40 µl) were collected

every 20 minutes. To obtain a balanced fluid exchange before baseline sampling began the rats were perfused with Ringer solution for two hours. Animals were killed directly after the experiment, brains were removed and probe placements were verified.

Neurochemical assay - dopamine

For the separation and detection of dopamine in dialysate samples a high-pressure lipid chromatography system (HPLC) with electrochemical detection was used. Besides the electrochemical detector (Decade, Kovalent AB), operated at 0.40 V versus the cell, this system consisted of a pump (Dionex P580, Kovalent AB, Sweden) and a stainless steel column 2x150 mm packed with Nucleosil, 5 µM SA 100A (Phenomenex Skandinaviska Genetec, Sweden). An external standard containing 3.25 fmol/µl was used to identify the dopamine peak and the time of analysis for each sample was 5 minutes. Results are presented as % of baseline.

Neurochemical assay - taurine

All samples received an addition of 10 mM sodium azide equal to the sample volume in order to maintain stability. Taurine was derivatized with *o*-phtaldialdehyde (OPA), separated by HPLC and detected by fluorescence. The signal was recorded and integrated with Chromeleon software. All equipment was from Dionex, Gothenburg, Sweden. The sample was allowed to react for 10 seconds with 5 µl of an OPA derivatization reagent before injection into the reverse-phase Chromolith Performance column, 100 x 4.6 mm, with a Chromolith Guard column, 5 x 4.6 mm in front (Merck KGaA, Germany). To separate the amino acid the HPLC utilised a gradient in mobile phase with a constant flow rate of 3.0 ml/min. The total cycle time was 12 minutes and the amino acid was eluted within 6.5 min. An external standard in two concentrations (0.5-1.0 µM) was used to identify the taurine peak. As for dopamine the results are presented as % of baseline.

PROTEIN EXPRESSION

Immunohistochemistry

To complement, and/or confirm mRNA results, protein expression in the tissue was examined using immunohistochemical methods. Similar to PCR, immunohistochemistry (IHC) is based on specific detection; the success of IHC relies on the specific interaction of an antibody and the antigen or protein (antigenic determinant) it is designed to recognise. This interaction is then visualised by a marker, in this case a secondary antibody with a coupled fluorescent dye. Animals used for immunohistochemical preparation were perfused with a 4% paraformaldehyde solution. After post-fixation in the same solution brains were cryopreserved in sucrose and frozen in a mixture of isopentane and dry ice. The tissue was cut in 40 or 50 µm (Paper II and III respectively) sections using a Leica CM1950 cryostat, placed in cryoprotective solution (30% glycerol, 30% ethylene glycol, 40% phosphate buffered saline), and kept at −20 °C until further processing. Brain regions were defined and identified using the rat brain atlas of Paxinos and Watson (Paxinos and Watson, 2007). Free-floating sections were washed in 1x Tris Buffered Saline (TBS), before and after, antigen recovery was performed in Tris–EDTA at 85 °C. For permeabilisation, sections were kept in −20 °C methanol before being immerged in blocking solution (10% bovine serum albumin and 0.5% Triton X-100, in TBS). The sections were then incubated with primary antibody overnight at 4 °C, followed by washing (3x5 min) in TBS and incubation in secondary antibody for one hour followed by an additional washing step. The marked secondary antibody binds to the primary antibody raised in a certain species, and the antigen is thus indirectly visualised, via detection of the marker. Before mounting in antifade DTG mounting medium (2.5% DABCO (D-2522), 50mM Tris pH 8.6, 90% glycerol) sections were incubated in 4',6-diamidino-2-phenylindole (DAPI, 1:2000) which stains DNA in the nucleus. Immunostained structures were photographed with a Zeiss LSM Inverted 700 Laser Scanning Microscope (Centre for Cellular Imaging, Sahlgrenska Academy, University of Gothenburg). As mentioned above, a critical component is the specificity of the primary antibody. The monoclonal mAb4a GlyR antibody, which detects α 1-3 but not β-subunits, was used (Synaptic Systems). This antibody allows detection of all functional GlyRs in the sectioned area but cannot determine subunit composition or whether receptors are heteromeric or homomeric. Apart from the common use of blocking solution, negative controls (omitting incubation with the primary antibody) were performed to recognise and avoid unspecific binding.

Retrograde tracing

While IHC allows detection of a certain protein in a cross section, retrograde tracing permits mapping of neuronal connections from their termination point to their origin by visualisation of axonal transport. This is achieved by injecting a traceable compound, marked with a fluorescent dye, in a certain area of the brain. This tracer is then transported from the synapse to the soma of neurons projecting to the area of the injection. The axonal transport enables visualisation of whole neurons and with additional staining other features of these neurons may be revealed. Animals used for retrograde tracing were anaesthetised with isofluorane and placed in a stereotaxic instrument on a heating pad (See *Microdialysis technique*). After exposing the skull a small hole was drilled above the target area; aVTA, coordinates A/P: -5.2, M/L: -0.7, V/D -8.4 relative to dura (Paxinos and Watson, 2007). Cholera toxin B subunit (CTB) labeled with Alexa Fluor 488 (Life Technologies) was dissolved in phosphate-buffered saline, pH 7.4, to a 0.2 % solution. To administer CTB a 10 µl syringe (Hamilton Co) was placed in a micro-perfusion pump (U-864 Syringe Pump AgnTho's). Via polythene tubing a microinjection cannula was attached to the syringe. The cannula was lowered into the aVTA under stereotaxic control and 1 µl CTB was slowly administered during a period of 2 min. To allow for diffusion of the tracer this was followed by a waiting period before the cannula was extracted. Animals were kept in separate cages until they woke from the anesthesia and were then housed together for 13 days until perfusion. To identify the exact size and location of the injection site may be difficult due to intense staining of the tracer in the immediate surrounding area. As always with fluorescent staining there is a risk of photobleach when the labeled tissue is viewed in the microscope.

ELECTRICAL ACTIVITY

Electrophysiology

Neuronal signalling is dependent on electrical activity (in particular action potential activity) which can be studied using electrophysiology. The physiological contact between the electrode and the tissue allows direct monitoring of the electrical activity of neurons in the tissue. In the present study (Paper IV) field potential recordings were used to investigate potential effects of strychnine on drug-induced (ethanol, nicotine and THC) changes in accumbal neurotransmission. Coronal brain slices were prepared from drug-naïve adult Wistar rats and allowed to equilibrate at room temperature in artificial cerebrospinal fluid before

being transferred to the recording chamber. Briefly, stimulation was delivered as constant current pulses (every 20 s) via a monopolar stimulation electrode located in the nAc shell. Stimulation was activated at a frequency of 0.05 Hz and the intensity of the stimuli was set to result in an evoked population spike (PS) amplitude about half the size of the maximal evoked response (Adermark et al., 2011a). Since ethanol is known to affect striatal activity through modulation of GABAergic neurotransmission, $GABA_A$ receptor inhibitors were omitted in all recordings (Adermark et al., 2011b; Mishra et al., 2012). Recorded PS were amplified by a custom made amplifier, filtered and digitized before transfer to a PC for further analysis. In strychnine-treated slides, 1 μM of strychnine was administered for at least 20 min before, and continuously during, drug treatment. After monitoring a stable baseline for at least 15 min slices (both control and strychnine-treated) were perfused with ethanol (50 mM), nicotine (1 μM) or THC (1 μM). Limitations of this method include the potential damage caused by the invasive electrode and a lack of selectivity in activation of structures and cell types. However, the method is suitable for studying involvement of different neuronal and cellular mechanisms, e.g. receptor activation, in a restricted brain area, as electrical stimulation can be applied to isolated regions/preparations.

STATISTICAL ANALYSIS

All values are expressed as means ± standard error of the mean (SEM). A probability value (p) less than 0.05 was considered statistically significant. With the exception of gene expression data from the RT² Profiler PCR Array, which was analysed with the RT² Profiler PCR Array Data Analysis Template v3.3, statistical analyses were performed using SPSS (SPSS Statistics 17.0 for Windows).

The following statistical methods were used:

- Independent samples t-tests (Paper I-III)
- Non-parametric Spearman correlation (Paper I)
- One-Way ANOVA (analysis of variance) followed by a Bonferroni (Paper II) or Tukey (Paper III) *post hoc* test
- Paired samples t-tests (Paper III)
- Linear mixed models (Paper IV)

RESULTS AND DISCUSSION

PAPER I

Glycine receptor expression in the forebrain of male AA/ANA rats

Earlier studies had demonstrated the involvement of accumbal glycine receptors in the dopamine elevating and reinforcing effects of ethanol (Molander et al., 2005). Since manipulations of this receptor alter ethanol consumption behaviour in rats it was hypothesised that differences in the glycine system might be involved in the variation of spontaneous consumption behaviour in rats. Selective breeding based on ethanol consumption should result in refinement and accumulation of such differences, creating two separate glycine profiles; mediating high and low ethanol consumption respectively. Selective breeding of Wistar rats based on ethanol preference has resulted in the high-preferring AA and low-preferring ANA rats (Eriksson, 1968). Since Wistar is the rat strain commonly used in our research AA/ANA rats were the natural choice for this experiment. To establish that these animals differed in ethanol consumption a two-bottle choice model was used to measure voluntary intake. Animals were also divided into groups with and without access to ethanol, to determine if tentative differences in the glycine system were due to predisposing factors or induced by ethanol exposure. Gene expression was used as a measure of change in the glycinergic system and mRNA expression of the α1-3 and β GlyR subunits was analysed in eight forebrain regions using qPCR.

Measurements of ethanol consumption and preference confirmed the dissimilar consumption behaviours of AA and ANA animals. The impact of selective breeding and/or alcohol exposure on gene expression proved to be more modest than expected. Effects of ethanol consumption on GlyR expression were primarily found in the hypothalamic region which was somewhat surprising, especially since there was a complete lack of changes in the nAc. Collectively the results indicate that subunit composition is more likely to be affected by ethanol-induced alterations than the number of receptors. Based on genetic differences, and the assumed effects of ethanol exposure, alcohol-consuming AA and ANA rats should differ the most. As anticipated a majority of the differences between subgroups were found here, but mainly in regions not directly related to reward e.g. anterior hypothalamus and cingulate gyrus. Although GlyR expression was largely unaffected by selective breeding and/or ethanol consumption, this does not exclude glycinergic involvement. It is possible that alterations in posttranslational modifications, protein synthesis, degradation and/or transportation have occurred and that these affect consumption behaviour. However, based on the results of this

study the diverse ethanol consumption behaviours of AA and ANA rats cannot be explained by differences in forebrain GlyR gene expression.

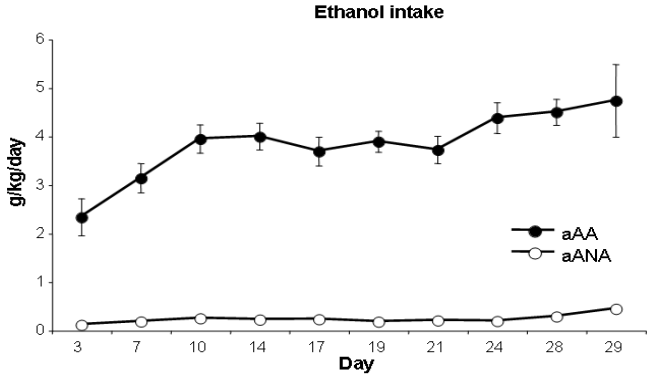


Figure 9. Ethanol intake of AA/ANA rats The presumed diverse ethanol consumption behaviour of AA and ANA animals was confirmed in a two-bottle choice paradigm. Abbreviations: AA; Alko Alcohol, ANA; Alko Non-Alcohol.

To investigate another possible ethanol-GlyR interaction qPCR results were compared with consumption and preference data in order to determine possible correlations. Interestingly, a positive correlation between ethanol intake and GlyRα1 expression was found in the nAc of alcohol-consuming AA rats. This could suggest that ethanol is more prone to interact with this than other subunits, and perhaps an increase in the proportion of α1-subunits would contribute to elevated ethanol consumption. Indeed, electrophysiological studies have recorded a higher sensitivity to ethanol in α1 than in α2 homomers (Mascia et al., 1996a; Mascia et al., 1996b). For alcohol consumption two negative correlations were also found in AA rats; with α2 expression in the anterior hypothalamus and with α3 expression in cingulate gyrus, regions not commonly associated with alcohol intake.

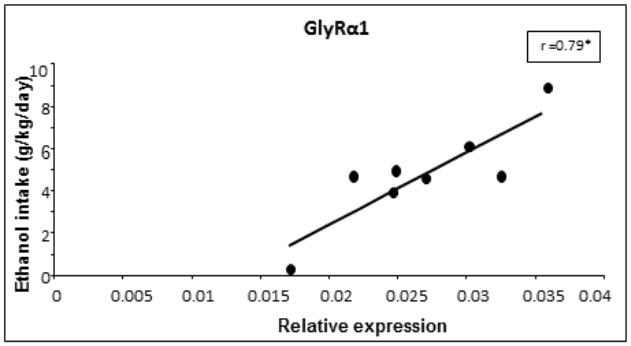


Figure 10. Correlation between intake and α1 expression A positive correlation between gene expression of the GlyR α1 subunit and ethanol intake was found in the nucleus accumbens of AA rats.

An overview of mRNA expression revealed that the differences in expression between GlyR subunits were not the expected. In contrast to previous reports, proclaiming α1 as the most abundant α-subunit in the adult brain and considering the α2 subunit as primarily neonatal (Becker et al., 1988; Malosio et al., 1991a; Kirsch, 2006; Lynch, 2009), we found that α2 expression was higher than both α1 and α3 expression in five of eight brain regions studied. Hence, based on the GlyR subunit expression we propose that α2 is the most widely expressed GlyR α-subunit and that α2β is the most likely receptor configuration in the forebrain of adult AA/ANA rats.

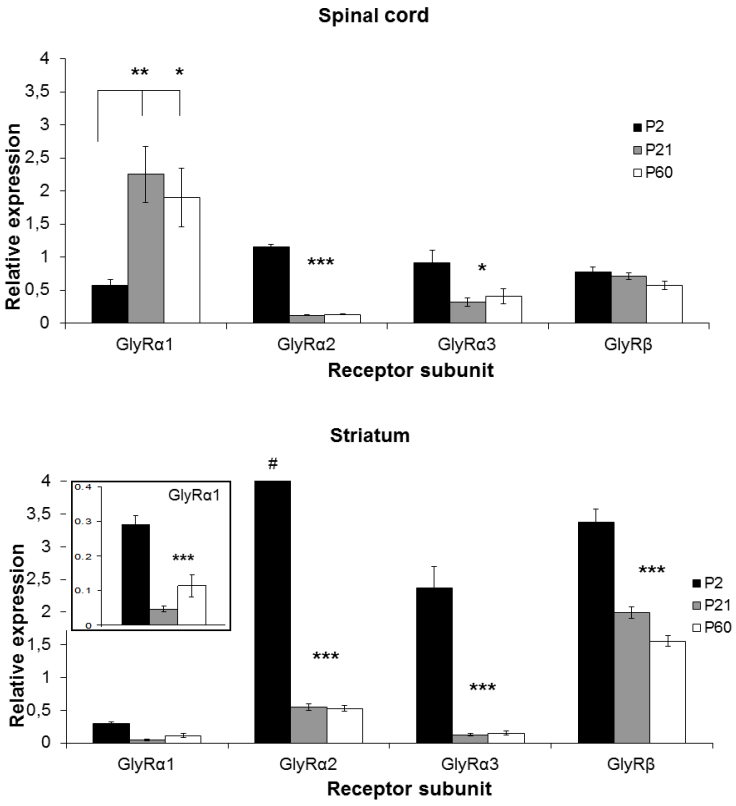
PAPER II

Changes in glycine receptor expression in forebrain regions of the Wistar rat over development

An of encountered phenomenon when reading about the GlyR is the developmental shift from α2 dominance during development to the α1β subtype that prevails in the adult brain (Becker et al., 1988; Hoch et al., 1989; Akagi et al., 1991). Both the mechanism behind and the functional importance of this switch are unknown but similar processes seem to occur in both $GABA_A$ and glutamate receptors (Fritschy et al., 1994; Davis et al., 2000; Ritter et al., 2002). Findings from Paper I appeared to contradict some aspects of this theory. Since our results were derived from selectively bred animals and did not include analysis of the spinal cord (the region the theory is primarily based on) we felt the need to complement these findings. Thus, a study was designed to confirm the previous results, and to investigate the developmental alterations in GlyR gene expression, in outbred Wistar rats. Since the shift is supposedly initiated around birth and completed 2-3 weeks later, tissue from animals at postnatal day 2 (neonatal), 21 (juvenile), and 60 (adult) was analysed. Similar to the previous study levels of α1-3 and β expression were measured using qPCR. The regions examined were spinal cord, hippocampus, striatum, nucleus accumbens and frontal cortex. These areas were chosen to give a functional and developmental variation, compared to Paper I which was focused on forebrain regions. In addition, immunohistochemistry was used to determine the localisation of receptor protein in the tissue.

