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CONTRIBUTIONS OF μ- AND κ-OPIOIDERIC SYSTEMS TO ETHANOL INTAKE AND ADDICTION

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CONTRIBUTIONS OF \( \mu \)- AND \( \kappa \)-OPIOIDERIC SYSTEMS TO ETHANOL INTAKE AND ADDICTION

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“Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.”

- Marie Curie -
ABSTRACT

Ethanol use disorders affect a vast number of people worldwide. In some individuals, controlled ethanol intake can gradually progress via ethanol abuse into addiction, characterized by escalated, uncontrolled and compulsive ethanol seeking and intake despite its negative consequences. A negative emotional state is common when ethanol is not available. The relapsing nature of this chronic disease also makes it difficult to treat. As the clinical efficiency of the currently available pharmacotherapies is relatively low, new treatment strategies are needed.

The μ- and κ-opioidergic systems interacting with the brain’s reward pathway have been suggested to be central in controlling ethanol intake. The μ-opioidergic system is attributed to the rewarding and positive reinforcing effects of ethanol while the κ-opioidergic system is attributed to its negative reinforcing effects. It has been suggested that the μ-opioidergic system is more important in controlling ethanol intake while intake is still under control, while the role of the κ-opioidergic system increases as ethanol intake becomes more chronic, compulsive and relapsing.

The main aim of this thesis was to clarify the role of μ- and κ-opioidergic mechanisms in the nucleus accumbens shell, a main brain area of the reward pathway, in controlling intermittent and relapse-like ethanol intake in rats. The used paradigms can roughly be considered to model aspects of ethanol intake before and after addiction has developed. Selective μ- or κ-opioid receptor agonists and antagonists were administered locally into the nucleus accumbens shell and systemic injections were used to elucidate this brain area’s overall role in controlling ethanol intake. These studies were undertaken as there is a gap in the knowledge on how the μ- and κ-opioidergic systems interacting with the nucleus accumbens shell affect ethanol intake and addiction-related behaviors per se.

A high innate μ-opioidergic tone in the nucleus accumbens shell of alcohol-preferring Alko Alcohol (AA) rats has been proposed to account for their high ethanol preference but pharmacological studies are lacking. As local infusions of a selective μ-opioid receptor antagonist increased and agonist tended to decrease intermittent ethanol intake, the results support the notion that nucleus accumbens shell μ-opioidergic mechanisms participate in controlling ethanol intake and reward in AA rats.

The role of nucleus accumbens shell κ-opioidergic mechanisms in controlling intermittent ethanol intake has not been extensively studied. Intra-accumbens shell administration of a selective κ-opioid receptor agonist had no effect but JDTic, a selective κ-opioid receptor antagonist, showed a weak long-term ethanol intake decreasing effect in AA rats. When these results are combined with the long-term decreasing effects shown after systemic JDTic administration, the results suggest that κ-opioid receptors are
indeed able to control intermittent ethanol intake and the nucleus accumbens shell is one site participating in mediating these effects.

The effects of JDTic on relapse-like ethanol intake in Long-Evans rats was examined because of the positive results from the previous study, earlier reports suggesting an increased tone of the accumbal κ-opioidergic system as ethanol addiction evolves and the lack of knowledge on what role the nucleus accumbens shell κ-opioid receptors have in relapse to ethanol intake. Both intra-accumbens shell and systemic JDTic attenuated relapse-like ethanol intake. These results suggest that the κ-opioidergic system interacting at least with the nucleus accumbens shell participates in controlling relapse-like ethanol intake. As the reference drug naltrexone, a non-selective antagonist, administered systemically also inhibited relapse-like ethanol intake, μ- and possibly also δ-opioidergic systems seem to have a role in mediating relapse.

Taken together, these findings suggest that μ- and κ-opioidergic mechanisms are important in controlling intermittent ethanol intake and relapse to ethanol intake and the nucleus accumbens shell is one anatomical site mediating these effects. The results also suggest that selective κ-opioid receptor antagonism could be a feasible treatment strategy for ethanol use disorders.
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ABBREVIATIONS

AA rat  Alko Alcohol rat
ADE      alcohol deprivation effect
ANA rat  Alko Non-Alcohol rat
ANOVA    analysis of variance
cAMP     cyclic adenosine monophosphate
CTOP     D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2
DAMGO    D-Ala2, N-Me-Phe4,Glyol5-enkephalin
GABA     γ-aminobutyric acid
Gi/Go -protein quanine-nucleotide binding regulatory protein
HAD rat  high alcohol-drinking rat
ICV      intracerebroventricular
JDTic    (3R)-7-hydroxy-N-((1S)-1-[(3R,4R)-4-(3-
               hydroxyphenyl)-3,4-dimethyl-1-
               piperidinyl]methyl]-2-methylpropyl)-1,2,3,4-
               tetrahydro-3-isoquinolinecarboxamide
LAD rat  low alcohol-drinking rat
nor-BNI  nor-binaltorphimine
NP rat   ethanol non-prefering rat
6-OHDA   6-hydroxydopamine
P rat    ethanol-prefering rat
sc       subcutaneously
U50488H  (±)-U-50488 hydrochloride
VTA      ventral tegmental area
1 INTRODUCTION

Ethanol is consumed worldwide, which is why ethanol use disorders, comprising of ethanol abuse, addiction and physical dependence, also affect a vast number of people (Heilig and Egli 2006; Herz 1997). Ethanol use disorders are primarily a disease of the brain, especially the reward pathway, even though ethanol itself also severely affects other organs and their functions. As ethanol use disorders are attributed to major health, economic and social burdens, effective pharmacotherapies for its treatment are crucially needed. The development of effective drug treatments, however, relies on the understanding of the neuronal mechanisms that participate in controlling ethanol intake and the development of addiction.

As a distinction from most other substances of abuse, ethanol does not have specific target receptors (Herz 1997). Instead, it is able to interact with various neuromodulators and neurotransmitters, such as dopamine, histamine, glutamate and γ-aminobutyric acid (GABA), and cast its effects via these interactions. Because of the wide range of ethanol’s actions in the brain, despite vast research efforts, it has proven to be difficult to specify the key mechanisms that control ethanol intake and addiction development. The complex nature of the human condition, which is an interplay of various environmental, developmental and genetical factors, also promotes challenges for animal models of ethanol intake and addiction, as all features are not possible to encompass into one model (Hansson et al. 2018; Spanagel 2000).

The interactions of the opioidergic systems with ethanol, especially in the brain’s reward pathway, have been shown to have an important role in mediating ethanol’s rewarding effects (Herz 1997). Currently, two commercially available drugs for the treatment of ethanol use disorders, the non-selective opioid receptor antagonists naltrexone and nalmefene, are based on their actions on the opioidergic systems (Mann et al. 2013; Soyka 2016; Volpicelli et al. 1992). Unfortunately, their clinical efficiency is relatively low, which is why new treatment strategies are urgently needed (Jonas et al. 2014). A deeper understanding of the opioidergic mechanisms that participate in controlling ethanol intake is, however, necessary to achieve this.

Out of the three µ-, δ- and κ-opioidergic systems, the μ- and κ-opioidergic systems have attracted the most attention partly due to their opposite roles in mediating hedonic states (Amalric et al. 1987; Funada et al. 1993; Mucha and Herz 1985; Shippenberg et al. 1987). This bidirectional function is hypothesized to also cast opposite effects on controlling ethanol intake (Koob 2013a; Sirohi et al. 2012). It has also been suggested that the balance in controlling ethanol intake between these two opioidergic systems changes as controlled ethanol intake gradually becomes uncontrolled and compulsive.
The main focus of this thesis was set on examining the contributions of the µ- and κ-opioidergic systems on ethanol intake and addiction, which are also extensively reviewed in the literature review. The experiments were designed to clarify the role of a central brain area of the reward pathway, the nucleus accumbens shell, in controlling intermittent ethanol intake and relapse to ethanol intake, a characteristic of ethanol addiction. To give a general idea of the role of the δ-opioidergic system in controlling ethanol intake, results from systemic δ-opioid receptor agonist and antagonist studies are briefly overviewed in the literature review.
2 REVIEW OF THE LITERATURE

2.1 ETHANOL INTAKE AND ADDICTION

2.1.1 FROM ETHANOL INTAKE TO ADDICTION

Initially ethanol is consumed due to its positive reinforcing and rewarding effects (Herz 1997; Koob 2013a; Sirohi et al. 2012). However, in addition to positive reinforcement, ethanol is known to also cause negative reinforcement. Reward as a term is defined as a hedonic state (Stolerman 1992). Positive reinforcement occurs when the frequency of certain behavior (e.g. operant lever pressing) towards a stimulus (e.g. ethanol solution) increases after the stimulus has been presented. In negative reinforcement, the frequency of a certain behavior (e.g. operant lever pressing for ethanol solution) increases aiming at preventing a stimulus or an event (e.g. aversion caused by ethanol withdrawal) from happening.

Controlled ethanol intake can gradually progress into ethanol abuse, during which the individual starts to experience negative consequences associated with ethanol intake (Heilig and Egli 2006). For subsequent ethanol addiction to develop, occasional and relatively limited use of ethanol has to develop into escalated, uncontrolled and compulsive seeking and intake which is relapsing and chronic in nature (Koob 2013a; Sirohi et al. 2012). A negative emotional state is often present when ethanol is not available. It is, however, important to note that not all individuals who abuse of ethanol become addicted. Further, some, but not all, addicted individuals may also become physically dependent of ethanol, in which the body needs ethanol to function properly. However, psychological dependence to ethanol, which is the hallmark of addiction, is the most difficult to treat (Heilig and Egli 2006; Polich 1981).

Physical ethanol dependence is often accompanied by tolerance to the effects of ethanol, in addition to aversive physical withdrawal symptoms, which immerse when ethanol is not available (Heilig and Egli 2006). Some drugs can induce physical dependence without inducing addiction. However, at least clinically if a patient is physically dependent of ethanol, the patient is also most likely addicted to ethanol. Laboratory animals can, however, be forced into physical ethanol dependence without the development of ethanol addiction.

Signs of physical dependence, such as withdrawal symptoms dissipate relatively quickly, within weeks after abstinence; however, addiction can persist for prolonged periods of time and cause relapse to ethanol intake months and years after the beginning of abstinence (Polich 1981; Sinclair et al. 1973b). It has even been shown that 90% of recovering alcoholics relapse within four years after a treatment period (Polich 1981).
As the transition from controlled to uncontrolled drug intake takes place, changes have been shown to occur in the brain’s reward pathway (Koob 2013a; Sirohi et al. 2012). Simultaneously, it is speculated that the positive reinforcing effects of ethanol decrease and the negative reinforcing effects of ethanol increase as ethanol addiction evolves. Negative reinforcement may even be one of the main factors that fuel ethanol addiction and it is regarded to be accompanied by an increased motivation to crave and thus seek ethanol (Figure 1). It is, however, important to note that even though positive reinforcement decreases, it does not entirely diminish, as severely addicted and physically ethanol dependent individuals also report reward from ethanol (Hansson et al. 2018).

![Graph of interplay between positive and negative reinforcement and motivation to seek ethanol as a function of the progression of ethanol addiction](modified from Hansson et al. 2018; Koob 2013b)

**2.1.2 ANIMAL MODELS OF ETHANOL INTAKE AND ADDICTION**

As the mechanisms mediating ethanol’s reinforcement and reward cannot be studied ethically in human subjects, animal models need to be used. However, as the behaviors that humans express are complex, as are the physiological processes related to the control of ethanol intake and addiction, no one model is able to encapsulate all of the features in itself. Here the most commonly used ethanol intake and addiction models are presented. The models are then discussed in 2.1.3 on how they relate to the progression of ethanol addiction.

**Voluntary ethanol intake paradigms**

In order to study the effects of treatments on maintenance ethanol intake, meaning ethanol intake that has stabilized to a certain level, the laboratory
animals have to first reliably consume ethanol. Several different voluntary ethanol intake paradigms are used in rodents for this purpose, with the most common being continuous, limited and intermittent ethanol access paradigms. In these free-choice based paradigms, rodents are given both water and ethanol solutions which they either consume from bottles or acquire by lever pressing in operant conditions. After stable ethanol intake has been established, the effects of different treatments on ethanol intake can be examined.

Differences exist between the different voluntary ethanol intake paradigms. In continuous ethanol access paradigms, ethanol is constantly available for 24 h per day. In limited ethanol access paradigms ethanol is available daily but only for restricted time periods, e.g. for 4 h per day (Sinclair et al. 1992). The amount of ethanol consumed in these two paradigms is, however, relatively low especially in unselected rodents. Additionally, a sucrose-fade paradigm, in which the amount of sucrose in the solution is gradually decreased while the amount of ethanol is increased, needs to often be used to initiate ethanol intake (Samson 1986).

In the intermittent ethanol access paradigm, ethanol is available generally every other day for 24 h or for shorter time periods (Carnicella et al. 2014; Simms et al. 2008, 2010). It has been shown that rats voluntarily consume higher amounts of ethanol in intermittent than continuous ethanol access paradigms (Simms et al. 2008). Ethanol intake initiation procedures such as sucrose-fade or water deprivation are often also not needed. Sometimes this paradigm is also referred to as a binge drinking model due to its ability to increase ethanol intake and elevate blood ethanol levels relatively quickly once ethanol is accessible (Crabbe et al. 2011).

**Ethanol craving and seeking paradigms**

It has been shown that cues associated with ethanol are able to induce craving and subsequent seeking for ethanol, and ultimately relapse to ethanol intake (Le and Shaham 2002; Spanagel 2000). In animals ethanol seeking can be modeled in operant conditions, in which the animal is first taught to lever press for ethanol, after which responding for the ethanol-associated lever is extinct. During the initial conditioning phase, ethanol availability can be paired with a certain cue (light, sound, smell) or context (environment). During the reinstatement test, the ethanol-associated cue is once again presented and the recovery of responding for the ethanol-associated lever is measured without ethanol being available. Ethanol seeking has also been shown to be triggered by priming injections of ethanol or stress, to which the animal is subjected to before the reinstatement test.

**Ethanol relapse paradigms**

Relapse to ethanol intake after a period of abstinence is common in recovering alcoholics (Polich 1981). In animals relapse-like ethanol intake can be modeled using the alcohol deprivation effect (ADE) paradigm.
(Sinclair and Senter 1967). After a stable ethanol intake baseline has been established, the animals are subjected to a period of ethanol deprivation, after which ethanol is once again re-accessible. Upon ethanol re-access, ethanol intake is transiently increased over baseline intake levels. This phenomenon is referred to as the ADE. Multiple cycles of ethanol access and deprivation have been shown to enhance the ADE, which also persists even after long deprivation periods (Sinclair et al. 1973b; Spanagel 2000; Spanagel et al. 1996). The ADE has not only been shown in rats and monkeys but also in humans (Burish et al. 1981; Sinclair 1971; Sinclair and Senter 1967). This model has been suggested to have high face validity to the human condition as it measures actual ethanol intake after deprivation, as opposed to ethanol reinstatement models that only measure responding for the ethanol-associated lever without the possibility to consume ethanol (Le and Shaham 2002; Meinhardt and Sommer 2015; Samson and Chappell 2001; Sinclair and Senter 1967; Spanagel 2000; Vengeliene et al. 2014).

**Forced ethanol administration paradigms**

Different forced ethanol administration paradigms have been used to be able to study the effects of long-term intermittent exposure to high blood ethanol levels seen in alcoholics and for physical ethanol dependence induction (Meinhardt and Sommer 2015). Such paradigms consist of ethanol being administered systemically or orally, forced ethanol intake via drinking water or as the only source of calories and intermittent ethanol vapor exposure (Goldstein and Pal 1971; Majchrowicz 1975; Meinhardt and Sommer 2015; Rogers et al. 1979; Walker and Koob 2008; Wise 1973). The intermittent ethanol vapor exposure paradigm is nowadays the most widely used.

In the intermittent ethanol vapor exposure paradigm, before rats are exposed to ethanol vapor, they first have to show stable voluntary ethanol intake either by lever-pressing for ethanol or via two-bottle choice ethanol access (Meinhardt and Sommer 2015; Walker and Koob 2008). After this initiation phase, physical ethanol dependence is induced by exposing rats long-term (at least for 4 weeks) to intermittent ethanol vapor, e.g. with ethanol vapor on for 14 h and off for 10 h. This method allows blood ethanol levels to be kept at high target levels when exposed to ethanol vapor. After physical dependence on ethanol has been induced, rats are let once again to self-administer ethanol and the effects of treatments on ethanol intake are tested.

None of these forced ethanol administration paradigms model ethanol addiction *per se*, however, they model aspects of behaviors that are attributed to ethanol dependence and they are also suggested to cause changes in the brain that, to some extent, resemble changes that may also occur in alcoholics (Hansson et al. 2018; Meinhardt and Sommer 2015).
2.1.3 DIFFERENT ANIMAL MODELS IN THE PROGRESSION OF ETHANOL ADDICTION

In humans, ethanol addiction evolves over a long period of time and occasional and limited use of ethanol gradually develops into escalated, uncontrolled and compulsive ethanol seeking and intake (Hansson et al. 2018; Koob 2013a; Meinhardt and Sommer 2015). Therefore, it is more useful to consider the progression of ethanol addiction as a non-linear gradient over time rather than a distinct line stating a time before and after the onset of addiction. However, none of the most commonly used ethanol intake and addiction models entirely mimic the human condition. To be able to make the correct interpretation from the results obtained from a certain paradigm, the paradigm itself should be roughly put onto the timeline of ethanol addiction progression. The consumed amounts of ethanol, the pattern of ethanol intake (stable or escalated), blood ethanol levels, duration of ethanol access or exposure and the purpose of the model should all be considered while doing this (Hansson et al. 2018; Meinhardt and Sommer 2015; Spanagel 2000). A proposal for what stages of ethanol addiction progression the most commonly used ethanol intake and addiction paradigms relate to are shown in Figure 2.

![Figure 2](image)

**Figure 2** Progression of ethanol addiction and to what stage of addiction the most commonly used ethanol intake and addiction models most probably relate to. ADE = alcohol deprivation effect, EA = ethanol access, EtOH = ethanol

All of the ethanol intake and addiction paradigms can be put on the ethanol addiction progression timeline, even though not all paradigms model addiction. The continuous and limited ethanol access paradigms are able to produce stable ethanol intake levels, but since, by definition ethanol addiction is defined as a loss of control rather than just high ethanol intake, the animals in these models are generally not regarded to be addicted, even though prolonged exposure to such conditions may promote addiction to evolve (Spanagel 2000). These maintenance ethanol intake models can, therefore, be considered to model very early stages of ethanol addiction. Because the intermittent ethanol access paradigm increases ethanol intake
levels higher than the continuous ethanol access paradigm (Simms et al. 2008), but ethanol intake levels eventually stabilize, it is sometimes difficult to determine to which stage of ethanol addiction progression it relates to, the very early stages or stages when escalated ethanol intake starts to occur. The implementation of the model (e.g. duration of ethanol access, means of use) largely determines this.

Ethanol craving and seeking, ADE and forced ethanol administration paradigms, such as the intermittent ethanol vapor exposure paradigm, try to encompass different aspects of ethanol addiction and some also try to model features of physical ethanol dependence (Hansson et al. 2018; Meinhardt and Sommer 2015; Spanagel 2000). These paradigms do not, and cannot, model ethanol addiction *per se*. Nevertheless, they can be considered to be models of the situation when ethanol addiction and/or physical dependence have been established. None of the paradigms presented here model severe late stage ethanol addiction accompanied by physical ethanol dependence.

### 2.1.4 SELECTIVELY BRED RAT LINES

Rodent lines selected on the basis of their opposite ethanol preference, meaning ratio of voluntarily consumed ethanol over total fluid intake when given the choice between ethanol and water solutions, have proven to be valuable tools when trying to identify the neural mechanisms underlying ethanol addiction (Sommer et al. 2006). The leading hypothesis in selected rodent lines is that the trait of interest (high or low voluntary ethanol intake) and the alleles promoting it are gradually enriched in the population upon selection. Therefore, detection of neurobiological factors attributing to high ethanol preference is possible by comparing the differences between opposite phenotypes. By using selectively bred rodent lines in different ethanol intake and addiction paradigms, more information on the role the neurobiological factors behind high ethanol preference on a specific ethanol-related behavior can be obtained.

Some of the first rodent lines produced by selective bidirectional outbreeding were the alcohol-preferring Alko Alcohol (AA) and alcohol-avoiding Alko Non-Alcohol (ANA) lines of rats (Eriksson 1968; Sommer et al. 2006). The AA rats voluntarily consume ten-times more ethanol than ANA rats. Other similar rodent lines are the ethanol-preferring (P) and non-preferring (NP) rats (Li et al. 1991, 1993), and the high alcohol-drinking (HAD) and low alcohol-drinking (LAD) rats (Hansen and Spuhler 1984).

### 2.1.5 THE REWARD PATHWAY

Many drugs of abuse, including ethanol, activate the reward pathway, which is an anatomically and functionally connected circuit of brain areas (Koob 1992; Wise 2002). The ventral tegmental area (VTA), nucleus accumbens, ventral pallidum, amygdala, hypothalamus, hippocampus, septal area and
frontal cortex are all brain areas associated with the reward system (Gianoulakis 2004; Koob 1992; Wise 2002). The principal components of the midbrain – forebrain – extrapyramidal circuit are the mesolimbic dopaminergic neurons that project form the VTA to the nucleus accumbens, prefrontal cortex and amygdala. From the nucleus accumbens, the medium spiny neurons project back to the VTA to provide feedback via at least two pathways, either the direct pathway from the nucleus accumbens or the indirect pathway via the ventral pallidum (Smith et al. 2009; Wise 2002; Zahm et al. 1985) (Figure 3). The ventral pallidum is often regarded as the endpoint of the reward pathway.

The accumbal feedback projections have vast implications on the activity of VTA dopaminergic neurons (Hjelmstad et al. 2013; Xia et al. 2011). Through the direct pathway, the accumbens shell GABAergic medium spiny neurons synapse onto non-dopaminergic VTA neurons, some of which are presumably also GABAergic interneurons (Johnson and North 1992; Kalivas et al. 1993; Nauta et al. 1978; Xia et al. 2011). These VTA interneurons in part control the activity of VTA’s dopaminergic output via release of the inhibitory neurotransmitter GABA. Thus, the direct pathway can disinhibit the dopaminergic neurons and increase their firing by decreasing the activity of the interneurons by releasing GABA. Through the indirect pathway, accumbal GABAergic medium spiny neurons first project to the ventral pallidum, which further project GABAergic neurons to the VTA (Haber et al. 1985; Hjelmstad et al. 2013; Kalivas et al. 1993; Wise 2002). These neurons
either target dopaminergic VTA neurons directly or they synapse onto non-

dopaminergic neurons, possibly also the GABAergic interneurons (Hjelmstad

et al. 2013). This enables the pallidal medium spiny neurons to either directly
decrease the firing of VTA dopaminergic neurons or to indirectly increase
dopamine release by disinhibiting the GABAergic interneurons by releasing

The role of dopamine in mediating ethanol’s reward

A common feature for all drugs of abuse is an increase in the release of
dopamine in the nucleus accumbens, an event which is regarded to be central
for mediating reward (Koob 1992). It is also one reason why addiction
research has extensively directed its focus on dopamine and this brain area.
Ethanol has been shown to directly interact with VTA dopaminergic neurons
and increase their firing (Brodie et al. 1990, 1999; Gessa et al. 1985). Also
acute systemic injections (Imperato and Di Chiara 1986; Kiiianmaa et al.
1995), voluntary self-administration (Weiss et al. 1993) and reverse intra-
accumbal microdialysis of ethanol (Tuomainen et al. 2003; Yoshimoto et al.
1992a) all have been shown to increase accumbal dopamine levels.
Accordingly, dopamine antagonists administered both systemically (Files
et al. 1998) and into the nucleus accumbens (Rassnick et al. 1992) were able to
decrease ethanol intake. Indeed, dopamine receptors and presynaptic
dopamine transporters controlling accumbal dopamine clearance are highly
abundant in the nucleus accumbens (Gerfen and Surmeier 2011; Hansson et
al. 2018; Kupchik et al. 2015). The reward-attributed, postsynaptic dopamine
D1 receptors are located both on medium spiny neurons of the direct and
indirect pathways, while the pre- and postsynaptic dopamine D2 receptors
considered to decrease dopamine transmission are abundant on neurons of
the indirect pathway.

Dopamine indisputably participates in mediating the rewarding effects of
ethanol (Koob 1992). However, ethanol may also cast its effects independent
of dopamine. It has been shown in rats with prior ethanol intake experience
that voluntary ethanol intake was not affected when accumbal dopaminergic
nerve terminals were destroyed with 6-hydroxydopamine (6-OHDA) (Fahlke
et al. 1994; Ikemoto et al. 1997; Kiiianmaa et al. 1979; Koistinen et al. 2001;
Rassnick et al. 1993; Shoemaker et al. 2002). Selected rat lines with opposing
ethanol preferences also support mechanisms other than dopamine to be the
major discriminators behind the phenotypes, as accumbal dopamine levels
were increased in a similar manner in both the ethanol-preferring and non-
preferring counterparts after systemic or intra-gastric administration of
ethanol (Kiiianmaa et al. 1995; Nurmi et al. 1996; Yoshimoto et al. 1992b).

The role of opiodergic systems in mediating ethanol’s reward

The opiodergic systems interacting with the reward pathway are considered
to be central mediators of the rewarding effects of ethanol (Herz 1997). They
are not only able to mediate ethanol’s reward by modulating accumbal
dopamine release, but they are also suggested to affect this in a dopamine non-dependent manner (Koob 2013a). Even though accumbal 6-OHDA lesions did not affect ethanol intake, naltrexone, a non-selective opioid receptor antagonist, was able to decrease ethanol intake in these rats (Koistinen et al. 2001). Also the differences in ethanol preferences in selected rodent lines have been suggested to be attributed to their innate differences in the opioidergic systems (further discussed in 2.6) (de Waele et al. 1995; Gianoulakis et al. 1992; Jamensky and Gianoulakis 1997; Marinelli et al. 2000; McBride et al. 1998; Soini et al. 2002). In line with this, opioid receptor antagonists have also been shown to decrease ethanol intake in humans and non-lesioned animals (Barson et al. 2009; Froehlich et al. 1990; Herz 1997; Heyser et al. 2003; Hubbell et al. 1991; Hyytä and Kiiianmaa 2001; Mann et al. 2013; Nealey et al. 2011; Soyka 2016; Volpicelli et al. 1992). It therefore seems that the opioidergic systems, which act as orchestrators of the activity of several different neurotransmitter systems including dopamine, may even be more important than dopamine alone in mediating the rewarding effects of ethanol (Herz 1997; Koistinen et al. 2001; Koob 2013a). The role of the opioidergic systems in mediating the effects of ethanol are extensively discussed in 2.3 and 2.4.

2.2 THE OPIOIDERGIC SYSTEMS

2.2.1 OPIOID RECEPTORS AND THEIR ENDOGENOUS LIGANDS
The brain’s endogenous opioid systems have been shown to participate in mediating pain relief, mood and reward (Gianoulakis 2004). The opioidergic systems were initially discovered in the 1970s during the quest to find an explanation to why opiates, when used long-term, cause addiction (Pert and Snyder 1973; Simon et al. 1973; Terenius 1973). After the discovery of binding sites for opiates in the brain, also opioid receptors and their endogenous ligands were discovered.

Currently, four opioid receptor classes have been indentified and cloned, the \(\mu\)-, \(\delta\)- and \(\kappa\)-opioid receptors and the nociceptin opioid peptide receptor (previously called the opioid receptor-like receptor) (Chen et al. 1993a, 1993b; Evans et al. 1992; Henderson and McKnight 1997; Kieffer et al. 1992; Meunier et al. 1995; Wang et al. 1993; Yasuda et al. 1993). Subtypes of the different opioid receptors have also been proposed (Akil and Watson 1994; Jiang et al. 1991; Negri et al. 1991; Pasternak 1986), however, different genes encoding these subtypes have not been characterized. As opioid receptors are able to regulate their signaling and ligand binding by homo- and/or heterodimerization as well as oligomerization (Cvejic and Devi 1997; George et al. 2000; Jordan and Devi 1999), and postranslational modifications as well as alternative RNA splicing are possible (Pasternak 2001), the different subtypes may be the product of these modifications. The relevancy of these
subtypes is still to be clarified; however, it seems that they may even have an effect on behavior. For example, the δ1-opioid receptor subtype has been proposed to be a δ- and µ-opioid receptor heterodimer, while the δ2-opioid receptor subtype is proposed to be a δ-opioid receptor homodimer (Porreca et al. 1992; van Rijn and Whistler 2009). Their effects on controlling ethanol intake may be different (Mitchell et al. 2014; van Rijn and Whistler 2009).

Opioid receptors belong to the superfamily of the inhibitory acting G-protein-coupled receptors with structurally similar (60% homology) seven-transmembrane helices, which form a helical bundle (Akil et al. 1998; Chen et al. 1993b; Law et al. 2000). When a ligand binds to the extracellular site of the opioid receptor causing conformational changes of the receptor, the intracellular domain interacts with pertussis toxin-sensitive guanine-nucleotide binding regulatory proteins (Gi/Go -proteins), which then interact with effector systems. This results in a decrease in adenylate cyclase, which further decreases intracellular levels of cyclic adenosine monophosphate (cAMP). Additionally, increased potassium efflux hyperpolarizes the cell and a decrease in calcium influx prevents the release of neurotransmitters (Figure 4).

Table 1.  

<table>
<thead>
<tr>
<th>Opioid peptide precursor</th>
<th>Endogenous opioid peptide</th>
<th>Affinity for opioid receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro-enkephalin</td>
<td>[Met]enkephalin, [Leu]enkephalin, Metorphamide</td>
<td>δ &gt;&gt; µ</td>
</tr>
<tr>
<td>Pro-opiomelanocortin</td>
<td>β-endorphin</td>
<td>µ &gt; δ</td>
</tr>
<tr>
<td>Pro-dynorphin</td>
<td>Dynorphin A, dynorphin A(1-8), Dynorphin B, α-Neoendorphin, β-Neoendorphin, [Leu]enkephalin</td>
<td>κ &gt;&gt; µ and δ</td>
</tr>
<tr>
<td>Pro-nociceptin</td>
<td>Nociceptin</td>
<td>NOR</td>
</tr>
</tbody>
</table>

The endogenous ligands that have been identified for the opioid receptors are presented in Table 1. The endogenous opioid peptides are derived from their precursors and synthesized widely throughout the brain, with major sites being in the arcuate nucleus of the hypothalamus and to some extent also the nucleus tractus solitarii (Curran and Watson 1995; Fallon et al. 1985; Gianoulakis 2004). Opioid peptides can then be released at the projection end points of these brain areas, such as the nucleus accumbens and VTA. Opioid peptides can also be synthesized locally, e.g. dynorphin and
enkephalin can be synthesized in neurons of the nucleus accumbens (Curran and Watson 1995). No one opioid peptide binds specifically to a single opioid receptor, instead they show different affinity for the different opioid receptors, as shown in Table 1 (Herz 1997; Janecka et al. 2004). This may be due to a structurally common ligand binding pocket located within the helical bundle (Waldhoer et al. 2004). The highly divergent extracellular loops between the helices partially cover the binding pockets and, in an extent, participate in ligand specificity.

Figure 4  Simplified representation of cellular processes that participate in mediating the inhibitory effects of opioid receptors once activated (modified from Chen et al. 1993b; Law et al. 2000). cAMP = cyclic adenosine monophosphate, GDP = guanosine diphosphate, Gi/Go proteins = pertussis toxin-sensitive guanine-nucleotide binding regulatory proteins, GTP = guanosine triphosphate

### 2.2.2 MODULATION OF OPIOID RECEPTOR SIGNALING

Characteristical of the opiodergic systems are that they are considered to show either very little or no activity during normal physiological conditions (Herz 1997). However, food, stress and pain activate them, as well as drugs which are either able to act as ligands for opioid receptors or release endogenous opioid peptides (Gianoulakis 2004). Opioid receptor agonists can have differing effects on secondary messaging of opioid receptors, depending on whether they affect the receptors acutely or chronically (Waldhoer et al. 2004). Signaling via opioid receptors is modulated at least
by receptor desensitization, endocytosis, downregulation and/or resensitization. In the complex process of desensitization, the opioid receptor activated by a ligand is uncoupled from its G-protein by G-protein-coupled receptor kinases and subsequently by β-arrestin or by other kinases, thus preventing further signaling. This is followed by endocytosis of the receptor into the intracellular compartment, from where it can be recycled back to the membrane for re-activation (resensitization). The receptor may also be degraded, which results in a decrease in ligand binding sites, also called receptor downregulation. Not all opioid receptors are downregulated following endocytosis, e.g. μ-opioid receptors are resensitized while δ-opioid receptors are downregulated (Finn and Whistler 2001; Whistler et al. 2002). Opioid receptors can also modify their signaling by dimerization, which alters ligand binding and receptor trafficking (Cvejic and Devi 1997; George et al. 2000; Jordan and Devi 1999; Waldhoer et al. 2004).

Tolerance to the effects of a chronically administered opioid receptor agonist has been hypothesized to result from a decreased amount of functional receptors available on the cell surface, a result presumably due to desensitization or downregulation (Waldhoer et al. 2004). Tolerance may also be due to cellular adaptive changes, which result in an elevated level of cAMP. This robust and difficultly reversed state is referred to as cAMP superactivation. In this state, animals seem tolerant to the effects of an opioid receptor agonist, which is due to cAMP levels no longer being regulated as effectively by the agonist compared to when the receptor was in its naïve state. At the same time the opioid receptors themselves remain functionally coupled to the Gi/Go-proteins. The same mechanisms responsible for tolerance of opioid receptor agonists may also be involved in cellular processes involved in the development of physical dependence (Bonci and Williams 1997).

2.2.3 THE INTERACTIONS OF THE OPIOIDERIC SYSTEMS WITH THE REWARD PATHWAY

The opioidergic systems can have various effects on the activity of the reward pathway and, thus, also on intake of ethanol and other drugs of abuse (Herz 1997). The inhibitory acting μ-, δ- and κ-opioid receptors are distributed widely around the central brain areas, including the VTA, nucleus accumbens and ventral pallidum (Lahti et al. 1989; Mansour et al. 1987, 1988, 1995, 1996; Mitrovic and Napier 1995; Moskowitz and Goodman 1984) (Figure 5). These brain areas also receive dense opioidergic innervations or the opioid peptides can be synthesized locally, e.g. dynorphin is synthesized in accumbal medium spiny neurons (Al-Hasani et al. 2015; Curran and Watson 1995; Fallon et al. 1985; Gianoulakis 2004). The VTA has a moderate density of μ-opioid receptors, a lesser density of κ-opioid receptors and undetectable or very low density of δ-opioid receptors (Lahti et al. 1989; Mansour et al. 1987, 1988, 1995, 1996; Mitrovic and Napier 1995; Moskowitz and Goodman...
In the nucleus accumbens, however, all three opioid receptors are densely found but distributed in a heterogenous manner. The \( \mu \)-opioid receptors are the most abundant in the ventral pallidum.

**Figure 5**  Simplified diagram of the reward pathway and the distribution of opioid receptors. For references please see text. VTA = ventral tegmental area, NAcc = nucleus accumbens, VP = ventral pallidum, PFC = prefrontal cortex, IN = interneuron

In the VTA, \( \mu \)- and \( \kappa \)-opioid receptors are located in a manner that enables them to oppose each other’s actions on the dopaminergic neurons (Johnson and North 1992; Margolis et al. 2003) (Figure 5). \( \mu \)-Opioid receptors are predominantly found on the GABAergic interneurons, and upon activation they decrease the release of GABA from these neurons, due to which the activity of the dopaminergic neurons is increased. However, as
κ-opioid receptors are found directly on a portion (25%) of dopaminergic neurons, κ-opioid receptors can also directly inhibit them (Margolis et al. 2003). Both μ- and κ-opioid receptors are also found presynaptically on VTA neurons, which also bind serotonin, as well as on glutamatergic neurons (Cameron et al. 1997; Margolis et al. 2003; 2005). δ-Opioid receptors are presumably found on tegmental GABAergic terminals (Margolis et al. 2008). Additionally, the GABAergic feedback neurons projecting to the VTA from either the nucleus accumbens shell (direct pathway) or via the ventral pallidum (indirect pathway) have been shown to express μ-opioid receptors both on their terminals and somas (Hjelmstad et al. 2013; Xia et al. 2011).

In the nucleus accumbens, μ-opioid receptors are predominantly found postsynaptically on GABAergic medium spiny neurons, even though μ-opioid receptors are also found presynaptically on GABAergic terminals (Dilts and Kalivas 1989; Mansour et al. 1995; Svingos et al. 1996; 1997). As accumbens shell κ-opioid receptors are located mainly presynaptically on dopaminergic, GABAergic and glutamatergic nerve terminals, they are not only able to modulate the release of these neurotransmitters but they can also oppose the effects of postsynaptic opioid receptors (Hjelmstad and Fields 2001; 2003; Svingos et al. 1999b, 2001). Indeed, in the nucleus accumbens shell it has been shown that κ-opioid receptor agonists are able to oppose the effects of μ-opioid receptor agonists on dopamine release (Di Chiara and Imperato 1988). Accumbal δ-opioid receptors are mainly found presynaptically on dopaminergic or GABAergic terminals but also on postsynaptic GABAergic neurons (Svingos et al. 1999a).

The ventral pallidum receives GABAergic innervations from the nucleus accumbens, which also co-expresses enkephalin and dynorphin (Reiner and Anderson 1990; Zahm et al. 1985). μ-Opioid receptors are found both pre- and postsynaptically on enkephalin expressing neurons, while δ-opioid receptors are found postsynaptically (Olive et al. 1997). Further, the postsynaptic δ-opioid receptors are predominantly located on neurons that have recurrent projections to the neurotransmitter-releasing neurons, which enable them to act as postsynaptic autoreceptors. The exact localization of pallidal κ-opioid receptors is still unclear.

2.3 OPIOIDERIC SYSTEMS IN ETHANOL INTAKE AND ADDICTION

Ethanol’s rewarding effects are considered to be mediated by the opioidergic systems (Herz 1997). Earlier results show that acute ethanol administration is able to release opioid peptides in the main brain areas of the reward pathway, in the VTA, nucleus accumbens, ventral pallidum and amygdala (Jarjour et al. 2009; Lam et al. 2008; Marinelli et al. 2003, 2005, 2006; Olive et al. 2001). The released opioid peptides could then interact with the local opioid receptors, which further control the activity of the brain area and...
those downstream of it by affecting neurotransmitter release (Herz 1997). This chain of events could then participate in controlling ethanol intake. In line with this, non-selective opioid receptor antagonists, naltrexone, naloxone and nalmefene, have been shown to decrease ethanol intake in a wide variety of paradigms assessing ethanol intake (Barson et al. 2009; Froehlich et al. 1990; Herz 1997; Heyser et al. 2003; Hubbell et al. 1991; Hyytiä and Kiianmaa 2001; Koistinen et al. 2001; Nealey et al. 2011; Shoemaker et al. 2002).

The roles of the different opioidergic systems are considered to vary. The µ- and δ-opioidergic systems have been shown to participate in mediating hedonic states (Amalric et al. 1987; Shippenberg et al. 1987), while the κ-opioidergic system is attributed to mediating anhedonic states (Funada et al. 1993; Mucha and Herz 1985). The bidirectional function of the opioidergic systems may thus also translate to differing roles in controlling ethanol intake (Koob 2013a; Sirohi et al. 2012). It has been proposed that µ- and δ-opioidergic systems may be more important in initiating and maintaining ethanol intake, while the role of the κ-opioidergic system in controlling ethanol intake may increase as ethanol intake becomes more chronic, compulsive and relapsing (Herz 1997; Sirohi et al. 2012; Walker et al. 2012). It is speculated that during controlled ethanol intake, the µ- and κ-opioidergic systems balance the affective states of one another with the aim of maintaining homeostasis. However, following chronic ethanol intake, due to repeated overactivation of the µ-opioidergic system, the hedonic effects are decreased and the anhedonic effects of the κ-opioidergic system are increased with an attempt to regain balance (Figure 6).

![Figure 6](image.png)

**Figure 6** Suggested role of the µ- and κ-opioidergic systems in controlling affective states before and after ethanol addiction has developed. Ethanol’s initial hedonic states mediated by the µ-opioidergic system are balanced by anhedonic states mediated by the κ-opioidergic systems. Once ethanol intake becomes chronic, compulsive and relapsing, in an attempt to regain balance, µ-opioidergic mechanisms are decreased and κ-opioidergic mechanisms are increased (modified from Walker et al. 2012).

As ethanol intake is considered to be initially commenced due to its rewarding effects, the reward-attributed µ-opioidergic system has received most of the attention in ethanol addiction research (Herz 1997). This is partly due to the boom of opioidergic research that began in the 1970s. During this time it was shown that β-endorphin is intracranially self-administered by
rats and monkeys (van Ree et al. 1979). More importantly, focus was set on the μ-opioidergic system because morphine, an opioid receptor agonist with high μ-opioid receptor affinity, and ethanol produce common acute effects and somewhat similar withdrawal syndromes, in addition to both causing addiction and tolerance (Herz 1997). Within the last decade, however, interest for the role of the κ-opioidergic system in controlling ethanol intake has grown, partly due to the need for new treatment strategies for ethanol use disorders (Sirohi et al. 2012).

In this section 2.3, to enable discussion of what role the opioidergic systems have in controlling ethanol intake-related behaviors and to simplify comparison of the results from different studies, the studies have been divided into two categories according to the paradigm used in them and how they relate to the timeline of ethanol addiction progression. They are, thus, divided into models of either earlier or later stages of ethanol addiction. Those categorized into models of earlier stages of ethanol addiction comprise of models in which ethanol addiction has most probably not yet been established but ethanol intake levels have stabilized (maintenance ethanol intake paradigms: mainly continuous, limited and intermittent ethanol access paradigms). Those models categorized into paradigms of later stages of ethanol addiction are considered to model aspects of ethanol addiction and also physical ethanol dependence (mainly intermittent ethanol vapor exposure, ADE and ethanol craving and seeking paradigms). It is important to note that this is a very crude division of the different paradigms, and some paradigms, depending on the implementation of the model, could also be categorized differently. To give a general idea on how non-selective and selective opioid receptor agonists and antagonists affect ethanol intake, only the results from studies using systemic or intracerebroventricular (ICV) routes of administration, which allow the drugs to be spread widely around the body and/or brain, are covered here.

### 2.3.1 OPIOIDERGIC SYSTEMS IN EARLIER STAGES OF ETHANOL ADDICTION

**Effects of non-selective opioid receptor antagonists**

Even though the majority of research has set out to disentangle the exact role of each of the opioidergic systems in controlling ethanol intake, non-selective opioid receptor antagonists have still been the most widely used ligands in different ethanol intake paradigms. Indeed, when the non-selective opioid receptor antagonists naltrexone, nalmefene or naloxone were administered systemically and ICV, voluntary operant responding for ethanol and ethanol intake were decreased in various maintenance ethanol access paradigms (Calleja-Conde et al. 2016; Critcher et al. 1983; Franck et al. 1998; Hyytiä and Kiianmaa 2001; Le et al. 1999; Le et al. 1993; Nestby et al. 1999; Stromberg et al. 1998). Even though these results highlight the importance of the
opioidergic systems in controlling ethanol intake, they cannot give answers to what role each individual opioidergic system has in the matter.

**The μ-opioidergic system**
The μ-opioidergic system has been attributed to the positive reinforcing effects of ethanol (Herz 1997). When selective μ-opioid receptor antagonists naloxonazine, β-funaltrexamine (irreversible antagonist), D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2 (CTOP) or GSK1521498 were administered systemically or ICV, the results are relatively unanimous, as ethanol intake and operant responding for ethanol decreased in intermittent and limited ethanol access paradigms in rats (Giuliano et al. 2015; Honkanen et al. 1996; Hyytiä 1993; Hyytiä and Kiianmaa 2001; Krishnan-Sarin et al. 1998; Stromberg et al. 1998). However, a few reports in similar paradigms in rats and mice state that no effects on ethanol intake were found after systemic β-funaltrexamine or naloxonazine treatment (Franck et al. 1998; Le et al. 1993).