In agreement with former studies a shift from α2 to α1 predominance was demonstrated in the spinal cord. However, this pattern was observed in neither of the other regions. Clearly α2 was the most highly expressed α-subunit in all regions in neonatal animals and, despite significant decreases in expression, it was not replaced by α1 but kept its position as the most highly expressed α-subunit also in adult animals. These results suggest a region-specific pattern of GlyR gene expression rather than a homogenous effect and they also support the hypothesis that α2β is the most likely GlyR subunit composition in the brain of adult rats. As expected the greatest differences were observed between neonatal and adult animals, whereas juvenile and adult animals were largely similar. This indicates that hormonal alterations associated with sexual maturity have a modest, if any, effect on GlyR expression. The high α2 expression, in combination with the pattern of β expression, suggests that homomers make up a larger proportion of GlyRs in the brain of neonatal rats than in juvenile or adult animals. This should affect glycinergic signalling since it has been proposed that homomers (extrasynaptic GlyRs) mediate slow and tonic modulation of neuronal activity

while heteromers (synaptic GlyRs) mainly mediate phasic and fast neurotransmission (Muller et al., 2008). As the CNS matures the number of cells decreases and paracrine signalling is reduced. This should mainly affect extrasynaptically located homomers primarily relying on paracrine and autocrine signalling while heteromers are located in the synapse where neurotransmitter concentrations are high and activation is more likely to occur (Deleuze et al., 2005; Muller et al., 2008; Le-Corronc et al., 2011). Due to these circumstances a general decrease in cells could help explain the substantial α2 decrease and the stable β expression. An interesting aspect is why α2-expression has achieved such a strong position; involvement in a time-restricted process during development could explain the high expression levels and the substantial decreases shortly after birth. The fact that α2 homomers have been implicated in the differentiation of interneurons in zebrafish could supply such an explanation (McDearmid et al., 2006), provided that this applies also to other CNS regions and species. An important role for GlyRs has also been proposed based on other grounds. In the developing cortex taurine is in abundance and the agonist most likely to activate GlyRs. Since deprivation of taurine may disturb neocortical development it is possible that taurine affects these processes via GlyR activation (Flint et al., 1998).



Figures 11 and 12. Gene expression of GlyR subunits in the spinal cord and striatum A shift from high α2 to high α1 expression did occur in the spinal cord, but not in any of the other regions studied. In the striatum, and several other regions, α2 kept its position as the α-subunit with the highest expression despite a massive reduction from P2 to P21. Error bars show SEM. Significant differences are indicated by *p<0.05, **p<0.01; ***p<0.001. # bar truncated at 4.0 for GlyRα2 in the striatum, actual relative expression value was 6.8. Abbreviations: GlyR; glycine receptor.

Provided that mRNA expression reflects protein expression the region-specific pattern of GlyR expression should affect glycinergic neurotransmission. So should developmental changes, but to what extent seems to be largely dependent on receptor

composition and localisation which are not possible to determine using qPCR. Since specific antibodies for all subunits, not to mention the splice variants, are not yet available immunohistochemical studies are also a blunt instrument in investigations of this matter. In line with the qPCR data IHC experiments indicated a decrease in GlyR proteins when comparing neonatal and adult animals. Previous studies have demonstrated the presence of functional GlyRs in the nAc and using IHC it was shown that cells positive for GlyRs are primarily found in the shell region of this area (see Paper II for IHC images; Molander and Soderpalm, 2005a).

PAPER III

The effects of long-term ethanol consumption on the expression of neurotransmitter receptor genes in the rat nucleus accumbens

After investigating the impact of selective breeding and alcohol (Paper I), and development (Paper II), on GlyR expression the effect of long-term ethanol exposure was the focus of Paper III. AUDs are conditions that normally develop over long periods of regular, and excessive, consumption of alcohol. In an animal model rats were single housed and given continuous access to ethanol in a two-bottle choice paradigm for an extended period of time (up to 10 months). With this study the aim was to monitor the effect of long-term ethanol intake on both consumption behaviour and gene expression. Furthermore, to see if long-term consumption would lead to a transition from voluntary controlled intake to compulsive intake and the loss of control that is a hallmark of addiction. Besides GlyRs several neurotransmitter receptors are primary targets of ethanol and long-term exposure has been shown to affect neurotransmission in many systems (Casamenti et al., 1993; Darstein et al., 1998; Vengeliene et al., 2008; Spanagel, 2009), alterations that have been suggested to contribute to the development of AUDs. Since ethanol intake is closely connected with the nAc, and previous studies of ours have investigated receptor populations here (Jonsson et al., 2009; Soderpalm et al., 2009), this area was chosen for gene expression analysis. To examine progressive changes a time series was created where tissue was analysed after 2, 4 or 10 months of consumption. A predesigned array, containing genes representative of multiple neurotransmitter systems (e.g. GABA, acetylcholine, glycine and dopamine), was used to analyse tissue from animals after 10 months of consumption. The results from this targeted screening of the material were used to guide qPCR analysis of tissue from animals after 2 and 4 months of consumption. No significant differences were observed in GlyR subunit expression between ethanol consumers and controls following 10 months of ethanol intake, a further indication of the robustness of this system suggested by previous results (see *Paper I*). After adjusting for multiple comparisons in the results of the PCR array no significant differences between ethanol consumers and controls were observed in the material. However, trends towards differential expression led to the continued analysis of genes translating into nAChR α5 (Chrna5), $GABA_A$ receptor subunit α1 (Gabra1), somatostatin receptor 4 (Sstr4) and tachykinin receptor 3 (Tacr3). In addition a selection of genes encoding dopamine D_2 and D_3 receptors (DRD2 and DRD3), NR2A and NR2B subunits of the NMDA receptor (NMDAR2a and NMDAR2b), metabotropic glutamate receptor 5 (Grm5) and the μ-opioid receptor (Oprm) were chosen for

analysis, based on their proposed involvement in ethanol consumption and addiction (Allgaier, 2002; Gass and Olive, 2008; Le Foll et al., 2009; Ramchandani et al., 2011).

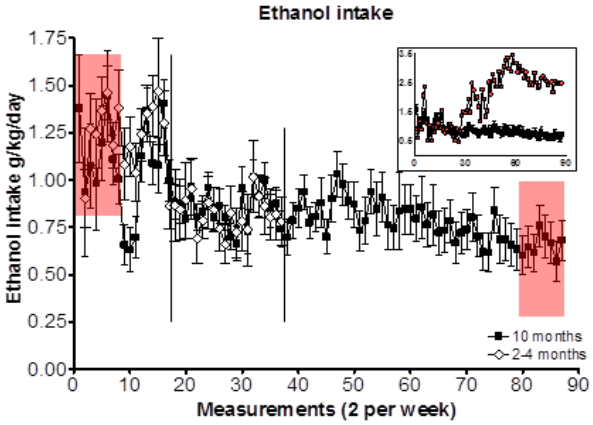


Figure 13. Ethanol intake
A decreasing trend in ethanol consumption was observed throughout the 10 months of the study. One individual differed from this pattern by increasing its intake (insert). The vertical lines indicate the 2 and 4 month time points and the shaded boxes show the data points used for statistical calculations. Bars show ±SEM.

Following long-term (2-4 month) ethanol consumption expression of DRD2, Oprm and Sstr4 was reduced. A down-regulation in expression of the D_2 receptor gene is in line with the reduced $D_{2/3}$ receptor levels observed in both alcohol addicts and ethanol-preferring animals (Stefanini et al., 1992; Volkow et al., 1996a; Volkow et al., 1996b; Phillips et al., 1998; Volkow et al., 2002; Tupala et al., 2003; Heinz et al., 2004; Tupala and Tiihonen, 2004), which has led to the suggestion that reduced D_2 levels increase susceptibility to AUDs (Volkow et al., 2002). Since animals were randomly divided into ethanol consumers and controls it is unlikely that a pre-existing difference would be the reason for the effects observed here. In human studies it cannot be determined whether the alteration in D2 expression is induced or pre-existing but these results suggest that ethanol causes the reduction in DRD2 expression in these animals. Furthermore, since DRD3 expression was unaltered the ethanol effect seems to be specific for DRD2, at least in the nAc. Ethanol consumption has also been reported to decrease μ-opioid receptor expression (Turchan et al., 1999). This receptor appears to be involved in e.g. control of ethanol intake and dopamine levels in the nAc (Mansour et al., 1995; Oswald and Wand, 2004; Ramchandani et al., 2011), and it is possible that ethanol-induced increases in endogenous opioids in nAc leads to decreased receptor levels. Somatostatin and Sstr4 are not as commonly studied in this context but following administration of somatostatin in nAc an increased dopamine release has been registered (Pallis et al., 2001). A plausible explanation to the reduced expression of receptor genes is that this was an adaptive response to increased levels of agonists, directly or indirectly induced by ethanol (Di Chiara and Imperato, 1988; Hathway et al., 1998; Olive et al., 2001).

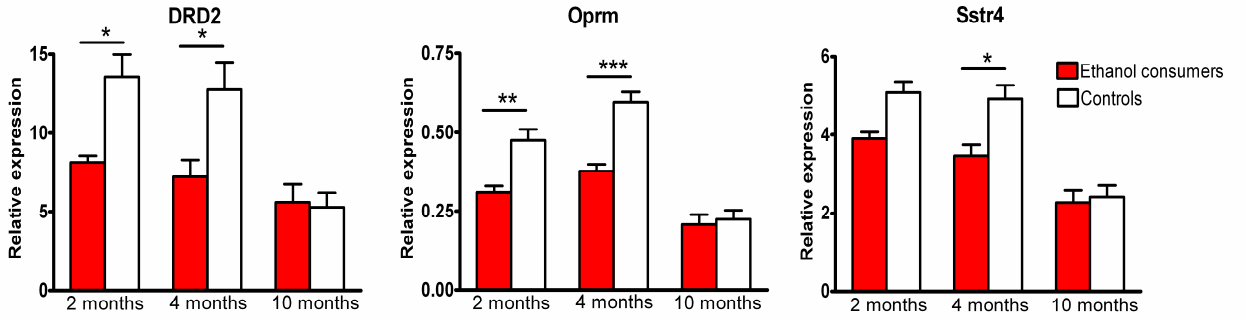


Figure 14. Alterations in gene expression in nucleus accumbens Animals had continuous access to ethanol for 2, 4 or 10 months. Tissue from the nucleus accumbens was analysed using qPCR. Ethanol intake resulted in reduced DRD2, Oprm and Sstr4 expression after 2 and/or 4 months of consumption. Bars show ±SEM. *p<0.05, **p<0.01 and ***p<0.001.

After 10 months of ethanol intake any previous difference between animals consuming ethanol and controls had disappeared, presumably due to the age-related decrease in expression observed in most of the analysed genes. These results in combination with the 2 and 4 month data suggest that ethanol accelerates, but does not add to, the age-related effect. That animals would consume ethanol on a daily basis for 2-10 months without being neurochemically affected is highly unlikely, especially since the genes examined after 10 months of consumption include genes coding for proteins known to be primary targets for ethanol. Adaptations ought to have taken place, perhaps on other levels than gene expression or in other regions than the nAc. The great subunit diversity of proteins involved in forming ligand-gated ion channels creates variety in expression patterns and sensitivity to ethanol, in agreement with reports of region-specific effects of ethanol (Barrios et al., 1990; Grobin et al., 2000; Allgaier, 2002; Simonyi et al., 2004; Kumar et al., 2009; Spanagel, 2009). It is possible that, under the present conditions and in this region, the genes analysed were not the ones affected. The average daily intake of ethanol was quite low, 0.85 g/kg/day for the entire study, and in general animals decreased their consumption over time. Only one of the 24 animals consuming ethanol for 10 months increased its intake in a manner that may be comparable with loss of control and addiction. Studies have revealed that rats display individual variations in behaviour, similar to a personality in humans (Blanchard et al., 1988; Blanchard et al., 1993; Wolffgramm and Heyne, 1995). A certain behavioural composition, combined with preference for the effects of the drug and a particular response to the environment (single housing etc), may have resulted in a predisposition for ethanol consumption/addiction in this rat (Wolffgramm, 1990; Wolffgramm and Heyne, 1995).

PAPER IV.

Glycine receptors are involved in mesolimbic dopamine release induced by drugs of abuse

The dopamine-increasing action of addictive drugs is well established, but how e.g. ethanol produces this effect still remains to be determined. Thus, a hypothesis concerning the elevation of dopamine in nAc has been put forward. This theory is based on studies demonstrating the importance of ventral tegmental nAChRs and accumbal GlyRs in the mediation of ethanol's dopamine activating and reinforcing effects (reviewed in Soderpalm et al., 2000; Soderpalm et al., 2009). In this study several methods were used to investigate the proposed neuronal circuit these receptor populations are part of. The effect of local GlyR blockade on the dopamine increasing effect of different drugs was examined to determine if involvement of accumbal GlyRs is specific for ethanol. Immunohistochemistry and retrograde tracing were used to survey the neuroanatomy of the circuit, in particular the proposed connection between nAc and aVTA.

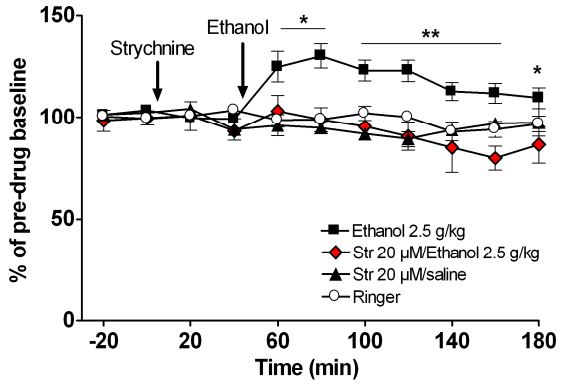
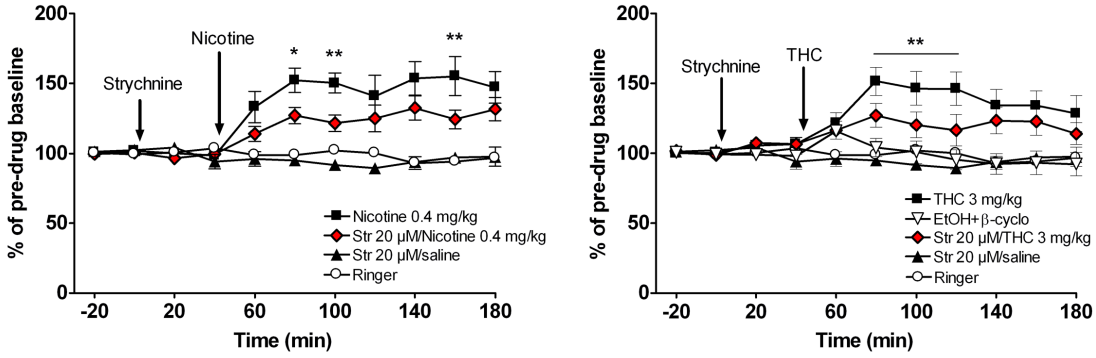


Figure 15. Strychnine blocks ethanol's effect on dopamine levels Local application of strychnine abolished the accumbal dopamine increase after a systemic injection of 2.5 g/kg ethanol. Bars show ±SEM. *p<0.05, **p<0.01 Abbreviation: Str; strychnine

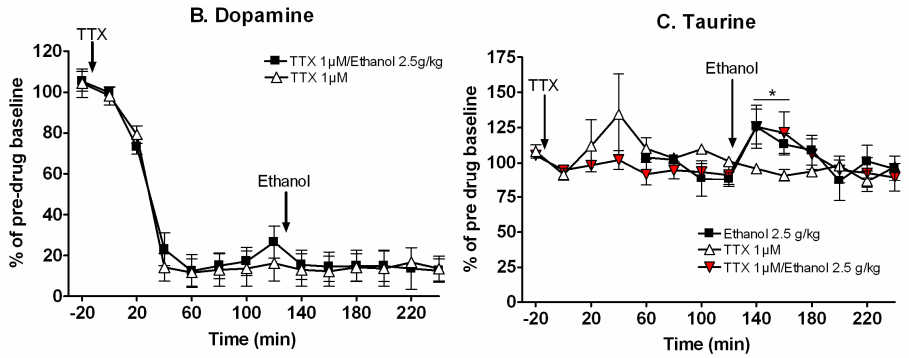
As in previous studies local application of the GlyR antagonist strychnine in nAc prevented the dopamine increase following systemic administration of ethanol (Molander et al., 2005; Molander and Soderpalm, 2005b). Strychnine also partially blocked the dopamine increase after administration of nicotine and THC while effects of morphine and cocaine were unaffected. GlyRs seemed to affect drug-induced changes in accumbal transmission as well, based on the effect of strychnine in the presence of ethanol, nicotine and THC. In field potential recordings the effects of strychnine in the presence of ethanol, nicotine and THC indicate that that GlyRs modulate drug-induced changes in accumbal transmission.

The similar, substantial, decreases in dopamine observed after local administration of strychnine and tetrodotoxin suggest that GlyRs control the majority of impulse dependent dopamine yield in nAc (Saigusa et al., 2001; Molander and Soderpalm, 2005a).