Generally, μ-opioid receptor activation increases ethanol intake (Critcher et al. 1983; Hubbell et al. 1993; Linseman and Harding 1990; Sabino et al. 2007; Sinclair 1974; Sinclair et al. 1973a; Stromberg et al. 1997; Vacca et al. 2002; Wild and Reid 1990; Volpicelli et al. 1991). Systemically and ICV administered morphine, an opioid receptor agonist with more affinity for the μ- than δ-opioid receptor, has been shown to have bidirectional effects on ethanol intake (Critcher et al. 1983; Hubbell et al. 1993; Linseman and Harding 1990; Sinclair 1974; Sinclair et al. 1973a; Stromberg et al. 1997; Vacca et al. 2002; Wild and Reid 1990; Volpicelli et al. 1991). Low doses have been shown to increase ethanol intake while high doses have an opposite effect in various voluntary ethanol access paradigms. Both systemic and ICV administrations of a μ-opioid receptor agonist 14-methoxymetopon have also been shown to have biphasic effects on ethanol intake in a continuous ethanol access paradigm, with an initial decrease followed by an ultimate increase in ethanol intake (Sabino et al. 2007).

Taken together, these studies suggest that μ-opioid receptor activation generally participates in increasing maintenance ethanol intake and ethanol’s rewarding effects, which could be counteracted by selective μ-opioid receptor antagonism. This is further supported by findings that both μ-opioid receptor and β-endorphin knock-out mice consume less ethanol in two-bottle choice and operant paradigms than their wild-type counterparts (Hall et al. 2001; Racz et al. 2008; Roberts et al. 2000). μ-Opioid receptor knock-out mice also exhibited less ethanol-induced reward in the conditioned place preference paradigm (Hall et al. 2001), which is considered to measure the rewarding effects of a drug.

**The κ-opioidergic system**
The κ-opioidergic system has been shown to have various, sometimes even contradictory effects on ethanol intake (Karkhanis et al. 2017; Sirohi et al.
When examining the results of studies assessing these effects, if the ethanol intake history, state of the \( \kappa \)-opioidergic system and timing of \( \kappa \)-opioidergic agent administration are taken into consideration, the results seem to make more sense. The effects of \( \kappa \)-opioid receptor agonists and antagonists on ethanol intake seem to differ depending on whether the drugs are administered while ethanol intake is considered to be under control or after ethanol addiction and/or physical dependence have been established (Anderson et al. 2016; Karkhanis et al. 2017; Lindholm et al. 2001; Mitchell et al. 2005; Rose et al. 2016; Walker et al. 2011). These differences in the effects on ethanol intake relative to ethanol addiction may even be more pronounced for the \( \kappa \)-opioidergic system than for the \( \mu \)-opioidergic system. This is hypothesized to be a reflection of the changes that the progression of ethanol addiction and also possible physical dependence afflicts on the \( \kappa \)-opioidergic system (Karkhanis et al. 2017; Sirohi et al. 2012).

It seems that before ethanol addiction and physical dependence have been established, systemic \( \kappa \)-opioid receptor agonism is able to decrease ethanol intake (Henderson-Redmond and Czachowski 2014; Lindholm et al. 2001; Nestby et al. 1999; Zhou et al. 2017). Systemic administration of the \( \kappa \)-opioid receptor agonists \((\pm)-U-50488\) hydrochloride (U50488H) decreased ethanol intake in a continuous ethanol access paradigm (Nestby et al. 1999), as did Mesyl Salvinorin B dose-dependently in an intermittent ethanol access paradigm, an effect which was reversed by nor-binaltorphimine (nor-BNI) pretreatment (Zhou et al. 2017). Consistently, chronic systemic U50488H dose-dependently decreased ethanol intake in rats in a limited ethanol access paradigm, an effect which was blocked with a selective \( \kappa \)-opioid receptor antagonist nor-BNI (Lindholm et al. 2001). U50488H decreased operant responding for and intake of ethanol in rats, however, the effects were not selective for ethanol as responding for sucrose solution were also decreased (Henderson-Redmond and Czachowski 2014).

In line with the effects of \( \kappa \)-opioid receptor agonism during maintenance ethanol intake, acutely administered U50488H was able to block ethanol-induced conditioned place preference, a measure of ethanol-induced reward, in mice (Logrip et al. 2009). Acutely administered \( \kappa \)-opioid receptor agonists have been shown to cause both taste and conditioned place aversion (Funada et al. 1993; Mucha and Herz 1985; Suzuki et al. 1992). Because of this, it should be determined if the ethanol intake decreasing effects of \( \kappa \)-opioid receptor agonism are due to general aversion or due to effects on mechanisms specifically controlling ethanol intake. Nevertheless, it may be that when \( \kappa \)-opioid receptor agonists are administered during maintenance ethanol intake, the aversive state that they produce may reduce intake of ethanol as well as other drugs of abuse, such as cocaine (Karkhanis et al. 2017).

In contrast to \( \kappa \)-opioid receptor agonist studies, no consistent effects have been shown after systemic \( \kappa \)-opioid receptor antagonist administration during maintenance ethanol intake. In the continuous ethanol access
paradigm, nor-BNI and CERC-501 (previously LY2456302) decreased ethanol intake in mice and rats (Logrip et al. 2008; Rorick-Kehn et al. 2014). However, nor-BNI has also been shown to increase ethanol intake for several days in rats that consumed high amounts of ethanol (Mitchell et al. 2005). Opposite to the results from continuous access paradigms, in the intermittent ethanol access paradigm CERC-501 decreased ethanol intake only of rats which showed an escalated increase in ethanol intake (Domi et al. 2018). In limited ethanol access paradigms, nor-BNI and JDTic have either been shown not to affect (Deehan et al. 2012; Doyon et al. 2006; Lindholm et al. 2001) or to decrease (Schank et al. 2012) operant responding for ethanol. However, in the experiment by Lindholm and co-workers (2001), as nor-BNI was administered only 30 min before ethanol was available, µ-opioid receptor directed effects cannot be ruled out, as systemic nor-BNI has been shown to transiently inhibit µ-opioid receptors for the first few hours after administration (Endoh et al. 1992).

In addition to opioidergic agents, genetic modulation has been used to try to clarify the role of the κ-opioidergic system in controlling ethanol intake (Blednov et al. 2006; Femenia and Manzanares 2012; Kovacs et al. 2005; Racz et al. 2013). However, the results have not been consistent. κ-Opioid receptor and preprodynorphin knock-out mice have been shown to consume less ethanol than their wild-type littermates (Blednov et al. 2006; Kovacs et al. 2005). In contrast, dynorphin and prodynorphin knock-out mice consumed more ethanol than wild-type mice (Femenia and Manzanares 2012; Racz et al. 2013). It is speculated that compensatory changes in the brain, especially of the remaining components of the κ-opioidergic system, are the main reason behind these discrepancies in results and definite conclusions are difficult to make.

The δ-opioidergic system
The role of the δ-opioidergic system in controlling maintenance ethanol intake is still to be established and the results are less consistent than those for the µ-opioidergic system. The systemically or ICV administered δ-opioid receptor antagonists ICI174864, naltrindole, N,N(CH₃)₂Dmt-Tic-OH, SoRI-9410 and naltriben (δ₂ selective) have either been shown not to have an effect on ethanol intake (Honkanen et al. 1996; Hyytiä 1993; Ingman et al. 2003; Stromberg et al. 1998) or to decrease ethanol intake and operant responding for ethanol (Franck et al. 1998; Froehlich et al. 1991; Hyytiä and Kianmaa 2001; June et al. 1999; Krishnan-Sarin et al. 1995a, 1995b; Le et al. 1993; Nielsen et al. 2008; van Rijn and Whistler 2009) (Table 2). The most consistent results on a decrease in ethanol intake have been obtained with the δ₂-selective opioid receptor antagonist naltriben (June et al. 1999; Krishnan-Sarin et al. 1995b; van Rijn and Whistler 2009). The δ₁-selective opioid receptor agonist tan-67 also dose-dependently decreased ethanol intake, while the δ₁-selective antagonist BNTX did not affect ethanol intake in mice (van Rijn and Whistler 2009).
Other strategies have also been used to clarify the role of the δ-opioidergic system in controlling ethanol intake. As enkephalins are degraded relatively quickly (Bayon et al. 1983), enkephalinase inhibitors have been used to potentiate the effects of enkephalins released by ethanol on δ-opioid receptors (Froehlich et al. 1991; George et al. 1991; Jarjour et al. 2009; Lam et al. 2008; Marinelli et al. 2005; Olive et al. 2001). However, both an increase and decrease in ethanol intake have been shown after enkephalinase inhibitor treatment (Froehlich et al. 1991; George et al. 1991). δ-Opioid receptor knock-out mice have also been used, which surprisingly consumed more ethanol than their wild-type littermates (Roberts et al. 2001).

Generally, it seems that δ-opioidergic systems do have a role in controlling ethanol intake. The differing results cannot fully be explained or predicted by the genetic background of the animals, since discrepancies have also been seen within the same selected animal strains (Honkanen et al. 1996; Hyytiä 1993; Hyytiä and Kiijanmaa 2001; Ingman et al. 2003). Nevertheless, it has been suggested that δ-opioid receptors are not central mediators of ethanol-induced reward in AA rats (Honkanen et al. 1996; Hyytiä 1993; Ingman et al. 2003). However, only universal δ-opioid receptor antagonists have been used in ethanol intake experiments in AA rats. Therefore, subtype selective δ-opioid receptor antagonists and agonists should also be tested, as it has been proposed that δ1- and δ2-opioid receptor subtypes have opposite roles in controlling ethanol intake (Mitchell et al. 2014; van Rijn and Whistler 2009). It has even been proposed that either selective δ1-receptor agonism or selective δ2-opioid receptor antagonism or both could be a beneficial strategy for decreasing ethanol intake. However, more research is required to verify this.
Table 2. Effects of systemic δ-opioid receptor antagonists or agonists on ethanol intake. ↑ = increased, ↓ = decreased or ↔ = no effect on ethanol intake.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Effect on ethanol intake</th>
<th>Model</th>
<th>Strain</th>
<th>Region</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Universal naltrindole</td>
<td>↔</td>
<td>intermittent</td>
<td>AA rat</td>
<td>systemic</td>
<td>Honkanen et al. 1996</td>
</tr>
<tr>
<td>δ-antagonists</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>naltrindole</td>
<td>↔</td>
<td>limited</td>
<td>Wistar rat</td>
<td>systemic</td>
<td>Stromberg et al. 1998</td>
</tr>
<tr>
<td>naltrindole</td>
<td>↔</td>
<td>intermittent</td>
<td>Long-Evans rat</td>
<td>systemic</td>
<td>Nielsen et al. 2008</td>
</tr>
<tr>
<td>naltrindole</td>
<td>↓</td>
<td>continuous</td>
<td>Long-Evans rat</td>
<td>systemic</td>
<td>Nielsen et al. 2008</td>
</tr>
<tr>
<td>naltrindole</td>
<td>↓</td>
<td>limited</td>
<td>Sprague-Dawley rat</td>
<td>systemic</td>
<td>Franck et al. 1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>limited, operant</td>
<td>AA rat</td>
<td>systemic</td>
<td>Hyytiä and Kiianmaa 2001</td>
</tr>
<tr>
<td>naltrindole</td>
<td>↓</td>
<td>limited</td>
<td>C57BL/6 mice</td>
<td>systemic</td>
<td>van Rijn and Whistler 2009</td>
</tr>
<tr>
<td>ICI174864</td>
<td>↔</td>
<td>limited</td>
<td>Sprague-Dawley rat</td>
<td>systemic</td>
<td>Franck et al. 1998</td>
</tr>
<tr>
<td>ICI174864</td>
<td>↓</td>
<td>limited</td>
<td>HAD rat</td>
<td>systemic</td>
<td>Froehlich et al. 1991</td>
</tr>
<tr>
<td>ICI174864</td>
<td>↓</td>
<td>intermittent</td>
<td>P rat</td>
<td>systemic</td>
<td>Krishnan-Sarin et al. 1995a</td>
</tr>
<tr>
<td>N,N(CH3)2-Dmt-Tic-OH</td>
<td>↔</td>
<td>intermittent</td>
<td>AA</td>
<td>systemic</td>
<td>Ingman et al. 2003</td>
</tr>
<tr>
<td>SoRI-9409</td>
<td>↓</td>
<td>intermittent</td>
<td>Long-Evans rat</td>
<td>systemic</td>
<td>Nielsen et al. 2008</td>
</tr>
<tr>
<td>SoRI-9410</td>
<td>↓</td>
<td>continuous</td>
<td>Long-Evans rat</td>
<td>systemic</td>
<td>Nielsen et al. 2008</td>
</tr>
<tr>
<td>δ2-antagonist</td>
<td>naltriben</td>
<td>↓</td>
<td>limited, operant</td>
<td>P rat</td>
<td>systemic</td>
</tr>
<tr>
<td></td>
<td>naltriben</td>
<td>↓</td>
<td>limited</td>
<td>P rat</td>
<td>systemic</td>
</tr>
<tr>
<td></td>
<td>naltriben</td>
<td>↓</td>
<td>limited</td>
<td>C57BL/6 mice</td>
<td>systemic</td>
</tr>
<tr>
<td>δ1-antagonist</td>
<td>BNTX</td>
<td>↔</td>
<td>limited</td>
<td>C57BL/6 mice</td>
<td>systemic</td>
</tr>
<tr>
<td>δ1-agonist</td>
<td>tan-67</td>
<td>↓</td>
<td>limited</td>
<td>C57BL/6 mice</td>
<td>systemic</td>
</tr>
</tbody>
</table>
2.3.2 OPIOIDERIC SYSTEMS IN LATER STAGES OF ETHANOL ADDICTION

Effects of non-selective opioid receptor antagonists
Systemically administered non-selective opioid receptor antagonists attenuate ethanol seeking, relapse-like ethanol intake and ethanol intake after forced ethanol exposure relatively unanimously (Burattini et al. 2006; Ciccocioppo et al. 2002, 2003; Harshberger et al. 2016; Heyser et al. 2003; Höltter and Spanagel 1999; Le et al. 1999; Marinelli et al. 2007; Orrico et al. 2014; Walker and Koob 2008; Zhou et al. 2018). Naltrexone has been shown to inhibit the ADE in mice and rats (Heyser et al. 2003; Höltter and Spanagel 1999; Orrico et al. 2014; Zhou et al. 2018). Additionally, naltrexone has been shown to attenuate context-induced, cue-induced, U50488H-induced and ethanol-primed reinstatement of ethanol seeking (Burattini et al. 2006; Ciccocioppo et al. 2002, 2003; Harshberger et al. 2016; Le et al. 1999; Marinelli et al. 2007). Interestingly, stress-induced reinstatement of ethanol seeking was not affected by naltrexone (Le et al. 1999; Liu and Weiss 2002). Oral naltrexone and nalmefene have also been shown to reduce ethanol intake in alcoholics (Mann et al. 2013; Soyka 2016; Volpicelli et al. 1992).

Nalmefene and naltrexone were both able to decrease ethanol intake of rats made physically dependent of ethanol by intermittent ethanol vapor exposure, though ethanol intake was decreased also in ethanol non-dependent animals (Walker and Koob 2008). However, the effects of nalmefene were more pronounced than those of naltrexone in physically ethanol-dependent rats, which was postulated to be due to the higher κ-opioid receptor affinity of nalmefene and the increased κ-opioidergic tone of the animals (Michel et al. 1985; Sirohi et al. 2012; Walker and Koob 2008). In line with this, naltrexone’s ability to attenuate cue-induced reinstatement of ethanol seeking was shown to be reduced in rats that had been exposed to repeated cycles of ethanol vapor and that also showed higher motivation to seek ethanol (Ciccocioppo et al. 2003).

The µ-opioidergic system
It has been suggested that as ethanol addiction and physical dependence develop, the positive reinforcing effects of ethanol decrease but they are not entirely abolished (Hansson et al. 2018; Sirohi et al. 2012). Interestingly, most of the studies examining the effects of the µ-opioidergic system on aspects of ethanol addiction have been conducted with naltrexone, even though naltrexone is not a selective µ-opioid receptor antagonist despite high affinity for µ-opioid receptors (Michel et al. 1985). One contributing factor for this discrepancy may be that some commonly used selective µ-opioid receptor agonists and antagonists, such as D-Ala2, N-Me-Phe4,Glyol5-enkephalin (DAMGO) and CTOP, respectively, do not readily cross the blood-
brain barrier (Van Dorpe et al. 2010), which complicates experimental setups.

Only few studies have been conducted using selective µ-opioid receptor antagonists in paradigms modeling aspects of ethanol addiction. Systemic naloxonazine prevented context-induced reinstatement of ethanol seeking in rats (Ciccocioppo et al. 2002). This is in line with the effects of ICV administered CTOP, which was able to attenuate context-induced but not cue-induced reinstatement of ethanol seeking (Marinelli et al. 2009).

Systemic GSK1521498, a novel µ-opioid receptor antagonist, has been shown to reduce ethanol intake in rats in two novel paradigms assessing either cue-controlled ethanol seeking and subsequent ethanol intake or punishment-resistant ethanol seeking and subsequent ethanol intake (Giuliano et al. 2015, 2018). GSK1521498 was more effective than naltrexone in preventing cue-controlled ethanol seeking and subsequent ethanol intake (Giuliano et al. 2015).

When the results from the selective µ-opioid receptor antagonist studies and those of naltrexone are combined, as is often done in studies addressing µ-opioidergic mechanisms, it seems that µ-opioidergic mechanisms also drive ethanol craving and seeking and, to some extent, relapse-like ethanol intake (Burattini et al. 2006; Ciccocioppo et al. 2002, 2003; Giuliano et al. 2015, 2018; Harshberger et al. 2016; Heyser et al. 2003; Höltér and Spanagel 1999; Le et al. 1999; Marinelli et al. 2007, 2009; Orrico et al. 2014; Walker and Koob 2008; Zhou et al. 2018). This is probably due to ethanol’s acute positive reinforcing effects even though these effects might not be as strong as during maintenance ethanol intake (Ciccocioppo et al. 2003; Walker and Koob 2008). Support for this also comes from clinical studies in alcoholics, which state that naltrexone was the most effective in reducing ethanol intake when paired with ethanol drinking but the effects of naltrexone did not differ from placebo if administered during abstinence (Sinclair 2001). Thus, naltrexone seemed to target the rewarding effects of ethanol with the aim of slowly extincting these effects when used concomitantly. However, not all alcoholics benefit from naltrexone treatment, which may partly be due to reduced reward from ethanol as addiction progresses (Jonas et al. 2014).

**The κ-opioidergic system**

As ethanol addiction and possible physical dependence gradually evolve, the role of the κ-opioidergic system in controlling ethanol intake seems to change and become more pronounced (Karkhanis et al. 2017; Sirohi et al. 2012; Walker et al. 2012). This shift in the κ-opioidergic tone also seems to be reflected on the outcome of different ethanol intake experiments, as the effects of κ-opioid receptor agonists and antagonists on ethanol intake may even turn out opposite to those reported before ethanol addiction or physical dependence have been established (Anderson et al. 2016; Karkhanis et al. 2017; Lindholm et al. 2001; Mitchell et al. 2005; Rose et al. 2016; Walker et al. 2011).
Forced ethanol exposure

Once animals have been exposed to either prolonged time periods of chronic ethanol or physical ethanol dependence has been induced by intermittent ethanol vapor exposure paradigms, κ-opioid receptor antagonists generally decrease ethanol intake (Cashman and Azar 2014; Rose et al. 2016; Sandi et al. 1990; Walker and Koob 2008; Walker et al. 2011). In intermittent ethanol vapor exposure paradigms, ICV or systemic nor-BNI decreased ethanol intake selectively in physically ethanol-dependent rats or mice without affecting intake of non-dependent animals (Rose et al. 2016; Walker and Koob 2008; Walker et al. 2011). However, a new short-acting κ-opioid receptor antagonist, a 6-naltrexamine analog, was able to decrease ethanol intake of both physically ethanol-dependent and non-dependent rats (Cashman and Azar 2014). In contrast to these results, the κ-opioid receptor antagonist LY2444296 did not affect ethanol intake in intermittent ethanol vapor treated mice (Anderson et al. 2016). The authors suspected that the single used dose of the novel drug LY2444296 may not have been high enough to reduce ethanol intake in physically ethanol-dependent mice, even though stress-induced ethanol intake was decreased specifically in these animals with the same dose. They propose a wider dose-range to be tested before definite conclusions are drawn. Additionally, in a forced ethanol intake paradigm the κ-opioid receptor antagonist MR-2266-BS decreased ethanol preference of rats pretreated with forced ethanol intake but not in non-force treated controls (Sandi et al. 1990).

As opposed to studies conducted during maintenance ethanol intake (Henderson-Redmond and Czachowski 2014; Lindholm et al. 2001; Nestby et al. 1999; Zhou et al. 2017), systemic κ-opioid receptor agonism seems to increase ethanol intake after physical ethanol dependence has been induced (Anderson et al. 2016; Rose et al. 2016). U50488H has been shown to increase ethanol intake in mice exposed to intermittent ethanol vapor both in a selective (Rose et al. 2016) and non-selective manner in regard to exposure to ethanol vapor (Anderson et al. 2016). The increase in ethanol intake induced by U50488H was blocked with the κ-opioid receptor antagonist LY2444296 (Anderson et al. 2016).

In conclusion, once physical ethanol dependence has been induced, κ-opioid receptor antagonists generally decrease and κ-opioid receptor agonists increase ethanol intake.

Relapse to ethanol intake

There have only been a few studies assessing the effects of κ-opioid receptor agonists and antagonists on relapse-like ethanol intake and the results are somewhat contradicting (Deehan et al. 2012; Hölter et al. 2000; Zhou et al. 2018). Using the ADE paradigm in rats, chronic treatment with CI-977, a κ-opioid receptor agonist, increased home-cage ethanol intake during re-access to ethanol (Hölter et al. 2000). Interestingly, acute administration of CI-977 decreased operant responding for ethanol during
the first hour of ethanol re-access, but during the whole 23 h operant session, responding for ethanol was increased. However, Zhou and co-workers (2018) have reported a single acute dose of the κ-opioid receptor agonist Mesyl Salvinorin B to prevent the ADE in mice. The authors speculated that Mesyl Salvinorin B may have different cellular properties than classical κ-opioid receptor agonists, which could partly explain the somewhat contradicting results to those reported earlier. It is also possible that the effects of the κ-opioid receptor agonists on the ADE differed in these two experiments because of the very different ethanol pre-exposure times used (Hölter et al. 2000; Zhou et al. 2018), which could also differently affect the tone of the κ-opioidergic system (Karkhanis et al. 2017; Sirohi et al. 2012). While the rats in the experiments by Hölter and co-workers (2000) were pre-exposed to ethanol for 10-18 months before a two week deprivation period, the mice in the experiments of Zhou and co-workers (2018) were first exposed only to three weeks of intermittent ethanol access before a one week deprivation period, after which the ADE was determined.

The effects of κ-opioid receptor antagonism on relapse-like ethanol intake have given somewhat conflicting results (Deehan et al. 2012; Hölter et al. 2000). A single acute systemic dose of JDTic administered 25 days prior to relapse testing decreased operant responding for ethanol in rats (Deehan et al. 2012). However, nor-BNI only tended to decrease lever pressing for ethanol during the first hour of ethanol re-access of a 23 h session (Hölter et al. 2000).

**Ethanol craving and seeking**

Several reports suggest κ-opioid receptor antagonism is able to decrease ethanol relapse-related behaviors (Deehan et al. 2012; Domi et al. 2018; Funk et al. 2014; Rorick-Kehn et al. 2014; Schank et al. 2012). CERC-501 reduced the motivation to lever press and consume ethanol, which was shown in a progressive ratio schedule of reinforcement (Rorick-Kehn et al. 2014). JDTic also reduced operant responding for ethanol in a Pavlovian Spontaneous Recovery paradigm, a model of ethanol-seeking behavior (Deehan et al. 2012). Additionally, JDTic and nor-BNI both blocked ethanol-associated cue-induced reinstatement of ethanol seeking (Funk et al. 2014; Schank et al. 2012) while CERC-501 did not have such effects (Domi et al. 2018).

Ethanol and stress both activate the κ-opioidergic system, increase dynorphin release and induce anxiety-like behaviors (Anderson et al. 2016; Gillett et al. 2013; Jarjour et al. 2009; Karkhanis et al. 2017; Lam et al. 2008; Marinelli et al. 2006; Shirayama et al. 2004; Sperling et al. 2010). Nor-BNI blocked stress and prolonged U50488H pretreatment-induced potentiation of ethanol-conditioned place preference (Sperling et al. 2010) and chronic ethanol exposure induced anxiety-like behaviors (Gillett et al. 2013). Prolonged κ-opioid receptor agonist pretreatment has been shown to mimic stress in potentiating ethanol-induced conditioned place preference and also
in increasing ethanol intake (Sperling et al. 2010). Both JDTic and CERC-501 reduced acute ethanol withdrawal-induced anxiety in rats (Domi et al. 2018; Schank et al. 2012). Also LY2444296 decreased stress-induced ethanol intake in physically ethanol-dependent animals (Anderson et al. 2016). Because of these findings, an interaction between ethanol and stress via the κ-opioidergic system has been proposed. In clinic, stress is also a major factor contributing to relapse to ethanol intake in recovering alcoholics (Sinha et al. 2009). Accordingly, CERC-501 and nor-BNI have been shown to block stress-induced reinstatement of ethanol seeking (Domi et al. 2018; Funk et al. 2014). However, JDTic did not have similar effects (Schank et al. 2012).

Even though less studied, the results of κ-opioid receptor agonists are in line with the results from κ-opioid receptor antagonist studies in ethanol-seeking paradigms. Systemic U50488H was able to induce reinstatement of ethanol-seeking behavior in rats (Funk et al. 2014; Harshberger et al. 2016; Le et al. 2018), which was attenuated by pre-treatment with nor-BNI (Funk et al. 2014; Harshberger et al. 2016). It has also been shown that intermittent ethanol vapor exposed rats are more sensitive to U50488H-induced reinstatement of ethanol seeking than non-vapor exposed controls (Funk et al. 2019).

### 2.4 ETHANOL INTAKE AND ADDICTION: THE INTERACTION OF THE OPIOIDERGIC SYSTEMS WITH DISTINCT PARTS OF THE REWARD PATHWAY

Evidence from systemic administration studies suggests that, as a function of ethanol addiction progression, the role of the µ-opioidergic system in controlling ethanol intake somewhat declines and the role of the κ-opioidergic system increases (Ciccocioppo et al. 2003; Hansson et al. 2018; Karkhanis et al. 2017; Sirohi et al. 2012; Walker and Koob 2008). In other words, anhedonic states seem to increase while hedonic states are decreased. However, while systemic studies give a general idea on how opioidergic agents affect ethanol intake, they do not allow assumptions to be made on the role of different brain areas and their locally acting opioidergic systems in mediating ethanol’s positive and negative reinforcing effects.

Since the brain areas of the reward pathway and opioidergic systems vastly interact (Herz 1997), the role of this interaction in mediating ethanol’s reward and positive and negative reinforcement needs to be examined more closely. The focus here is set on the VTA, nucleus accumbens and the ventral pallidum of the reward pathway. The VTA and nucleus accumbens are of interest, because rats have been shown to self-infuse ethanol into these brain areas, suggesting the local effect to be perceived as rewarding (Engleman et al. 2009; Gatto et al. 1994; Rodd et al. 2004b). The ventral pallidum is of interest because it has been shown to participate in reward-related behaviors.
and it is regarded as the endpoint of the reward pathway (Koob 1992; Smith et al. 2009; Wise 2002; Zahm et al. 1985). The role of these brain areas and their interactions with the \( \mu \)- and \( \kappa \)-opioidergic systems will be discussed both in earlier and later stages of ethanol addiction, as defined in 2.3.

### 2.4.1 EARLIER STAGES OF ETHANOL ADDICTION

**Effects of non-selective opioid receptor antagonists**

Non-selective opioid receptor antagonists have been frequently used to study the role of local opioidergic systems in controlling ethanol intake. During maintenance ethanol intake, intra-VTA and intra-accumbal administrations of non-selective opioid receptor antagonists have given uniform results in decreasing ethanol intake (Heyser et al. 1999; June et al. 2004; Mitchell et al. 2009). Intra-VTA naltrexone decreased ethanol intake in a continuous ethanol access paradigm (Mitchell et al. 2009) and intra-VTA nalmefene reduced operant responding for ethanol in a limited ethanol access paradigm (June et al. 2004). However, the magnitude in reduced ethanol responding was lower and the intra-VTA dose of nalmefene needed was higher than when nalmefene was administered into the nucleus accumbens (June et al. 2004). The authors postulated this to be due to the smaller density of \( \mu \)-opioid receptors found in the VTA as compared to the nucleus accumbens (June et al. 2004; Mansour et al. 1987; McBride et al. 1998). Intra-accumbal administrations of both non-selective methylnaloxonium and naloxone methiodide decreased ethanol intake or operant responding for ethanol also in limited ethanol access paradigms (Barson et al. 2009; Heyser et al. 1999). Interestingly, ethanol-induced conditioned place preference was blocked with intra-VTA but not intra-accumbal infusions of methylnaloxonium (Bechtholt and Cunningham 2005).

As intra-ventral pallidal naloxone has been shown to produce conditioned place aversion (Skoubis and Maidment 2003), it could be assumed that local opioid receptor antagonism would decrease ethanol intake in this brain area. However, naltrexone administered into the ventral pallidum did not affect ethanol intake in a limited ethanol access paradigm (Kemppainen et al. 2012b).

**The \( \mu \)-opioidergic system**

Both the nucleus accumbens and the VTA have been suggested to mediate ethanol’s rewarding effects as acute ethanol administration increased \( \beta \)-endorphin levels in both of these brain areas (Jarjour et al. 2009; Marinelli et al. 2003; Olive et al. 2001). Additionally, rats voluntarily self-infuse ethanol into these sites (Engleman et al. 2009; Gatto et al. 1994; Rodd et al. 2004b). Intra-VTA infusions of \( \mu \)-opioid receptor agonists morphine and DAMGO have also been shown to produce conditioned place preference, suggesting that the VTA’s \( \mu \)-opioidergic mechanisms mediate reward (Bals-Kubik et al.
1993; Olmstead and Franklin 1997; Phillips and LePiane 1980). Surprisingly, μ-opioid receptor ligands, such as endomorphin-1, endomorphin-2, DAMGO and morphine have yielded mixed results after intra-accumbal infusions, as they either produced conditioned place preference or conditioned place aversion or had no effect (Bals-Kubik et al. 1993; Olmstead and Franklin 1997; Terashvili et al. 2004; van der Kooy et al. 1982). Intra-accumbally administered selective μ-opioid receptor antagonists CTOP and CTAP either had no effect or, at high CTOP doses, produced conditioned place aversion (Soderman and Unterwald 2008; Terashvili et al. 2004).

The effects of intra-VTA and intra-accumbal administrations of μ-opioidergic agents on ethanol intake are generally in line with the results from systemic administration studies stating that μ-opioid receptor agonists increase and antagonists decrease maintenance ethanol intake (Critcher et al. 1983; Giuliano et al. 2015; Honkanen et al. 1996; Hubbell et al. 1993; Hyytiä 1993; Hyytiä and Kihanmaa 2001; Krishnan-Sarin et al. 1998; Linseman and Harding 1990; Sabino et al. 2007; Sinclair 1974; Sinclair et al. 1973a; Stromberg et al. 1997, 1998; Vacca et al. 2002; Wild and Reid 1990; Volpicelli et al. 1991). In a continuous ethanol access paradigm, intra-VTA administration of the μ-opioid receptor agonist DAMGO had no effect, but the μ-opioid receptor antagonist CTOP reduced ethanol intake in Lewis rats (Margolis et al. 2008). Yet in Wistar rats, intra-VTA CTOP did not affect operant responding for ethanol (Hyytiä and Kihanmaa 2001). Seemingly in line with the former study, local downregulation of VTA μ-opioid receptors by RNA interference decreased ethanol intake in mice (Lasek et al. 2007).

At the level of the nucleus accumbens, intra-accumbally administered morphine increased ethanol intake, while DAMGO has either been shown not to have an effect (Barson et al. 2009) or to increase ethanol intake and operant responding for ethanol in a limited ethanol access paradigm (Richard and Fields 2016; Zhang and Kelley 2002). Intra-accumbally infused CTOP has been shown not to effect operant ethanol self-administration (Hyytiä and Kihanmaa 2001). However, ethanol intake decreased after intra-accumbal infusions of antisense oligonucleotides, which selectively downregulated local μ-opioid receptors (Myers and Robinson 1999). Taken together, both the VTA and nucleus accumbens seem to centrally mediate ethanol’s rewarding effects via μ-opioidergic interactions.

The ventral pallidum is often considered as a convergent point for hedonic and motivational signaling and it is also implicated in mediating the reinforcing effects of drugs of abuse (Koob 1992; Smith et al. 2009). Additionally, the ventral pallidum expresses opioid receptors and receives opioidergic innervations also from the nucleus accumbens (Lahti et al. 1989; Moskowitz and Goodman 1984; Zahm et al. 1985). Due to its location downstream from the nucleus accumbens, accumbal μ-opioid receptor activation by ethanol-evoked β-endorphin could result also in an increased activation of the ventral pallidum due to inhibition of GABA release from the medium spiny neurons (Kemppainen et al. 2010; Marinelli et al. 2003).
Opioidergic mechanisms within the ventral pallidum could possibly also trigger changes to ethanol intake independently (Smith et al. 2009). Only one group has addressed the role of the ventral pallidum in controlling maintenance ethanol intake (Kemppainen et al. 2012a, 2012b). In a limited ethanol access paradigm, intra-ventral pallidal infusions of DAMGO and morphine both suppressed ethanol intake whereas CTOP increased ethanol intake, suggesting that pallidal μ-opioidergic mechanisms indeed are able to affect ethanol consumption. Somewhat surprisingly, intra-ventral pallidal infusions of CTOP have also been shown to produce conditioned place aversion while morphine had no effect (Olmstead and Franklin 1997; Skoubis and Maidment 2003). Since the ventral pallidum has been shown to poses hedonic hotspots, the site of infusions could significantly affect the results (Smith and Berridge 2005).

**The κ-opioidergic system**

The effects of intra-VTA administrations of selective κ-opioid receptor agonists and antagonists on maintenance ethanol intake have to date not been studied. However, it has been shown that acute intra-VTA infusions of κ-opioid receptor agonists cause conditioned place aversion (Bals-Kubik et al. 1993). This seems logical as it has been shown that subsets (25%) of dopaminergic neurons within the VTA are sensitive to κ-agonism, thus resulting in a direct inhibition of dopaminergic firing upon activation (Margolis et al. 2003). Contrasting these findings, VTA κ-opioid receptor agonist induced changes in dopamine levels in the nucleus accumbens were not shown in anesthetized animals (Spanagel et al. 1992). However, it is not clear if VTA dopaminergic neurons fire spontaneously under anesthesia and the firing may significantly differ from awake and behaving animals (Margolis et al. 2003). Conditioned place preference mediated by the VTA-nucleus accumbens-axis is generally considered to be attributed to changes in the release of accumbal dopamine as 6-OHDA lesions of dopaminergic neurons prevented systemic κ-opioid receptor agonist-induced conditioned place aversion (Bals-Kubik et al. 1993; Shippenberg et al. 1993). Therefore, as acute administration of ethanol has been shown to increase dynorphin release in the VTA (Jarjour et al. 2009), this released dynorphin could to some extent directly activate postsynaptic κ-opioid receptors and decrease dopaminergic firing to the nucleus accumbens (Margolis et al. 2003), and ultimately also affect ethanol intake. This may also be one of the possible mechanism by which systemic κ-opioid receptor agonists are able to decrease maintenance ethanol intake (Henderson-Redmond and Czachowski 2014; Lindholm et al. 2001; Nestby et al. 1999; Zhou et al. 2017). However, ethanol intake studies using intra-VTA administrations of κ-opioidergic agents need to verify this hypothesis.

In the nucleus accumbens, acute systemic administrations of high doses of ethanol have been shown to increase dynorphin levels (Marinelli et al. 2006). However, acute intra-accumbal administration of U50488H did not
affect ethanol intake in a limited ethanol access paradigm (Barson et al. 2009), even though intra-accumbal administration of U50488H has been shown to cause conditioned place aversion (Bals-Kubik et al. 1993). When the nucleus accumbens was perfused with κ-opioid receptor agonists, dialysate dopamine concentrations have been shown to decrease, an effect which was blocked by systemic pre-treatment with nor-BNI (Doyon et al. 2006; Spanagel et al. 1992). Systemic nor-BNI has also been shown to cause a latent increase in accumbal dialysate dopamine levels (Doyon et al. 2006). These effects are logical considering the mainly presynaptic localization of κ-opioid receptors on dopaminergic neuron terminals within the nucleus accumbens (Svingos et al. 1999b, 2001). Nevertheless, it does seem that even though the κ-opioidergic mechanisms within the nucleus accumbens provide a route by which systemic κ-opioid receptor agonists decrease maintenance ethanol intake (Henderson-Redmond and Czachowski 2014; Lindholm et al. 2001; Nestby et al. 1999; Zhou et al. 2017), the local effects of κ-opioidergic drugs do not seem pronounced enough to be able to actually affect maintenance ethanol intake on their own (Barson et al. 2009).

The effects of κ-opioidergic mechanisms within the ventral pallidum on ethanol intake have received very little attention. One study showed that acute intra-ventral pallidal administrations of either U50488H or nor-BNI had no effects on ethanol intake in a limited ethanol access paradigm (Kemppainen et al. 2012b). The transient μ-opioidergic effects of nor-BNI may, however, have affected the results (Endoh et al. 1992). Nevertheless, these results cannot exclusively state that ventral pallidal κ-opioidergic mechanisms do not have any role in controlling maintenance ethanol intake. However, it might be that while acting alone the local κ-opioidergic mechanisms are not pronounced enough to change maintenance ethanol intake. More research is needed to verify this.

2.4.2 LATER STAGES OF ETHANOL ADDICTION

Effects of non-selective opioid receptor antagonists

Studies are few using non-selective opioid receptor antagonists to target the central brain areas of the reward pathway once ethanol addiction and/or physical dependence have developed. Intra-accumbens shell administered naltrexone has been shown to decrease ethanol intake in both physically ethanol-dependent and non-dependent rats after intermittent ethanol vapor exposure (Nealey et al. 2011). However, the effects of naltrexone were more pronounced in the physically ethanol-dependent animals. This may be due to the high κ-opioid receptor affinity of naltrexone and the suggested increased κ-opioidergic activity of the nucleus accumbens after exposure to intermittent ethanol vapor (Gulya et al. 1993; Karkhanis et al. 2016; Lindholm et al. 2000; Michel et al. 1985; Przewlocka et al. 1997; Rose et al. 2016; Siciliano et al. 2015; Walker and Koob 2008). These results also mirror
those from systemic administration studies with nalmefene and naltrexone using a similar study designs (Walker and Koob 2008). In alcoholics, naltrexone was also able to reduce ethanol cue-induced activation of the ventral striatum, as shown by functional brain imaging (Myrick et al. 2008).

**The µ-opioidergic system**

It is still largely unclear how chronic ethanol exposure affects the local µ-opioidergic systems. Most of the studies addressing the matter have concentrated on µ-opioid receptor binding or functional coupling in central brain areas of the reward pathway, and the results are highly contradictory (Hansson et al. 2018). Prolonged ethanol exposure seems to cause oscillations in µ-opioid receptor binding sites, which causes discrepancies in the results of studies addressing the issue (Hansson et al. 2018). When µ-opioid receptor binding or functional coupling were tested immediately after chronic ethanol exposure, either a decrease or no change in the nucleus accumbens (Chen and Lawrence 2000; Djouma and Lawrence 2002; He and Whistler 2011; Leriche and Mendez 2010; Turchan et al. 1999) or no change in the VTA (Djouma and Lawrence 2002; Leriche and Mendez 2010) µ-opioid receptor binding or coupling have been reported. Similarly during deprivation, accumbal and VTA µ-opioid receptor binding or functional coupling either increased, decreased or it did not change (Chen and Lawrence 2000; Djouma and Lawrence 2002; Sim-Selley et al. 2002; Turchan et al. 1999).

In the ventral striatum of deceased alcoholics, however, µ-opioid receptor binding sites have been shown to be decreased (Hermann et al. 2017). It is worth noting that these samples might represent severe cases of ethanol addiction accompanied with physical dependence and, thus, the results might not apply to less severe cases. In support of a reduction in µ-opioidergic function after chronic ethanol intake, naltrexone has been shown to be able to restore nucleus accumbens shell µ-opioid receptor binding after chronic ethanol intake had reduced it (Oliva and Manzanares 2007). Interestingly though, increased ventral striatal density of µ-opioid receptors has been shown in abstinent alcoholics, which also correlated with an increase in ethanol craving (Heinz et al. 2005). In accordance, patients that showed high ethanol-associated cue reactivity in the ventral striatum before treatment were shown to respond favorably to naltrexone, which might reflect the ability of naltrexone to be able to attenuate the acute positive effects of ethanol via the µ-opioidergic system also in addicted individuals (Mann et al. 2014). However, as no clear pattern of accumbal µ-opioid receptor density or functional coupling has been established after either chronic ethanol intake or during deprivation, it is possible that individual differences account for a high degree of variability in the results (Hansson et al. 2018). Additionally, individual variation in µ-opioid receptor activity may also in part account for the high variation in the effects of naltrexone in recovering alcoholics (Hansson et al. 2018; Jonas et al. 2014).
Only a few studies have addressed the role of local \( \mu \)-opioidergic systems in paradigms addressing aspects of ethanol addiction. Context-induced, but not ethanol primed, reinstatement of ethanol seeking was blocked by intra-accumbens shell infusions of the \( \mu \)-opioid receptor antagonist CTAP in Long-Evans rats (Perry and McNally 2013a). This is in line with ICV administered CTOP which also attenuated context-induced reinstatement of ethanol seeking (Marinelli et al. 2009), suggesting the nucleus accumbens shell to mediate some of these \( \mu \)-opioid receptor-mediated effects. Additionally, intra-accumbens shell infused DAMGO potentiated cue-induce reinstatement of ethanol seeking but it did not reinsert ethanol seeking on its own in the absence of ethanol-associated cues in Long-Evans rats (Richard and Fields 2016). Interestingly, intra-ventral pallidal infusions of CTAP, however, prevented both context-induced and ethanol primed reinstatement of ethanol seeking in Long-Evans rats (Perry and McNally 2013b).

**The \( \kappa \)-opioidergic system**

As ethanol addiction evolves, changes in the \( \kappa \)-opioidergic systems are seen locally in relevant brain areas of the reward tract (Gulya et al. 1993; Karkhanis et al. 2016; Lindholm et al. 2000; Przewlocka et al. 1997; Rose et al. 2016; Siciliano et al. 2015; Walker and Koob 2008). Further, these changes seem to affect ethanol intake (Nealey et al. 2011; Walker and Koob 2008).

The effects of the interactions of the \( \kappa \)-opioidergic system in the VTA on ethanol intake behavior have not been extensively studied in paradigms modeling aspects of ethanol addiction or physical dependence and no local VTA-infusion studies are available. In the VTA, \( \kappa \)-opioid receptor mRNA levels have been shown to be downregulated following repeated ethanol administrations (Rosin et al. 1999). Also a non-significant tendency to decrease VTA dynorphin B concentrations has been reported during acute withdrawal 30 min after repeated ethanol administrations has ceased but not at later time points (Lindholm et al. 2000). Unfortunately, too little data are available to make any clear conclusions on the effects of these local changes on ethanol intake.