Figures 16 and 17. Pre-treatment with strychnine alters the dopamine response to nicotine and THC Local administration of strychnine in the nAc reduced the dopamine-elevating effects of both nicotine and THC, indicating the involvement of accumbal GlyRs in the reinforcing effects of these drugs. Bars show ±SEM. *p<0.05, **p<0.01. Abbreviations: β-cyclo; 2-hydroxypropyl-β-cyclodextrin, EtOH; ethanol, Str; strychnine, THC; $^{\Delta 9}$-Tetrahydrocannabinol

That tetrodotoxin prevented the dopamine increasing effect of ethanol without affecting taurine levels indicates that taurine release is not dependent on neuronal activity, and that ethanol elevates dopamine and taurine via different processes. As in paper II immunohistochemical experiments showed that GlyRs were mainly expressed in the nAc shell, in some instances in ChAT positive neurons but also on what presumably are GABAergic cells. Retrograde tracing revealed that more cells projecting to the aVTA originated in the lateral septum than in nAc. Also, these projecting cells appear to be GABAergic and lack somatic GlyR expression. One possibility is that the lateral septum is a relay station for the transmission between nAc and VTA believed to participate in ethanol's dopamine elevating actions. Further studies are needed to examine how THC and nicotine interacts with accumbal GlyRs and the connection between GlyRs in nAc and ventral tegmental nAChRs (Soderpalm et al., 2009).



Figures 18 and 19. Pre-treatment with TTX alters dopamine but not taurine levels Local administration of TTX, which inhibits synaptic transmission, markedly reduces dopamine levels in nucleus accumbens but does not affect taurine levels. This indicates that dopamine, but not taurine, is dependent on neuronal signalling for release into the extracellular space. Bars show ±SEM. *p<0.05 Abbreviations: TTX; tetrodotoxin

SUMMARY OF RESULTS

- The divergent ethanol consumption behaviours of selectively bred AA and ANA rats cannot be explained by alterations in forebrain GlyR gene expression.

- A significant positive correlation between ethanol consumption and α1 expression in nAc indicates an association between ethanol intake and this subunit.

- The proposed developmental shift from α2 to α1β dominance during the first postnatal weeks is not a general effect for the entire CNS but rather a pattern of region-dependent transitions.

- Based on mRNA expression α2 is the most abundantly expressed α-subunit and α2β should be the dominating subunit composition of GlyRs in the brain of adult AA/ANA and Wistar rats.

- Long-term (2-4 months) ethanol exposure reduces expression of genes encoding the D_2, μ-opioid and somatostatin 4 receptors.

- After 10 months of ethanol intake any previous difference between animals consuming ethanol and controls had disappeared, presumptively due to an age-related decrease in gene expression.

- As previously demonstrated local application of the GlyR antagonist strychnine in nAc prevented the dopamine increase following systemic administration of ethanol.

- Accumbal glycine receptors also appear to be involved in the dopamine-increasing effect of THC and nicotine.

- Immunohistochemical experiments show that GlyRs are mainly expressed in the shell region of the nAc.

- Based on immunohistochemical results it seems likely that the connection between nAc and aVTA is relayed via the lateral septum. Thus, the lateral septum could be a component in the circuit believed to mediate the reinforcing effects of ethanol.

GENERAL DISCUSSION

Accumulating evidence supports a prominent role for accumbal GlyRs in the dopamine-elevating and reinforcing properties of ethanol and a hypothesis of a neuronal circuit mediating these effects has been proposed. The work of this thesis aimed to further investigate the glycine receptor, its involvement in alcohol-related behaviour and the proposed neuronal circuit. The main findings were; (1) that based on gene expression the glycinergic system seems robust, (2) α2 appears to be the dominating α-subunit in the rat brain, (3) the proposed developmental shift from α2 to α1β is not a general effect, (4) that accumbal GlyRs are involved in the dopamine-elevating effect of nicotine and THC and (5) the possible inclusion of the lateral septum in the neuronal circuit mediating ethanol's dopamine-elevating effect.

Gene expression of glycine receptor subunits has been investigated in selectively bred animals (with and without exposure to ethanol), during development, and following long-term ethanol consumption. The results indicate that gene expression of this receptor is fairly stable. Surprisingly few differences were observed between AA and ANA animals (Paper I), despite the disparate consumption behaviour in these animals. Microdialysis results have indicated that a greater accumbal dopamine response to ethanol, presumably mediated by GlyRs, would be predisposing for high ethanol preference (Honkanen et al., 1994; Katner and Weiss, 2001). Differences in GlyR expression would have been in agreement with this suggestion. However, dissimilarities in nAc dopamine response of this kind have not always been observed in AA and ANA rats (Kiianmaa et al., 1995). To examine other aspects of the glycinergic system the expression of glycine 1 and taurine transporters was analysed, without revealing any alterations (unpublished results). In all, these results do not support addition of glycine to the list of systems (DA, serotonin, NMDA, neuropeptide Y and endogenous opioids) proposed to contribute to the differential ethanol consumption of AA and ANA rats (Bell et al., 2012). There may still be a glycinergic component in the diverse behaviour observed, due to e.g. effects on protein levels, if not in AA/ANA animals perhaps in other selectively bred strains, as several pathways seem able to produce alcohol-preferring phenotypes (Bell et al., 2012).

Few alterations in gene expression were observed after long periods of ethanol consumption in common outbred animals (Paper III). Since not only GlyRs but also $GABA_A$, nACh and NMDA receptors seemed unaffected by ethanol exposure the brain area that was analysed might be the reason for this lack of effects. In Paper I, nAc was the only region (out of eight) without alterations in GlyR gene expression. That nAc belongs to the oldest part of the forebrain (the extended amygdala) may contribute to the stability of this region as it has

not been subjected to any major evolutionary adaptation, in contrast to other more recent regions like e.g. PFC. Interestingly, glycine is the primary inhibitory neurotransmitter in the oldest parts of the CNS; the spinal cord and the brain stem. These regions are essential for development and survival and the centre for vital functions including cardiovascular control and respiration. The danger of non-functioning glycine receptors in the CNS, particularly in these regions, is illustrated by the effects of strychnine poisoning, where seizures and asphyxiation caused by GlyR blockade can be lethal. One would presume that stability, and a robust neurotransmitter system, would be preferable in these regions. Whereas in the brain, a more dynamic region where development continues well into adult life in e.g. humans and plasticity is a more useful quality, GABA is the major inhibitory neurotransmitter.

Even though accumulating evidence supports that the developmental shift is not a general maturation effect but a region-dependent pattern of alterations (Paper II; Piechotta et al., 2001; Garcia-Alcocer et al., 2008; Delaney et al., 2010), it is evident that major changes in GlyR gene expression occur throughout the CNS. The mechanism behind these processes remains to be established, as well as the functional consequences. The switch from α2 to α1β dominance is observed in the spinal cord and brain stem (Paper II; Becker et al., 1988; Malosio et al., 1991a; Watanabe and Akagi, 1995). Such alterations in receptor expression should lead to a transition from slow and tonic modulation by axonal receptors at extrasynaptic locations to fast and phasic neurotransmission via activation of synaptic receptors. The proposed involvement of α2 homomers in differentiation of interneurons, its extrasynaptic location and activation by paracrine and autocrine signaling (Flint et al., 1998; Mangin et al., 2003; Deleuze et al., 2005; McDearmid et al., 2006; Le-Corronc et al., 2011), may all contribute to the general high levels of expression associated with development (Paper II). The expression pattern of GlyRs after the shift may be a reflection of the demands on that particular region, presumably influenced by other inhibitory (GABA) elements. This would explain the switch in spinal cord and brain stem, where the importance of fast transmission is evident and glycine is the primary inhibitory neurotransmitter (while taurine is proposed to be the primary agonist in the brain) (Le-Corronc et al., 2011). In other CNS regions, where $GABA_A$ receptors are probably responsible for a majority of the fast inhibitory neurotransmission, it is possible that (α2) homomers (and α2β heteromers) function as a complement and/or a modulator. Co-release of glycine and GABA from the same presynaptic terminal occurs; activating co-aggregated postsynaptic GlyRs and $GABA_A$ receptors, inducing cross-modulation of their respective activation properties (Muller et al., 2008).

Based on the present results the developmental switch induces great alterations while the glycinergic neurotransmitter system seems less affected by influence of external factors. What if these events were to coincide? A young, developing, brain is vulnerable and early exposure to ethanol is a strong predictor of future abuse and dependence in humans (Hawkins et al., 1997). Studies in rodents support these findings as higher levels of intake have been observed in animals exposed to the drug at an early age (Slawecki and Betancourt, 2002; Barbier et al., 2008; Schramm-Sapyta et al., 2008; Barbier et al., 2009). The developmental switch in GlyR subunits takes place during the first few weeks after birth, before sexual maturity. This period seems to be critical since, in addition to GlyRs, both $GABA_A$ and glutamate receptors are subjected to subunit alterations (Fritschy et al., 1994; Davis et al., 2000; Ritter et al., 2002), presumably affecting organisation and communication in the CNS. Whether ethanol interferes with these alterations in receptor composition, and if this is related to the increased risk of abuse and addiction, remains to be investigated. However, ethanol is known to interact with these receptors and some subunits are reported to be extra sensitive to the drug e.g. GlyRα1, NR2A, NR2B, $GABA_A$ δ (and α4/6β3δ) (Mascia et al., 1996b; Wallner et al., 2006; Perkins et al., 2008; Vengeliene et al., 2008; Yevenes et al., 2010; Nie et al., 2011). Genetic and environmental factors would surely be of importance as well, but the main issue is if these shifts also occur in humans. Presumably yes, but based on the region-dependent alterations in rodents and interspecies differences dissimilarities are to be expected.

The complete lack of effect on gene expression after 10 months of ethanol consumption was puzzling. Chronic alcohol intake is known to affect most of the neurotransmitter systems analysed and long-term exposure to addictive drugs has been shown to induce alterations in nAc (Hope et al., 1992; Robinson and Kolb, 1999; Nestler, 2001; Li et al., 2004). Even more intriguing was that the differences (and trends) observed after 2 and 4 months of consumption had disappeared (Paper III). That gene expression in ethanol consumers' displayed the same pattern after 2 and/or 4 months as in control animals after 10 months led to the assumption that ethanol consumption accelerated an age-related decrease. Indeed age-related decreases in gene expression have been reported for neurotransmitters and receptors (e.g (Kalaria et al., 1989; Araujo et al., 1990; Kowalski et al., 1992; Dournaud et al., 1996; Gutierrez et al., 1996) and studies of alcohol addicted humans have resulted in the age-accelerating hypothesis of alcoholism (Blusewicz et al., 1977; Ryan and Butters, 1980; Noonberg et al., 1985). Analysis of material from additional regions will clarify if the observed effects are general or restricted to nAc.

Apart from the age-related decreases in gene expression, results from Paper III illustrated that even low levels of ethanol consumption (the average daily intake over 10 months of consumption being 0.85 g/kg) was enough to induce alterations in gene expression. In adult Wistar rats blood alcohol levels (BALs) after 0.75 g/kg ethanol were around 50 mg% (Walker and Ehlers, 2009). However this dose was administered via gavage. Voluntarily consumed ethanol is commonly ingested in several drinking bouts during the animal's active period, and a daily dose of 0.85 g/kg should therefore result in lower BALs than in the above mentioned study. How animals experienced the effect of ethanol during this extended period of consumption is uncertain. Development of tolerance to the sedative (GABAergic), but not to the dopamine-elevating/reinforcing (glycinergic), effects of ethanol has been observed in animals following repeated administration of the drug (Kiianmaa and Tabakoff, 1983; Diana et al., 1992; Bassareo et al., 2003; Liang et al., 2009) and is also reported in humans (Brower et al., 2001; Roehrs and Roth, 2001). Like the modest effects on gene expression these reports indicate a robustness of the glycinergic system in nAc.

The involvement of GlyRs in the dopamine elevating effect of ethanol has been repeatedly demonstrated, and in Paper IV the dopamine increase following administration of other addictive drugs was examined. In addition to preventing the effect of alcohol, pre-treatment with strychnine also reduced the dopamine increase of nicotine and THC. While THC is believed to directly potentiate GlyRs (Hejazi et al., 2006; Xiong et al., 2011; Yevenes and Zeilhofer, 2011a), nicotine induces release of glycine via nAChRs (Zappettini et al., 2011; Zappettini et al., 2012). Blockade of GlyRs should prevent the potentiating effects of both these actions, which could explain the outcome observed. Even though the drugs were administered in doses commonly used and known to be potent in rats (e.g. (Ericson et al., 2000; Ahmadi et al., 2007; Shram et al., 2007; Solinas et al., 2007; Cortright et al., 2011; Panlilio et al., 2012), additional doses must be tested to confirm the results.

After using qPCR to detect and give a quantitative measure of GlyR gene expression in the nAc, immunohistochemistry was employed to provide information on the presence and location of functional receptors in this region. Previous studies had demonstrated the existence of functional GlyRs in this region (Woodruff et al., 1976; Martin and Siggins, 2002; Molander and Soderpalm, 2005b), results that were confirmed. Findings from both Paper III and IV showed that GlyRs were located mainly in the shell, the subregion which seems to be the primary site for dopamine increase after administration of addictive drugs in the rat (Pontieri et al., 1995; Pontieri et al., 1996; Ikemoto et al., 1997; Tanda et al., 1997; McBride et al., 1999; Di Chiara et al., 2004). Although this result was in line with the

hypothesis of a circuit mediating ethanol's dopamine elevating effect, retrograde tracing studies found only a few neurons projecting directly from nAc to aVTA. A larger population of neurons was found to project from the lateral septum (LS) to aVTA. The septal area is one of the regions Olds and Milner found that animals would self-stimulate in their classic studies identifying the brain reward system (Olds and Milner, 1954). The LS is interconnected with regions in the mesolimbic dopamine system, including nAc (Conrad and Pfaff, 1976; Nauta et al., 1978; Groenewegen and Russchen, 1984; Sheehan et al., 2004). Through these connections it is proposed that LS can stimulate the midbrain dopaminergic neurons, as well as mediating the effects of this stimulation in the nAc (Sheehan et al., 2004). LS appears to be involved in processes mediating temper and motivation and has been implicated in regulation of stress, anxiety and depression (Thomas and Evans, 1983; Contreras et al., 1989; Contreras et al., 1990; Duncan et al., 1996; Sheehan et al., 2004; Singewald et al., 2011). Additional studies are needed to investigate if the lateral septum is in fact a component in the proposed neuronal circuit. This seems plausible since pharmacological manipulations in LS alters dopamine release in nAc (Louilot et al., 1989), and this region has been suggested to be involved in alcohol consumption (Bachtell et al., 2003; Ryabinin and Weitemier, 2006; Ryabinin et al., 2008).

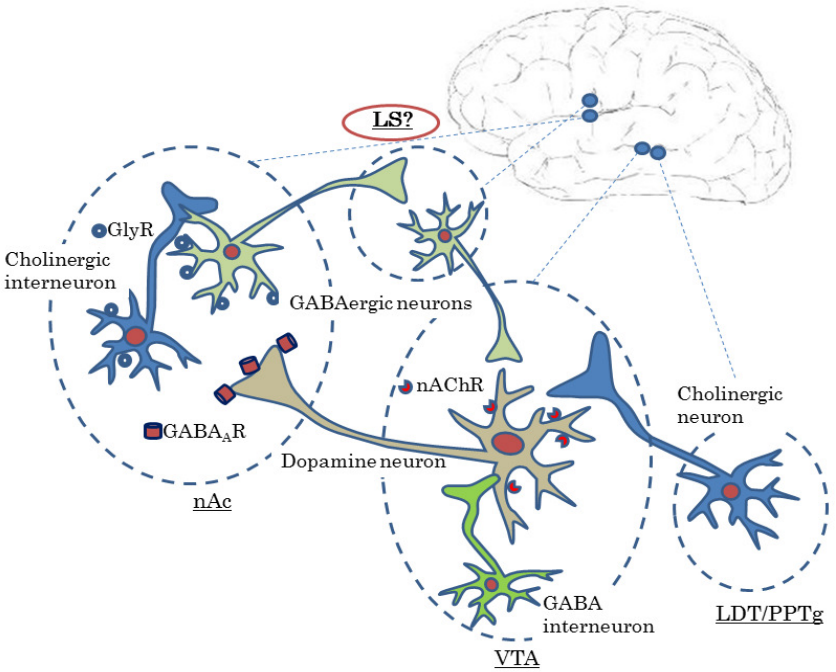


Figure 20. A neuronal circuit mediating ethanol's actions. Results from Paper IV suggests that the neuronal circuit mediating the dopamine-elevating and reinforcing effects of ethanol includes the lateral septum. Modified from Chau 2011. Abbreviations: $GABA_AR$; GABA $_A$ type receptors, GlyR; glycine receptor, LDTg/PPTg; laterodorsal/pedunculopontine tegmental nuclei, LS; lateral septum, nAc; nucleus accumbens, nAChR; nicotinic acetylcholine receptor, VTA; ventral tegmental area.

SWEDISH SUMMARY

Glycinreceptorer i det centrala nervsystemet

-utveckling, distribution och förhållande till alkoholens effekter

Alkoholism är en kronisk sjukdom och ett betydande samhällsproblem. De sociala och ekonomiska kostnaderna för den uppskattningsvis dryga miljon svenskar som tros lida av alkoholrelaterade problem är enorma. För personer som är beroende av alkohol har de positiva effekterna; känslan av välmående, ångestdämpning och avslappning, ersatts av ett tvångsmässigt behov att få i sig drogen. Vad det är som skiljer dessa individer från andra är okänt. Ingen gemensam nämnare har identifierats som kan förklara varför vissa människor utvecklar ett beroende och andra inte. Det faktum att risken att utveckla sjukdomen till viss del är ärftlig är vedertaget och med hjälp av olika genetiska studier och djurmodeller har man lyckats identifiera ett stort antal gener som kan vara inblandade i alkoholberoende. Men att bevisa att de har en given roll och bidrar till att sjukdomen utvecklas har visat sig vara mycket svårt, troligtvis för att varje enskild gen har liten betydelse och kräver samverkan med en mängd andra gener och faktorer för att ge någon effekt.