Intra-accumbens shell infusions of nor-BNI were able to reduce ethanol intake in rats exposed to an intermittent ethanol vapor paradigm (Nealey et al. 2011). The effect was specific to physically ethanol-dependent rats, as nor-BNI had no effect on ethanol intake in air exposed animals, unlike intra-accumbal nalmefone, which decreased ethanol intake in both groups. These results are similar to those reported after systemic and ICV nor-BNI administrations on decreasing ethanol intake specifically in physically ethanol-dependent animals (Rose et al. 2016; Walker and Koob 2008; Walker et al. 2011). The results are also seemingly in line with the ability of systemic \( \kappa \)-opioid receptor antagonists to decrease a variety of other ethanol-related behaviors attributed to ethanol addiction (Cashman and Azar 2014;
Increased activity of the accumbal κ-opioidergic system has been suggested following repeated and prolonged exposure to ethanol as well as withdrawal (Gulya et al. 1993; Karkhanis et al. 2016; Lindholm et al. 2000, 2007; Przewlocka et al. 1997; Rose et al. 2016; Siciliano et al. 2015). The function of accumbal κ-opioid receptors has been suggested to increase after prolonged ethanol and intermittent ethanol vapor exposure, suggesting increased sensitivity of the receptors (Karkhanis et al. 2016; Rose et al. 2016; Siciliano et al. 2015). This is supported by findings that U50488H decreased accumbal dopamine release more in physically ethanol-dependent animals than in non-dependent controls. Additionally, after repeated ethanol administrations, accumbens shell dynorphin B levels were increased up to 21 days after ethanol withdrawal (Lindholm et al. 2000). Accumbal prodynorphin mRNA levels have also been shown to be elevated up to 48 h after withdrawal from forced ethanol intake (Gulya et al. 1993; Przewlocka et al. 1997). Interestingly, chronic ethanol administration has also been shown to downregulate κ-opioid receptor mRNA levels (Rosin et al. 1999), an effect that was suspected to be due to an adaptive response to increased levels of dynorphin (Lindholm et al. 2000).

2.5 NEURONAL MECHANISMS CONTROLLING ETHANOL INTAKE

2.5.1 THE µ-OPIOIDERGIC SYSTEM

The µ-opioidergic mechanisms affecting maintenance ethanol intake have been immensely studied but many uncertainties remain. According to the most common theory, ethanol’s reward is mediated via the dopaminergic neurons that project from the VTA to the nucleus accumbens (Herz 1997; Spanagel et al. 1992; Xiao et al. 2007) (Figure 5). At the level of the VTA, ethanol is able to release β-endorphin, which can bind to the µ-opioid receptors on GABAergic interneurons (Jarjour et al. 2009; Johnson and North 1992; Margolis et al. 2003). Upon µ-opioid receptor activation, GABA release is decreased, which disinhibits the dopaminergic neuron and increases its firing to the nucleus accumbens. An increase and decrease in accumbal dopamine levels has also been shown after intra-VTA infusions of either the µ-opioid receptor agonist DAGO or antagonist CTOP, respectively (Spanagel et al. 1992). The application of the µ-opioid receptor agonist DAMGO to VTA containing brain slices in patch-clamp studies has also been shown to have the same effect on increasing dopamine firing as ethanol (Xiao et al. 2007).

Another pathway suggested to contribute to increasing accumbal dopamine levels and drug reinforcement is the activation of µ-opioid
receptors on the direct pathway of the nucleus accumbens shell which synapse on the GABAergic interneurons in the VTA (Cui et al. 2014; Xia et al. 2011) (Figure 5). Upon activation of μ-opioid receptors on these GABAergic medium spiny neurons, the VTA interneurons are disinhibited, which would ostensibly lead to a decrease in dopamine release (Cui et al. 2014; Kalivas et al. 1993; Nauta et al. 1978). However, it has been suggested that dopaminergic neurons in the VTA are controlled in a multilayer manner, in which μ-opioidergic mechanisms in the VTA have a more dominant role over accumbal μ-opioidergic mechanisms, which is why activation of the direct pathway ultimately results in increasing dopamine levels (Cui et al. 2014).

An increase in accumbal dopamine and, further, its interactions with dopaminergic receptors has been suggested to be sufficient to produce reward and increase ethanol intake (Koob 1992). However, it has also been proposed that ethanol can mediate reward in a dopamine non-dependent manner (Fahlke et al. 1994; Ikemoto et al. 1997; Kiianmaa et al. 1979; Koistinen et al. 2001; Rassnick et al. 1993; Shoemaker et al. 2002). One proposed pathway is downstream of the nucleus accumbens (Smith et al. 2009; Wise 2002; Zahm et al. 1985). Ethanol increases the release of β-endorphin in the nucleus accumbens which can bind to the postsynaptic μ-opioid receptors on GABAergic medium spiny neurons (Marinelli et al. 2003; Svingos et al. 1996). This results in activation of the ventral pallidum due to a decrease in local GABA and enkephalin release (Kemppainen et al. 2010; Zahm et al. 1985). As activation of the ventral pallidum is implicated in an increase in the reinforcing properties of drugs of abuse (Wise 2002), this could also result in an increase in ethanol intake. Indeed, intra-pallidal administrations of GABA receptor antagonists have been shown to increase and agonists to decrease ethanol intake (Kemppainen et al. 2012a). Furthermore, intra-pallidal administrations of DAMGO and CTOP were able to modulate ethanol intake in rats (Kemppainen et al. 2012a, 2012b). The exact pre- or postsynaptic site on which μ-opioid receptor ligands act to affect ethanol intake remains to be established.

Support for a non-dopaminergic or non-VTA-centric view has recently been shown in a study in which μ-opioid receptors were conditionally knocked out from GABAergic forebrain neurons (including the nucleus accumbens and ventral pallidum) while leaving midbrain (including VTA) μ-opioid receptors intact (Ben Hamida et al. 2019). In these mice, ethanol intake was reduced and ethanol-induced conditioned place preference was not shown, paralleling the results of total μ-opioid receptor knockout mice when their results were compared to wild type littermates. These results show in a very elaborate way the relevance of forebrain μ-opioidergic mechanisms in mediating ethanol reward.

It seems that the μ-opioidergic mechanisms controlling maintenance ethanol intake function basically in a similar manner also after ethanol addiction has developed (Hansson et al. 2018). However, as it has been shown that μ-opioid receptor binding sites decrease in at least severe cases...
alcoholics (Hermann et al. 2017), it may be that the rewarding effects of ethanol are substantially also decreased. Since the amount of accumbal μ-opioid receptor binding sites, and possibly also opioid peptide levels, seem to fluctuate after ethanol withdrawal following prolonged ethanol intake (Hansson et al. 2018), it is difficult to make concise conclusions. Nevertheless, it does seem that the role of positive reinforcement as a driving force of excess ethanol consumption somewhat decreases as ethanol addiction evolves. This could also explain why many alcoholics do not benefit from naltrexone treatment (Jonas et al. 2014).

2.5.2 THE κ-OPIOIDERIC SYSTEM

The neuronal mechanisms by which the κ-opioidergic system is able to affect ethanol intake during the course of maintenance ethanol intake to addiction and physical dependence are still largely unclear. The discussion has extensively centered on how κ-opioidergic mechanisms affect the activity of mesolimbic dopamine neurons projecting to the nucleus accumbens (Karkhanis et al. 2016, 2017; Rose et al. 2016; Sirohi et al. 2012; Walker et al. 2012). Even though the κ-opioidergic system is attributed to mediating stress and negative reinforcement also via the extended amygdala, a neurocircuitry of which the nucleus accumbens is also part of, and projections from this circuitry influence the activity of the nucleus accumbens (Alheid and Heimer 1988; Koob 2013a; Walker et al. 2012), here the discussion will focus on mechanisms related to the reward pathway.

Dynorphin can be released from dynorphinergic projection neurons or it can be locally synthesized in a subset of accumbal medium spiny neurons which co-express the dopamine D1 receptor (Al-Hasani et al. 2015; Curran and Watson 1995; Gianoulakis 2004). Ethanol has been shown to increase accumbal dynorphin levels possibly by activation of local dopamine D1 receptors by ethanol-evoked dopamine (Al-Hasani et al. 2015; Curran and Watson 1995; Imperato and Di Chiara 1986; Karkhanis et al. 2017; Kiianmaa et al. 1995; Marinelli et al. 2006). The released dynorphin is then able to decrease further release of dopamine from dopaminergic neurons by binding to presynaptic κ-opioid receptors (Svingos et al. 1999b, 2001) (Figure 5). At the level of the VTA, ethanol-evoked release of dynorphin could directly bind to κ-opioid receptors located on dopaminergic neurons and, as a result, also further decrease the release of dopamine into the nucleus accumbens (Jarjour et al. 2009; Margolis et al. 2003). The role of the κ-opioidergic mechanisms in the ventral pallidum in controlling maintenance ethanol intake is to date uncertain (Kemppainen et al. 2012b). Nevertheless, during maintenance ethanol intake when ethanol intake is not yet out of control, the increase in VTA and accumbal dynorphin and decrease in accumbal dopamine release would be part of a homeostasis mechanism aiming at keeping ethanol intake under control (Karkhanis et al. 2017).
As ethanol addiction and possible physical dependence develop, it has been suggested that the κ-opioidergic system becomes overactivated (Gulya et al. 1993; Karkhanis et al. 2016, 2017; Lindholm et al. 2000; Przewlocka et al. 1997; Rose et al. 2016; Siciliano et al. 2015; Walker and Koob 2008). Once addiction and physical dependence have been established, dynorphin is presumably released by the same mechanisms as during controlled ethanol intake. However, the amount of dynorphin released may be greater and, as the function of accumbal κ-opioid receptors have been shown to increase following chronic ethanol exposure, accumbal dopamine release is presumably decreased to a greater extent (Karkhanis et al. 2016, 2017; Rose et al. 2016). It has been proposed that this results in a hypodopaminergic state of the nucleus accumbens, which also possibly drives further ethanol intake due to negative reinforcement. The reason why κ-opioid receptor antagonists seem to be able to decrease ethanol intake once ethanol addiction and physical dependence have been established may be due to blocking of the overactivated κ-opioid receptors, which would result in a relative rise in accumbal dopamine levels.

In addition to modulating dopamine release, κ-opioid receptors have, upon activation by agonists, also been shown to form complexes with dopamine transporters, which are coexpressed with κ-opioid receptors presynaptically on accumbal dopaminergic terminals (Kivell et al. 2014; Svingos et al. 2001; Thompson et al. 2000). κ-Opioid receptor agonists have been suggested to increase dopamine transporter function possibly via activating the extracellular signal regulated kinase-½ pathway (Kivell et al. 2014). Therefore, this still less studied interaction may also have a role in controlling ethanol intake both during maintenance ethanol intake and also after ethanol intake has become out of control.

It is important to note that the neuroadaptations imposed by ethanol addiction are complex and this is only one oversimplified theory proposed to explain the role of κ-opioidergic mechanisms in controlling ethanol intake. The clinical relevancy of these presumable mechanisms is still to be verified and the significance needs to be evaluated. In deceased alcoholics, ventral striatal κ-opioid receptor binding sites were shown to be unchanged (Hermann et al. 2017). However, the possible sensitized state of these receptors is unknown. Also a hyperdopaminergic state of the nucleus accumbens has been proposed after protracted abstinence (Hirth et al. 2016). As κ-opioid receptors also inhibit the release of other neurotransmitters, such as glutamate and GABA in the nucleus accumbens (Hjelmstad and Fields 2001, 2003), their role in ethanol addiction and physical dependence has to also be evaluated. Further research is needed to understand the mechanisms driving compulsive ethanol intake in addicted individuals.
2.6 OPIOIDERGIC SYSTEMS IN SELECTED RODENT LINES

Studies in the selected AA and ANA lines of rats, as well as other selected rodent lines, suggest that differences in their opioidergic systems could to some extent contribute to their opposite ethanol preferences (de Waele et al. 1995; Gianoulakis et al. 1992; Jamensky and Gianoulakis 1997; Marinelli et al. 2000; McBride et al. 1998; Nylander et al. 1994; Soini et al. 2002). Differences have been shown in opioid receptor density and distribution, in the levels of propeptide mRNA as well as in the G-protein coupled receptor function in relevant brain areas of the reward pathway between opposite ethanol-preferring counterparts (de Waele et al. 1995; Gianoulakis et al. 1992; Jamensky and Gianoulakis 1997; Marinelli et al. 2000; McBride et al. 1998; Soini et al. 2002). Additionally, opioid peptide levels have been shown to differ in several brain areas under baseline and ethanol intake-induced conditions (Nylander et al. 1994).

The relationships between innate differences in opioidergic systems and ethanol preference are most probably governed by complicated mechanisms, many of which are still not understood (McBride et al. 1998). Therefore, conclusions on the roles of different opioidergic systems in controlling ethanol intake based solely on innate differences between opposite ethanol preferring lines cannot be drawn. They can, however, be used as clues of factors that contribute to controlling ethanol intake.

2.6.1 THE µ-OPIOIDERGIC SYSTEM AND ETHANOL PREFERENCE

Studies in selected rodent lines have given indicative results that the ethanol-preferring lines have a higher innate and ethanol-induced µ-opioidergic (AA and P rats), possibly also δ-opioidergic (ethanol preferring C57BL/6 mice) tone in central mesolimbic brain areas in regard to their ethanol non-preferring counterparts (de Waele and Gianoulakis 1997; de Waele et al. 1995; Gianoulakis et al. 1992; Lam et al. 2010; Marinelli et al. 2000; McBride et al. 1998; Nylander et al. 1994; Soini et al. 2002; Winkler et al. 1998). The density of µ-opioid receptors has been shown to be higher in the nucleus accumbens shell in AA rats as compared to ANA rats (de Waele et al. 1995; Marinelli et al. 2000), as well as in P rats as compared to NP rats (McBride et al. 1998). However, no differences were found in the nucleus accumbens shell between ethanol-preferring C57BL/6 and non-preferring DBA/2 mice (de Waele and Gianoulakis 1997). The µ-opioid receptor density in the VTA has been shown to be either higher in AA than ANA rats (de Waele et al. 1995) or no differences have been found (Marinelli et al. 2000). Differences in VTA µ-opioid receptor densities have not been found between P and NP rats or C57BL/6 and DBA/2 mice (de Waele and Gianoulakis 1997; McBride et al. 1998). The levels of nucleus accumbens shell β-endorphin have been
shown to be increased in AA rats but not in ANA rats following systemic ethanol administration (Lam et al. 2010). However, no differences in accumbal tissue concentrations of β-endorphin or pro-opiomelanocortin mRNA have been found between AA and ANA rats (Gianoulakis et al. 1992; Lam et al. 2010). It would be interesting to know what differences these lines have in the μ-opioidergic system in the ventral pallidum.

These differences in the μ-opioidergic systems between the opposite ethanol-preferring lines give rise to speculation that the μ-opioidergic system plays a role in high ethanol preference. Further support is given by findings that AA rats voluntarily consume more μ-opioid receptor agonist etonitazene solution than ANA rats (Hyytiä and Sinclair 1993), and they are more susceptible to the opioid agonist morphine-induced sensitization than ANA rats (Honkanen et al. 1999; Mikkola et al. 2002; Ojanen et al. 2003, 2007).

### 2.6.2 THE Κ-OPIOID ERGIC SYSTEM AND ETHANOL PREFERENCE

A higher innate κ-opioidergic tone in central mesolimbic brain areas can be correlated with the low ethanol preference in the non-preferring rodent lines as compared to their ethanol-preferring counterparts (Jamensky and Gianoulakis 1997; Marinelli et al. 2000; Nylander et al. 1994; Winkler and Spanagel 1998). This correlation seems logical since activation of the κ-opioidergic system is perceived as aversive (Funada et al. 1993; Mucha and Herz 1985).

Generally, the levels of dynorphin peptides have been shown to be higher in the nucleus accumbens than in the VTA in both AA and ANA rats (Nylander et al. 1994). Also κ-opioid receptor binding sites are more abundant in the nucleus accumbens than in the VTA (Mansour et al. 1996; Mansour et al. 1987). Ethanol-naïve ethanol non-preferring DBA/2 mice have been shown to have more κ-opioid receptor binding sites, higher prodynorphin mRNA levels and a higher content of dynorphin peptides in the nucleus accumbens than ethanol-preferring C57BL/6 mice (Jamensky and Gianoulakis 1997). Interestingly in ethanol-naïve AA and ANA rats, only accumbal pro-dynorphin derived peptide levels were higher in ANA rats (Nylander et al. 1994), while no differences between the lines were found in the density of accumbal κ-opioid receptor binding sites or prodynorphin mRNA levels (Marinelli et al. 2000).

In the VTA, no differences have been found in the density of κ-opioid receptor binding sites and prodynorphin mRNA levels of ethanol-naïve C57BL/6 and DBA/2 mice or AA and ANA rats (Jamensky and Gianoulakis 1997; Marinelli et al. 2000). However, higher levels of pro-dynorphin derived peptides were shown in the VTA of ethanol-naïve ANA rats as compared to AA rats (Nylander et al. 1994). Gene mRNA levels and peptide expression do not always correlate, which may explain this discrepancy. Interestingly, ethanol-naïve C57BL/6 mice expressed more dynorphin A 1-8 in the VTA.
than DBA/2 mice even though the levels of dynorphin A 1-13 were similar between the strains (Jamensky and Gianoulakis 1997). The significance of this difference is unclear.

In conclusion, these differences in the κ-opioidergic systems between opposite ethanol-preferring counterparts in the nucleus accumbens and VTA may contribute to why ethanol non-preferring rodent strains do not voluntarily consume ethanol. However, future studies need to further examine these differences to unravel the significance of these differences on controlling ethanol intake.
3 AIMS OF THE STUDY

Previous studies have demonstrated that the hedonic μ- and the anhedonic κ-opioidergic systems participate in mediating the positive and negative reinforcing effects of ethanol, respectively, during situations of controlled ethanol intake and also when ethanol addiction has been established. Despite vast research, it is not exactly known what role these opioidergic systems interacting with the reward pathway, especially the nucleus accumbens shell, have in controlling ethanol intake and behaviors related to addiction.

Systemic routes of administration as well as non-selective opioidergic agents have been vastly used; however, they cannot bring answers to what role the μ- and κ-opioidergic systems have in specific brain areas in controlling ethanol intake. In studies addressing specific brain areas, most often indirect measurements have been used (e.g. opioid receptor binding studies, mRNA levels, fast scan cyclic voltammetry), which give important information on the changes that the used ethanol intake or addiction model imposes on the local opioidergic systems. However, these studies cannot directly answer to what role the observed local changes have in regard to behavior, that is in controlling ethanol intake itself. Additionally, the high innate μ-opioidergic tone in the nucleus accumbens shell of alcohol-preferring AA rats has been proposed to partly explain their high ethanol preference but pharmacological studies verifying this are lacking.

In an attempt to bridge this gap in knowledge, the major goal of this thesis was to bring clarification on the role of μ- and κ-opioidergic systems in the nucleus accumbens shell in controlling intermittent and relapse-like ethanol intake by using selective μ- and κ-opioid receptor agonists and antagonists in rats.

The specific aims of the studies were:

1. To elucidate the role of μ-opioidergic mechanisms in the nucleus accumbens shell in controlling intermittent ethanol intake in AA rats (I).

2. To clarify the role of κ-opioidergic mechanisms in the nucleus accumbens shell in controlling intermittent ethanol intake in AA rats (I & II).

3. To determine the effects of κ-opioid receptor antagonism on relapse-like ethanol intake and clarify what role the nucleus accumbens shell κ-opioid receptors have in it by using the alcohol deprivation effect paradigm in Long-Evans rats (III).
4 MATERIALS AND METHODS

4.1 ANIMALS

Male alcohol-preferring AA rats (University of Helsinki, Helsinki, Finland) were used in the ethanol intake experiments (I, II) and male Long-Evans rats (HsdBlu:LE, Envigo, Indianapolis, IN, USA) were used in the ADE experiments (III). The AA rats were from generations F\textsubscript{108} and F\textsubscript{110} and they were 3 months old and weighed at least 250 g in the beginning of the experiments. The Long-Evans rats were 6–7 weeks old upon arrival, they were let to settle for 2 weeks and weighed 270–300 g before the onset of experiments. Water and standard rat chow (SDS RM1 [E] SQC; Witham, Essex, UK) were available ad libitum. All experiments were conducted in a reversed 12/12 h light/dark cycle (lights off at 9 AM). The rats were housed either individually in wire mesh cages (38 cm x 21 cm x 19 cm) (I, II) or in groups of 2 to 3 rats per cage in individually ventilated cages (III). Group housed rats were transferred to the individual wire mesh cages (38 cm x 21 cm x 19 cm) for monitoring of ethanol intake. Animal experiments were conducted according to the 3R principles of the EU directive 2010/63/EU governing the care and use of experimental animals, and following local laws and regulations (Finnish Act on the Protection of Animals Used for Scientific or Educational Purposes (497/2013), Government Decree on the Protection of Animals Used for Scientific or Educational Purposes (564/2013)). The protocols were authorized by the national Animal Experiment Board of Finland (license numbers ESAVI/2073/04.10.03/2012 and ESAVI/5705/04.10.07/2013).

4.2 DRUGS

The µ-opioid receptor antagonist CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH\textsubscript{2}), the µ-opioid receptor agonist DAMGO (D-Ala\textsubscript{2}, N-Me-Phe\textsubscript{4},Gly\textsubscript{ol}\textsubscript{5}-enkephalin), the κ-opioid receptor agonist U50488H [(±)-U-50488 hydrochloride] and the κ-opioid receptor antagonist nor-BNI (nor-binaltorphimine dihydrochloride) were purchased from Tocris (Bristol, UK). The opioid receptor agonist morphine hydrochloride was purchased from the University Pharmacy (Helsinki, Finland). The non-selective opioid receptor antagonist naltrexone hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO, USA). The κ-opioid receptor antagonist JDTic [(3R)-7-hydroxy-N-((1S)-1-[[[(3R,4R)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl]-2-methylpropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide] was a generous gift from RTI International (Research Triangle Park, NC, USA). Ethanol (Etax A, 96% v/v; Altia, Rajamäki, Finland) was diluted into
tape water. All drugs were dissolved in sterile saline. The subcutaneously (sc) injected drugs were administered at a volume of 1 ml/kg and the intracranially infused drugs were administered at a volume of 0.3 µl.

4.3 ETHANOL INTAKE

4.3.1 INTERMITTENT ETHANOL INTAKE (I & II)

An intermittent and time-restricted, two-bottle choice ethanol access paradigm was used in the studies I and II (method modified from Simms et al. 2010). This paradigm was also used to teach rats to drink ethanol in the beginning of the ADE experiments (III).