Även om det fortfarande är oklart hur ett beroende uppstår så är det väl etablerat att flera olika regioner och kommunikationssystem i hjärnan är inblandade. Det är inte bara själva drogen utan även gener, miljö, sociala och psykologiska faktorer, och samspel däremellan som kan ha betydelse för utvecklingen av ett beroendetillstånd. Att ta hänsyn till detta försvårar arbetet med att utreda och behandla drogmissbruk. Trots att alkohol har konsumerats, och missbrukats, i de flesta civilisationer i tusentals år saknar vi fortfarande kunskap och verktyg för att framgångsrikt behandla majoriteten av de drabbade. En bättre förståelse av vad som händer när en individ exponeras för alkohol behövs, både för att begripa hur ett beroende uppkommer och för att få fram mer effektiva behandlingar. Eftersom diagnosen alkoholberoende inte baseras på mätningar eller provtagningar utan på hur en individ beter sig och upplever sin situation är det väldigt svårt att återskapa det här sjukdomsförloppet i en modell. I likhet med studier av andra psykiatriska sjukdomar som schizofreni och depression försöker man härma ett tillstånd som tros vara unikt för människan i djur. Att beroende är ett komplicerat tillstånd gör att man får koncentrera sig på vissa aspekter eftersom hela sjukdomsbilden inte kan återskapas och studeras i en enda modell.

Det syns kanske inte på ytan att alla däggdjur är nära besläktade, men om man jämför genuppsättningar eller fosterutveckling hos olika arter är det tydligt att vi har ett gemensamt ursprung. Likadant är det med våra hjärnor, särskilt de delar som anläggs tidigt i

utvecklingen och rymmer livsnödvändiga funktioner som muskelkontroll, andning och hjärtrytm. Det här utnyttjas flitigt inom forskningen och i jakten på drogberoendets gåta är det ofta hjärnans belöningssystem som står i fokus. Belöningssystemet finns hos alla ryggradsdjur och har bevarats väl genom evolutionen vilket gör att det ser väldigt likt ut i exempelvis människa och råtta. Det här systemet är en samling hjärnregioner som grupperats ihop för att de, vid aktivering, ger upphov till en känsla av välbefinnande som beror på att signalsubstansen dopamin frisätts. Detta sker i samband med aktiviteter som att äta, dricka, träna och ha sex, så kallade naturliga belöningar. Ökningen av dopamin gör att dessa beteenden, som gynnar individens och därmed artens överlevnad, upplevs som belönande vilket motiverar en upprepning av aktiviteten. Problemet är bara att beroendeframkallande droger, inklusive alkohol, också aktiverar belöningssystemet. Dessutom på ett mycket mer kraftfullt sätt än naturliga belöningar, vilket gör att individen motiveras att upprepa beteenden som inte alls främjar dess överlevnad. Snarare tvärtom. Strävan att uppnå den starka positiva känslan som associeras med intag av drogen kan leda till ökad konsumtion och i förlängningen till ett beroendetillstånd.

En nyckelregion i belöningssystemet är nukleus accumbens, det är här som ökningen av dopamin är mest markant och regionen har därför tilldragit sig stort intresse. Tidigare studier utförda av vår forskargrupp har visat att man på olika sätt kan förhindra att alkohol ökar utsöndringen av dopamin i belöningssystemet. Man kan exempelvis blockera glycinreceptorerna genom att tillföra stryknin lokalt i nukleus accumbens, vilket leder till att ökningen av dopamin uteblir. Det här är ett tydligt tecken på att glycinreceptorerna i nukleus accumbens behövs för att ge en dopaminökning, och därmed en känsla av välbefinnande efter alkoholkonsumtion. Att uppleva intag av en drog som något positivt och behagligt är ofta vad som motiverar fortsatt konsumtion, vilket i förlängningen alltså kan leda till missbruk och beroende. Trots den betydelse som den här effekten av droger verkar ha så är mekanismerna som orsakar dopaminökningen oklara för exempelvis alkohol. Baserat på många års forskning har vår grupp lanserat en teori om hur alkohol orsakar en frisättning av dopamin i belöningssystemet, och här verkar glycinreceptorerna i nukleus accumbens vara av stor betydelse. Syftet med mitt avhandlingsarbete var att vidare undersöka glycinreceptorn, hur den påverkas av alkohol och dess inblandning i beroendeframkallande drogers förmåga att förmedla sin belönande effekt.

Hjärnan rymmer uppskattningsvis mer än 100 miljarder nervceller. Dessa celler är förbundna i nätverk och kommunicerar genom att frige så kallade signalsubstanser som fäster till mottagarprotein, så kallade receptorer, på närliggande nervcellers yta. Genom den

här kommunikationen aktiveras mottagarcellen, vilket leder till att den i sin tur frigör signalsubstanser till sina omgivande celler och på så sätt förs signaler vidare. Varje nervcell kan kommunicera med tusentals andra celler på det här sättet. Därigenom påverkas nervceller och regioner i olika konstellationer, information tas emot, behandlas och ger upphov till en lämplig reaktion. Varje signalsubstans har en eller flera receptorer som de binder in till och både substans och receptor har speciella egenskaper som påverkar den typ av signal de förmedlar och den effekt de har på mottagarcellen. Generellt så finns det två typer av signalsubstanser; excitatoriska som underlättar att celler aktiveras (exempelvis glutamat) och inhibitoriska som försvårar aktivering av celler (glycin och gamma amino smörsyra, GABA). De kan liknas vid en gas och en broms som tillsammans ser till att hjärnan är i balans och fungerar optimalt. Alkohol (och även andra droger och substanser) påverkar receptorernas funktion och därmed signaleringen mellan nervceller. Medan många andra droger har specifika receptorer har alkohol visat sig interagera med ett flertal olika mottagarprotein. Receptorer för signalsubstanserna acetylkolin, GABA, glycin, glutamat och serotonin påverkas alla direkt av alkohol, vilket leder till en mängd effekter då både gasen och bromsen i hjärnan påverkas.

Av särskilt intresse i det här sammanhanget är alltså glycinreceptorn och dess relation till alkohol. Glycinreceptorn består av fem delar (subenheter); antingen fem stycken α -subenheter (homomer) eller en blandning av två α och tre β subenheter (heteromer). Hittills har fem olika subenheter identifierats för den här receptorn; α1-4 och β. Till viss del influeras receptorernas egenskaper av vilka subenheter som den består av. I glycinreceptors fall verkar särskilt skillnaden mellan homomerer och heteromerer vara av betydelse och påverka både receptorns funktion och placering på cellen. För var och en av dessa subenheter finns det en särskild gen. Dessa gener kopieras i cellkärnan och används som en ritning för att producera subenheterna som sätts samman till den funktionella receptorn vilken sedan placeras på cellens yta. Eftersom antalet kopior som produceras av varje gen (genuttrycket) varierar beroende på behov och som svar på förändringar kan det användas som ett mått på vilken effekt en behandling eller någon annan faktor har.

Liksom hos människor finns det stora individuella variationer i råttors alkoholkonsumtion och genom avel kan man skapa en population med ett visst drickbeteende. Djur med hög respektive låg alkoholkonsumtion, orsakat av genetiska skillnader, har tagits fram och användes i avhandlingens första delarbete. Eftersom den belönande känslan tros ha stor betydelse för fortsatt alkoholintag var tanken att djur skiljer sig åt i drickbeteende på grund av skillnader i dopaminökning, eventuellt orsakade av skillnader i glycinreceptorn som

verkar vara nödvändig för den effekten. Genuttrycket av de olika receptorsubenheterna undersöktes i ett flertal regioner i främre delen av hjärnan (bland andra nukleus accumbens) utan att hitta några klara skillnader. Detta tyder på att skillnaden i alkoholkonsumtion mellan dessa hög- och lågkonsumerande djur inte kan förklaras av förändringar i genuttryck av glycinreceptorn. Däremot visade resultaten att α2 var den mest förekommande α-subenheten i främre delen av hjärnan och att α2β-heteromerer borde vara den vanligaste receptorformen, inte α1 respektive α1β som föreslagits i andra studier.

Detta följdes upp i nästa studie (delarbete II) där åldersrelaterade förändringar av glycinreceptorn undersöktes i olika delar av hjärnan och i ryggmärgen. Dessa resultat, från djur som inte utsatts för avel med avseende på alkoholkonsumtion, överensstämde med de från delarbete I. Genuttrycket av glycinreceptorns subenheter var klart högre i nyfödda än i vuxna djur. Trots en rejäl minskning i uttryck av α2-subenheten så behöll den sin position som den α-subenhet med högst uttryck i de flesta regioner som undersöktes. Det verkar som om den förändring i glycinreceptorns sammansättning som föreslagits tidigare, där α2-receptorer dominerar under utvecklingen för att sedan ersättas av α1β-receptorer, inte är allmän utan varierar beroende på region. Baserat på genuttryck borde α2β-receptorer vara de vanligast förekommande i hjärnan hos vuxna djur.

Nästa steg, delarbete III, var att undersöka hur genuttryck av bland annat glycinreceptorn reagerade på långvarig alkoholkonsumtion. Övergången från konsument till alkoholberoende är ofta en utdragen process där det exakta förloppet är osäkert men som med största säkerhet inleds med att individen frivilligt intar alkohol. För att försöka återskapa omständigheterna kring en sådan process fick råttor (på frivillig basis) konsumera alkohol under lång tid (upp till tio månader). På så sätt undersöktes vilka förändringar som kan bli följden av långvarig alkoholexponering. För att kunna spåra förändringar på cellnivå som den här behandlingen gav upphov till analyserades uttrycket av ett stort antal gener i nukleus accumbens. Dessa gener representerade många av de kommunikationssystem som påverkas av alkohol (bland andra GABA, glycin, serotonin och dopamin) och förhoppningen var att få en uppfattning om hur alkoholen påverkar aktiviteten i dessa system, och i förlängningen individens beteende.

Trots daglig alkoholkonsumtion under tio månader fanns det inga skillnader i genuttryck mellan råttor som konsumerade alkohol och kontroller. Ett mindre antal gener analyserades i material från djur efter två och fyra månaders konsumtion. Här fanns ett fåtal förändringar, bland annat ett minskat uttryck av DRD2. Den här genen översätts till dopamin D_2-receptorn som tilldragit sig stor uppmärksamhet eftersom flera studier har visat att

alkoholister har lägre nivåer av D_2 receptorn i hjärnan jämfört med friska kontroller. Märkligt nog så hade den här, och övriga skillander, försvunnit efter tio månaders konsumtion. De flesta gener uppvisade ett minskat uttryck med ökad ålder och eftersom minskningen hos alkoholkonsumerande djur efter två och fyra månader var jämförbar med den hos kontrollerna efter tio månader så verkar det som om alkoholen accelererade den åldersrelaterade processen. Även om de verktyg som användes i den här studien inte kunde identifiera några effekter så borde rimligtvis tio månaders alkoholkonsumtion ha konsekvenser. Kanske i uttryck av andra gener, i reglering av själva proteinerna eller i andra hjärnregioner.

I delarbete IV undersöktes effekten av alkohol på glycinreceptorn. Förutom att åter visa att den ökning i dopamin som alkohol ger upphov till kan förhindras med lokal blockad av glycinreceptorn i nukleus accumbens så testades stryknin även i kombination med andra droger. Resultaten visade att glycinreceptorer i nukleus accumbens även verkar vara inblandade i både nikotins och cannabis förmåga att höja nivåerna av dopamin i belöningssystemet. Däremot påverkade inte lokal behandling med stryknin effekterna av vare sig kokain eller morfin.

Eftersom analyser av genuttryck inte ger någon information om den fungerande receptorn vad gäller mängd och placering så använde vi oss av immunohistokemi. Dessa studier visade att glycinreceptorerna i nukleus accumbens främst finns i regionens yttre del, vilket också är den del som antas vara av störst betydelse för drogers effekt. Enligt vår hypotes om alkoholens belönande effekter skulle det finnas en direkt förbindelse mellan den främre delen av ventrala tegementala arean och nukleus accumbens. Något förvånande tyder våra resultat på att laterala septum har en starkare koppling till ventrala tegmentala arean än vad nukleus accumbens har. Laterala septum är en region som verkar vara inblandad i reglering av stress, ångest och depression. Den är även sammanlänkad med både nukleus accumbens och ventrala tegementala arean och påstås vara involverad i reglering av alkoholkonsumtion. Fler studier krävs dock för att bekräfta om laterala septum är en komponent i den krets som förmedlar alkoholens belönande effekt.

Sammanfattningsvis tyder avhandlingens arbete på att det glycinerga systemet är stabilt hos vuxna djur men genomgår omfattande förändringar under utvecklingen, att glycinreceptorer är inblandade i den dopaminökande effekten av alkohol, nikotin och cannabis och att laterala septum kan komma att adderas till den neuronala krets som tros mediera alkoholens belönande effekt. Vidare studier behövs för att följa upp dessa fynd och utreda om de kan sprida ljus över de frågeställningar som fortfarande omger alkoholberoende.

ACKNOWLEDGEMENTS

During the years that I have spent working on what was to become this thesis contributions have been made by a number of people. I would like to acknowledge, and express my gratitude to, the following:

My supervisor professor Bo Söderpalm, for your ability to inspire and motivate, to see opportunities not obstacles, and for having confidence in me as a researcher. Over the years I have much appreciated our interesting conversations, not necessarily of scientific character as there are always sporting events in need of discussion.

Mia Ericson, my co-supervisor, for leading by example, for having an open door, a minute to spare and a sense of humor. I have learned a lot from working with you.

PeiPei Chau and Helga H Lidö, my ever supportive and sympathetic partners in crime, for sharing frustration and success as well as knowledge and experiences at the office, on trips and over dinners.

Rosita Stomberg and Anne Fagerberg for making life as a PhD-student easier with your invaluable technical expertise and willingness to help.

My fellow PhD-students and office-occupants Rhona Clarke, Emma Persson and Julia Morud for great company and cooperation, contributing to the sorority that is the Addiction Biology Unit.

Chris Pickering for linguistic assistance, methodological discussions and scientific input.

Louise Adermark for scientific discussions and collaborations.

Colleagues at Blå Stråket (Anna Söderpalm-Gordh, Elin Löf, Andrea de Bejczy, Sejla Brkic, Barbro Askerup and Cecilia Nilsson-Wallmark) for pleasant meetings and social events. Through your work you provide a glimpse of the reality that the laboratory cannot offer, reminding me of the importance of what we do.

My pre-PhD supervisors Kim Fejgin and Johan Ruud for enthusiasm, encouragement and good advice.

All my friends for taking an interest in my work, and then distracting me from it by being inspiring, interesting and considerate people.

Oscar, for constantly reminding me what friends are for, and for believing in me more than I do myself.

Elisabeth, for countless laughs and endless conversations. Often out of sight, never out of mind.

My grandparents, for the immense impact they have had on me.

My parents, Carl-Erik and Britt-Marie, for encouraging my interests and aspirations. But most of all for always being there, lovingly supporting me in any way possible.

My sister Catharina, whom I could never do without.

The work presented in this thesis was financially supported by from the Swedish Medical Research Council, governmental support under the LUA/ALF agreement, the Swedish Brain foundation, the National Board of Health and Welfare, the Lars Hiertha Memorial Foundation, the Bror Gadelius Memorial Foundation, the Wilhelm and Martina Lundgren Scientific Foundation, the Fredrik and Ingrid Thuring Foundation, the Sigurd and Elsa Goljes Foundation, the Greta Johansson and Brita Andersson Memorial Foundation, and the Gunnar and Märta Bergendahl Foundation.

REFERENCES

Adermark L, Clarke RB, Ericson M, Soderpalm B (2011a) Subregion-Specific Modulation of Excitatory Input and Dopaminergic Output in the Striatum by Tonically Activated Glycine and GABA(A) Receptors. Front Syst Neurosci 5:85.

Adermark L, Clarke RB, Soderpalm B, Ericson M (2011b) Ethanol-induced modulation of synaptic output from the dorsolateral striatum in rat is regulated by cholinergic interneurons. Neurochem Int 58:693-699.

Adermark L, Clarke RB, Olsson T, Hansson E, Soderpalm B, Ericson M (2011c) Implications for glycine receptors and astrocytes in ethanol-induced elevation of dopamine levels in the nucleus accumbens. Addict Biol 16:43-54.

Aguayo LG, Tapia JC, Pancetti FC (1996) Potentiation of the glycine-activated Cl- current by ethanol in cultured mouse spinal neurons. J Pharmacol Exp Ther 279:1116-1122.

Ahmadi S, Zarrindast MR, Nouri M, Haeri-Rohani A, Rezayof A (2007) N-Methyl-D-aspartate receptors in the ventral tegmental area are involved in retrieval of inhibitory avoidance memory by nicotine. Neurobiol Learn Mem 88:352-358.