Briefly, the rats had access to 10% ethanol solution (v/v) in the wire mesh cages three days a week - on Mondays, Wednesdays and Fridays – in the beginning of the dark phase within 30 to 60 min after the lights went out. A water containing tube was always present and the positions of the tubes were changed before each session to prevent side preference. Food was available ad libitum. Initially, the time of ethanol access was 24 h, which was continued at least for 3 weeks. After this, ethanol was accessible in Richter tubes for 90 min per session, which was also carried on for at least 3 weeks or until stable ethanol intake baseline levels (g/kg/90 min) had been established. The rats were habituated to ethanol intake being monitored (in ml) for 90 min either every 30 min (II) or at 10 to 20 min intervals (I, II). Body weight and food intake were measured three times per week.

After the initial ethanol intake baseline had been established, the rats assigned for guide cannula placements underwent surgery. After surgery and during the 2 week recovery period, the ethanol intake baselines were re-established, according to which the rats were assigned to treatments groups and one intra-accumbal sham and one training vehicle infusion was given (see 4.4.) either 5 min (I, II) or 24 h (II) before ethanol access. Thereafter, the effects of bilateral intra-accumbens shell infusions of µ- and κ-opioid receptor agonists and antagonists on intermittent ethanol intake were examined.

In Study I, CTOP (0, 0.3 and 1 µg/0.3 µl/site, n=9), DAMGO (0, 0.03 and 0.1 µg/0.3 µl/site, n=8) or U50488H (0, 0.3 and 1 µg/0.3 µl/site, n=8) were administered 5 min before ethanol was accessible in a within-subjects Latin square design. An additional group of rats (n=8) received intra-accumbal morphine (30 µg/0.3 µl/site), CTOP (1 µg/0.3 µl/site) or vehicle (0.3 µl/site) with the same study design. Between infusions there were always two ethanol intake sessions without infusions to monitor that the baseline levels remained stable. A method control group received 3 infusions of vehicle (0.3 µl/site, n=7) to determine the effects of multiple infusions per se on intermittent ethanol intake. The doses were selected based on previous published and our unpublished data (Hyytia and Kiianmaa 2001;

In Study II, both the acute and long-term effects of intra-accumbens shell and systemic administrations of κ-opioid receptor antagonists on intermittent ethanol intake were examined. Intra-accumbens shell JDTic (15 µg/0.3 µl/site, n=8), nor-BNI (3 µg/0.3 µl/site, n=10) or vehicle (0.3 µl/site, n=8) were administered 24 h before ethanol was accessible and the effects on ethanol intake were examined on days 1, 3 and 5 after administration. JDTic was also administered intra-accumbally 5 min before ethanol was accessible to determine its acute effects on ethanol intake (JDTic 0 µg/0.3 µl/site, n=9; JDTic 1 µg/0.3 µl/site, n=9; JDTic 5 µg/0.3 µl/site, n=7; JDTic 15 µg/0.3 µl/site, n=8). Both the acute and long-term effects of systemic JDTic (10 mg/kg, sc, n=10) or vehicle (n=8, sc) on ethanol intake were examined on days 0, 2 and 4 after administration (administration 10 min prior access to ethanol). In Study II, each rat received only one dose of the drugs and a between-subjects design was used due to the long-term actions of the used κ-opioid receptor antagonists (Carroll et al. 2004; Deehan et al. 2012; Endoh et al. 1992). The doses were based on previous reports (Deehan et al. 2012; Kemppainen et al. 2012b; Knoll et al. 2011; Nealey et al. 2011; Rorick-Kehn et al. 2014; Schank et al. 2012; Varaschin and Morato 2009; Walker and Koob 2008).

4.3.2 ALCOHOL DEPRIVATION EFFECT (III)

The rats were initially trained to drink ethanol using the same intermittent and time-restricted, two-bottle choice ethanol access paradigm as used in the Studies I and II. A saccharin-fade paradigm was used in the beginning to initiate ethanol intake, starting with 0.2% (w/v) saccharin in 10% ethanol solution (modified from Samson 1986). After stable 90 min ethanol intake levels (g/kg/90 min) were established (8 to 10 weeks of total ethanol access), the ethanol access and deprivation cycles were initiated.

One ethanol access and deprivation cycle consisted of 10 days of access to ethanol for 90 min per day starting within 1 h of the beginning of the dark phase in the wire mesh cages. This was followed by a 6 day deprivation period in the home cages. After this, a new cycle was initiated, in the beginning of which (first 2 days) the ADE was determined from the cumulative 90 min ethanol intake data. The average 90 min baseline ethanol intake of the last 3 days before the deprivation period was compared to ethanol intake on days 1 and 2 after deprivation. For a rat to be accepted for use in the experiments, ethanol intake had to increase at least 20% over baseline ethanol intake levels during the ADE. Additionally, baseline ethanol intake had to be at least 0.2 g/kg/90 min. Repeated cycles were used to habituate the rats to the used experimental procedures. Water intake was measured at the 90 min time point. Body weight was measured three times per week.
The manifestation of the ADE in the used paradigm was tested in non-treated rats after the first ethanol access and deprivation cycle using a between-subjects design. One group of rats (n=10) was deprived of ethanol for 6 days and ethanol intake during ethanol re-access days 1 and 2 was compared to a group of rats (n=10) that had had daily 90 min access to ethanol.

The effects of both intra-accumbens shell and systemic κ-opioid receptor antagonism by JDTic on the ADE were examined using a between-subjects design. The rats were assigned to treatment groups according to the ethanol intake levels and magnitude of the ADE (expressed as percentage from baseline) of the last cycle. Intra-accumbens shell JDTic (15 µg/0.3 µl/site, n=9) or vehicle (0.3 µl/site, n=7) or systemic JDTic (10 mg/kg, n=9) or vehicle (n=9) were administered 24 h before ethanol re-access during the deprivation period during either the fourth (intracranial, 9-10 weeks after surgery) or third (systemic) cycle. For habituation purposes, one sham and two training vehicle infusions were given to the rats during the three previous cycles in the intracranial infusion experiment, and two training vehicle injections were given during the two previous cycles in the systemic injection experiment.

With one wash-out ethanol access and deprivation cycle in between (4.5 weeks), the same rats used for systemic JDTic administration experiments were reused to determine the effects of the non-selective antagonist naltrexone on the ADE. In a within-subjects design, naltrexone (0.3 mg/kg, sc, n=12) or vehicle were administered 20 min prior to re-access to ethanol in random order in two consecutive cycles.

The doses of the drugs used in the ADE experiments were chosen based on previous reports (Deehan et al. 2012; Heyser et al. 2003; Hölter and Spanagel 1999; Knoll et al. 2011; Schank et al. 2012). Due to the long-term κ-antagonistic effects of JDTic, only one dose was given per rat (Carroll et al. 2004; Deehan et al. 2012).

### 4.4 SURGICAL PROCEDURES AND INFUSIONS

For implantation of guide cannulas for the intra-accumbens infusions in Studies I, II and III, the rats were anesthetized with isoflurane (4% during induction and 2-2.5% for anesthesia maintenance) and attached to a stereotaxic frame. Dual guide cannulas constructed either from microdialysis guide cannulas (OD = 1.0 mm) (CMA, CMA Microdialysis, Stockholm, Sweden) (I, II) or cut from stainless steel hypodermic tubes (23 G, Component Supply Company, Sparta, TN, USA) (III) were implanted bilaterally 2 mm above the nucleus accumbens shell according to the stereotaxic coordinates by Paxinos and Watson (1998). The coordinates used were AP +1.7; ML ±1.2; DV -5.2 from the dura, which were selected based on earlier studies (Castro and Berridge 2014; Hyytiä and Kiianmaa 2001; Nealey...
et al. 2011; Smith and Berridge 2007). The guide cannulas were fastened to the skull with the help of 2 to 3 stainless steel screws and dental cement. Dummy stylets, either microdialysis guide cannula dummy stylets (CMA, CMA Microdialysis, Stockholm, Sweden) (I, II) or dummy stylets cut out of stainless steel wire (Component Supply Company, Sparta, TN, USA) (III), were used to prevent occlusion. During surgery, the rat’s body temperature was kept constant with a heating pad attached to a thermostatic sensor. Intraperitoneal saline was given to prevent dehydration. Lidocain (Lidocain c. adrenalin 10 mg/ml + 10 µg/ml, Orion Oyj, Espoo, Finland) was used as a local anesthetic and carprofen (5 mg/kg, Rimadyl, sc, Vericore, Dundee, UK) was given for analgesia 30 min before the surgery and for two consecutive days. After surgery the rats were let to recover in their home cages.

The bilateral infusions were given to awake rats held lightly by the experimenter. The rats were habituated to the infusion procedure by first removing and replacing the dummy stylets at least during 4 habituation sessions. After this, the rats received a sham infusion, during which the injection needle [tip OD = 0.3 mm (I, II) or 30 G (III)] extending 2 mm beyond the ventral tip of the guide, was placed into the guide cannulas but no infusions were given. Thereafter, the rats received vehicle infusions either 5 min (I, II) or 24 h (II, III) before access to ethanol according to the specific requirements of the Studies I, II and III (see 4.3) in a volume of 0.3 µl at a rate of 0.3 µl/min with a infusion pump (CMA, Stockholm, Sweden). The injection needle was left in place for 1 min before the infusion and after the infusion for 2 min to avoid leakage up the cannula track. The sham and training vehicle infusions were used to ensure that the infusion procedure itself would not confound with interpretation of the results.

4.5 HISTOLOGY

To verify correct guide placement and site of intra-cranial infusions (I, II & III), the brains were fixed with 10% formalin and cut into 100 µm coronal sections. When brain slices were compared to the brain atlas (Paxinos and Watson 1998), the marks of the injection needle tips had to be in the nucleus accumbens shell or otherwise that rat was excluded. Rats were also excluded if the guide cannulas loosened during the experiments.

4.6 STATISTICAL ANALYSIS

For data analysis, ethanol intake (ml) data was first converted into grams of 100% ethanol/body weight (kg). In Study I, the acute effects of the different drug doses on intermittent ethanol intake (cumulated intake at 30, 60 or 90 min) were analyzed from the percentage of baseline values, calculated from the average of the two ethanol intake sessions which preceded each
treatment session. This was to control for the possible effects of repeated intracranial infusions on ethanol intake over time. For these experiments, a within-subjects one-way analysis of variance (ANVOA) with repeated measures on dose was used. For analysis of uncumulated ethanol intake (g/kg) during a 90 min ethanol intake session, a within-subjects two-way ANOVA with repeated measures on treatment over time was used. Following a significant treatment x time interaction, simple main effects were analyzed by separate one-way repeated measures ANOVAs on treatment at different time-points (e.g. the 10 min or the 20 min time point). The paired t-test with the Bonferroni’s adjustment was used for post hoc means.

To determine the effects of systemic or intra-accumbal κ-opioid receptor antagonist treatments (in g/kg) on either long-term intermittent ethanol intake (II) or on the ADE (III), as well as to determine the effects of deprivation vs. continuous ethanol access on the ADE in non-treated rats (III), a mixed-model two-way ANOVA with treatment as the between-groups factor and ethanol intake session as the within-subjects factor was used. The effects of naltrexone on the ADE (III) were determined with the within-subjects two-way ANOVA with repeated measures on treatment over time. The acute effects of the different doses of JDTic on ethanol intake (II) were analyzed with the one-way ANOVA (at the 90 min time point). For post hoc comparisons, the unpaired or paired two-sample t-test with the Holm-Bonferroni correction for multiple comparisons was applied when appropriate.

The ethanol intake data (g/kg) during the re-access days 1 and 2 for each treatment group in the ADE experiments (III) were also converted for percentages of baseline ethanol intake data, which were then compared (drug vs. vehicle) to one another either by the unpaired or paired two-samples t-test, where appropriate, with the Holm-Bonferroni correction for multiple comparisons. The baseline ethanol intake was determined as the average of total 90 min ethanol intake of the last 3 ethanol intake sessions before ethanol deprivation.

Water (I, II, III) and food intake (I, II) (ml or g) were also first converted to grams of consumed amount/body weight (kg) and analyzed similarly to ethanol intake.

In all statistical analyses, the accepted level of significance was set at p<0.05. The statistical software used was IBM SPSS Statistics V22.0 (IBM Corp., Armonk, NY, USA).
5 RESULTS

5.1 INTERMITTENT ETHANOL INTAKE (I, II & III)

The average intermittent ethanol intake of all rats before the onset of the ethanol access and deprivation cycles and/or drug administrations in Studies I, II and III assigned either for the nucleus accumbens shell infusions or systemic injections are shown in Table 3. Surgery did not affect ethanol intake baselines significantly in any experiment.

Table 3. The average intermittent ethanol intake of all rats before the onset of Studies I, II and III assigned either for the nucleus accumbens shell infusions or systemic injections.

<table>
<thead>
<tr>
<th>Nucleus accumbens shell infusions</th>
<th>Pre-surgery ethanol intake g/kg/90 min</th>
<th>Post-surgery ethanol intake g/kg/90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>Rat strain</td>
<td></td>
</tr>
<tr>
<td>I, acute</td>
<td>AA</td>
<td>0.87 ± 0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.82 ± 0.07</td>
</tr>
<tr>
<td>II, long-term</td>
<td>AA</td>
<td>0.87 ± 0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.84 ± 0.08</td>
</tr>
<tr>
<td>II, acute</td>
<td>AA</td>
<td>0.83 ± 0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.10 ± 0.05</td>
</tr>
<tr>
<td>III, ADE</td>
<td>Long-Evans</td>
<td>0.43 ± 0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.50 ± 0.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Systemic injections</th>
<th>Ethanol intake g/kg/90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>Rat strain</td>
</tr>
<tr>
<td>II, acute &amp; long-term</td>
<td>AA</td>
</tr>
<tr>
<td></td>
<td>0.94 ± 0.07</td>
</tr>
<tr>
<td>III, ADE</td>
<td>Long-Evans</td>
</tr>
<tr>
<td></td>
<td>0.46 ± 0.10</td>
</tr>
</tbody>
</table>

Mean intake of ethanol ± SEM. Long-term/acute/ADE = rats assigned for accumbal infusions/systemic injections assessing long-term/acute effects of drugs on intermittent ethanol intake or on the ADE.

No differences in ethanol intake levels were detected between the treatment sessions in Study I in the method control group, which received multiple acute intra-accumbal vehicle infusions. This suggests that multiple infusions per se do not affect ethanol intake (see Fig. 1D in Study I). Also in Study II, intra-accumbal vehicle infusions kept ethanol intake levels comparable to baseline levels. Additionally, multiple intra-accumbal infusions of various doses of CTOP, DAMGO and U50488H did not have an effect on ethanol intake baseline levels between treatments in any group (see Table 1 in Study I). Blood ethanol levels were not measured in the Studies I-III, however, it has previously been shown that similar consumption levels shown here in AA rats (I, II in Table 3) and in vehicle and non-treated Long-Evans rats during re-access to ethanol (0.7 to 0.8 g/kg, III) reach pharmacologically significant blood ethanol levels (Nurmi et al. 1999).
Results

None of the used opioidergic drugs or paradigms itself affected water intake (I, II & III) or food consumption (I, II).

5.2 EFFECTS OF NUCLEUS ACCUMBENS SHELL µ-OPIOID RECEPTOR AGONISM AND ANTAGONISM ON INTERMITTENT ETHANOL INTAKE (I)

Intra-accumbally infused CTOP increased ethanol intake at the 30 min time point, F(2, 16)=7.183, p=0.019 (repeated measures ANOVA). The 1 µg dose of CTOP increased ethanol intake (p=0.011) when compared to the vehicle treatment (Figure 7). DAMGO decreased ethanol intake at the 30 min time point, F(2, 14)=4.207, p=0.037 (repeated measures ANOVA). However, post hoc tests failed to differentiate between the doses (p<0.1 for both doses vs. vehicle), even though a tendency for dose dependency was evident in decreasing ethanol intake. By 90 min no differences were evident.

To verify that the intra-accumbally infused CTOP is indeed able to increase ethanol intake, an additional group of rats received the highest dose of CTOP (1 µg), morphine (30 µg) and vehicle. The two-way repeated measures ANOVA revealed a significant treatment and time interaction during the 90 min ethanol intake session from the uncumulated ethanol intake data, F(10, 70)=4.789, p=0.005. Post hoc tests revealed that CTOP increased ethanol intake at the 10 min time point (p=0.035) (Figure 8). Morphine did not affect ethanol intake.
The acute effects of the opioid receptor agonist morphine (30 µg/0.3 µl/site), µ-opioid receptor antagonist CTOP (1 µg/0.3 µl/site) and vehicle (VEH, 0.3 µl/site) on intermittent ethanol intake in AA rats (n=8) when infused into the nucleus accumbens shell. Ethanol intake is expressed as g/kg (mean±SEM) in intervals of 10 to 20 min. Asterisks indicate a significant difference of CTOP from vehicle treatment (*p<0.05) at 10 min following a significant within-subjects two-way ANOVA interaction with repeated measures on treatment over time.

5.3 EFFECTS OF Κ-OPIOID RECEPTOR AGONISM AND ANTAGONISM ON INTERMITTENT ETHANOL INTAKE (I & II)

5.3.1 EFFECTS OF NUCLEUS ACCUMBENS SHELL Κ-OPIOID RECEPTOR AGONISM AND ANTAGONISM ON ETHANOL INTAKE (I & II)

Intra-accumbally infused JDTic showed a weak long-term ethanol intake decreasing effect in the intermittent ethanol intake paradigm (II). The mixed-model ANOVA showed a significant interaction on treatment and ethanol intake session from the 90 min data, F(3,42)=3.41, p=0.026. However, the post hoc test revealed that the reduction in ethanol intake only approached significance (p=0.058) 3 days post-infusion (Figure 9). Intra-accumbally infused nor-BNI showed a tendency to decrease ethanol intake. The mixed-model ANOVA showed a significant interaction on treatment and ethanol intake session from the 90 min data, F(3,48)=3.547, p=0.021. Still, the post hoc test did not distinguish between nor-BNI and vehicle at any specific ethanol intake session post-infusion (Figure 9).

In addition to the long-term effects of κ-opioid receptor antagonism, also the acute effects of intra-accumbally infused κ-opioid receptor agonist U50488H (I) and antagonist JDTic (II) on ethanol intake were examined. None of the examined doses of either U50488H (0.3 and 1 µg) (see Study I, Fig. 1C) or JDTic (1, 5 and 15 µg) (see Study II, Fig. 3) had acute effects on 90 min ethanol intake.
5.3.2 EFFECTS OF SYSTEMIC K-OPIOID RECEPTOR ANTAGONISM ON ETHANOL INTAKE (II)

Systemically administered JDTic decreased intermittent ethanol intake in a long-term manner (II). The mixed-model ANOVA revealed a significant treatment and ethanol intake session interaction from the 30 min data, F(3,48)=2.847, p=0.047. Ethanol intake was decreased 2 days (p=0.0073) after the JDTic injection and showed a tendency for reducing ethanol intake also 4 days post-injection (p=0.08) relative to vehicle treatment (Figure 10). Systemic JDTic did not have any acute effects on ethanol intake.

Figure 9  Intermittent ethanol intake in AA rats after bilateral intra-accumbens shell infusions of the \( \kappa \)-opioid receptor antagonists JDTic (n=8) or nor-BNI (n=10) or vehicle (VEH, n=8). The infusions were given 24 h before ethanol intake. Ethanol intake is expressed as g/kg (mean±SEM). The doses were administered in a volume of 0.3µl/site. Baseline (BL) = average intake of the last two ethanol intake sessions before treatments.

Figure 10  Intermittent ethanol intake after the \( \kappa \)-opioid receptor antagonist JDTic (n=10) or vehicle (VEH, n=8) administered subcutaneously 10 min prior ethanol access in AA rats. Ethanol intake is expressed as g/kg (mean±SEM). Asterisks indicate a significant difference from vehicle treatment (**p<0.01) after a significant two-way mixed-model ANOVA. At 4 days post-injection, JDTic tended to decrease ethanol intake, p<0.1. Baseline (BL) = average intake of the last two ethanol intake sessions before treatments.
5.4 EFFECTS OF K-OPIOID RECEPTOR ANTAGONISM ON THE ALCOHOL DEPRIVATION EFFECT (III)

5.4.1 EXPRESSION OF THE ALCOHOL DEPRIVATION EFFECT (III)

A significant increase in ethanol intake (67±12% higher than baseline levels) was evident on the first day of ethanol re-access in the deprived rats as compared to rats with continuous access to ethanol, as shown by the post hoc test (p=0.0334) after a significant two-way mixed-model ANOVA treatment and ethanol intake session interaction from the g/kg data, F(2,36)=3.982, p=0.027 (Figure 11A). This is also supported by the percentage of baseline data (p=0.0019) (Figure 12A).

In all ADE experiments, the vehicle treated or non-treated but ethanol deprived rats increased their ethanol intake significantly on the first day of ethanol access after the deprivation period, as shown by a paired t-test (baseline vs. day 1 in g/kg) (Table 4).

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Paired t-test</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline vs. Day 1 (g/kg)</td>
</tr>
<tr>
<td>Non-treated but ethanol deprived</td>
<td>10</td>
<td>p&lt;0.0005</td>
</tr>
<tr>
<td>Intra-accumbens shell vehicle 24 h prior to ethanol re-access</td>
<td>7</td>
<td>p=0.019</td>
</tr>
<tr>
<td>Systemic vehicle 24 h prior to ethanol re-access</td>
<td>9</td>
<td>p=0.001</td>
</tr>
<tr>
<td>Systemic vehicle 20 min prior to ethanol re-access</td>
<td>12</td>
<td>p&lt;0.0005</td>
</tr>
</tbody>
</table>

5.4.2 EFFECTS OF NUCLEUS ACCUMBENS SHELL K-OPIOID RECEPTOR ANTAGONISM ON THE ALCOHOL DEPRIVATION EFFECT (III)

Intra-accumbally administered JDTic attenuated the ADE on the first day of ethanol re-access as compared to vehicle treated rats (p=0.0034), when the percentage of baseline ethanol intake data was examined (Figure 12B). The g/kg data also suggested that JDTic attenuated ethanol intake, as shown by a significant two-way mixed-model ANOVA treatment and ethanol intake session interaction, F(2,28)=4.179, p=0.026. However, the post hoc test showed that JDTic only tended to attenuate the ADE on the first day of ethanol re-access (p=0.077) (Figure 11B).
Results

Figure 11  Effects of A) a 6 day deprivation period (deprived, Depr, n=10) or continuous ethanol access (Cont, n=10), B) bilateral intra-accumbens shell infusions of the κ-opioid receptor antagonist JDTic (15 µg/0.3 µl/site, n=9) or vehicle (0.3 µl/site, VEH, n=7) administered 24 h prior to ethanol re-access, C) systemic injections of JDTic (10 mg/kg, sc, n=9) or vehicle (n=9) administered 24 h prior to ethanol re-access and D) systemic injections of naltrexone (0.3 mg/kg, sc) or vehicle (n=12, within-design) injected 20 min prior to ethanol re-access on the alcohol deprivation effect of Long-Evans rats. Ethanol intake is expressed as g/kg (mean±SEM). Baseline (BL) ethanol intake is the average of the last three 90 min ethanol intake sessions (days) prior to the deprivation phase. Asterisks indicate a significant difference from vehicle treatment (p<0.05, ***p<0.0005) after a significant mixed-model ANOVA treatment x ethanol intake session (day) interaction (A) or a within-subjects repeated ANOVA treatment x day interaction (D). Intra-accumbally infused JDTic tended to decrease ethanol intake (p=0.077 on Day 1 of ethanol re-access after a significant mixed-model ANOVA) (B).

5.4.3 EFFECTS OF SYSTEMIC K-OPIOID RECEPTOR ANTAGONISM ON THE ALCOHOL DEPRIVATION EFFECT (III)

The percentage of baseline ethanol intake data showed that systemically administered JDTic attenuated the ADE on the first day of ethanol re-access (p=0.0257) (Figure 12C). However, the two-way mixed-model ANOVA did not show a significant interaction for treatment and ethanol intake session from the g/kg data (Figure 11C).
Figure 12  Effects of A) a 6 day deprivation period (deprived, Depr, n=10) or continuous ethanol access (Cont, n=10), B) bilateral intra-accumbens shell infusions of the κ-opioid receptor antagonist JDTic (15 µg/0.3 µl/site, n=9) or vehicle (0.3 µl/site, VEH, n=7) administered 24 h prior to ethanol re-access, C) systemic injections of JDTic (10 mg/kg, sc, n=9) or vehicle (n=9) administered 24 h prior to ethanol re-access and D) systemic injections of naltrexone (NTX, 0.3 mg/kg, sc) or vehicle (n=12, within-design) injected 20 min prior to ethanol re-access on the alcohol deprivation effect of Long-Evans rats. Ethanol intake on Days 1 and 2 after deprivation is expressed as percentage of baseline ethanol intake, which is the average of the last three 90 min ethanol intake sessions (days) prior to the deprivation phase. Asterisks indicate a significant difference from vehicle treatment (*p<0.05, **p<0.01, Holm-Bonferroni’s paired or unpaired t-test).

5.4.4 EFFECTS OF SYSTEMIC NALTREXONE ON THE ALCOHOL DEPRIVATION EFFECT (III)

Naltrexone decreased ethanol intake by 64±5.5% and inhibited the ADE significantly on the first day of ethanol re-access, as shown by the post hoc test (p<0.0005) from the g/kg data after a significant within-subjects repeated ANOVA treatment and ethanol intake session interaction, F(2,22)=76.14, p<0.0005 (Figure 11D). This was also demonstrated by the percentage of baseline data (p=0.0021) (Figure 12D). Naltrexone did not show a rebound ADE on the re-access days 2 to 4 (data not shown).
Summary of the studies

The main findings from the intermittent ethanol access and alcohol deprivation effect studies are summarized below.

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<th>Intermittent</th>
<th>ADE</th>
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<td></td>
<td>systemic</td>
<td>NAcc shell</td>
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<tr>
<td>Selective μ-agonist DAMGO</td>
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<tr>
<td>Selective μ-antagonist CTOP</td>
<td>-</td>
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<tr>
<td>Opioid agonist morphine</td>
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<tr>
<td>Opioid antagonist naltrexone</td>
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<tr>
<td>Selective κ-antagonist JDTic</td>
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<tr>
<td>Selective κ-antagonist nor-BNI</td>
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<tr>
<td>Selective κ-agonist U50488H</td>
<td>-</td>
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↑ = increased ethanol intake, ↓ = decreased ethanol intake,
leftrightarrow = no effect on ethanol intake, - = not studied

ADE = alcohol deprivation effect paradigm, Intermittent = intermittent ethanol access paradigm, NAcc shell = nucleus accumbens shell
6 DISCUSSION

6.1 ROLE OF NUCLEUS ACCUMBENS SHELL µ-OPIOID RECEPTORS IN CONTROLLING INTERMITTENT ETHANOL INTAKE

It is widely acknowledged that the µ-opioidergic system mediates a large portion of the rewarding effects of ethanol (Herz 1997). The role of the µ-opioidergic system has especially been suggested to be important in situations when ethanol intake is under control and the individual is not yet addicted to ethanol (Herz 1997; Sirohi et al. 2012). As the µ-opioidergic system vastly interacts with the reward pathway, focus has been set on this circuit when trying to understand the mechanisms behind ethanol’s reward. As a main brain area of the reward pathway, the nucleus accumbens shell is considered to be central for mediating reward from several drugs of abuse, including ethanol (Koob 1992). In alcohol-preferring AA rats, which represent individuals that have a genetic susceptibility to consume high amounts of ethanol (Sommer et al. 2006), a high tone of the µ-opioidergic system especially in the nucleus accumbens shell has been associated with high ethanol preference (de Waele et al. 1995; Lam et al. 2010; Marinelli et al. 2000; McBride et al. 1998). However, the role of the nucleus accumbens shell µ-opioidergic mechanisms in controlling intermittent ethanol intake has previously not been examined in AA rats using selective µ-opioid receptor agonists or antagonists, which is why the experiments were undertaken.

According to the current results, intra-accumbens shell infusions of the µ-opioid receptor antagonist CTOP increased ethanol intake in AA rats. Previously it has been shown in Wistar rats, however, that intra-accumbal CTOP did not affect operant responding for ethanol (Hyytiä and Kiianmaa 2001). Both of these results are somewhat surprising as it has also been shown that acute ICV CTOP decreased operant responding for ethanol in both AA and Wistar rats (Hyytiä and Kiianmaa 2001). The means of ethanol access do not seem to confound at least the ICV CTOP results, as also home cage ethanol intake from bottles was decreased following consecutive (over 3 days) ICV CTOP (1 µg) administrations in AA rats (Hyytiä 1993). Also systemically administered selective µ-opioid receptor antagonists decreased maintenance ethanol intake in AA, Wistar and P rats (Honkanen et al. 1996; Krishnan-Sarin et al. 1998; Stromberg et al. 1998), despite some contradicting reports from Sprague-Dawley rats and C57BL/6 mice (Franck et al. 1998; Le et al. 1993). Intra-accumbally administered non-selective opioid receptor antagonists decrease ethanol intake in several ethanol access paradigms, even though the exact part played by µ-opioid receptor
antagonism is difficult to determine from these studies (Barson et al. 2009; Heyser et al. 1999; June et al. 2004).

It is important to note that in the intra-accumbal Wistar rat study, the different regions of the nucleus accumbens, the core and the shell, were not distinguished from one another (Hyytiä and Kiianmaa 2001). The two nucleus accumbens compartments have been shown to have different functional properties and they also project to different sites (Saddoris et al. 2013). Only one other study using intra-accumbens shell CTOP administrations has been conducted in Wistar rats but unfortunately in that study CTOP was coadministered with naltrindole, a universal δ-opioid receptor antagonist (Nealey et al. 2011). According to their results, the CTOP and naltrindole cocktail decreased ethanol intake. However, as intra-accumbally administered naltrindole is able to decrease responding for ethanol also on its own (Hyytiä and Kiianmaa 2001), the decrease in ethanol intake reported following the µ- and δ-antagonist cocktail administration may largely be due to the effects of δ-opioid receptor antagonism (Nealey et al. 2011).

The pattern of ethanol intake after CTOP administration in the current study is interesting, as it is the opposite from the pattern that acute ICV CTOP causes on operant ethanol responding in AA rats (Hyytiä and Kiianmaa 2001). In the current study, the increase in ethanol intake occurred during the first 10 min of the 90 min ethanol intake sessions after intra-accumbal CTOP (1 µg). In contrast, acute ICV CTOP tended to slow the initiation of operant responding for ethanol, an effect which was evident during the first 5 to 10 min of the operant session especially for higher doses of CTOP (3 µg) (Hyytiä and Kiianmaa 2001). The current results seem to suggest that intra-accumbally administered CTOP initially increased ethanol’s rewarding effects while the general effect of CTOP is the opposite in AA rats. It would be interesting to infuse CTOP into the nucleus accumbens shell of AA rats before a conditioned place preference experiment to gain more information on CTOP’s rewarding effects in this rat line. Caudal intra-accumbens shell infusions of high doses of CTOP have been shown to produce conditioned place aversion in outbred rats (Terashvili et al. 2004), while medial nucleus accumbens shell infusions of CTAP, also a µ-opioid receptor antagonist, did not have any effects in Sprague-Dawley rats (Soderman and Unterwald 2008). According to the histological verification of the site of infusions, the accumbens shell infusions were given into the rostral to medial shell.

The intermittent ethanol access paradigm was used here to model maintenance ethanol intake of individuals that consume high amounts of ethanol. Given that the role of the µ-opioidergic system has been suggested to somewhat decline as ethanol addiction evolves but no results suggest that these changes turn the effects of µ-opioidergic drugs on ethanol intake completely opposite (Ciccocioppo et al. 2002; Hansson et al. 2018; Honkanen et al. 1996; Hyytiä 1993; Koob 2013a; Marinelli et al. 2009; Sirohi
et al. 2012), as what seems to happen to the effects of κ-opioidergic drugs
(Anderson et al. 2016; Karkhanis et al. 2017; Lindholm et al. 2001; Mitchell
et al. 2005; Rose et al. 2016; Walker et al. 2011), also the results from studies
addressing aspects of ethanol addiction can loosely be compared to those
presented here despite different aims of the paradigms. Intra-accumbens
shell CTAP blocked context-induced reinstatement of ethanol seeking in
Long-Evans rats (Perry and McNally 2013a), which is in line with ICV CTOP
in attenuating context-induced reinstatement of ethanol seeking in Wistar
rats (Marinelli et al. 2009). It would be interesting to test the effects of intra-
accumbens shell CTAP or CTOP in AA rats in similar settings since our
current results would predict opposite results.

Because no previous reports suggest that intra-accumbens shell CTOP
would increase ethanol intake, it can be speculated that CTOP revealed some
characteristics of the AA rat itself, for example characteristical distribution of
µ-opioid receptors within the nucleus accumbens shell (see discussion in
6.6), which could explain the increase in ethanol intake reported here. The
other explanation could be that the infusions were given to a specific hotspot
of the nucleus accumbens shell which promotes such effects (Castro and
Berridge 2014). This is, however, somewhat unlikely regarding the site to
where the infusions were given (see discussion below).

In the current study, the selective µ-opioid receptor agonist DAMGO
tended to dose-dependently decrease ethanol intake when administered into
the nucleus accumbens shell while the opioid receptor agonist morphine had
no effect. The high level of ethanol intake after the vehicle treatment in the
DAMGO experiment may, however, somewhat complicate result
interpretation, even though baseline levels between the treatments remained
stable. Individual variation is the most probable reason behind this variation.

A biphasic inhibitory–stimulatory effect on ethanol intake has previously
been shown for intra-accumbens shell infused DAMGO in limited ethanol
access paradigms in Sprague-Dawley and Long-Evans rats (Richard and
Fields 2016; Zhang and Kelley 2002), which could possibly explain our
results. DAMGO has been suggested to increase ethanol intake after a
prolonged delay possibly due to a delayed pharmacological effect of the drug
itself (Richard and Fields 2016). It is possible that the delayed increase in
ethanol intake was missed in the current study due to termination of ethanol
access at 90 min, which warrants replication of the current studies with a
longer follow-up period. Previously, the increase in ethanol intake has been
shown between 60 to 120 min (Zhang and Kelley 2002) or 75 to 110 min
(Richard and Fields 2016) post-infusion. On the contrary, intra-accumbens
shell DAMGO has also been reported not to affect ethanol intake during a 3 h

Previous hedonic hotspot mapping experiments of the nucleus accumbens
shell suggests rostral sites to be regarded as hedonic after DAMGO
administration (Castro and Berridge 2014). Interestingly, µ-opioid receptor
agonists infused into the nucleus accumbens have either been shown to
produce conditioned place preference, aversion or have no effects (Bals-Kubik et al. 1993; Olmstead and Franklin 1997; Terashvili et al. 2004; van der Kooy et al. 1982). It would be interesting to examine the rewarding effects of intra-accumbens shell DAMGO in conditioned place preference experiments also in AA rats.

Morphine has been shown to have bidirectional, dose-related effects on ethanol intake when administered systemically (Critcher et al. 1983; Hubbell et al. 1993; Linseman and Harding 1990; Sinclair 1974; Sinclair et al. 1973a; Stromberg et al. 1997; Vacca et al. 2002; Volpicelli et al. 1991; Wild and Reid 1990). This could also explain why effects on ethanol intake were not seen after nucleus accumbens shell infusions in the current study. It has also been suggested that the ethanol intake increasing effects of morphine are mediated by δ-opioid receptors when administered into the nucleus accumbens shell (Barson et al. 2009). Considering the suggestion that δ-opioid receptors are not central mediators of ethanol-induced reward in AA rats (Honkanen et al. 1996; Hyytiä 1993; Ingman et al. 2003), this would also be somewhat in line with the current results showing that intra-accumbally infused morphine did not affect ethanol intake. AA rats have been shown to have a low density of δ-opioid receptors in the nucleus accumbens shell and whole brain δ-opioid receptor levels were especially low as compared to Sprague-Dawley rats (de Waele et al. 1995).

Taken together, a selective µ-opioid receptor antagonist increased and agonist tended to decrease intermittent ethanol intake when administered into the nucleus accumbens shell of AA rats. These results bring new knowledge on the role of nucleus accumbens shell µ-opioid receptors in controlling intermittent ethanol intake in AA rats. The results also add to the existing knowledge on the role of the nucleus accumbens shell in controlling ethanol intake during high but controlled ethanol intake. These results support the notion that µ-opioidergic mechanisms in the nucleus accumbens shell mediate the positive reinforcing and rewarding effects of ethanol and participate in controlling ethanol intake.

6.2 ROLE OF Κ-OPIOID RECEPTORS IN CONTROLLING INTERMITTENT ETHANOL INTAKE

The role of the κ-opioidergic system in controlling ethanol intake before ethanol addiction has been established is still unclear. κ-Opioidergic mechanisms have suggested to balance the hedonic effects of ethanol mediated by the µ-opioidergic system in situations when ethanol intake is under control (Sirohi et al. 2012; Walker et al. 2012). Especially κ-opioidergic mechanisms interacting with the nucleus accumbens shell have suggested to take part in this due to their ability to counteract the local µ-opioidergic effects (Di Chiara and Imperato 1988). By administering κ-opioid receptor antagonists both systemically and into the nucleus accumbens shell, the role
of the nucleus accumbens shell κ-opioid receptors in controlling voluntary ethanol intake could be clarified. Interestingly, the local effects of κ-opioid receptor antagonism in the nucleus accumbens shell on ethanol intake have previously only been studied in rats exposed to intermittent ethanol vapor (Nealey et al. 2011). However, as forced ethanol exposure paradigms mainly model aspects of physical dependence and such models have been proposed to inflict changes on the κ-opioidergic system interacting with the reward pathway (Karkhanis et al. 2016, 2017; Rose et al. 2016), the results from those studies cannot be directly generalized to models in which voluntary ethanol intake gradually stabilizes to a certain level.

According to the current intra-accumbens shell infusion results, JDTic caused a weak long-term ethanol intake decreasing effect 3 days after the infusion while nor-BNI only tended to decrease intermittent ethanol intake. The systemic effects of JDTic seem to be more pronounced than those after intra-accumbens shell administration on ethanol intake, as ethanol intake was significantly decreased 2 days after the systemic injection. Four days after administration systemic JDTic showed an ethanol intake decreasing trend. The long-term κ-antagonistic effects of JDTic are in line with previous reports (Carroll et al. 2004; Deehan et al. 2012).

In continuous or limited ethanol access paradigms, the results of κ-antagonists on ethanol intake have been inconsistent as it has been reported that systemic nor-BNI, CERC-501 and JDTic either decreased (Logrip et al. 2008; Rorick-Kehn et al. 2014; Schank et al. 2012), parallel to the results reported here, or had no effect (Deehan et al. 2012; Doyon et al. 2006; Lindholm et al. 2001) on ethanol intake or operant responding. A long-term increase in ethanol intake after systemic nor-BNI has also been shown in a continuous ethanol access paradigm (Mitchell et al. 2005). The delayed and long-term decreasing effect of JDTic on ethanol intake shown in the current study is in contrast to the results reported by Schank et al. (2012) stating that only the pretreatment time of 2 h but not longer for both JDTic and nor-BNI was able to decrease ethanol intake in the limited ethanol access paradigm. Neither acute intra-accumbal infusions nor systemic injections of JDTic had any immediate effects on 90 min ethanol intake in the current study.

It has been proposed that ethanol intake in the intermittent ethanol access paradigm may partly be driven by similar mechanisms that drive the ADE, a model of relapse-like ethanol intake attributed to ethanol addiction (Rosenwasser et al. 2013). This is because of the concurrent short deprivation periods in the intermittent ethanol access paradigm, while in the ADE paradigm the deprivation periods are only longer. In line with this, ethanol intake has been shown to be higher in intermittent than continuous ethanol access paradigms (Simms et al. 2008). Additionally, AA rats have been shown to increase ethanol intake after short deprivation periods (under 24 h) even though they are considered not to express an ADE after longer deprivation periods (1 week) (Sinclair and Li 1989; Sinclair and Tiibonen 1988; Vengeliene et al. 2003). Given that changes in the κ-opioidergic system
have been suggested as ethanol addiction evolves, with an increased κ-opioidergic tone in the nucleus accumbens (Gulya et al. 1993; Karkhanis et al. 2016; Lindholm et al. 2000; Nealey et al. 2011; Przewlocka et al. 1997; Rose et al. 2016; Siciliano et al. 2015; Walker and Koob 2008), some of these changes might also contribute to the results presented here.

Indeed, the current κ-antagonist results showing a decrease in intermittent ethanol intake are seemingly in line with those reported from paradigms addressing aspects of ethanol addiction and physical dependence. Previously, a single systemic dose of JDTic has been shown to attenuate both ethanol seeking and relapse-like ethanol intake (Deehan et al. 2012). Nor-BNI, however, did not affect relapse-like ethanol intake in rats (Hölter et al. 2000). Some studies suggest that for nor-BNI to be able to reduce ethanol intake, the subjects have to first be made physically dependent of ethanol with intermittent ethanol vapor procedures (Nealey et al. 2011; Rose et al. 2016; Walker and Koob 2008; Walker et al. 2011). In these studies, intra-accumbens shell, ICV and systemic nor-BNI decreased operant ethanol responding only in animals considered physically ethanol-dependent but had no effect in non-dependent animals. These results are interesting when compared to the current results, as the rats in the current study were not physically dependent of ethanol but still a decrease in ethanol intake was evident after κ-opioid receptor antagonist administration. However, a short-acting κ-opioid receptor antagonist, a 6-naltrexamine analog, decreased ethanol intake irrespective of the induced physical dependence state (Cashman and Azar 2014). With more similarity to the paradigm used here, CERC-501 has been shown to selectively decrease ethanol intake only in rats which showed escalated ethanol intake in the intermittent ethanol access paradigm (Domi et al. 2018). It is unclear whether the differences in the used paradigms or the used κ-opioid receptor antagonists themselves or both are the reason for the discrepancies in the previous reported and also the current results.

In the current study, acute U50488H had no effects on intermittent ethanol intake in AA rats. This is in line with earlier reports stating that acute intra-accumbens shell U50488H did not affect ethanol intake in a limited ethanol access paradigm in Sprague-Dawley rats (Barson et al. 2009). In contrast to these results, systemically administered κ-opioid receptor agonists generally decrease maintenance ethanol intake (Henderson-Redmond and Czachowski 2014; Lindholm et al. 2001; Nestby et al. 1999; Zhou et al. 2017). In models addressing aspects of ethanol addiction and physical dependence, ethanol intake and ethanol seeking generally increased after systemic κ-agonist administration (Anderson et al. 2016; Funk et al. 2014, 2019; Harshberger et al. 2016; Hölter et al. 2000; Le et al. 2018; Rose et al. 2016) (however, see Zhou et al. 2018). It seems that nucleus accumbens shell κ-opioid receptor agonism per se is not sufficient to alter ethanol intake.

Despite similarity of the current results to those from ethanol addiction and physical ethanol dependence models, the rats in the current study were
most probably neither addicted nor physically dependent of ethanol despite high ethanol intake levels. As not all patients with ethanol use disorders are addicted or physically dependent of ethanol, despite abuse of ethanol, the current results may have some translational value to these situations. As the intermittent ethanol access paradigm has also been referred to as a binge drinking model, it could in that sense also somewhat model ethanol abuse-behavior seen in patients (Crabbe et al. 2011). In regard to this aspect, the current results suggest that also those who abuse of ethanol could benefit from selective κ-opioid receptor antagonism. However, whether the current model afflicts negative consequences to the rats as ethanol abuse does in humans is debatable.

The current results give new knowledge on the role of the κ-opioidergic system in controlling ethanol intake. Especially the role of the nucleus accumbens shell κ-opioid receptors in controlling intermittent ethanol intake was addressed. Previous studies have not addressed this by using selective κ-opioid receptor antagonists in voluntary ethanol access paradigms. The results suggest that κ-opioid receptor antagonism is able to decrease intermittent ethanol intake in AA rats. Additionally, nucleus accumbens shell κ-opioid receptors have a role in mediating these κ-antagonistic effects on intermittent ethanol intake, even though direct agonism of the κ-opioid receptors did not affect ethanol intake. Selective κ-opioid receptor antagonism could be a feasible strategy for decreasing ethanol intake in patients who abuse of ethanol.