Akagi H, Hirai K, Hishinuma F (1991) Cloning of a glycine receptor subtype expressed in rat brain and spinal cord during a specific period of neuronal development. FEBS Lett 281:160-166.

Allgaier C (2002) Ethanol sensitivity of NMDA receptors. Neurochem Int 41:377-382.

Allgaier C, Franke H, Sobottka H, Scheibler P (2000) Acamprosate inhibits Ca2+ influx mediated by NMDA receptors and voltage-sensitive Ca2+ channels in cultured rat mesencephalic neurones. Naunyn Schmiedebergs Arch Pharmacol 362:440-443.

Anton RF, Oroszi G, O'Malley S, Couper D, Swift R, Pettinati H, Goldman D (2008) An evaluation of mu-opioid receptor (OPRM1) as a predictor of naltrexone response in the treatment of alcohol dependence: results from the Combined Pharmacotherapies and Behavioral Interventions for Alcohol Dependence (COMBINE) study. Arch Gen Psychiatry 65:135-144.

Aprison MH, Werman R (1965) The distribution of glycine in cat spinal cord and roots. Life Sci 4:2075-2083.

Araujo DM, Lapchak PA, Meaney MJ, Collier B, Quirion R (1990) Effects of aging on nicotinic and muscarinic autoreceptor function in the rat brain: relationship to presynaptic cholinergic markers and binding sites. J Neurosci 10:3069-3078.

Arias-Carrion O, Stamelou M, Murillo-Rodriguez E, Menendez-Gonzalez M, Poppel E (2010) Dopaminergic reward system: a short integrative review. Int Arch Med 3:24.

Bachtell RK, Weitemier AZ, Galvan-Rosas A, Tsivkovskaia NO, Risinger FO, Phillips TJ, Grahame NJ, Ryabinin AE (2003) The Edinger-Westphal-lateral septum urocortin pathway and its relationship to alcohol consumption. J Neurosci 23:2477-2487.

Barbier E, Pierrefiche O, Vaudry D, Vaudry H, Daoust M, Naassila M (2008) Long-term alterations in vulnerability to addiction to drugs of abuse and in brain gene expression after early life ethanol exposure. Neuropharmacology 55:1199-1211.

Barbier E, Houchi H, Warnault V, Pierrefiche O, Daoust M, Naassila M (2009) Effects of prenatal and postnatal maternal ethanol on offspring response to alcohol and psychostimulants in long evans rats. Neuroscience 161:427-440.

Barrios V, Rodriguez-Sanchez MN, Arilla E (1990) Effects of acute and chronic ethanol administration and its withdrawal on the level and binding of somatostatin in rat brain. Clin Sci (Lond) 79:451-456.

Bassareo V, De Luca MA, Aresu M, Aste A, Ariu T, Di Chiara G (2003) Differential adaptive properties of accumbens shell dopamine responses to ethanol as a drug and as a motivational stimulus. Eur J Neurosci 17:1465-1472.

Bayard M, McIntyre J, Hill KR, Woodside J, Jr. (2004) Alcohol withdrawal syndrome. Am Fam Physician 69:1443-1450.
Beaulieu JM, Gainetdinov RR (2011) The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol Rev 63:182-217.
Becker CM, Hoch W, Betz H (1988) Glycine receptor heterogeneity in rat spinal cord during postnatal development. EMBO J 7:3717-3726.
Bell RL, Sable HJ, Colombo G, Hyytia P, Rodd ZA, Lumeng L (2012) Animal models for medications development targeting alcohol abuse using selectively bred rat lines: Neurobiological and pharmacological validity. Pharmacol Biochem Behav 103:119-155.
Betz H, Becker CM (1988) The mammalian glycine receptor: biology and structure of a neuronal chloride channel protein. Neurochem Int 13:137-146.
Biala G, Kotlinska J (1999) Blockade of the acquisition of ethanol-induced conditioned place preference by N-methyl-D-aspartate receptor antagonists. Alcohol Alcohol 34:175-182.
Bjork K, Hansson AC, Sommer WH (2010) Genetic variation and brain gene expression in rodent models of alcoholism implications for medication development. Int Rev Neurobiol 91:129-171.
Blanchard DC, Sakai RR, McEwen B, Weiss SM, Blanchard RJ (1993) Subordination stress: behavioral, brain, and neuroendocrine correlates. Behav Brain Res 58:113-121.
Blanchard RJ, Flannelly KJ, Blanchard DC (1988) Life-span studies of dominance and aggression in established colonies of laboratory rats. Physiol Behav 43:1-7.
Blomqvist O (1996) Ethanol and central nicotinic acetylcholine receptors, a behavioral and neurochemical study in rodents. Thesis ISBN:91-628-2130-X.
Blomqvist O, Engel JA, Nissbrandt H, Soderpalm B (1993) The mesolimbic dopamine-activating properties of ethanol are antagonized by mecamylamine. Eur J Pharmacol 249:207-213.
Blomqvist O, Ericson M, Engel JA, Soderpalm B (1997) Accumbal dopamine overflow after ethanol: localization of the antagonizing effect of mecamylamine. Eur J Pharmacol 334:149-156.
Blomqvist O, Hernandez-Avila CA, Van Kirk J, Rose JE, Kranzler HR (2002) Mecamylamine modifies the pharmacokinetics and reinforcing effects of alcohol. Alcohol Clin Exp Res 26:326-331.
Blusewicz MJ, Schenkenberg T, Dustman RE, Beck EC (1977) WAIS performance in young normal, young alcoholic, and elderly normal groups: an evaluation of organicity and mental aging indices. J Clin Psychol 33:1149-1153.
Boileau I, Assaad JM, Pihl RO, Benkelfat C, Leyton M, Diksic M, Tremblay RE, Dagher A (2003) Alcohol promotes dopamine release in the human nucleus accumbens. Synapse 49:226-231.
Bormann J, Rundstrom N, Betz H, Langosch D (1993) Residues within transmembrane segment M2 determine chloride conductance of glycine receptor homo- and hetero-oligomers. EMBO J 12:3729-3737.
Brog JS, Salyapongse A, Deutch AY, Zahm DS (1993) The patterns of afferent innervation of the core and shell in the "accumbens" part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. J Comp Neurol 338:255-278.
Brower KJ, Aldrich MS, Robinson EA, Zucker RA, Greden JF (2001) Insomnia, self-medication, and relapse to alcoholism. Am J Psychiatry 158:399-404.
Brown ZW, Gill K, Abitbol M, Amit Z (1982) Lack of effect of dopamine receptor blockade on voluntary ethanol consumption in rats. Behav Neural Biol 36:291-294.

Cadoni C, Di Chiara G (2000) Differential changes in accumbens shell and core dopamine in behavioral sensitization to nicotine. Eur J Pharmacol 387:R23-25.
Cadoni C, Solinas M, Di Chiara G (2000) Psychostimulant sensitization: differential changes in accumbal shell and core dopamine. Eur J Pharmacol 388:69-76.
Carlsson A (1993) Thirty years of dopamine research. Adv Neurol 60:1-10.
Carlsson A, Lindqvist M, Magnusson T (1957) 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature 180:1200.
Casamenti F, Scali C, Vannucchi MG, Bartolini L, Pepeu G (1993) Long-term ethanol consumption by rats: effect on acetylcholine release in vivo, choline acetyltransferase activity, and behavior. Neuroscience 56:465-471.
Charlton ME, Sweetnam PM, Fitzgerald LW, Terwilliger RZ, Nestler EJ, Duman RS (1997) Chronic ethanol administration regulates the expression of GABAA receptor alpha 1 and alpha 5 subunits in the ventral tegmental area and hippocampus. J Neurochem 68:121-127.
Chau P (2011) Brain glycine receptors as a common target for alcohol and the relapse-preventing drug acamprosate- a preclinical study. Thesis ISBN 978-91-628-8256-3.
Chau P, Hoifodt-Lido H, Lof E, Soderpalm B, Ericson M (2010) Glycine receptors in the nucleus accumbens involved in the ethanol intake-reducing effect of acamprosate. Alcohol Clin Exp Res 34:39-45.
Chen X, Webb TI, Lynch JW (2009) The M4 transmembrane segment contributes to agonist efficacy differences between alpha1 and alpha3 glycine receptors. Mol Membr Biol 26:321-332.
Chi H, de Wit H (2003) Mecamylamine attenuates the subjective stimulant-like effects of alcohol in social drinkers. Alcohol Clin Exp Res 27:780-786.
Cloninger CR, Bohman M, Sigvardsson S (1981) Inheritance of alcohol abuse. Cross-fostering analysis of adopted men. Arch Gen Psychiatry 38:861-868.
Cohen C, Perrault G, Sanger DJ (1998) Preferential involvement of D3 versus D2 dopamine receptors in the effects of dopamine receptor ligands on oral ethanol self-administration in rats. Psychopharmacology (Berl) 140:478-485.
Conrad LC, Pfaff DW (1976) Autoradiographic tracing of nucleus accumbens efferents in the rat. Brain Res 113:589-596.
Contreras CM, Alcala-Herrera V, Marvan ML (1989) Action of antidepressants on the septal nuclei of the rat. Physiol Behav 46:793-798.
Contreras CM, Marvan ML, Alcala-Herrera V, Guzman-Saenz MA (1990) Chronic clomipramine increases firing rate in lateral septal nuclei of the rat. Physiol Behav 48:551-554.
Corrigall WA, Franklin KB, Coen KM, Clarke PB (1992) The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. Psychopharmacology (Berl) 107:285-289.
Cortright JJ, Lorrain DS, Beeler JA, Tang WJ, Vezina P (2011) Previous exposure to delta9-tetrahydrocannibinol enhances locomotor responding to but not self-administration of amphetamine. J Pharmacol Exp Ther 337:724-733.
Crabbe JC (2008) Review. Neurogenetic studies of alcohol addiction. Philos Trans R Soc Lond B Biol Sci 363:3201-3211.
Crabbe JC (2010) Consilience of rodent and human phenotypes relevant for alcohol dependence. Addict Biol 15:103-108.
Curtis DR, Hosli L, Johnston GA (1968) A pharmacological study of the depression of spinal neurones by glycine and related amino acids. Exp Brain Res 6:1-18.
Dani JA, Heinemann S (1996) Molecular and cellular aspects of nicotine abuse. Neuron 16:905-908.

Darstein M, Albrecht C, Lopez-Francos L, Knorle R, Holter SM, Spanagel R, Feuerstein TJ (1998) Release and accumulation of neurotransmitters in the rat brain: acute effects of ethanol in vitro and effects of long-term voluntary ethanol intake. Alcohol Clin Exp Res 22:704-709.

Davies M (2003) The role of GABAA receptors in mediating the effects of alcohol in the central nervous system. J Psychiatry Neurosci 28:263-274.

Davis AM, Penschuck S, Fritschy JM, McCarthy MM (2000) Developmental switch in the expression of GABA(A) receptor subunits alpha(1) and alpha(2) in the hypothalamus and limbic system of the rat. Brain Res Dev Brain Res 119:127-138.

Davis KM, Wu JY (2001) Role of glutamatergic and GABAergic systems in alcoholism. J Biomed Sci 8:7-19.

Davis TJ, de Fiebre CM (2006) Alcohol's actions on neuronal nicotinic acetylcholine receptors. Alcohol Res Health 29:179-185.

Delaney AJ, Esmaeili A, Sedlak PL, Lynch JW, Sah P (2010) Differential expression of glycine receptor subunits in the rat basolateral and central amygdala. Neurosci Lett 469:237-242.

Deleuze C, Runquist M, Orcel H, Rabie A, Dayanithi G, Alonso G, Hussy N (2005) Structural difference between heteromeric somatic and homomeric axonal glycine receptors in the hypothalamo-neurohypophysial system. Neuroscience 135:475-483.

Devaud LL, Smith FD, Grayson DR, Morrow AL (1995a) Chronic ethanol consumption differentially alters the expression of gamma-aminobutyric acidA receptor subunit mRNAs in rat cerebral cortex: competitive, quantitative reverse transcriptase-polymerase chain reaction analysis. Mol Pharmacol 48:861-868.

Devaud LL, Morrow AL, Criswell HE, Breese GR, Duncan GE (1995b) Regional differences in the effects of chronic ethanol administration on [3H]zolpidem binding in rat brain. Alcohol Clin Exp Res 19:910-914.

Di Chiara G (1999) Drug addiction as dopamine-dependent associative learning disorder. Eur J Pharmacol 375:13-30.

Di Chiara G (2000) Role of dopamine in the behavioural actions of nicotine related to addiction. Eur J Pharmacol 393:295-314.

Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci U S A 85:5274-5278.

Di Chiara G, North RA (1992) Neurobiology of opiate abuse. Trends Pharmacol Sci 13:185-193.

Di Chiara G, Bassareo V, Fenu S, De Luca MA, Spina L, Cadoni C, Acquas E, Carboni E, Valentini V, Lecca D (2004) Dopamine and drug addiction: the nucleus accumbens shell connection. Neuropharmacology 47 Suppl 1:227-241.

Diana M, Gessa GL, Rossetti ZL (1992) Lack of tolerance to ethanol-induced stimulation of mesolimbic dopamine system. Alcohol Alcohol 27:329-333.

Dick DM, Foroud T (2003) Candidate genes for alcohol dependence: a review of genetic evidence from human studies. Alcohol Clin Exp Res 27:868-879.

Dodd PR, Beckmann AM, Davidson MS, Wilce PA (2000) Glutamate-mediated transmission, alcohol, and alcoholism. Neurochem Int 37:509-533.

Dournaud P, Jazat-Poindessous F, Slama A, Lamour Y, Epelbaum J (1996) Correlations between water maze performance and cortical somatostatin mRNA and high-affinity binding sites during ageing in rats. Eur J Neurosci 8:476-485.

Drevets WC, Gautier C, Price JC, Kupfer DJ, Kinahan PE, Grace AA, Price JL, Mathis CA (2001) Amphetamine-induced dopamine release in human ventral striatum correlates with euphoria. Biol Psychiatry 49:81-96.

Drews E, Zimmer A (2010) Modulation of alcohol and nicotine responses through the endogenous opioid system. Prog Neurobiol 90:1-15.
Duncan GE, Knapp DJ, Breese GR (1996) Neuroanatomical characterization of Fos induction in rat behavioral models of anxiety. Brain Res 713:79-91.
Dyr W, McBride WJ, Lumeng L, Li TK, Murphy JM (1993) Effects of D1 and D2 dopamine receptor agents on ethanol consumption in the high-alcohol-drinking (HAD) line of rats. Alcohol 10:207-212.
Eggers ED, Berger AJ (2004) Mechanisms for the modulation of native glycine receptor channels by ethanol. J Neurophysiol 91:2685-2695.
Eggers ED, O'Brien JA, Berger AJ (2000) Developmental changes in the modulation of synaptic glycine receptors by ethanol. J Neurophysiol 84:2409-2416.
Elkins IJ, King SM, McGue M, Iacono WG (2006) Personality traits and the development of nicotine, alcohol, and illicit drug disorders: prospective links from adolescence to young adulthood. J Abnorm Psychol 115:26-39.
Engel JA, Fahlke C, Hard E, Johannessen K, Svensson L, Soderpalm B (1992) Serotonergic and dopaminergic involvement in ethanol intake. Clin Neuropharmacol 15 Suppl 1 Pt A:64A-65A.
Engel SR, Lyons CR, Allan AM (1998) 5-HT3 receptor over-expression decreases ethanol self administration in transgenic mice. Psychopharmacology (Berl) 140:243-248.
Ericson M, Engel JA, Soderpalm B (2000) Peripheral involvement in nicotine-induced enhancement of ethanol intake. Alcohol 21:37-47.
Ericson M, Blomqvist O, Engel JA, Soderpalm B (1998) Voluntary ethanol intake in the rat and the associated accumbal dopamine overflow are blocked by ventral tegmental mecamylamine. Eur J Pharmacol 358:189-196.
Ericson M, Molander A, Stomberg R, Soderpalm B (2006) Taurine elevates dopamine levels in the rat nucleus accumbens; antagonism by strychnine. Eur J Neurosci 23:3225-3229.
Ericson M, Molander A, Lof E, Engel JA, Soderpalm B (2003) Ethanol elevates accumbal dopamine levels via indirect activation of ventral tegmental nicotinic acetylcholine receptors. Eur J Pharmacol 467:85-93.
Ericson M, Lof E, Stomberg R, Chau P, Soderpalm B (2008) Nicotinic acetylcholine receptors in the anterior, but not posterior, ventral tegmental area mediate ethanol-induced elevation of accumbal dopamine levels. J Pharmacol Exp Ther 326:76-82.
Ericson M, Clarke RB, Chau P, Adermark L, Soderpalm B (2010) beta-Alanine elevates dopamine levels in the rat nucleus accumbens: antagonism by strychnine. Amino Acids 38:1051-1055.
Ericson M, Chau P, Clarke RB, Adermark L, Soderpalm B (2011) Rising taurine and ethanol concentrations in nucleus accumbens interact to produce dopamine release after ethanol administration. Addict Biol 16:377-385.
Eriksson K (1968) Genetic selection for voluntary alcohol consumption in the albino rat. Science 159:739-741.
Fahlke C, Hansen S, Engel JA, Hard E (1994) Effects of ventral striatal 6-OHDA lesions or amphetamine sensitization on ethanol consumption in the rat. Pharmacol Biochem Behav 47:345-349.
Farris SP, Miles MF (2012) Ethanol modulation of gene networks: implications for alcoholism. Neurobiol Dis 45:115-121.
Flint AC, Liu X, Kriegstein AR (1998) Nonsynaptic glycine receptor activation during early neocortical development. Neuron 20:43-53.
Florin SM, Kuczenski R, Segal DS (1994) Regional extracellular norepinephrine responses to amphetamine and cocaine and effects of clonidine pretreatment. Brain Res 654:53-62.

Follesa P, Ticku MK (1995) Chronic ethanol treatment differentially regulates NMDA receptor subunit mRNA expression in rat brain. Brain Res Mol Brain Res 29:99-106.
Fritschy JM, Paysan J, Enna A, Mohler H (1994) Switch in the expression of rat GABAA-receptor subtypes during postnatal development: an immunohistochemical study. J Neurosci 14:5302-5324.
Garcia-Alcocer G, Mejia C, Berumen LC, Miledi R, Martinez-Torres A (2008) Developmental expression of glycine receptor subunits in rat cerebellum. Int J Dev Neurosci 26:319-322.
Garcia-Colunga J, Miledi R (1999) Modulation of nicotinic acetylcholine receptors by strychnine. Proc Natl Acad Sci U S A 96:4113-4118.
Gass JT, Olive MF (2008) Glutamatergic substrates of drug addiction and alcoholism. Biochem Pharmacol 75:218-265.
George DT, Gilman J, Hersh J, Thorsell A, Herion D, Geyer C, Peng X, Kielbasa W, Rawlings R, Brandt JE, Gehlert DR, Tauscher JT, Hunt SP, Hommer D, Heilig M (2008) Neurokinin 1 receptor antagonism as a possible therapy for alcoholism. Science 319:1536-1539.
Gessa GL, Muntoni F, Collu M, Vargiu L, Mereu G (1985) Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. Brain Res 348:201-203.
Gilpin NW, Smith AD, Cole M, Weiss F, Koob GF, Richardson HN (2009) Operant behavior and alcohol levels in blood and brain of alcohol-dependent rats. Alcohol Clin Exp Res 33:2113-2123.
Goldman D, Oroszi G, Ducci F (2005) The genetics of addictions: uncovering the genes. Nat Rev Genet 6:521-532.
Grant KA (1994) Emerging neurochemical concepts in the actions of ethanol at ligand-gated ion channels. Behav Pharmacol 5:383-404.
Grenhoff J, Svensson TH (1989) Pharmacology of nicotine. Br J Addict 84:477-492.
Grenningloh G, Pribilla I, Prior P, Multhaup G, Beyreuther K, Taleb O, Betz H (1990) Cloning and expression of the 58 kd beta subunit of the inhibitory glycine receptor. Neuron 4:963-970.
Grenningloh G, Rienitz A, Schmitt B, Methfessel C, Zensen M, Beyreuther K, Gundelfinger ED, Betz H (1987) The strychnine-binding subunit of the glycine receptor shows homology with nicotinic acetylcholine receptors. Nature 328:215-220.
Grobin AC, Papadeas ST, Morrow AL (2000) Regional variations in the effects of chronic ethanol administration on GABA(A) receptor expression: potential mechanisms. Neurochem Int 37:453-461.
Groenewegen HJ, Russchen FT (1984) Organization of the efferent projections of the nucleus accumbens to pallidal, hypothalamic, and mesencephalic structures: a tracing and immunohistochemical study in the cat. J Comp Neurol 223:347-367.
Groenewegen HJ, Wright CI, Beijer AV (1996) The nucleus accumbens: gateway for limbic structures to reach the motor system? Prog Brain Res 107:485-511.
Grudzinska J, Schemm R, Haeger S, Nicke A, Schmalzing G, Betz H, Laube B (2005) The beta subunit determines the ligand binding properties of synaptic glycine receptors. Neuron 45:727-739.
Gutierrez A, Khan ZU, Ruano D, Miralles CP, Vitorica J, De Blas AL (1996) Aging-related subunit expression changes of the GABAA receptor in the rat hippocampus. Neuroscience 74:341-348.
Han Y, Li P, Slaughter MM (2004) Selective antagonism of rat inhibitory glycine receptor subunits. J Physiol 554:649-658.
Handford CA, Lynch JW, Baker E, Webb GC, Ford JH, Sutherland GR, Schofield PR (1996) The human glycine receptor beta subunit: primary structure, functional

characterisation and chromosomal localisation of the human and murine genes. Brain Res Mol Brain Res 35:211-219.
Harris RA (1999) Ethanol actions on multiple ion channels: which are important? Alcohol Clin Exp Res 23:1563-1570.
Harris RA, Trudell JR, Mihic SJ (2008) Ethanol's molecular targets. Sci Signal 1:re7.
Harvey RJ, Schmieden V, Von Holst A, Laube B, Rohrer H, Betz H (2000) Glycine receptors containing the alpha4 subunit in the embryonic sympathetic nervous system, spinal cord and male genital ridge. Eur J Neurosci 12:994-1001.
Hathway GJ, Emson PC, Humphrey PP, Kendrick KM (1998) Somatostatin potently stimulates in vivo striatal dopamine and gamma-aminobutyric acid release by a glutamate-dependent action. J Neurochem 70:1740-1749.
Hawkins JD, Graham JW, Maguin E, Abbott R, Hill KG, Catalano RF (1997) Exploring the effects of age of alcohol use initiation and psychosocial risk factors on subsequent alcohol misuse. J Stud Alcohol 58:280-290.
Heffner TG, Hartman JA, Seiden LS (1980) A rapid method for the regional dissection of the rat brain. Pharmacol Biochem Behav 13:453-456.
Heimer L, Alheid GF, de Olmos JS, Groenewegen HJ, Haber SN, Harlan RE, Zahm DS (1997) The accumbens: beyond the core-shell dichotomy. J Neuropsychiatry Clin Neurosci 9:354-381.
Heinz A (2002) Dopaminergic dysfunction in alcoholism and schizophrenia--psychopathological and behavioral correlates. Eur Psychiatry 17:9-16.
Heinz A, Siessmeier T, Wrase J, Hermann D, Klein S, Grusser SM, Flor H, Braus DF, Buchholz HG, Grunder G, Schreckenberger M, Smolka MN, Rosch F, Mann K, Bartenstein P (2004) Correlation between dopamine D(2) receptors in the ventral striatum and central processing of alcohol cues and craving. Am J Psychiatry 161:1783-1789.
Heinze L, Harvey RJ, Haverkamp S, Wassle H (2007) Diversity of glycine receptors in the mouse retina: localization of the alpha4 subunit. J Comp Neurol 500:693-707.
Hejazi N, Zhou C, Oz M, Sun H, Ye JH, Zhang L (2006) Delta9-tetrahydrocannabinol and endogenous cannabinoid anandamide directly potentiate the function of glycine receptors. Mol Pharmacol 69:991-997.
Hietala J, West C, Syvalahti E, Nagren K, Lehikoinen P, Sonninen P, Ruotsalainen U (1994) Striatal D2 dopamine receptor binding characteristics in vivo in patients with alcohol dependence. Psychopharmacology (Berl) 116:285-290.
Hoch W, Betz H, Becker CM (1989) Primary cultures of mouse spinal cord express the neonatal isoform of the inhibitory glycine receptor. Neuron 3:339-348.
Hoffman PL (1995) Glutamate receptors in alcohol withdrawal-induced neurotoxicity. Metab Brain Dis 10:73-79.
Honkanen A, Chrapusta SJ, Karoum F, Korpi ER (1994) Alterations in dopamine metabolism by intraperitoneal ethanol in rats selected for high and low ethanol preference: a 3-methoxytyramine study. Alcohol 11:323-328.
Hope B, Kosofsky B, Hyman SE, Nestler EJ (1992) Regulation of immediate early gene expression and AP-1 binding in the rat nucleus accumbens by chronic cocaine. Proc Natl Acad Sci U S A 89:5764-5768.
Ikemoto S (2007) Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. Brain Res Rev 56:27-78.
Ikemoto S, Glazier BS, Murphy JM, McBride WJ (1997) Role of dopamine D1 and D2 receptors in the nucleus accumbens in mediating reward. J Neurosci 17:8580-8587.

Iorio KR, Reinlib L, Tabakoff B, Hoffman PL (1992) Chronic exposure of cerebellar granule cells to ethanol results in increased N-methyl-D-aspartate receptor function. Mol Pharmacol 41:1142-1148.

Ito S, Cherubini E (1991) Strychnine-sensitive glycine responses of neonatal rat hippocampal neurones. J Physiol 440:67-83.

Jacobs EH, Smit AB, de Vries TJ, Schoffelmeer AN (2003) Neuroadaptive effects of active versus passive drug administration in addiction research. Trends Pharmacol Sci 24:566-573.

Jerlhag E, Grotli M, Luthman K, Svensson L, Engel JA (2006a) Role of the subunit composition of central nicotinic acetylcholine receptors for the stimulatory and dopamine-enhancing effects of ethanol. Alcohol Alcohol 41:486-493.

Jerlhag E, Egecioglu E, Dickson SL, Andersson M, Svensson L, Engel JA (2006b) Ghrelin stimulates locomotor activity and accumbal dopamine-overflow via central cholinergic systems in mice: implications for its involvement in brain reward. Addict Biol 11:45-54.

Jiang ZL, Ye JH (2003) Protein kinase C epsilon is involved in ethanol potentiation of glycine-gated Cl(-) current in rat neurons of ventral tegmental area. Neuropharmacology 44:493-502.

Jonsson S, Kerekes N, Hyytia P, Ericson M, Soderpalm B (2009) Glycine receptor expression in the forebrain of male AA/ANA rats. Brain Res 1305 Suppl:S27-36.

Kalaria RN, Andorn AC, Tabaton M, Whitehouse PJ, Harik SI, Unnerstall JR (1989) Adrenergic receptors in aging and Alzheimer's disease: increased beta 2-receptors in prefrontal cortex and hippocampus. J Neurochem 53:1772-1781.

Kalivas PW, Churchill L, Klitenick MA (1993) GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to the ventral tegmental area. Neuroscience 57:1047-1060.

Katner SN, Weiss F (2001) Neurochemical characteristics associated with ethanol preference in selected alcohol-preferring and -nonpreferring rats: a quantitative microdialysis study. Alcohol Clin Exp Res 25:198-205.

Kelley AE, Berridge KC (2002) The neuroscience of natural rewards: relevance to addictive drugs. J Neurosci 22:3306-3311.

Kiianmaa K, Tabakoff B (1983) Neurochemical correlates of tolerance and strain differences in the neurochemical effects of ethanol. Pharmacol Biochem Behav 18 Suppl 1:383-388.

Kiianmaa K, Andersson K, Fuxe K (1979) On the role of ascending dopamine systems in the control of voluntary ethanol intake and ethanol intoxication. Pharmacol Biochem Behav 10:603-608.

Kiianmaa K, Nurmi M, Nykanen I, Sinclair JD (1995) Effect of ethanol on extracellular dopamine in the nucleus accumbens of alcohol-preferring AA and alcohol-avoiding ANA rats. Pharmacol Biochem Behav 52:29-34.

King AC, Volpicelli JR, Frazer A, O'Brien CP (1997) Effect of naltrexone on subjective alcohol response in subjects at high and low risk for future alcohol dependence. Psychopharmacology (Berl) 129:15-22.

Kirsch J (2006) Glycinergic transmission. Cell Tissue Res 326:535-540.

Kirsch J, Betz H (1995) The postsynaptic localization of the glycine receptor-associated protein gephyrin is regulated by the cytoskeleton. J Neurosci 15:4148-4156.

Kirsch J, Meyer G, Betz H (1996) Synaptic Targeting of Ionotropic Neurotransmitter Receptors. Mol Cell Neurosci 8:93-98.

Kobayashi T, Ikeda K, Kojima H, Niki H, Yano R, Yoshioka T, Kumanishi T (1999) Ethanol opens G-protein-activated inwardly rectifying K+ channels. Nat Neurosci 2:1091-1097.

Koe BK (1976) Molecular geometry of inhibitors of the uptake of catecholamines and serotonin in synaptosomal preparations of rat brain. J Pharmacol Exp Ther 199:649-661.

Koob GF (1992) Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol Sci 13:177-184.

Koob GF (1996) Drug addiction: the yin and yang of hedonic homeostasis. Neuron 16:893-896.

Koob GF (2003) Neuroadaptive mechanisms of addiction: studies on the extended amygdala. Eur Neuropsychopharmacol 13:442-452.

Koob GF (2010) The role of CRF and CRF-related peptides in the dark side of addiction. Brain Res 1314:3-14.

Koob GF, Bloom FE (1988) Cellular and molecular mechanisms of drug dependence. Science 242:715-723.

Koob GF, Volkow ND (2010) Neurocircuitry of addiction. Neuropsychopharmacology 35:217-238.

Koob GF, Rassnick S, Heinrichs S, Weiss F (1994) Alcohol, the reward system and dependence. EXS 71:103-114.

Kowalski C, Micheau J, Corder R, Gaillard R, Conte-Devolx B (1992) Age-related changes in cortico-releasing factor, somatostatin, neuropeptide Y, methionine enkephalin and beta-endorphin in specific rat brain areas. Brain Res 582:38-46.

Krystal JH, Petrakis IL, Mason G, Trevisan L, D'Souza DC (2003) N-methyl-D-aspartate glutamate receptors and alcoholism: reward, dependence, treatment, and vulnerability. Pharmacol Ther 99:79-94.

Kuhse J, Schmieden V, Betz H (1990) Identification and functional expression of a novel ligand binding subunit of the inhibitory glycine receptor. J Biol Chem 265:22317-22320.

Kuhse J, Laube B, Magalei D, Betz H (1993) Assembly of the inhibitory glycine receptor: identification of amino acid sequence motifs governing subunit stoichiometry. Neuron 11:1049-1056.

Kuhse J, Kuryatov A, Maulet Y, Malosio ML, Schmieden V, Betz H (1991) Alternative splicing generates two isoforms of the alpha 2 subunit of the inhibitory glycine receptor. FEBS Lett 283:73-77.

Kumar S, Porcu P, Werner DF, Matthews DB, Diaz-Granados JL, Helfand RS, Morrow AL (2009) The role of GABA(A) receptors in the acute and chronic effects of ethanol: a decade of progress. Psychopharmacology (Berl) 205:529-564.

Kumari M, Ticku MK (2000) Regulation of NMDA receptors by ethanol. Prog Drug Res 54:152-189.

Larsson A, Engel JA (2004) Neurochemical and behavioral studies on ethanol and nicotine interactions. Neurosci Biobehav Rev 27:713-720.

Larsson A, Jerlhag E, Svensson L, Soderpalm B, Engel JA (2004) Is an alpha-conotoxin MII-sensitive mechanism involved in the neurochemical, stimulatory, and rewarding effects of ethanol? Alcohol 34:239-250.

Laube B, Maksay G, Schemm R, Betz H (2002) Modulation of glycine receptor function: a novel approach for therapeutic intervention at inhibitory synapses? Trends Pharmacol Sci 23:519-527.

Le-Corronc H, Rigo JM, Branchereau P, Legendre P (2011) GABA(A) receptor and glycine receptor activation by paracrine/autocrine release of endogenous agonists: more than a simple communication pathway. Mol Neurobiol 44:28-52.
Le AD, Corrigall WA, Harding JW, Juzytsch W, Li TK (2000) Involvement of nicotinic receptors in alcohol self-administration. Alcohol Clin Exp Res 24:155-163.
Le Foll B, Gallo A, Le Strat Y, Lu L, Gorwood P (2009) Genetics of dopamine receptors and drug addiction: a comprehensive review. Behav Pharmacol 20:1-17.
Le Moal M, Simon H (1991) Mesocorticolimbic dopaminergic network: functional and regulatory roles. Physiol Rev 71:155-234.
Lejoyeux M, Solomon J, Ades J (1998) Benzodiazepine treatment for alcohol-dependent patients. Alcohol Alcohol 33:563-575.
Lesch OM, Walter H (1996) Subtypes of alcoholism and their role in therapy. Alcohol Alcohol Suppl 1:63-67.
Lewohl JM, Wilson WR, Mayfield RD, Brozowski SJ, Morrisett RA, Harris RA (1999) G-protein-coupled inwardly rectifying potassium channels are targets of alcohol action. Nat Neurosci 2:1084-1090.
Levy AD, Murphy JM, McBride WJ, Lumeng L, Li TK (1991) Microinjection of sulpiride into the nucleus accumbens increases ethanol drinking in alcohol-preferring (P) rats. Alcohol Alcohol Suppl 1:417-420.
Li P, Slaughter M (2007) Glycine receptor subunit composition alters the action of GABA antagonists. Vis Neurosci 24:513-521.
Li Y, Acerbo MJ, Robinson TE (2004) The induction of behavioural sensitization is associated with cocaine-induced structural plasticity in the core (but not shell) of the nucleus accumbens. Eur J Neurosci 20:1647-1654.
Liang J, Spigelman I, Olsen RW (2009) Tolerance to sedative/hypnotic actions of GABAergic drugs correlates with tolerance to potentiation of extrasynaptic tonic currents of alcohol-dependent rats. J Neurophysiol 102:224-233.
Lido HH, Marston H, Ericson M, Soderpalm B (2012) The glycine reuptake inhibitor Org24598 and acamprosate reduce ethanol intake in the rat; tolerance development to acamprosate but not to Org24598. Addict Biol 17:897-907.
Linseman MA (1990) Effects of dopaminergic agents on alcohol consumption by rats in a limited access paradigm. Psychopharmacology (Berl) 100:195-200.
Liu X, Weiss F (2002) Reversal of ethanol-seeking behavior by D1 and D2 antagonists in an animal model of relapse: differences in antagonist potency in previously ethanol-dependent versus nondependent rats. J Pharmacol Exp Ther 300:882-889.
Lof E, Ericson M, Stomberg R, Soderpalm B (2007a) Characterization of ethanol-induced dopamine elevation in the rat nucleus accumbens. Eur J Pharmacol 555:148-155.
Lof E, Chau PP, Stomberg R, Soderpalm B (2007b) Ethanol-induced dopamine elevation in the rat--modulatory effects by subchronic treatment with nicotinic drugs. Eur J Pharmacol 555:139-147.
Lof E, Olausson P, deBejczy A, Stomberg R, McIntosh JM, Taylor JR, Soderpalm B (2007c) Nicotinic acetylcholine receptors in the ventral tegmental area mediate the dopamine activating and reinforcing properties of ethanol cues. Psychopharmacology (Berl) 195:333-343.
Louilot A, Le Moal M, Simon H (1989) Opposite influences of dopaminergic pathways to the prefrontal cortex or the septum on the dopaminergic transmission in the nucleus accumbens. An in vivo voltammetric study. Neuroscience 29:45-56.
Lovinger DM (1991) Ethanol potentiates ion current mediated by 5-HT3 receptors on neuroblastoma cells and isolated neurons. Alcohol Alcohol Suppl 1:181-185.