6.3 ROLE OF K-OPIOID RECEPTOR ANTAGONISM IN RELAPSE-LIKE ETHANOL INTAKE

The role of the κ-opioidergic system in controlling ethanol intake has been suggested to increase as ethanol intake becomes compulsive, chronic and relapsing (Karkhanis et al. 2017; Sirohi et al. 2012; Walker et al. 2012). The activational state of the κ-opioidergic system especially in the nucleus accumbens has been proposed to increase as ethanol addiction and physical dependence develop (Gulya et al. 1993; Karkhanis et al. 2016; Lindholm et al. 2000; Nealey et al. 2011; Przewlocka et al. 1997; Rose et al. 2016; Rosin et al. 1999; Siciliano et al. 2015). To date, however, the majority of research has addressed the question by indirectly measuring what effects various addiction and physical dependence paradigms cast on the κ-opioidergic system interacting with the nucleus accumbens e.g. by using fast scan cyclic voltammetry in brain slices or by measuring dynorphin mRNA levels. The pharmacological verification of these results on addiction- and physical dependence-related behaviors are often inadequate. The role of the nucleus accumbens shell in controlling ethanol intake has only been addressed in physically ethanol dependent animals (Nealey et al. 2011). Alternatively, selective κ-opioid receptor antagonists have been administered systemically
or ICV (Cashman and Azar 2014; Deehan et al. 2012; Domi et al. 2018; Funk et al. 2014; Höltel et al. 2000; Rorick-Kehn et al. 2014; Rose et al. 2016; Sandi et al. 1990; Schank et al. 2012; Walker and Koob 2008; Walker et al. 2011), which cannot give answers to how the κ-opiodergic system in specific brain areas of the reward pathway affect behaviors related to ethanol addiction or physical dependence.

The current study aimed at clarifying what effects κ-opiod receptor antagonism has on relapse-like ethanol intake in Long-Evans rats as evaluated by the ADE paradigm. The role of the nucleus accumbens shell κ-opiod receptors in mediating these effects was especially addressed, as to our knowledge no previous behavioral data is available. Thus, this is the first study to address the role of the nucleus accumbens shell κ-opiod receptors on the ADE. The incentive for these experiments also came from the results of Study II in which JDTic decreased intermittent ethanol intake both after systemic and intra-accumbens shell administrations.

JDTic was able to attenuate the ADE after both systemic and intra-accumbens shell administrations. The current results are parallel to the one previous report for JDTic stating that a single systemic injection (1 to 10 mg/kg) given 25 days prior during deprivation was able to prevent ethanol relapse responding in operant conditions (Deehan et al. 2012). Previous studies using systemic nor-BNI, however, showed that the ADE was not significantly affected by the treatment in rats with over a year of ethanol experience (Höltel et al. 2000). However, a non-significant trend for decreasing ethanol intake was evident during the first 60 min of ethanol re-access.

The current results are generally in line with the ability of κ-opiod receptor antagonists to decrease a variety of other ethanol intake-related behaviors attributed to ethanol addiction. Systemic JDTic and nor-BNI, but not CERC-501, have been shown to block ethanol-associated cue-induced reinstatement of ethanol seeking (Domi et al. 2018; Funk et al. 2014; Schank et al. 2012). Accordingly, systemic JDTic has also been shown to reduce operant responding for the ethanol-associated lever in the Pavlovian Spontaneous Recovery Paradigm, an ethanol-seeking model which measures spontaneous recovery to responding for the ethanol-associated lever without the presence of ethanol after both extinction training and a break from it (Deehan et al. 2012). CERC-501 has been shown to reduce the motivation to lever press for ethanol in a progressive ratio schedule of reinforcement (Rorick-Kehn et al. 2014). Accordingly, nor-BNI attenuated U50488H-induced reinstatement of ethanol-seeking (Funk et al. 2014; Harshberger et al. 2016). In comparison to nor-BNI and CERC-501, JDTic, however, did not attenuate stress-induced ethanol seeking (Domi et al. 2018; Funk et al. 2014; Schank et al. 2012). The current results are also seemingly in line with the ability of intra-accumbens shell, ICV and systemic nor-BNI to selectively attenuate ethanol intake in physically ethanol-dependent animals (Nealey et al. 2011; Rose et al. 2016; Walker and Koob 2008; Walker et al. 2011) and the
ability of a 6-naltrexamine analog, a new short-acting κ-opioid receptor antagonist, to decrease ethanol intake regardless of the physical dependent state of the animal (Cashman and Azar 2014).

In the current ADE paradigm, systemic naltrexone was used as a reference substance. The ability of naltrexone to inhibit the ADE is in line with the results of previous reports (Heyser et al. 2003; Hölter and Spanagel 1999; Orrico et al. 2014; Zhou et al. 2018). No rebound ADE was observed on days 2 to 4 after administration, which also parallels the results of a previous study which used similar small naltrexone doses (Heyser et al. 2003). These results are seemingly in line with the effects of systemic naltrexone on attenuating cue-induced, context-induced, U50488H-induced and ethanol-primed reinstatement of ethanol seeking as well as decreasing ethanol intake in intermittent ethanol vapor exposed rats (Burattini et al. 2006; Ciccocioppo et al. 2002, 2003; Harshberger et al. 2016; Le et al. 1999; Marinelli et al. 2007; Walker and Koob 2008). However, nalmefene has been shown to be more effective than naltrexone in intermittent ethanol vapor paradigms, possibly due to its κ-opioid receptor directed effects (Michel et al. 1985; Walker and Koob 2008). Additionally, prolonged intermittent ethanol vapor exposure has been shown to reduce naltrexone’s ability to attenuate cue-induced reinstatement of ethanol seeking (Ciccocioppo et al. 2003), which could be a reflection of changes in the µ-opioidergic system as physical dependence evolves (Hansson et al. 2018).

In the current study, systemic naltrexone was more effective than JDTic in inhibiting the ADE, as also ethanol intake levels decreased below baseline levels on the day of ethanol re-access. Previously systemic naltrexone has been shown to be more effective than nor-BNI in preventing U50488H-induced reinstatement of ethanol seeking (Harshberger et al. 2016). Even though naltrexone is not a selective µ-opioid receptor antagonist, these results are in line with the notion that as ethanol addiction evolves, the role of the reward-attributed µ-opioidergic system presumably decreases but does not entirely abolish (Hansson et al. 2018; Hermann et al. 2017; Mann et al. 2014; Sinclair 2001). Therefore, reward mediated by the µ-opioidergic system, and possibly also the δ-opioidergic system, seem to also contribute to relapse to ethanol intake. Regarding the ADE, however, this would need to be verified with selective µ-opioid receptor antagonist and agonist studies, which have to date not been conducted.

Considering that the ADE has been suggested to have high clinical relevancy (Spanagel 2000; Vengeliene et al. 2014), the current results on JDTic are encouraging. The previously reported lack of effect of nor-BNI on the ADE might be more attributed to the properties of nor-BNI itself, as discussed in 6.5, rather than to κ-opioid receptor antagonism not generally having an effect on ethanol relapse (Hölter et al. 2000). To further elucidate the role of κ-opioid receptor antagonists on the ADE, other κ-opioid receptor antagonists, such as the short-acting CERC-501 should also be tested.
In conclusion, the current results suggest that negative reinforcement mediated by the κ-opioidergic system is able to mediate relapse to ethanol intake, given that selective κ-opioid receptor antagonism was able to attenuate relapse-like ethanol intake in rats. The nucleus accumbens shell seems to be one anatomical site which mediates these effects. Additionally, naltrexone was able to inhibit the ADE and decrease ethanol intake, suggesting that reward mediated at least by the μ-opioidergic system may also mediate ethanol relapse. Selective κ-opioid receptor antagonism could be used to prevent ethanol relapse in addicted individuals.

6.4 CONSIDERATIONS REGARDING THE ALCOHOL DEPRIVATION EFFECT PARADIGM

The ADE as a phenomenon is interesting, as genetic susceptibility to voluntarily consume high amounts of ethanol and the expression of an ADE do not seem to be related, since not all rat lines bred on the basis of high ethanol consumption show an ADE, including the AA rats (Sinclair and Tiihonen 1988; Vengeliene et al. 2003). Additionally, the occurrence, duration and robustness of the ADE can vary considerably between individual animals, as well as the magnitude of the ADE between different rodent strains (Vengeliene et al. 2014).

Physical ethanol dependence is not required for the ADE to be evident, as the ADE is based on voluntary ethanol intake, which is hardly able to keep blood ethanol concentrations elevated long enough for physical dependence to develop (McKinzie et al. 1998; Rodd et al. 2004a; Sinclair and Senter 1967). The ADE is also not a consequence of withdrawal, a symptom associated with physical dependence, since an ADE can be seen long after withdrawal symptoms have dissipated (Sinclair et al. 1973b). Therefore, the ADE can be considered to be a model of relapse attributed to ethanol addiction (Meinhardt and Sommer 2015; Spanagel 2000). Withdrawal may, however, be a factor to take into account when using shorter deprivation periods. In operant conditions, a significant ADE was shown after a 5 day deprivation period without any signs of withdrawal (Heyser et al. 1997). In the current experiment, the deprivation period was 6 days. An initial voluntary ethanol intake period of 6 to 8 weeks is considered necessary for a reliable subsequent ADE to develop after a deprivation period (Hölter and Spanagel 1999; Hölter et al. 2000; Spanagel and Hölter 1999; Vengeliene et al. 2014; Wolffgramm and Heyne 1995). In the current study, the initial ethanol intake period was 8 to 10 weeks. The current ADE paradigm caused similar effects on ethanol intake during ethanol re-access as has previously been reported in different variations of the ADE paradigm regarding both the magnitude and duration of the ADE (Heyser et al. 1997, 2003; Hölter et al. 2000; McKinzie et al. 1998; Rodd et al. 2004a).
6.5 CONSIDERATIONS REGARDING THE USE OF LONG-ACTING Κ-OPIOID RECEPTOR ANTAGONISTS

In experimental procedures, the most commonly used κ-opioid receptor antagonist is nor-BNI, which is derived from naltrexone (Portoghese et al. 1988). JDTic is a newer phenylpiperidine-based, non-opioid derived κ-opioid receptor antagonist (Thomas et al. 2001). Both antagonists have in common a slow onset of κ-antagonism, which starts at 6 h for JDTic and 4 h for nor-BNI after administration (Carroll et al. 2004; Endoh et al. 1992). Also a prolonged duration of κ-antagonistic activity has been shown for both drugs, lasting up to 4 weeks after JDTic administration (Carroll et al. 2004; Endoh et al. 1992). JDTic is more selective and potent towards the κ-opioid receptor than nor-BNI (Thomas et al. 2001, 2003), which has partly contributed to the increased interest in JDTic and similar compounds.

Both nor-BNI and JDTic have been shown to have transient effects which correlate with plasma levels of the drug and only last for a few hours (Endoh et al. 1992; Munro et al. 2012, 2013). Nor-BNI, but not JDTic, has been shown to have μ-opioid receptor antagonistic effects at least during the first 30 min after administration (Carroll et al. 2004; Endoh et al. 1992; Schank et al. 2012). In one study, both nor-BNI and JDTic decreased ethanol-related behaviors only at 2 h from administration (Schank et al. 2012). When considering possible transient effects of the drugs, the role of κ-opioid receptor antagonism in decreasing ethanol intake in that study is uncertain (Endoh et al. 1992; Munro et al. 2013; Schank et al. 2012). Due to the aforementioned properties of both drugs, 24 h pretreatment times and between-subjects designs have commonly been used in experimental settings (Deehan et al. 2012; Knoll et al. 2011; Rorick-Kehn et al. 2014; Walker et al. 2011).

Due to the positive outcomes in preclinical studies, κ-opioid receptor antagonism has also been considered to be clinically interesting in treating ethanol use disorders. JDTic has been tested in phase I clinical trials (Buda et al. 2015). Unfortunately, heart directed adverse effects (ventrical tachycardia) prevented further clinical trials. It was speculated that the reason for these adverse effects was due to the ability of JDTic to activate the c-Jun N-terminal kinase in an irreversible manner, which not only seems to be the mechanism by which JDTic and nor-BNI both disrupt κ-opioid receptor signaling long-term, but it is also possibly the reason why JDTic can affect the heart in humans (Bruchas et al. 2007; Buda et al. 2015). Therefore, κ-opioid receptor antagonists with less effect on this kinase could be clinically safer. CERC-501 is one such example and it has also been shown to be clinically well tolerated (Lowe et al. 2014). The short-acting effects of it have also been considered to increase its tolerability.
6.6 PLAUSIBLE NEURONAL MECHANISMS UNDERLYING THE \(\mu\)- AND \(\kappa\)-OPIOIDERIC RESULTS

The current results indicate that the nucleus accumbens shell is one brain area of the reward tract that mediates the effects of the \(\mu\)- and \(\kappa\)-opioidergic drugs both during intermittent and relapse-like ethanol intake. The nucleus accumbens shell and its GABAergic medium spiny neuron projections into the ventral pallidum have been suggested to participate in mediating the rewarding effects of ethanol (Ben Hamida et al. 2019; Kemppainen et al. 2010, 2012b; Smith et al. 2009; Wise 2002; Zahm et al. 1985). Disinhibition of the ventral pallidum has been suggested to increase ethanol and other drug intake (Kemppainen et al. 2012a; Smith et al. 2009; Wise 2002). At the level of the nucleus accumbens, \(\mu\)-opioid receptors are found presynaptically on GABAergic terminals and postsynaptically on the GABAergic medium spiny neurons (Dilts and Kalivas 1989; Mansour et al. 1995; Svingos et al. 1996, 1997) (Figure 5). For CTOP to be able to increase intermittent ethanol intake as reported here, the end result would supposedly have to be a decrease of GABA and opioid peptide co-release into the ventral pallidum (Smith et al. 2009; Wise 2002). Speculatively, this could result from a net-effect of CTOP blocking more accumbal inhibitory-acting \(\mu\)-opioid receptors pre- than postsynaptically. Whether intra-accumbally infused CTOP was able to reveal a distinct distribution of \(\mu\)-opioid receptors within the nucleus accumbens shell of AA rats as compared to outbred strains, however, needs to be determined. Speculatively, DAMGO could decrease ethanol intake also by the net-effect of activating more \(\mu\)-opioid receptors pre- than postsynaptically. When combined with previous results on the ability of pallidal opioidergic mechanisms in controlling ethanol intake (Kemppainen et al. 2012b), the current results can also give support to the hypothesis that mechanisms downstream of the nucleus accumbens participate in controlling ethanol intake.

At the level of the nucleus accumbens shell, \(\kappa\)-opioid receptors are located presynaptically on dopaminergic neuron terminals, thus being able to modulate dopamine release (Svingos et al. 1999b, 2001). While ethanol intake is still under control, ethanol-evoked dynorphin has been proposed to activate the presynaptic accumbal \(\kappa\)-opioid receptors, consequently causing ethanol intake to decrease due to a decrease in dopamine release (Al-Hasani et al. 2015; Curran and Watson 1995; Karkhanis et al. 2017; Marinelli et al. 2006). Therefore, it could be assumed that local \(\kappa\)-opioid receptor agonism would presumably result in a decrease and antagonism in an increase in ethanol intake during maintenance ethanol intake. Interestingly, intra-accumbally infused U50488H did not have any effects on intermittent ethanol intake and both systemic and intra-accumbens shell JDTic decreased ethanol intake. This gives implications that the intermittent ethanol intake
paradigm itself has possibly affected the tone of the nucleus accumbens shell κ-opioidergic system.

It has been suggested that once the individual becomes addicted and/or physically dependent of ethanol, the κ-opioidergic system interacting with the nucleus accumbens shell becomes overactivated, resulting in increased function of κ-opioid receptors and an increase in the release of dynorphin (Gulya et al. 1993; Karkhanis et al. 2016, 2017; Lindholm et al. 2000; Nealey et al. 2011; Przewlocka et al. 1997; Rose et al. 2016; Siciliano et al. 2015; Walker and Koob 2008). These changes could result in a hypodopaminergic state to predominate in the nucleus accumbens (Karkhanis et al. 2016, 2017; Rose et al. 2016), which could further drive ethanol intake and cause relapse due to negative reinforcement. This theory is supported by findings which showed that accumbens shell dopamine levels were lower during deprivation than 24 h after re-access to ethanol in rats exposed to repeated ethanol access and deprivation cycles (Hadar et al. 2017). Therefore, κ-opioid receptor antagonism would be able to normalize, in this case increase, the accumbal dopamine levels (Karkhanis et al. 2016, 2017; Rose et al. 2016). The results from the current ADE studies support this theory as intra-accumbally administered JDTic attenuate relapse-like ethanol intake in rats. Also the results from the intermittent ethanol access paradigm seem to support similar neuronal mechanisms to control intermittent ethanol intake.

6.7 SUMMARY AND FUTURE ASPECTS

The results from the current studies suggest that μ- and κ-opioidergic mechanisms are important in controlling intermittent ethanol intake and relapse-like ethanol intake and the nucleus accumbens shell is one anatomical site mediating these effects. These results are in line with the notion that μ-opioidergic mechanisms mediate the rewarding effects of ethanol during maintenance ethanol intake (Herz 1997). The negative reinforcing effects of ethanol mediated by the κ-opioidergic system also seem to control both controlled and uncontrolled ethanol intake. This is also in line with the hypothesis that the role of the κ-opioidergic system in controlling ethanol intake gradually increases as ethanol intake becomes chronic and ethanol addiction gradually develops (Karkhanis et al. 2017; Sirohi et al. 2012; Walker et al. 2012). Further, the results suggest that selective κ-opioid receptor antagonism could be a feasible treatment strategy for ethanol use disorders.

In future research it is important to address the various aspects of ethanol use disorders as ethanol can be abused without the individual being addicted to ethanol. Aspects of ethanol abuse can be addressed by, for example, modifications of the intermittent ethanol access paradigm (Crabbe et al. 2011). To further understand the role of the κ-opioidergic system in decreasing binge-like ethanol intake often associated with ethanol abuse,
JDTic could be examined in animals which do not have genetic susceptibility to high ethanol intake. Since in non-selected rat strains there is more variability between individuals in the amount of consumed ethanol than in ethanol-preferring rat strains, the rats could further be divided into low and high ethanol drinkers before JDTic administration. Thus, the effects of intra-accumbens shell and systemic JDTic could be compared between these two populations to clarify the role of the κ-opioidergic system in escalated and low ethanol intake. Systemic CERC-501 has previously been shown to selectively decrease ethanol intake in Wistar rats which escalated their ethanol intake in the intermittent ethanol access paradigm (Domi et al. 2018).

Because of the stark effects of the reference substance naltrexone in Study III, the role of the µ-opioidergic system, and possibly also δ-opioidergic system, in controlling relapse to ethanol intake needs to be further addressed as it is largely unclear. No previous studies seem to have addressed the question, which is somewhat astonishing considering that relapse is so common in recovering alcoholics. The current knowledge mostly relies on studies conducted with naltrexone, which cannot give exact answers to what role the µ-opioidergic system has during situations of ethanol relapse because naltrexone is a non-selective antagonist. Selective µ-opioid receptor agonists and antagonists would have to be used for that purpose.

The role of the nucleus accumbens shell µ-opioidergic mechanisms in mediating ethanol relapse also needs to be addressed. Implications for a role of accumbal µ-opioidergic mechanisms in behaviors associated with ethanol addiction have been shown in ethanol seeking experiments, in which intra-accumbens shell CTAP blocked context-induced reinstatement of ethanol seeking similarly to ICV CTOP or systemic naloxonazine, all selective µ-opioid receptor antagonists (Ciccocioppo et al. 2002; Marinelli et al. 2009; Perry and McNally 2013a). It seems that reward mediated by the µ-opioidergic system also contributes to ethanol addiction-related behaviors even though it has been suggested that the role of µ-opioidergic mechanisms in controlling ethanol intake somewhat decline concomitantly with the progression of ethanol addiction (Hansson et al. 2018; Sirohi et al. 2012).

Since both JDTic and naltrexone attenuated relapse-like ethanol intake, combinations of selective µ- and κ-opioid receptor antagonists should be investigated for relapse prevention, as such cocktails could simultaneously address the increased anhedonia mediated by the κ-opioidergic system as well as reward mediated by the µ-opioidergic system (Herz 1997; Sirohi et al. 2012; Walker et al. 2012). The clinically available nalmefene in part addresses this suggestion, as it is a partial κ-opioid receptor agonist in addition to being a µ-opioid receptor antagonist (Michel et al. 1985). However, to be able to separate the role of different opioiidergic systems on a given phenomenon, such as relapse-like ethanol intake, as well as to examine their possible additive effects, it is more feasible to use different combinations of selective antagonists instead of a non-selective antagonist.
7 CONCLUSIONS

The main findings of this thesis and the conclusions that can be drawn from them are:

1. A selective μ-opioid receptor antagonist increased and agonist tended to decrease intermittent ethanol intake when administered into the nucleus accumbens shell of AA rats. The results support the notion that μ-opioidergic mechanisms in the nucleus accumbens shell mediate the positive reinforcing and rewarding effects of ethanol and participate in controlling intermittent ethanol intake.

2. Systemic and nucleus accumbens shell administration of a selective κ-opioid receptor antagonist decreased intermittent ethanol intake long-term, though the effect was weak after intra-accumbens shell administration. Acute κ-opioid receptor agonism had no effects on ethanol intake. These results suggest that κ-opioid receptors are able to control intermittent ethanol intake and the κ-opioidergic mechanisms in the nucleus accumbens shell participate in mediating these effects. Selective κ-opioid receptor antagonism could be a feasible strategy for decreasing ethanol intake.

3. A selective κ-opioid receptor antagonist attenuated the ADE after both systemic and intra-accumbens shell administrations. These results suggest that the κ-opioidergic system participates in mediating relapse-like ethanol intake and nucleus accumbens shell κ-opioidergic mechanisms have a role in it. Selective κ-opioid receptor antagonism could be used to prevent ethanol relapse.
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