Lovinger DM (1997) Alcohols and neurotransmitter gated ion channels: past, present and future. Naunyn Schmiedebergs Arch Pharmacol 356:267-282.
Lovinger DM (1999) 5-HT3 receptors and the neural actions of alcohols: an increasingly exciting topic. Neurochem Int 35:125-130.
Lovinger DM, White G (1991) Ethanol potentiation of 5-hydroxytryptamine3 receptor-mediated ion current in neuroblastoma cells and isolated adult mammalian neurons. Mol Pharmacol 40:263-270.
Lovinger DM, White G, Weight FF (1989) Ethanol inhibits NMDA-activated ion current in hippocampal neurons. Science 243:1721-1724.
Lu T, Rubio ME, Trussell LO (2008) Glycinergic transmission shaped by the corelease of GABA in a mammalian auditory synapse. Neuron 57:524-535.
Luczak SE, Elvine-Kreis B, Shea SH, Carr LG, Wall TL (2002) Genetic risk for alcoholism relates to level of response to alcohol in Asian-American men and women. J Stud Alcohol 63:74-82.
Lynch JW (2004) Molecular structure and function of the glycine receptor chloride channel. Physiol Rev 84:1051-1095.
Lynch JW (2009) Native glycine receptor subtypes and their physiological roles. Neuropharmacology 56:303-309.
Maccioni P, Colombo G (2009) Role of the GABA(B) receptor in alcohol-seeking and drinking behavior. Alcohol 43:555-558.
Malosio ML, Marqueze-Pouey B, Kuhse J, Betz H (1991a) Widespread expression of glycine receptor subunit mRNAs in the adult and developing rat brain. EMBO J 10:2401-2409.
Malosio ML, Grenningloh G, Kuhse J, Schmieden V, Schmitt B, Prior P, Betz H (1991b) Alternative splicing generates two variants of the alpha 1 subunit of the inhibitory glycine receptor. J Biol Chem 266:2048-2053.
Mangin JM, Baloul M, Prado De Carvalho L, Rogister B, Rigo JM, Legendre P (2003) Kinetic properties of the alpha2 homo-oligomeric glycine receptor impairs a proper synaptic functioning. J Physiol 553:369-386.
Mansour A, Fox CA, Akil H, Watson SJ (1995) Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. Trends Neurosci 18:22-29.
Mardones J, Segovia-Riquelme N (1983) Thirty-two years of selection of rats by ethanol preference: UChA and UChB strains. Neurobehav Toxicol Teratol 5:171-178.
Marszalec W, Aistrup GL, Narahashi T (1999) Ethanol-nicotine interactions at alpha-bungarotoxin-insensitive nicotinic acetylcholine receptors in rat cortical neurons. Alcohol Clin Exp Res 23:439-445.
Martin G, Siggins GR (2002) Electrophysiological evidence for expression of glycine receptors in freshly isolated neurons from nucleus accumbens. J Pharmacol Exp Ther 302:1135-1145.
Mascia MP, Machu TK, Harris RA (1996a) Enhancement of homomeric glycine receptor function by long-chain alcohols and anaesthetics. Br J Pharmacol 119:1331-1336.
Mascia MP, Wick MJ, Martinez LD, Harris RA (1998) Enhancement of glycine receptor function by ethanol: role of phosphorylation. Br J Pharmacol 125:263-270.
Mascia MP, Mihic SJ, Valenzuela CF, Schofield PR, Harris RA (1996b) A single amino acid determines differences in ethanol actions on strychnine-sensitive glycine receptors. Mol Pharmacol 50:402-406.
Matsubayashi H, Alkondon M, Pereira EF, Swanson KL, Albuquerque EX (1998) Strychnine: a potent competitive antagonist of alpha-bungarotoxin-sensitive nicotinic acetylcholine receptors in rat hippocampal neurons. J Pharmacol Exp Ther 284:904-913.

Matzenbach B, Maulet Y, Sefton L, Courtier B, Avner P, Guenet JL, Betz H (1994) Structural analysis of mouse glycine receptor alpha subunit genes. Identification and chromosomal localization of a novel variant. J Biol Chem 269:2607-2612.

Mayo-Smith MF (1997) Pharmacological management of alcohol withdrawal. A meta-analysis and evidence-based practice guideline. American Society of Addiction Medicine Working Group on Pharmacological Management of Alcohol Withdrawal. JAMA 278:144-151.

McBride WJ, Li TK (1998) Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. Crit Rev Neurobiol 12:339-369.

McBride WJ, Murphy JM, Ikemoto S (1999) Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. Behav Brain Res 101:129-152.

McBride WJ, Chernet E, Dyr W, Lumeng L, Li TK (1993a) Densities of dopamine D2 receptors are reduced in CNS regions of alcohol-preferring P rats. Alcohol 10:387-390.

McBride WJ, Murphy JM, Gatto GJ, Levy AD, Yoshimoto K, Lumeng L, Li TK (1993b) CNS mechanisms of alcohol self-administration. Alcohol Alcohol Suppl 2:463-467.

McBride WJ, Kimpel MW, McClintick JN, Ding ZM, Hyytia P, Colombo G, Edenberg HJ, Lumeng L, Bell RL (2012) Gene expression in the ventral tegmental area of 5 pairs of rat lines selectively bred for high or low ethanol consumption. Pharmacol Biochem Behav 102:275-285.

McDearmid JR, Liao M, Drapeau P (2006) Glycine receptors regulate interneuron differentiation during spinal network development. Proc Natl Acad Sci U S A 103:9679-9684.

McGue M, Slutske W, Iacono WG (1999) Personality and substance use disorders: II. Alcoholism versus drug use disorders. J Consult Clin Psychol 67:394-404.

Mhatre MC, Ticku MK (1993) Alcohol: effects on GABAA receptor function and gene expression. Alcohol Alcohol Suppl 2:331-335.

Mihic SJ (1999) Acute effects of ethanol on GABAA and glycine receptor function. Neurochem Int 35:115-123.

Mihic SJ, Harris RA (1996) Inhibition of rho1 receptor GABAergic currents by alcohols and volatile anesthetics. J Pharmacol Exp Ther 277:411-416.

Mihic SJ, Whiting PJ, Harris RA (1994) Anaesthetic concentrations of alcohols potentiate GABAA receptor-mediated currents: lack of subunit specificity. Eur J Pharmacol 268:209-214.

Mihic SJ, Ye Q, Wick MJ, Koltchine VV, Krasowski MD, Finn SE, Mascia MP, Valenzuela CF, Hanson KK, Greenblatt EP, Harris RA, Harrison NL (1997) Sites of alcohol and volatile anaesthetic action on GABA(A) and glycine receptors. Nature 389:385-389.

Miles MF, Diaz JE, DeGuzman VS (1991) Mechanisms of neuronal adaptation to ethanol. Ethanol induces Hsc70 gene transcription in NG108-15 neuroblastoma x glioma cells. J Biol Chem 266:2409-2414.

Mishra D, Zhang X, Chergui K (2012) Ethanol Disrupts the Mechanisms of Induction of Long-Term Potentiation in the Mouse Nucleus Accumbens. Alcohol Clin Exp Res.

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

Molander A, Soderpalm B (2005b) Accumbal strychnine-sensitive glycine receptors: an access point for ethanol to the brain reward system. Alcohol Clin Exp Res 29:27-37.

Molander A, Lof E, Stomberg R, Ericson M, Soderpalm B (2005) Involvement of accumbal glycine receptors in the regulation of voluntary ethanol intake in the rat. Alcohol Clin Exp Res 29:38-45.

Molander A, Lido HH, Lof E, Ericson M, Soderpalm B (2007) The glycine reuptake inhibitor Org 25935 decreases ethanol intake and preference in male wistar rats. Alcohol Alcohol 42:11-18.

Morrow AL, Montpied P, Lingford-Hughes A, Paul SM (1990) Chronic ethanol and pentobarbital administration in the rat: effects on GABAA receptor function and expression in brain. Alcohol 7:237-244.

Morrow AL, Devaud LL, Bucci D, Smith FD (1994) GABAA and NMDA receptor subunit mRNA expression in ethanol dependent rats. Alcohol Alcohol Suppl 2:89-95.

Muller E, Le-Corronc H, Legendre P (2008) Extrasynaptic and postsynaptic receptors in glycinergic and GABAergic neurotransmission: a division of labor? Front Mol Neurosci 1:3.

Narahashi T, Aistrup GL, Marszalec W, Nagata K (1999) Neuronal nicotinic acetylcholine receptors: a new target site of ethanol. Neurochem Int 35:131-141.

Nauta WJ, Smith GP, Faull RL, Domesick VB (1978) Efferent connections and nigral afferents of the nucleus accumbens septi in the rat. Neuroscience 3:385-401.

Nestler EJ (2001) Molecular basis of long-term plasticity underlying addiction. Nat Rev Neurosci 2:119-128.

Nestler EJ (2004) Molecular mechanisms of drug addiction. Neuropharmacology 47 Suppl 1:24-32.

Nestler EJ, Aghajanian GK (1997) Molecular and cellular basis of addiction. Science 278:58-63.

Nevin ST, Cromer BA, Haddrill JL, Morton CJ, Parker MW, Lynch JW (2003) Insights into the structural basis for zinc inhibition of the glycine receptor. J Biol Chem 278:28985-28992.

Nie H, Rewal M, Gill TM, Ron D, Janak PH (2011) Extrasynaptic delta-containing GABAA receptors in the nucleus accumbens dorsomedial shell contribute to alcohol intake. Proc Natl Acad Sci U S A 108:4459-4464.

Nikolic Z, Laube B, Weber RG, Lichter P, Kioschis P, Poustka A, Mulhardt C, Becker CM (1998) The human glycine receptor subunit alpha3. Glra3 gene structure, chromosomal localization, and functional characterization of alternative transcripts. J Biol Chem 273:19708-19714.

Noonberg A, Goldstein G, Page HA (1985) Premature aging in male alcoholics: "accelerated aging" or "increased vulnerability"? Alcohol Clin Exp Res 9:334-338.

Nowak KL, McBride WJ, Lumeng L, Li TK, Murphy JM (2000) Involvement of dopamine D2 autoreceptors in the ventral tegmental area on alcohol and saccharin intake of the alcohol-preferring P rat. Alcohol Clin Exp Res 24:476-483.

Olds J, Milner P (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. J Comp Physiol Psychol 47:419-427.

Olive MF, Koenig HN, Nannini MA, Hodge CW (2001) Stimulation of endorphin neurotransmission in the nucleus accumbens by ethanol, cocaine, and amphetamine. J Neurosci 21:RC184.

Olsen RW, Hanchar HJ, Meera P, Wallner M (2007) GABAA receptor subtypes: the "one glass of wine" receptors. Alcohol 41:201-209.

Ortiz S, Oliva JM, Perez-Rial S, Palomo T, Manzanares J (2004) Chronic ethanol consumption regulates cannabinoid CB1 receptor gene expression in selected regions of rat brain. Alcohol Alcohol 39:88-92.

Oslin DW, Berrettini W, Kranzler HR, Pettinati H, Gelernter J, Volpicelli JR, O'Brien CP (2003) A functional polymorphism of the mu-opioid receptor gene is associated with naltrexone response in alcohol-dependent patients. Neuropsychopharmacology 28:1546-1552.

Oswald LM, Wand GS (2004) Opioids and alcoholism. Physiol Behav 81:339-358.
Pallis E, Thermos K, Spyraki S (2001) Chronic desipramine treatment selectively potentiates somatostatin-induced dopamine release in the nucleus
accumbens. European Journal of Neuroscience 14:763-767.
Panlilio LV, Ferre S, Yasar S, Thorndike EB, Schindler CW, Goldberg SR (2012) Combined effects of THC and caffeine on working memory in rats. Br J Pharmacol 165:2529-2538.
Pautassi RM, Camarini R, Quadros IM, Miczek KA, Israel Y (2010) Genetic and environmental influences on ethanol consumption: perspectives from preclinical research. Alcohol Clin Exp Res 34:976-987.
Paxinos G, Watson CR (2007) The Rat Brain in Stereotaxic Coordinates. San Diego, CA: Academic Press Inc.
Perkins DI, Trudell JR, Crawford DK, Alkana RL, Davies DL (2008) Targets for ethanol action and antagonism in loop 2 of the extracellular domain of glycine receptors. J Neurochem 106:1337-1349.
Perogamvros L, Schwartz S (2012) The roles of the reward system in sleep and dreaming. Neurosci Biobehav Rev 36:1934-1951.
Pettit HO, Ettenberg A, Bloom FE, Koob GF (1984) Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. Psychopharmacology (Berl) 84:167-173.
Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29:e45.
Pfeffer AO, Samson HH (1988) Haloperidol and apomorphine effects on ethanol reinforcement in free feeding rats. Pharmacol Biochem Behav 29:343-350.
Phillips TJ, Brown KJ, Burkhart-Kasch S, Wenger CD, Kelly MA, Rubinstein M, Grandy DK, Low MJ (1998) Alcohol preference and sensitivity are markedly reduced in mice lacking dopamine D2 receptors. Nat Neurosci 1:610-615.
Piechotta K, Weth F, Harvey RJ, Friauf E (2001) Localization of rat glycine receptor alpha1 and alpha2 subunit transcripts in the developing auditory brainstem. J Comp Neurol 438:336-352.
Pontieri FE, Tanda G, Di Chiara G (1995) 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 92:12304-12308.
Pontieri FE, Tanda G, Orzi F, Di Chiara G (1996) Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. Nature 382:255-257.
Pribilla I, Takagi T, Langosch D, Bormann J, Betz H (1992) The atypical M2 segment of the beta subunit confers picrotoxinin resistance to inhibitory glycine receptor channels. EMBO J 11:4305-4311.
Rage F, Arancibia S, Tapia-Arancibia L (1998) Effect of acute, but not chronic ethanol treatment on somatostatin secretion in rat hypothalamic neurons. Neurosci Lett 245:175-179.
Ramchandani VA, Umhau J, Pavon FJ, Ruiz-Velasco V, Margas W, Sun H, Damadzic R, Eskay R, Schoor M, Thorsell A, Schwandt ML, Sommer WH, George DT, Parsons LH, Herscovitch P, Hommer D, Heilig M (2011) A genetic determinant of the striatal dopamine response to alcohol in men. Mol Psychiatry 16:809-817.
Rassnick S, Pulvirenti L, Koob GF (1992) Oral ethanol self-administration in rats is reduced by the administration of dopamine and glutamate receptor antagonists into the nucleus accumbens. Psychopharmacology (Berl) 109:92-98.

Ray LA, Hutchison KE (2004) A polymorphism of the mu-opioid receptor gene (OPRM1) and sensitivity to the effects of alcohol in humans. Alcohol Clin Exp Res 28:1789-1795.

Rezvani AH, Overstreet DH, Mason GA, Janowsky DS, Hamedi M, Clark E, Jr., Yang Y (2000) Combination pharmacotherapy: a mixture of small doses of naltrexone, fluoxetine, and a thyrotropin-releasing hormone analogue reduces alcohol intake in three strains of alcohol-preferring rats. Alcohol Alcohol 35:76-83.

Richardson HN, Lee SY, O'Dell LE, Koob GF, Rivier CL (2008) Alcohol self-administration acutely stimulates the hypothalamic-pituitary-adrenal axis, but alcohol dependence leads to a dampened neuroendocrine state. Eur J Neurosci 28:1641-1653.

Ripley TL, Stephens DN (2011) Critical thoughts on current rodent models for evaluating potential treatments of alcohol addiction and withdrawal. Br J Pharmacol 164:1335-1356.

Ritter LM, Vazquez DM, Meador-Woodruff JH (2002) Ontogeny of ionotropic glutamate receptor subunit expression in the rat hippocampus. Brain Res Dev Brain Res 139:227-236.

Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ (1987) Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 237:1219-1223.

Roberts DC, Koob GF, Klonoff P, Fibiger HC (1980) Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. Pharmacol Biochem Behav 12:781-787.

Robinson TE, Kolb B (1999) Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. Eur J Neurosci 11:1598-1604.

Roehrs T, Roth T (2001) Sleep, sleepiness, sleep disorders and alcohol use and abuse. Sleep Med Rev 5:287-297.

Roman E, Stewart RB, Bertholomey ML, Jensen ML, Colombo G, Hyytia P, Badia-Elder NE, Grahame NJ, Li TK, Lumeng L (2012) Behavioral profiling of multiple pairs of rats selectively bred for high and low alcohol intake using the MCSF test. Addict Biol 17:33-46.

Russell RN, McBride WJ, Lumeng L, Li TK, Murphy JM (1996) Apomorphine and 7-OH DPAT reduce ethanol intake of P and HAD rats. Alcohol 13:515-519.

Ryabinin AE, Weitemier AZ (2006) The urocortin 1 neurocircuit: ethanol-sensitivity and potential involvement in alcohol consumption. Brain Res Rev 52:368-380.

Ryabinin AE, Yoneyama N, Tanchuck MA, Mark GP, Finn DA (2008) Urocortin 1 microinjection into the mouse lateral septum regulates the acquisition and expression of alcohol consumption. Neuroscience 151:780-790.

Ryan C, Butters N (1980) Learning and memory impairments in young and old alcoholics: evidence for the premature-aging hypothesis. Alcohol Clin Exp Res 4:288-293.

Saigusa T, Fusa K, Okutsu H, Koshikawa N (2001) Monitoring of extracellular dopamine levels in the dorsal striatum and the nucleus accumbens with 5-minute on-line microdialysis in freely moving rats. J Oral Sci 43:129-134.

Sarviharju M, Jaatinen P, Hyytia P, Hervonen A, Kiianmaa K (2001) Effects of lifelong ethanol consumption on drinking behavior and motor impairment of alcohol-preferring AA and alcohol-avoiding ANA rats. Alcohol 23:157-166.

Schmieden V, Kuhse J, Betz H (1992) Agonist pharmacology of neonatal and adult glycine receptor alpha subunits: identification of amino acid residues involved in taurine activation. EMBO J 11:2025-2032.

Schramm-Sapyta NL, Kingsley MA, Rezvani AH, Propst K, Swartzwelder HS, Kuhn CM (2008) Early ethanol consumption predicts relapse-like behavior in adolescent male rats. Alcohol Clin Exp Res 32:754-762.
Schuckit MA (1994) Low level of response to alcohol as a predictor of future alcoholism. Am J Psychiatry 151:184-189.
Schumann G (2007) Okey Lecture 2006: identifying the neurobiological mechanisms of addictive behaviour. Addiction 102:1689-1695.
Schuster CR, Thompson T (1969) Self administration of and behavioral dependence on drugs. Annu Rev Pharmacol 9:483-502.
Sebe JY, Eggers ED, Berger AJ (2003) Differential effects of ethanol on GABA(A) and glycine receptor-mediated synaptic currents in brain stem motoneurons. J Neurophysiol 90:870-875.
Shaham Y, Shalev U, Lu L, De Wit H, Stewart J (2003) The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology (Berl) 168:3-20.
Sheehan TP, Chambers RA, Russell DS (2004) Regulation of affect by the lateral septum: implications for neuropsychiatry. Brain Res Brain Res Rev 46:71-117.
Sher KJ, Grekin ER, Williams NA (2005) The development of alcohol use disorders. Annu Rev Clin Psychol 1:493-523.
Shram MJ, Funk D, Li Z, Le AD (2007) Acute nicotine enhances c-fos mRNA expression differentially in reward-related substrates of adolescent and adult rat brain. Neurosci Lett 418:286-291.
Simonyi A, Christian MR, Sun AY, Sun GY (2004) Chronic ethanol-induced subtype- and subregion-specific decrease in the mRNA expression of metabotropic glutamate receptors in rat hippocampus. Alcohol Clin Exp Res 28:1419-1423.
Sinclair J, Senter R (1967) Increased preference for ethanol in rats following
alcohol deprivation. Psychon Sci 8:11-12.
Singer JH, Talley EM, Bayliss DA, Berger AJ (1998) Development of glycinergic synaptic transmission to rat brain stem motoneurons. J Neurophysiol 80:2608-2620.
Singewald GM, Rjabokon A, Singewald N, Ebner K (2011) The modulatory role of the lateral septum on neuroendocrine and behavioral stress responses. Neuropsychopharmacology 36:793-804.
Slawecki CJ, Betancourt M (2002) Effects of adolescent ethanol exposure on ethanol consumption in adult rats. Alcohol 26:23-30.
Slutske WS, Heath AC, Madden PA, Bucholz KK, Statham DJ, Martin NG (2002) Personality and the genetic risk for alcohol dependence. J Abnorm Psychol 111:124-133.
Snell LD, Tabakoff B, Hoffman PL (1993) Radioligand binding to the N-methyl-D-aspartate receptor/ionophore complex: alterations by ethanol in vitro and by chronic in vivo ethanol ingestion. Brain Res 602:91-98.
Snell LD, Nunley KR, Lickteig RL, Browning MD, Tabakoff B, Hoffman PL (1996) Regional and subunit specific changes in NMDA receptor mRNA and immunoreactivity in mouse brain following chronic ethanol ingestion. Brain Res Mol Brain Res 40:71-78.
Soderpalm B, Lof E, Ericson M (2009) Mechanistic studies of ethanol's interaction with the mesolimbic dopamine reward system. Pharmacopsychiatry 42 Suppl 1:S87-94.
Soderpalm B, Ericson M, Olausson P, Blomqvist O, Engel JA (2000) Nicotinic mechanisms involved in the dopamine activating and reinforcing properties of ethanol. Behav Brain Res 113:85-96.

Solinas M, Scherma M, Tanda G, Wertheim CE, Fratta W, Goldberg SR (2007) Nicotinic facilitation of delta9-tetrahydrocannabinol discrimination involves endogenous anandamide. J Pharmacol Exp Ther 321:1127-1134.

Spanagel R (2009) Alcoholism: a systems approach from molecular physiology to addictive behavior. Physiol Rev 89:649-705.

Spanagel R, Holter SM (1999) Long-term alcohol self-administration with repeated alcohol deprivation phases: an animal model of alcoholism? Alcohol Alcohol 34:231-243.

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

Spanagel R, Heilig M (2005) Addiction and its brain science. Addiction 100:1813-1822.

Spanagel R, Kiefer F (2008) Drugs for relapse prevention of alcoholism: ten years of progress. Trends Pharmacol Sci 29:109-115.

Stefanini E, Frau M, Garau MG, Garau B, Fadda F, Gessa GL (1992) Alcohol-preferring rats have fewer dopamine D2 receptors in the limbic system. Alcohol Alcohol 27:127-130.

Tanda G, Pontieri FE, Di Chiara G (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. Science 276:2048-2050.

Thanos PK, Volkow ND, Freimuth P, Umegaki H, Ikari H, Roth G, Ingram DK, Hitzemann R (2001) Overexpression of dopamine D2 receptors reduces alcohol self-administration. J Neurochem 78:1094-1103.

Thellin O, Zorzi W, Lakaye B, De Borman B, Coumans B, Hennen G, Grisar T, Igout A, Heinen E (1999) Housekeeping genes as internal standards: use and limits. J Biotechnol 75:291-295.

Thomas E, Evans GJ (1983) Septal inhibition of aversive emotional states. Physiol Behav 31:673-678.

Ticku MK (1990) Alcohol and GABA-benzodiazepine receptor function. Ann Med 22:241-246.

Tobler PN, Fiorillo CD, Schultz W (2005) Adaptive coding of reward value by dopamine neurons. Science 307:1642-1645.

Tu GC, Israel Y (1995) Alcohol consumption by orientals in North America is predicted largely by a single gene. Behav Genet 25:59-65.

Tupala E, Tiihonen J (2004) Dopamine and alcoholism: neurobiological basis of ethanol abuse. Prog Neuropsychopharmacol Biol Psychiatry 28:1221-1247.

Tupala E, Hall H, Mantere T, Rasanen P, Sarkioja T, Tiihonen J (2003) Dopamine receptors and transporters in the brain reward circuits of type 1 and 2 alcoholics measured with human whole hemisphere autoradiography. Neuroimage 19:145-155.

Tupala E, Hall H, Bergstrom K, Sarkioja T, Rasanen P, Mantere T, Callaway J, Hiltunen J, Tiihonen J (2001) Dopamine D(2)/D(3)-receptor and transporter densities in nucleus accumbens and amygdala of type 1 and 2 alcoholics. Mol Psychiatry 6:261-267.

Turchan J, Przewlocka B, Toth G, Lason W, Borsodi A, Przewlocki R (1999) The effect of repeated administration of morphine, cocaine and ethanol on mu and delta opioid receptor density in the nucleus accumbens and striatum of the rat. Neuroscience 91:971-977.

Turek VF, Tsivkovskaia NO, Hyytia P, Harding S, Le AD, Ryabinin AE (2005) Urocortin 1 expression in five pairs of rat lines selectively bred for differences in alcohol drinking. Psychopharmacology (Berl) 181:511-517.

Waldvogel HJ, Baer K, Eady E, Allen KL, Gilbert RT, Mohler H, Rees MI, Nicholson LF, Faull RL (2010) 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 518:305-328.
Valenzuela CF (1997) Alcohol and neurotransmitter interactions. Alcohol Health Res World 21:144-148.
Walker BM, Ehlers CL (2009) Age-related differences in the blood alcohol levels of Wistar rats. Pharmacol Biochem Behav 91:560-565.
Wallner M, Olsen RW (2008) Physiology and pharmacology of alcohol: the imidazobenzodiazepine alcohol antagonist site on subtypes of GABAA receptors as an opportunity for drug development? Br J Pharmacol 154:288-298.
Wallner M, Hanchar HJ, Olsen RW (2003) Ethanol enhances alpha 4 beta 3 delta and alpha 6 beta 3 delta gamma-aminobutyric acid type A receptors at low concentrations known to affect humans. Proc Natl Acad Sci U S A 100:15218-15223.
Wallner M, Hanchar HJ, Olsen RW (2006) Low dose acute alcohol effects on GABA A receptor subtypes. Pharmacol Ther 112:513-528.
Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 3:RESEARCH0034.
Wang X, Wang G, Lemos JR, Treistman SN (1994) Ethanol directly modulates gating of a dihydropyridine-sensitive Ca2+ channel in neurohypophysial terminals. J Neurosci 14:5453-5460.
Watanabe E, Akagi H (1995) Distribution patterns of mRNAs encoding glycine receptor channels in the developing rat spinal cord. Neurosci Res 23:377-382.
Wells GB (2008) Structural answers and persistent questions about how nicotinic receptors work. Front Biosci 13:5479-5510.
Vengeliene V, Bilbao A, Molander A, Spanagel R (2008) Neuropharmacology of alcohol addiction. Br J Pharmacol 154:299-315.
Vengeliene V, Celerier E, Chaskiel L, Penzo F, Spanagel R (2009) Compulsive alcohol drinking in rodents. Addict Biol 14:384-396.
Vengeliene V, Leonardi-Essmann F, Sommer WH, Marston HM, Spanagel R (2010) Glycine transporter-1 blockade leads to persistently reduced relapse-like alcohol drinking in rats. Biol Psychiatry 68:704-711.
Vengeliene V, Leonardi-Essmann F, Perreau-Lenz S, Gebicke-Haerter P, Drescher K, Gross G, Spanagel R (2006) The dopamine D3 receptor plays an essential role in alcohol-seeking and relapse. FASEB J 20:2223-2233.
Williams RJ, Berry LJ, Beerstecher E (1949) Individual Metabolic Patterns, Alcoholism, Genetotrophic Diseases. Proc Natl Acad Sci U S A 35:265-271.
Wise RA (1998) Drug-activation of brain reward pathways. Drug Alcohol Depend 51:13-22.
Wise RA (2008) Dopamine and reward: the anhedonia hypothesis 30 years on. Neurotox Res 14:169-183.
Wise RA, Bozarth MA (1987) A psychomotor stimulant theory of addiction. Psychol Rev 94:469-492.
Wise RA, Rompre PP (1989) Brain dopamine and reward. Annu Rev Psychol 40:191-225.
Wolffgramm J (1990) Free choice ethanol intake of laboratory rats under different social conditions. Psychopharmacology (Berl) 101:233-239.
Wolffgramm J, Heyne A (1995) From controlled drug intake to loss of control: the irreversible development of drug addiction in the rat. Behav Brain Res 70:77-94.
Volkow ND, Li TK (2004) Drug addiction: the neurobiology of behaviour gone awry. Nat Rev Neurosci 5:963-970.
Volkow ND, Wang GJ, Fowler JS, Tomasi D (2012) Addiction circuitry in the human brain. Annu Rev Pharmacol Toxicol 52:321-336.

Volkow ND, Fowler JS, Gatley SJ, Logan J, Wang GJ, Ding YS, Dewey S (1996a) PET evaluation of the dopamine system of the human brain. J Nucl Med 37:1242-1256.
Volkow ND, Wang GJ, Fowler JS, Logan J, Hitzemann R, Ding YS, Pappas N, Shea C, Piscani K (1996b) Decreases in dopamine receptors but not in dopamine transporters in alcoholics. Alcohol Clin Exp Res 20:1594-1598.
Volkow ND, Wang GJ, Maynard L, Fowler JS, Jayne B, Telang F, Logan J, Ding YS, Gatley SJ, Hitzemann R, Wong C, Pappas N (2002) Effects of alcohol detoxification on dopamine D2 receptors in alcoholics: a preliminary study. Psychiatry Res 116:163-172.
Woodruff GN, McCarthy PS, Walker RJ (1976) Studies on the pharmacology of neurones in the nucleus accumbens of the rat. Brain Res 115:233-242.
Woodward JJ (2000) Ethanol and NMDA receptor signaling. Crit Rev Neurobiol 14:69-89.
Xiong W, Cheng K, Cui T, Godlewski G, Rice KC, Xu Y, Zhang L (2011) Cannabinoid potentiation of glycine receptors contributes to cannabis-induced analgesia. Nat Chem Biol 7:296-303.
Yang Z, Cromer BA, Harvey RJ, Parker MW, Lynch JW (2007) A proposed structural basis for picrotoxinin and picrotin binding in the glycine receptor pore. J Neurochem 103:580-589.
Yevenes GE, Zeilhofer HU (2011a) Allosteric modulation of glycine receptors. Br J Pharmacol 164:224-236.
Yevenes GE, Zeilhofer HU (2011b) Molecular sites for the positive allosteric modulation of glycine receptors by endocannabinoids. PLoS One 6:e23886.
Yevenes GE, Moraga-Cid G, Peoples RW, Schmalzing G, Aguayo LG (2008) A selective G betagamma-linked intracellular mechanism for modulation of a ligand-gated ion channel by ethanol. Proc Natl Acad Sci U S A 105:20523-20528.
Yevenes GE, Moraga-Cid G, Avila A, Guzman L, Figueroa M, Peoples RW, Aguayo LG (2010) Molecular requirements for ethanol differential allosteric modulation of glycine receptors based on selective Gbetagamma modulation. J Biol Chem 285:30203-30213.
Young AB, Snyder SH (1973) Strychnine binding associated with glycine receptors of the central nervous system. Proc Natl Acad Sci U S A 70:2832-2836.
Young EM, Mahler S, Chi H, de Wit H (2005) Mecamylamine and ethanol preference in healthy volunteers. Alcohol Clin Exp Res 29:58-65.
Zahm DS (1999) Functional-anatomical implications of the nucleus accumbens core and shell subterritories. Ann N Y Acad Sci 877:113-128.
Zappettini S, Mura E, Grilli M, Preda S, Salamone A, Olivero G, Govoni S, Marchi M (2011) Different presynaptic nicotinic receptor subtypes modulate in vivo and in vitro the release of glycine in the rat hippocampus. Neurochem Int 59:729-738.
Zappettini S, Grilli M, Olivero G, Mura E, Preda S, Govoni S, Salamone A, Marchi M (2012) Beta Amyloid Differently Modulate Nicotinic and Muscarinic Receptor Subtypes which Stimulate in vitro and in vivo the Release of Glycine in the Rat Hippocampus. Front Pharmacol 3:146.
Zeise ML, Kasparov S, Capogna M, Zieglgansberger W (1993) Acamprosate (calciumacetylhomotaurinate) decreases postsynaptic potentials in the rat neocortex: possible involvement of excitatory amino acid receptors. Eur J Pharmacol 231:47-52.
Zetterstrom T, Fillenz M (1990) Local administration of flurazepam has different effects on dopamine release in striatum and nucleus accumbens: a microdialysis study. Neuropharmacology 29:129-134.
Zhou FC, McKinzie DL, Patel TD, Lumeng L, Li TK (1998) Additive reduction of alcohol drinking by 5-HT1A antagonist WAY 100635 and serotonin uptake blocker fluoxetine in alcohol-preferring P rats. Alcohol Clin Exp Res 22:266-269.